

ISBT Science Series

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Special issue: Introduction to Blood Transfusion: From Donor to Recipient

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**Special issue: Introduction to Blood
Transfusion: From Donor to Recipient**

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First Edition

Introduction to Blood Transfusion Technology

In 1989, following several years of being national examiner for blood transfusion technology in South Africa, I wrote a book that was updated in 1992: *Introduction to Blood Transfusion Technology*. The aim was to provide written information as simply as possible, so that students who found themselves without adequate training and support were able to gain knowledge for themselves. The book was privately printed and distributed in South Africa for many years. Some copies also found their way to other parts of the world . . .

Technical information, such as that given in *Introduction to Blood Transfusion Technology* should be regularly reviewed and updated, but unfortunately this did not take place.

In April 2007 the International Society of Blood Transfusion (ISBT) made the rewriting of this original publication a possibility by offering to support its printing and dissemination by both physical and electronic means. In June 2007 the project began its journey towards becoming a reality, thanks to a small and select group of medical technologists specialized in blood transfusion technology, with a combined total of more than 170 years' experience. A short introduction follows: Rob Wilkinson, an expert in all aspects of quality, particularly within the management of a transfusion service, wrote the 'Quality' section as well as 'Equipment and materials management', and 'Safety, health and the environment', besides giving critical comment on all sections and coauthoring 'Genetics' and 'Compatibility testing'. Elizabeth Smart, an experienced immunohaematologist with international recognition, coauthored many sections; her major input being the 'Blood group systems'. Her contribution to the 'Principles of laboratory techniques', 'Donation testing', 'Haemolytic diseases' and 'Compatibility testing' has also been invaluable. Jonathan Hardwick, an expert in cold chain management and the preparation of blood components, has contributed outstanding value in writing the sections on 'Blood processing' and 'Blood storage and transportation'. Leesha Raman, a practicing training officer, coauthored 'Principles of laboratory techniques' and 'Donation testing' and provided important practical input into the other sections, particularly from the perspective of the student. I was involved in training and development for many years in South Africa, and recently had the privilege of working in Blood Transfusion Safety at the World Health Organization in Geneva, Switzerland.

The original publication of *Introduction to Blood Transfusion Technology* consisted of 150 pages and included the fundamentals of haematology, immunology, antigen-antibody

reactions, causes of false results, genetics, blood group and HLA systems, blood donation and storage, blood components, safety in the laboratory, quality control and assurance, proficiency testing, donor grouping and the detection of viral markers, crossmatching, transfusion reactions, blood transfusion regulations, haemolytic disease of the newborn, antenatal and postnatal testing, haemolytic anaemias, laboratory glassware and equipment and sterilization.

This publication is not an updated edition of the original texts that were written almost 16 years ago. Although it shares the same title as its predecessor, it takes into account the advances in technologies and presents introductory information for international benefit. The primary objective of this publication has been to meet the academic needs of students whose courses relate to blood transfusion technology, particularly when they find themselves in areas of the world where there is not ready access to information and facilities. It has not been written for experts; it has been written as a training manual to give access to the inexperienced. It contains a broad spectrum of information with sufficient detail to provide a platform for students to build on their knowledge and become experts themselves one day.

The information covers the transfusion chain and is supported by many illustrations, a comprehensive glossary, an index and list of abbreviations. Information that relates to more than one section has either been cross-referenced in the text or duplicated sufficiently in the section so that each can be studied as a stand-alone module. Each section lists the learning objectives, provides a content summary and suggests additional learning activities. The scope of this publication does not include some of the more recent technological advances, such as stem cell production, cord blood banking and detailed molecular techniques and findings.

Education is a crucial component of lifelong learning and continuous improvement. Without it, quality cannot be attained and maintained in a blood transfusion service, and professional capacity and leadership cannot be built for the future. Blood transfusion is about saving lives, and we should take this very seriously. Although a blood service needs to run as a business, it first needs to cherish and develop its scientific capacity.

Nelson Mandela has said on many occasions that 'education is the key', and the five of us fully endorse this philosophy.

BERYL ARMSTRONG

Second Edition

Introduction to Blood Transfusion: from donor to recipient

The wisdom of the International Society of Blood Transfusion (ISBT) in making expert resources available to update the 2008 publication, *Introduction to Blood Transfusion Technology*, which ISBT distributed at the Macau congress and thereafter, is to be admired. It is very much appreciated by the original authors, and especially by the two, from the original five, who were able to participate in the current review.

The decision to update the publication was prompted by the evidence that electronic versions of the various chapters of the first edition were downloaded from the ISBT Academy website more than 62 000 times since 2010, with more than 20 000 downloads in 2018 alone.

Two of the original authors, Rob Wilkinson and Beryl Armstrong, worked on the review with the editor of the second edition, Mindy Goldman and a dedicated group of ISBT members who provided updates within their areas of expertise. These expert reviewers are from Asia, the Middle East, Europe, North and South America, and Australia – and as the two original authors are from Africa – this is truly an international effort. The ISBT Publications Committee are thanked for their valuable inputs. This second publication reflects the global nature of the effort while remaining true to the original ideal of producing a text suitable for students whose first language may not be English, and who may not have access to expert tuition.

With the extended scope of the updated publication, it was felt that a name change should be considered. Therefore, the 2020 release is called *Introduction to Blood Transfusion: from donor to recipient*.

Beryl Armstrong: From my perspective, this project signifies the continued fulfilment of a dream – to bring introductory knowledge to students in the field of blood transfusion, in a way that could be understood without necessarily enjoying the guidance of an instructor. When

I began working in the laboratories in 1964 at the Natal Blood Transfusion Service in Durban, South Africa, there was a commemoration plaque at the entrance of the building, from which I drew inspiration. The plaque carried a message related to the opening of this new blood service and read: ‘*In the service of humanity.*’ Writing the first book, with this in mind, and with a South African team of five, and then being fortunate enough to work with a much wider group of professionals from around the world, in providing this update, means that the book is brought to you yet again, in the service of humanity.

Rob Wilkinson: In the twelve years since the release of the first edition of *Introduction to Blood Transfusion Technology* in June 2008 I have been gratified to see the extent to which the book has been used as a learning resource in Africa. As a member of the team from the Africa Society for Blood Transfusion working to strengthen blood services in Africa I was privileged to visit many less developed blood services throughout Africa, where easy access to training materials are sorely needed, and it was humbling to see “our book” being used and discussed by laboratory and collection staff alike. This has been motivation enough to participate in the preparation of this, the second edition, which I trust will prove to be at least as useful as the first edition, and which will, in some small way, contribute to the safety of blood transfusions in the developing world.

My personal thanks go to Beryl for her tireless and inspirational efforts in writing, editing and generally shepherding this work to its conclusion.

BERYL ARMSTRONG AND ROB WILKINSON
South Africa

Introduction to Blood Transfusion: from donor to recipient

Blood transfusion is an essential component of medical care, contributing to saving and enhancing the lives of millions of patients worldwide.

The ISBT is a global community of professionals sharing knowledge to enhance transfusion practice. Our strategic goals include playing a prominent role in transfusion medicine education and knowledge sharing.

We are therefore delighted to present this update of the much loved first edition of *Blood Transfusion Technology*, written by Beryl Armstrong with colleagues Rob Wilkinson, Elizabeth Smart, Jonathan Hardwick and Leesha Raman from South Africa, first printed in 2007 and much downloaded from the ISBT website ever since. We have renamed the second edition *Introduction to Blood Transfusion: from donor to recipient* to emphasize the entire transfusion chain that extends vein to vein from donor to recipient, encompassing the expertise of diverse professionals involved in all aspects of transfusion.

We thank the international team of transfusion medicine experts, including scientists, technologists, nurses and physicians from five continents for their assistance in updating the original text. We owe a particular thank you to Beryl and Rob for giving so generously of their time to review all the sections in their entirety.

In its updated version, the text will continue to be a valuable resource to all professionals who require a sound knowledge of the basic principles that underpin good practice in transfusion medicine. The material is useful in all settings, but particularly in low- and medium-income countries where specialist education in blood transfusion may be more difficult to access, bringing us closer to our vision of a world of safe and sufficient blood.

MINDY GOLDMAN, Editor and Chair, ISBT Publications Committee,
JENNY WHITE, Executive Director, ISBT and
ERICA WOOD, President, ISBT

ABBREVIATIONS

Abbreviation	Term in full	Abbreviation	Term in full
2,3 DPG	2,3 diphosphoglycerate	HBsAg	Hepatitis B surface antigen
2-ME	2-mercaptoethanol	HBV	Hepatitis B virus
Å	Angstrom units	Hct	Haematocrit
Ab	Antibody	HCV	Hepatitis C virus
ACD	Acid citrate dextrose	HDFN	Haemolytic disease of the fetus and newborn
Ag	Antigen	HES	Hydroxyethyl starch
AHF	Anti-haemophilic factor	HFA	High-frequency antigen
AHG	Antihuman globulin	HIV	Human immunodeficiency virus
AIDS	Acquired immune deficiency syndrome	HLA	Human leucocyte antigen
AIHA	Autoimmune haemolytic anaemia	HPA	Human platelet antigen
ATP	Adenosine triphosphate	HPA	Hybridisation protection assay
BC	Buffy coat	HTLA	High titre low avidity (antibodies)
BP	Blood pressure	HTLV	Human T-cell lymphotropic virus
BSE	Bovine spongiform encephalopathy	IAT	Indirect antiglobulin test
C'	Complement	IFA	Immunofluorescent antibody
CFC	Chlorofluorocarbon	IgG	Immunoglobulin G
CHAD	Cold haemagglutinin disease	IgM	Immunoglobulin M
CMV	Cytomegalovirus	ITP	Idiopathic (or immune) thrombocytopenic purpura
CPD	Citrate phosphate dextrose	IVIg	Intravenous immunoglobulin
CPD-A	Citrate phosphate dextrose adenine	LED	Light-emitting diode
CSF	Colony-stimulating factor	LFA	Low-frequency antigen
DAT	Direct antiglobulin test	LISS	Low ionic strength saline
DIC	Disseminated intravascular coagulation	mf	Mixed-field agglutination
DNA	Deoxyribonucleic acid	MHC	Major histocompatibility complex
dNTP	Deoxynucleotide triphosphate	mRNA	Messenger ribonucleic acid
DTT	Dithiothreitol	NAT	Nucleic acid testing
EBV	Epstein-Barr virus	NISS	Normal ionic strength saline
EDTA	Ethylenediaminetetraacetic acid	NT	Not tested
EIA	Enzyme immunoassay	PAS	Platelet additive solution
ELISA	Enzyme-linked immunosorbent assay	PBS	Phosphate-buffered saline
EQA	External quality assessment	PC	Platelet concentrate
EQAS	External quality assessment scheme	PCH	Paroxysmal cold haemoglobinuria
F	Factor (clotting)	PCR	Polymerase chain reaction
F1	Filial 1	PEG	Polyethylene glycol
Fab	Fragment antigen binding	pH	Degree of alkalinity or acidity (solution)
Fc	Fragment crystallisable or constant	PH	Partial haemolysis
FFP	Fresh frozen plasma	PMF	Plasma master file
FMAIT	Fetomaternal alloimmune thrombocytopenia	PNH	Paroxysmal nocturnal haemoglobinuria
FMH	Fetomaternal haemorrhage	PPE	Personal protective equipment
FTA	Fluorescent Treponemal antibody absorption	PRP	Platelet-rich plasma
G6PD	Glucose-6-phosphate dehydrogenase	PUBS	Percutaneous umbilical cord blood sampling
GLP	Good laboratory practice	PVC	Polyvinyl chloride
GMP	Good manufacturing practice	PVP	Polyvinylpyrrolidone
GvHD	Graft versus host disease	QA	Quality assurance
H	Haemolysis	QC	Quality control
HAV	Hepatitis A virus	RBC	Red blood cell
Hb	Haemoglobin	RCC	Red cell concentrate
HbA	Adult haemoglobin	rcf	Relative centrifugal force
HbF	Fetal haemoglobin	RIA	Radioimmunoassay

Abbreviation	Term in full	Abbreviation	Term in full
RIPA	Radioimmunoprecipitation assay	UV	Ultraviolet (light)
RNA	Ribonucleic acid	vCJD	Variant Creutzfeldt-Jakob disease
RPR	Rapid plasma reagin	VNRBD	Voluntary non-remunerated blood donor/donation
S	Svedberg units	VWD	von Willebrand's disease
SAGM	Saline adenine glucose mannitol	vWF	von Willebrand factor
SARS	Severe acute respiratory syndrome	VZ	Varicella zoster
SCD	Sterile connecting device	WNV	West Nile virus
S/D	Solvent/detergent	Symbol	Other miscellaneous abbreviations
SG	Specific gravity	°C	Degrees Celsius
SLE	Systemic lupus erythematosus	Ca ⁺⁺	Calcium ions
SOP	Standard operating procedure	CuSO ₄	Copper sulphate
TACO	Transfusion-associated circulatory overload	Da	Dalton
Taq	Thermus aquaticus	g	Gravitational force
TA-GVHD	Transfusion-associated graft vs. host disease	g/mL	Grams per millilitre
TMA	Transcription-mediated amplification	HCl	Hydrochloride
TPHA	Treponema pallidum haemagglutination test	Hg	Mercury
TQM	Total quality management	ID	Identification code
TRALI	Transfusion-related acute lung injury	IU	International units
TRIM	Transfusion-related immune modulation	Mg ⁺⁺	Magnesium ions
TSE	Transmissible spongiform encephalopathy	MSM	Men who have sex with men
TTI	Transfusion-transmissible infection	NaCl	Sodium chloride
TTP	Thrombotic thrombocytopenic purpura	rpm	Revolutions per minute
TTV	Transfusion-transmitted virus	SI	International system of units
UPS	Uninterrupted power supply	Vol	Volume

Original authors: Beryl Armstrong, Jonathan Hardwick, Leesha Raman, Elizabeth Smart & Rob Wilkinson

Reviewers for Second Edition: Beryl Armstrong & Rob Wilkinson

The entries are defined and described mainly from the perspective of transfusion medicine. Additional entries that do not necessarily appear in the text may be included; others within the text may have been omitted from this glossary. Some entries are cross-referenced; other entries are 'opposites', such as acute and chronic, or 'relate' to each other, such as immunodiffusion and precipitation, and although separately defined and described, these entries are flagged in the subject title as 'see also'.

2,3 diphosphoglycerate (2,3 DPG) – see also adenosine triphosphate (ATP)

2,3 diphosphoglycerate (2,3 DPG) is present in red cells and together with haemoglobin, controls the amount of oxygen released to the tissues of the body. 2,3 DPG levels deteriorate in stored red cells which then lose their ability to release oxygen rapidly at tissue level. Post-transfusion, it takes about 24 hours for the 2,3 BPG in stored red cells to fully regain functionality.

2-mercaptoethanol (2-ME) – see also dithiothreitol (DTT)

2-mercaptoethanol (2-ME) is a reducing agent used to differentiate between IgM and IgG antibodies. When mixed with serum containing IgM antibodies, some of the disulphide bonds are broken. As a result, the IgM antibodies become less efficient or lose their ability to agglutinate red cells carrying the corresponding antigen. 2-ME is extremely strong smelling and to minimise inhalation it should be used only in a well-ventilated area apart from other personnel. 2-ME may cause irritation of the eyes, skin and respiratory tract, so protective clothing should be worn. Safety protocols for the storage of potentially harmful chemicals should be followed.

Absorption – see adsorption

Accident – see also incident

This may be viewed from the occupational safety angle or from the perspective of transfusion. An accident is an unplanned event with an unfavourable outcome. For example, a brick falls from the building site, hitting an

individual on the head, or a unit of blood intended for another patient is accidentally transfused.

Accreditation

This is the process whereby an agency evaluates an organisation against a predetermined set of documented standards, and, if the organisation meets or exceeds the standards, accredits the organisation.

Acid citrate dextrose (ACD) – see also citrate phosphate dextrose A1 (CPDA-1)

Acid citrate dextrose (ACD) is an anticoagulant solution containing citric acid (A), sodium citrate (C) and dextrose (D).

Acquired immune deficiency syndrome (AIDS) – see also human immunodeficiency virus

Acquired immune deficiency syndrome (AIDS) is the result of untreated infection with the human immunodeficiency virus (HIV). Individuals who are seropositive become prone to infections. This is because the ability of the immune system to overcome micro-organisms becomes increasingly impaired by the actions of HIV. If an opportunistic infection such as tuberculosis (TB), hepatitis B, shingles or CMV is contracted, there is a serious impact on health and such an infection may become life-threatening.

Acute – see also chronic

When the medical condition of the patient is termed acute, it means that it is of sudden onset. For example, hypovolaemic shock may be the result of sudden haemorrhage.

Acute normovolaemic haemodilution

This is the deliberate reduction in the amount of haemoglobin in the blood, usually by the withdrawal of whole blood from a patient just prior to elective surgery. Blood volume is restored, but the number of circulating red cells is reduced. After surgery, the blood that was withdrawn may be re-infused if needed, and this prevents the use of allogeneic blood, which may be considered less safe.

Additive solution

Red cells separated from whole blood collected in CPD are suspended in an additive solution such as AS-3 for improved storage and shelf life extended up to 42 days.

Adenosine triphosphate (ATP) – see also 2,3 diphosphoglycerate (2,3 DPG)

Breakdown of ATP provides the energy required by cells to carry out energy requiring processes. Energy rich compounds (such as glucose or dextrose) are taken into the cell, and enzymes break them down to release their potential energy. The released energy must be stored in a form that the cell can utilise, and this is known as ATP.

Adjuvant

An adjuvant is a substance that may stimulate the immune system to enhance its response to an immunogen, increasing the speed and intensity of the response to a smaller dose of immunogen, without altering its nature.

Adsorption

This is the process by which an antibody is removed from serum/plasma through the addition of red cells carrying the corresponding antigen. Serum/plasma that has been rendered free of antibody in this way is said to have been adsorbed. Red cells that have bound an antibody from serum/plasma are said to have adsorbed that antibody. The term adsorb should be used in this context and not the term absorb.

Agammaglobulinaemia

This term means 'without globulin'. Patients with agammaglobulinaemia have an absence of serum immunoglobulin and this causes primary immunodeficiency. It may be sex-linked or inherited because of an autosomal gene and may be of early or late onset. Patients suffer recurrent infections, and often have chronic respiratory tract disease. Prophylactic, chronic treatment with intravenous immunoglobulin is usually helpful. Hypogammaglobulinaemia is the term used to describe a condition in which the amount of serum immunoglobulin is reduced rather than absent.

Agglutination

In the laboratory, this is a visible clumping reaction that may be seen with the naked eye when an antibody in the serum/plasma binds to its specific antigen. The reaction

is called haemagglutination when the antigen is found on a red blood cell, but the term agglutination is often used when referring to red cell agglutination.

Agglutinin

A substance that makes particles (such as red cells) for which it is specific, clump together or agglutinate in a visible way, is called an agglutinin. For example, anti-A (in the ABO blood group system) is an agglutinin.

Albumin – see also bovine serum albumin

This is a protein extracted (fractionated) from human plasma and used in place of whole plasma for the treatment of shock and burns or to restore blood volume. It is pasteurised to make it a viral-safe product.

Allele or Allelomorph

An allele is one of the alternate genes in a series of genes at the same locus. Each individual inherits two alleles for each gene at each autosomal locus, one from each parent. For example, *A* and *B* are alleles. Normally the gene products, expressed in terms of red cell antigens, are termed *antithetical*.

Allelomorph – see *Allele*

Allergen

This is a substance with which the corresponding antibody reacts (usually of the IgE class of immunoglobulin) to cause an allergic reaction in the host. For example, pet dander or pollen is an allergen and causes a reaction, such as hay fever in those with the allergy to the specific substance.

Alloantibody – see also *antibody*

Immunoglobulin that is produced against an antigen that is foreign to the individual, but in the same species.

Allogeneic blood

This is blood that is collected from a different member of the same species. Blood banks store allogeneic blood as available stock.

Alloimmunisation

Alloimmunisation is the production of antibodies in an individual against the antigens of another individual of

the same species. For example, a patient who is negative for the K antigen may become alloimmunised to K when transfused with K positive blood. Another older term that is sometimes used is isoimmunisation.

Amniocentesis

This is a process whereby a small sample of amniotic fluid is withdrawn from the sac surrounding the fetus in the uterus of a pregnant woman, in order to carry out tests to determine medical information about the fetus.

Amniotic fluid

This is the fluid that surrounds the fetus *in utero*. It supports and protects the fetus, allowing it to move around as it grows and develops. Amniotic fluid may be sampled to test for various conditions, such as the karyotyping of fetal epithelial cells to determine genetic information, or the measurement of bilirubin levels to determine the severity of haemolytic disease.

Amorph

An amorph is an allele that has no apparent gene product. For example, in the genes controlling the ABO antigens, the *O* gene does not result in a specific antigen being found on the red cells and may be referred to as an amorph.

Anaemia

This is the term used to describe a reduction in the number of circulating red blood cells and/or in the level of haemoglobin.

Anamnestic response

This is the memory response of the immune system when it has been primed (exposed) to an immunogen in the past, and when the same immunogen is presented again, there is a recall response to produce antibodies more rapidly than if the immunogen was being encountered for the first time.

Anaphylaxis

This is a severe allergic reaction that occurs with subsequent exposure to an immunogen that initially stimulated the production of immunoglobulin E (IgE) antibodies. This type of immune reaction leads to the release of powerful chemicals that cause vasodilatation, a drop in blood pressure and swelling of the bronchial tubes. This causes

restrictions to breathing and may be life-threatening without medical intervention. Anaphylactoid reactions resemble anaphylactic reactions clinically but are not caused by an IgE-mediated immune response. The two terms are sometimes used interchangeably.

Angstrom units (Å)

A unit of length equal to 10^{-10} m.

Antenatal

The antenatal period refers to the time during which the female is pregnant. The word antenatal literally means before birth.

Antibody (Ab)

During the immune response, antibodies that are able to react specifically with the foreign immunogens that stimulated their production are formed in the blood, with a view to destroying those specific immunogens. An alloantibody is specific for an antigen within the same species; an autoantibody is directed towards self, and a heterophile antibody is directed towards an antigen in another species.

Anticoagulant

An anticoagulant is a solution that prevents blood from clotting. Anticoagulants such as ACD and CPDA-1, used in the collection of donor blood, must be non-toxic in order to be safely transfused along with blood. Donated blood usually has additional preservatives added to assist in the storage of the blood and allow for a longer shelf life.

Antigen (Ag) – see also immunogen

An antigen is a substance that, although it is able to combine with specific antibody, does not provoke an immune response unless it is immunogenic. Although the terms antigen and immunogen are often viewed as synonyms, the term immunogen is correctly used in the place of antigen to describe a substance that stimulates the production of antibodies by the immune system.

Antigen-presenting cell (APC)

This is a cell that attaches to antigenic epitopes and locks them onto its surface. The antigen-presenting cell then transports the captured antigen to other cells within the immune system that can initiate an antibody

response. In other words, the antigen is 'presented' to immunocompetent cells for their response.

Anti-haemophilic factor (AHF)

AHF is an older term for clotting factor VIII. It can be extracted from fresh frozen plasma by the fractionation process and is used to replace factor VIII in those with FVIII deficiency, such as individuals with haemophilia A. It may also be produced using recombinant technology without human plasma as a starting material.

Antihuman globulin (AHG)

Antihuman globulin (AHG) consists of antibodies that are directed against human proteins. This reagent was originally produced by injecting human immunoglobulin or human complement into animals of a different species, such as rabbits or goats, to stimulate the production of antibodies. Once the AHG is standardised, it is used in laboratory tests to bring about the agglutination of red cells sensitised either with IgG antibodies or complement. AHG is now manufactured using monoclonal production technology.

Antiserum

This is the term commonly used for a blood grouping reagent containing specific red cell antibodies. Antibodies in antiserum against bacteria or viruses can bind with the infectious agent.

Antithetical – see *allele*

Anuria

This term describes the failure of the kidneys to produce urine. It is one of the signs of a haemolytic transfusion reaction, in which the kidneys may be damaged.

Apheresis

This refers to the automated process whereby whole blood is taken from the donor (while he/she is attached to the apheresis machine) and then immediately processed by the machine to harvest a selected component and re-infuse the rest. The machine is programmed according to the component of blood to be harvested, such as plasma or platelets.

Arachis hypogaea – see also *T-activation*

This is a lectin derived from groundnuts (peanuts) that will detect T-activation of red cells. Red cells may

become T-activated when their surfaces are eroded by the action of bacteria.

Assessment

This is a procedure used to evaluate the performance and effectiveness of a system, such as an organisation's quality management system.

ATP – see *adenosine triphosphate*

Attenuated

Attenuated literally means weakened. This is a term used to describe a micro-organism that has been weakened or 'subdued' by a laboratory process, while maintaining its immunogenic properties. Attenuated strains of viruses may be used to inoculate or vaccinate individuals to produce antibodies and become immune without causing illness.

Audit (Quality)

A quality audit is an independent evaluation in order to assess whether the operational activities of an organisation comply with the documented and planned provisions, such as the organisation's quality policy, operating procedures, general policies and any applicable legislation.

Autoimmune haemolytic anaemia (AIHA)

Autoimmune haemolytic anaemia (AIHA) is an anaemia caused by the action of autoantibodies, or antibodies against self. The red cells of the individual become sensitised and this may result in their reduced lifespan, leading to anaemia.

Autologous control

This is a control carried out (in parallel with laboratory tests) on the red cells and serum/plasma of an individual. There should be no reaction in this control for the test results carried out at the same time, to be valid.

Autologous transfusion

When patients scheduled for elective surgery can donate and later receive their own blood during or after the operation, this is referred to as autologous transfusion.

Autoimmune disease

An autoimmune disease occurs when the immune system fails to recognise one or more of the host's tissues as

“self” and mounts an immune attack on that tissue. Examples are autoimmune haemolytic anaemia (AIHA) and systemic lupus erythematosus (SLE).

Autosome

An autosome is any one of the chromosomes that are not involved in the determination of sex and are alike in both males and females. Humans have 22 pairs of autosomes.

Bactericidal

This describes an antiseptic solution or biological substance (e.g. white cells or plasma) that can kill bacteria.

Bilirubin

One of the waste products of haemoglobin breakdown is called bilirubin. It is a yellow-pigmented substance that gives newborn infants suffering from haemolytic disease of the fetus and newborn or physiological jaundice their characteristic jaundiced (yellow) colour.

Blood bank

The responsibility for collection, testing, processing and provision of safe blood for transfusion, within a defined area, region or country, is that of the blood service. The blood bank is the depot/laboratory in the hospital, where blood and blood products are stored prior to issue, and is part of the hospital transfusion service. Some products, such as red blood cells, must be crossmatched for specific patients before transfusion, to ensure that blood is compatible. These tests are usually the blood bank's responsibility.

Blood components

Blood components are those therapeutic parts of whole blood that are prepared by centrifugation, separation and specialised storage and which are prescribed specifically according to patients' needs. They may also be collected by apheresis.

Blood group system

A blood group system is comprised of one or more blood group antigens controlled at a single genetic locus or by two or more closely linked alleles.

Blood product

This is any therapeutic product derived from blood or plasma. Products include plasma, red cells, platelets,

and cryoprecipitate, which are derived from whole blood and sometimes called fresh components. Plasma contains different proteins such as albumin, fibrinogen, immunoglobulin, and clotting factors, some of which are separated by fractionation techniques.

Bovine serum albumin

This is a laboratory reagent that is used as a potentiator for the agglutination of sensitised red cells.

Bromelin

This is a proteolytic enzyme extracted from pineapples and used in blood group serology to enhance certain sensitisation/agglutination reactions.

Buffy coat (BC)

After centrifugation, the BC is the layer of white cells and platelets that is seen between the red cells (at the bottom) and the plasma (at the top) of anticoagulated blood.

Calibration

Calibration is the comparison of a standard or instrument against one of certified accuracy in order to detect and quantify any inaccuracy, and the process whereby the standard or instrument is adjusted to compensate for the inaccuracy. Laboratory equipment is adjusted to perform according to such a reference standard (e.g. a centrifuge rpm meter reading is adjusted to the actual speed of the rotor measured with an approved device, or the mass reading of a scale is set to match the mass of a certified set of standard weights). An external service agent usually performs the calibration at preset time intervals (often annually). Written records of calibration should be kept.

Cancer

A broad term used for any disease that results from cellular changes that cause the uncontrolled division and growth of cells.

Carbohydrate

A large group of organic compounds, including sugars, starches and cellulose. The A and B antigens of the ABO blood group system are examples of carbohydrate antigens. As foodstuff, carbohydrates are broken down to release energy in the human body,

Centrifugation

Special machines, called centrifuges, are used to centrifuge or 'spin' the blood bags (or specimen tubes) around at high speed, using centrifugal force to separate the components of the sample mixture based on differences in density or mass. The bags (or tubes) are placed in the centrifuge in buckets that swing out to a horizontal position when the centrifuge is centrifuging or 'spinning'. In so doing, the heaviest particles (red cells) are forced furthest away from the central spindle. This means that they are forced to the bottom of the bag or tube. The buffy coat of lighter platelets and white cells settles above the red cells and the plasma remains on the top.

Chagas' disease

Trypanosoma cruzi is a parasite transmitted by reduviid bugs that live in Central and South America. The actual bite does not transfer the parasite; when the bite itches and the host scratches it, the infected faeces that the bug has deposited on the skin are able to enter the host, either via the bite site or by hand contact transfer to the eyes or mouth. The disease may also be spread from accidentally ingesting infected bugs, by placental transfer of parasites from mother to fetus, or by transfusion.

Chemical mediators

A nonspecific term for a chemical agent which carries out a reaction or process. For example, chemical mediators such as histamine and serotonin increase vascular permeability and play an important role in the inflammatory response.

Chemokines

Chemokines are small cytokines, or signalling proteins, that are secreted by cells and are able to induce chemotaxis in nearby cells of the immune system.

Chikungunya virus

This virus is spread to humans by infected mosquitoes and outbreaks have occurred in Africa (mainly Central and Southern Africa), South America, Asia and parts of Europe. Symptoms include fever, joint pain, headache, and rash.

Chimera

A chimera is an individual or organism whose cells are derived from two or more zygotes. Twin chimerism in

humans occurs when some of the haemopoietic stem cells of one twin crosses over and develops in the bone marrow of the other twin. The blood of each of the twins may then contain a mixture of their own blood and that of the other twin. If the blood groups of the twins are different this may result in mixed field agglutination being detected in laboratory tests – the individual may appear to have two blood groups. Chimerism may also be seen after a bone marrow transplant.

Chromosome

A chromosome is the threadlike strand of DNA and other proteins found in the nucleus of animal and plant cells, and on which the genes are located. Chromosomes play a role in the passing on of hereditary information.

Chronic – see also acute

A chronic medical condition is one which persists or is ongoing over a prolonged period of time.

Citrate phosphate dextrose A1 (CPDA-1) – see also acid citrate dextrose (ACD)

This is an anticoagulant solution containing citric acid, sodium citrate (C), sodium phosphate (P), dextrose (D) and adenine (A). The amounts used in the formulation vary and variations are indicated by the number at the end, in this case '1'.

Clone

These are cells that are genetically identical in every way and are derived from a single ancestor. A colony of bacteria developed by asexual cell division is an example of a clone.

Closed system – see also open system

In the blood donation procedure, the venepuncture site on the donor's arm is thoroughly cleaned and the sterile needle on the end of the blood bag tubing is inserted into the vein of the donor. In this way a direct connection is made between the donor's circulation and the sterile blood pack. At the end of the donation the blood bag tubing is sealed, and the contents of the blood bag should be free of any bacterial contamination. Once the blood bag has been sealed it is considered to be a closed system and the integrity of the product is ensured while the system remains closed.

Clotting factors

Blood normally clots soon after being shed, or after collection into a test tube or container, unless an anticoagulant is added. There are 13 different proteins or other substances that comprise the clotting factors in the plasma, all of which are essential if normal clotting is to occur. These are the clotting factors.

Coagulation

Blood does not normally coagulate *in vivo*. The coagulation mechanism must be triggered for the coagulation cascade to commence, at the end of which coagulation or clotting occurs. Blood coagulation is the culmination of a series of complex reactions that follows one of two pathways initially; the extrinsic pathway (tissue damage) or the intrinsic pathway (contact activation). This is followed by a common pathway, and the remaining clotting factors activated, after which a clot is formed.

Co-dominant

Genes are co-dominant if both alleles are expressed equally when both are present. For example, in the ABO system the alleles *A* and *B* are co-dominant and when both are present, the red cells will carry both *A* and *B* antigens and the individual will be group AB.

Cold chain management

Management of storage conditions relating to the maintenance of temperature from the time of blood collection, through processing, testing, labelling, to transfusion into a patient is known as 'cold chain management'.

Colloid – see also crystalloid

These are intravenous fluids that contain large molecular mass molecules in suspension, such as proteins or large glucose polymers. Colloids do not pass through the capillary membranes into the tissues and therefore raise the osmotic pressure of blood. Colloids are either extracted from plasma (albumin) or are produced synthetically, such as dextrose starches (hydroxyethyl starches or HES), dextran and gelatines.

Compatible

A blood product is said to be compatible if it causes no adverse reaction in the recipient and if all the transfused components function and survive as expected. Compatibility between donated blood and the blood of an intended recipient is indicated when there is no observable reaction using appropriate laboratory tests with the

serum/plasma of the patient and with the red cells of the donation intended for transfusion.

Complement

Complement plays a role in immune defence and consists of a series of alpha, beta and gamma globulins that are present in all normal sera. Some antibodies activate complement once they have complexed with antigen. Activation of the complement pathway serves as the effector mechanism by which cells coated with complement components are removed from the circulation by macrophages or are immediately destroyed (ruptured or lysed).

Component therapy – see blood components

Computer software validation

This is the process of evaluating that computer programs, together with the computer hardware as configured in the local environment, conform to the specified requirements and perform as expected under all circumstances.

Coolant

Solutions with the ability of absorbing heat at a particular melting point are used as coolants in blood/component transport containers. The selected coolant reaches its melting point and then stays at that temperature for long periods as it is converting from solid phase to liquid phase, thus maintaining the blood/component temperature close to its melting point.

Coombs' test

The Coombs' test is an historical term for the antihuman globulin test, named after one of the scientists (Coombs) who together with Mourant and Race introduced the technique into laboratory use in 1945, although it was discovered many years earlier in 1908 by Moreschi.

Copper sulphate (CuSO₄)

Copper sulphate (CuSO₄) solution is used to qualitatively assess Hb levels of prospective blood donors. The solution is designed to be used with finger-prick samples of blood. Provided that the drop of blood added to the solution sinks to the bottom of the container within about 10 seconds, the individual has a sufficiently high Hb level to give a donation of blood. Failing the CuSO₄ test does not necessarily mean that the individual is anaemic, but

that the Hb level is not high enough to safely donate blood.

Cord cells

These are red cells derived from the umbilical cord at delivery of an infant. A cord blood sample is taken to test the blood of the newborn.

Cordocentesis

The procedure whereby a fetal blood sample is drawn, antenatally, from the umbilical cord. Ultrasound is used to determine the position of the fetus and the umbilical cord.

Corrective action

These are measures that are taken to correct conditions that are impacting adversely on quality in order to eliminate an existing cause of non-conformity.

Creutzfeldt-Jakob disease (CJD)

Creutzfeldt-Jakob disease (CJD) is a type of transmissible spongiform encephalopathy (TSE). These diseases are caused by prions which are misfolded proteins found (as the result of a disease process) in the body. They can transmit their misfolded shape onto normal protein. Their presence in the body leads to several types of fatal neurodegenerative disease, including familial CJD, sporadic CJD, iatrogenic CJD (caused by contaminated instruments or substances, such as human growth hormone), and variant CJD (vCJD). vCJD in humans is caused by the ingestion of cattle afflicted with bovine spongiform encephalopathy (BSE, or mad cow disease) and may rarely be transmissible by transfusion.

Crossmatch

These are the tests performed before transfusion to ensure that the donor's blood will not react adversely with that of the patient when it is transfused. The major crossmatch is performed, in which the patient's serum/plasma is mixed with the donor's red cells. For the blood of the donor to be considered compatible, there must be no reaction between the patient's serum/plasma and the donor's cells.

Cryoprecipitate – see also 'wet' cryoprecipitate

This is a plasma component prepared from frozen fresh plasma by slow thawing of the plasma which causes

precipitation of proteins. This precipitate will go back into solution if the temperature is not carefully controlled to remain sufficiently low. Cryoprecipitate is rich in factor VIII and fibrinogen.

Cryoprotective solution – see *glycerol*

Crystalloid – see also *colloid*

Crystalloids are aqueous (water-based) solutions of low molecular mass salts that may be used for rehydration. They are isotonic or slightly more hypotonic than blood and may contain dextrose. Although they are intravenous replacement fluids, they cross the capillary membrane from the bloodstream into the interstitial spaces and are rapidly distributed within the extracellular compartment.

Cyanosis

This is a bluish discolouration of the skin that is caused by poor oxygenation of the blood, or by a poor circulation.

Cytapheresis

When an individual donates specifically for his/her cells (usually platelets but occasionally white cells or red cells) the process is termed cytapheresis. The remaining components are immediately re-infused. Sophisticated programmable blood separating machines that are linked to the donor, are used. In this way, many more cells than those available from a whole blood donation may be gathered from one donor. The process of obtaining platelets from apheresis is called plateletpheresis. Harvesting of white cells is called leucapheresis or granulocytapheresis, and collecting red cells is called erythrocytapheresis.

Cytokine

These are anti-viral and anti-tumour agents produced by the body, as part of the system of immune defence. Cytokines (e.g. interleukins) are released into the blood by white cells and in transfused blood components can lead to immune modulation in the recipient.

Cytomegalovirus (CMV)

Cytomegalovirus (CMV) belongs to the herpes group of viruses and may be transmitted by blood transfusion (components that contain leucocytes). In immunocompromised CMV-negative patients, the transmission of CMV

infection can be very serious, causing morbidity or mortality. Selected blood donations may be tested for anti-CMV, so that newborn infants or other immunodeficient patients receive only CMV-negative blood transfusions. Alternatively, pre-storage leucocyte filtration may be carried out on all or selected donations. As CMV is usually found within the white cells, filtering the blood, particularly prior to storage before white cells start to disintegrate, reduces the risk of transmission.

Cytopenia

Cytopenia is a lower than expected number of circulating blood cells of all types.

Dalton

A Dalton (Da) is the unit used in expressing the molecular weight of, for example, proteins. It is equal to 1/12 of the mass of one atom of carbon-12.

Deoxyribonucleic acid (DNA)

DNA is the material found in the nucleus of cells that carries the genetic information. The chromosomes are made up largely of DNA.

Diastolic – *see also systolic*

The diastolic blood pressure refers to the measurement taken when the heart is relaxed. Diastole refers to the short period of time when the heart is at rest and dilated between each contraction.

Directed donation

Directed donations are those donated for a particular patient. It may be that the patient has been scheduled for elective surgery and will require blood. Friends or family members are recruited to donate blood, and this may be the result of the fact that the individual does not have confidence in the safety of the blood supply, or that there is not an adequate supply of blood from volunteer donors. In such cases, it has been found that directed donations are not as safe as voluntary donations, as friends and family coerced into giving blood are less likely to disclose risk factors. On the other hand, patients with antibodies to high incidence antigens may require directed donations from previously grouped individuals – who may be family members, or who have the same rare blood group as the individual requiring the blood transfusion (e.g. Bombay Oh).

Disseminated intravascular coagulation (DIC)

Disseminated intravascular coagulation (DIC) can be initiated by clinical conditions such as traumatic shock, haemolytic transfusion reactions, sepsis and complications of childbirth. It results from the presence of thrombin in the bloodstream, causing fibrin formation in the microcirculation. As a result, clotting factors such as fibrinogen, prothrombin, and FV and FVIII are consumed, as well as platelets. The result is widespread bleeding. The condition can be very serious and may require the transfusion of fresh plasma to replace consumed clotting factors. If the fibrinogen level falls very low, it may be necessary to transfuse cryoprecipitate or fibrinogen concentrate as a source of fibrinogen.

Dithiothreitol (DTT) – *see also 2-mercaptoethanol (2-ME)*

Dithiothreitol (DTT) has the same effect as adding 2-ME to serum but is not strong smelling. DTT may be harmful if it is swallowed or inhaled or comes into contact with skin, so protective clothing should be worn. The area where it is used should be well ventilated. Containers should be kept tightly sealed and safety protocols for the storage of potentially harmful chemicals followed.

Diuresis

This is the increased production of urine. Diuresis may be medically initiated by the use of diuretics to reduce oedema (fluid retention in the body tissues) or to flush out the kidneys (to help clear the body of unwanted waste substances).

Diuretics

These are drugs that increase the amount of urine produced by the kidneys, thus expelling more water and salt from the body.

Dizygotic twins

These are twins developed from two separate zygotes, and therefore non-identical.

Document control

Within a quality management system, the various policies and procedures that are used by an organisation are subject to document control, to ensure that its written policies, procedures and other documents are reviewed,

authorised, distributed, archived and retrieved in a manner that meets the organisation's requirements.

Dolichos biflorus

Dolichos biflorus is a lectin (plant agglutinin), which when suitably diluted shows anti-A₁ specificity. It can be used as a laboratory reagent to differentiate between group A₁ and group A₂ red cell types.

Dominant – see also recessive

This refers to an allele that is fully expressed even in the heterozygote. For example, the A and B alleles are co-dominant and therefore always expressed on the red blood cells, whether inherited from one or both parents.

Donath-Landsteiner test

A positive Donath-Landsteiner test result is obtained when testing the serum of a patient with paroxysmal cold haemoglobinuria (PCH). The Donath-Landsteiner antibody is a biphasic haemolysin and is associated with P specificity. The term *biphasic haemolysin* means that when the environment cools (either *in vivo* or *in vitro*) the antibody (plus complement) becomes cell bound, and when the environment is subsequently warmed, complement adherence causes the sensitised red cells to haemolyse. When this happens *in vivo*, it causes haemoglobinuria (blood in the urine).

Donor

This is an individual who donates blood, either as a voluntary non-remunerated blood donor (VNRBD), or as a family replacement donor, or as an individual who is paid in cash or in kind, for his/her blood. A VNRBD is considered to be the safest donor, as he/she donates for altruistic reasons only and is therefore least likely to be carrying transfusion transmissible infections.

Doppler ultrasound see also Ultrasound

A procedure that can be used to estimate blood flow through specific blood vessels using high-frequency sound waves.

Dosage effect

This refers to the number of copies of an allele present in the cell nucleus. Heterozygotes have a single dose of the allele; homozygotes have a double dose. The gene product (such as a blood group antigen) may be

quantitatively affected by the dosage; in other words a double dose of the gene may result in more antigens being found on the red cells than a single dose. Some antibodies react well with red cells carrying a double dose of antigen, i.e. from a homozygous individual, and poorly with cells carrying a single dose of the antigen, i.e. from a heterozygous individual. For example, anti-M sometimes reacts better with M⁺ N⁻ cells than with M⁺ N⁺ cells.

Dyspnoea

This is the term used to describe shortness of breath, and pain and difficulty in breathing.

Eculizumab

This is a monoclonal antibody that is used in the treatment of paroxysmal nocturnal haemoglobinuria (PNH). PNH is caused by a genetic defect in a natural complement inhibitor which protects the red cells from the complement membrane attack complex (MAC) and therefore the patient's red cells are destroyed. Eculizumab reacts with C5, effectively blocking the formation of the MAC and stopping the cell destruction.

Effector cells

Effector cells are cells that actively respond to a stimulus and bring about a change. For example, plasma cells are effector B cells that react to the stimulus of a foreign immunogen by producing specific antibodies.

Efficacy

This is a term used to describe the degree of desired effect that the administration of a blood component or product achieves in the recipient. It asks the question: 'did the treatment achieve the result expected?' For example, an efficacious dose of factor VIII into a haemophilic prevents or stops bleeding.

Electrolyte

An electrolyte is a substance containing ions (electrically charged molecules) in solution. Electrolyte balance is maintained in the healthy body, but in medical emergencies the intravenous intake of electrolyte-containing substances may be needed. Electrolyte imbalances, such as may be caused by dehydration or blood loss, may lead to serious medical complications unless corrected.

Electrophoresis

This is the term that refers to the separation of molecules (according to size and electrical charge) by applying an electric current to them in a porous medium. Gel electrophoresis is used to separate proteins based on their electrophoretic mobility. The substances under test are added to wells in the gel and a continuous electric charge is applied. The molecules migrate at different rates, depending on their nature, and are thus able to be separated, stained and identified in the laboratory.

Eluate

This is the substrate into which antibodies have been eluted or transferred during the elution process of separating antibodies from the red cells to which they were adhering.

Elution

This is a process whereby antibody molecules that have been adsorbed onto a red cell surface are transferred into a supernatant fluid (the eluate) and can be tested or used in the laboratory.

Endemic

An endemic disease is one that is always present, although perhaps to a varying extent, in the population of a particular geographical area.

Endothelium

The endothelium is the layer of cells lining the interior surface of blood vessels. They reduce the friction caused by the flow of blood through the circulatory system.

Endotoxin

An endotoxin is a toxin that is present inside a bacterial cell and which is released when the cell lyses.

Enzyme

An enzyme is sometimes used in blood grouping tests to enhance agglutination reactions. Enzymes used in the laboratory are plant-derived proteases such as ficin from figs, bromelain from pineapples and papain from papaya. Proteases act by the cleavage of sialic acid residues, bringing red cells in suspension closer together because of the consequent reduction in zeta potential

(repelling force). Cells sensitised by IgG antibodies are therefore able to agglutinate, giving a visible result in the laboratory test.

Enzyme linked immunosorbent assay (ELISA)

ELISA or enzyme immunoassay (EIA) is a family of tests designed to detect markers for various proteins, bacteria and viruses (including HIV), or antibodies to viruses such as hepatitis B and hepatitis C. Blood donations may be screened using this type of test. Donor plasma/serum is added to the wells of microtitre plates, in which the solid phase reagent is already captured. After incubation, washing and the addition of various reagents, according to manufacturer's instructions, tests are read for a colour change which is indicative of a reactive result. ELISA tests are usually automated.

Enzymopathy

This is a (usually) genetic disorder that causes a missing or defective enzyme.

Epithelial cells

Epithelial cells are the cells that form the surface of many organs, such as skin cells and the cells lining the blood vessels and urinary tract. As part of the innate immune system they form a barrier, protecting the internal organs from pathogens such as viruses.

Epitope

This is the extremely small, specific portion of the antigen with which the antibody molecules combine. A single antigen may have many epitopes, for example, the D antigen in the Rh system. Individuals who lack some of the epitopes of the D antigen may make antibodies to the missing epitopes and appear to be D positive with anti-D.

Erythroblastosis fetalis

This is also known as haemolytic disease of the fetus and newborn (HDFN). It literally means erythroblasts (immature red cells) present in the fetus. In anaemic states, the body releases immature red cells into the circulation in order to maintain oxygenation.

Erythrocyte

This is the formal name for a red blood cell, which contains haemoglobin and is primarily responsible to the transportation of oxygen from lungs to tissues.

Erythropoiesis

Erythropoiesis is the production of red blood cells.

Erythropoietin

Erythropoietin is a hormone that influences erythropoiesis. It is produced in the kidneys, and stimulates the bone marrow, causing the erythrocyte forming cells to differentiate and divide and eventually form erythrocytes. Erythropoietin also causes the release of reticulocytes (immature red cells) into the circulation. Synthetic erythropoietin or other erythropoiesis stimulatory agents (ESAs) are used in certain patient populations, such as patients with chronic renal failure, instead of red cell transfusions. ESAs may also be used as part of patient blood management in the perioperative setting.

Ethylenediaminetetraacetic acid (EDTA)

This is a type of anticoagulant, used in the collection of samples for a variety of laboratory tests. It chelates (binds) calcium and in so doing, prevents the action of complement. Therefore, cell lysis or complement-binding is not able to take place in tests if the anticoagulant used is EDTA.

Evaluation – see also validation

Evaluation is the process of assessing or examining a system, process or item to determine the extent to which a set of predetermined criteria are being met.

Exchange transfusion

Exchange transfusion is carried out with the aim of exchanging most of the blood of a patient with donor blood. It may be performed on a neonate suffering from HDFN, primarily to remove bilirubin and prevent it accumulating in the bloodstream, as this is able to cause irreversible brain damage (kernicterus). The exchange is performed by gradually exchanging the neonatal blood with fresh donor blood, until most of the blood of the neonate has been exchanged for donor blood.

External quality assessment scheme (EQAS)

This is a scheme whereby samples are received from an outside agency for assessment and the results returned to the outside agency for evaluation. To be effective, the quality samples should be assessed in precisely the same way as routine samples are assessed.

Extravascular – see also intravascular

The term extravascular refers to the environment outside of the bloodstream, but within the body tissues, lymphatic system or other areas such as the peritoneal space.

Factor VIII concentrate

FVIII is one of the clotting factors that may be conserved by controlled freezing and thawing of fresh plasma. Factor VIII concentrates are produced by bulk separation of FVIII from large pools of fresh frozen plasma by fractionation. FVIII concentrates may also be produced by recombinant technology, rather than from human plasma.

False positive or false negative

False positive is the term used for reporting that a sample contains a defined substance (e.g. an antibody or antigen) when in fact it does not. False negative is the term used for reporting that a sample does not contain a defined substance (e.g. an antibody or antigen) when in fact it does.

Fc Receptor

The Fc receptor is a molecule involved in antigen recognition which is located on the cell membrane of some of the cells of the immune system, such as B-lymphocytes, natural killer cells and mast cells. It derives its name from the fact that it binds to the Fc region of an antibody molecule.

Febrile

This refers to a fever-producing condition. It may be caused by an infection or as the result of an antigen-antibody reaction *in vivo* (such as a transfusion reaction).

Fetomaternal haemorrhage (FMH)

This is the term used to describe the passage of blood from a fetus, usually via the umbilical cord, into the maternal circulation.

Fibrin

These are long strands of web-like material formed from fibrinogen during the clotting process. Fibrin strands may be found in blood and/or plasma products if the initial whole blood donation is not thoroughly mixed

with the anticoagulant during collection. In the case of laboratory tests, fibrin strands may cause false results.

Fibroblast

A fibroblast is a cell found in connective tissue that produces collagen and other types of fibres.

Fibrinogen

Fibrinogen is a clotting factor essential in the completion of the clotting cascade. In component therapy a shortage of fibrinogen is replaced by transfusion of cryoprecipitate that has been stored frozen and is thawed just prior to infusion. Alternatively, fibrinogen concentrates, derived from plasma pools by the fractionation may be used.

Ficin

This is a proteolytic enzyme extracted from figs and used in blood group serology to enhance certain sensitisation/ agglutination reactions.

Forward (cell) grouping – see also reverse (serum) grouping

Forward grouping involves the testing of red cells of unknown ABO blood group, with reagent antibodies of known monospecificity, with a view to identifying the presence or absence of antigens on those red cells. For example, if a test using reagent anti-A and unknown cells is positive then one may conclude that those red cells contain A antigen. On the other hand, if the test result is negative (no reaction) one may conclude that those red cells lack A antigen.

Fractionation

The fractionation of plasma fractions may be achieved by using a prescribed concentration of ethanol, pH level and cold temperature, to isolate and separate (fractionate) plasma proteins. Plasma derived products include albumin, immunoglobulin (gamma globulin) and clotting factors. They may be made viral-safe in various ways, using heat or chemicals.

Fresh frozen plasma (FFP)

Fresh frozen plasma (FFP) is a component prepared from plasma separated from whole blood and rapidly frozen within a short time of donation. The component contains the labile clotting factors (such as FV and FVIII) and must

be kept frozen throughout storage to maintain viability. FFP may also be obtained during plasmapheresis.

Frozen red cells

Frozen storage of red cells is used mainly to preserve units of blood with rare blood types. The shelf life of red cells can be extended up to 10 years or longer if the units are adequately protected using glycerol during freezing and ultra-low freezing temperatures (colder than -65°C). are maintained during the entire storage period. Before use they are thawed and reconstituted by washing in various solutions, after which their shelf life is reduced.

G6PD (glucose-6-phosphate dehydrogenase) deficiency

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked characteristic seen mainly in Mediterranean, Asian and African individuals. It is a red cell enzyme deficiency and sufferers may be at risk of haemolytic anaemia. Although G6PD deficiency may remain hidden as asymptomatic (sub-clinical) chronic anaemia, infections or exposure to certain medicinal drugs may result in haemolysis and hyperbilirubinaemia and could lead to kernicterus in newborn infants.

Gamete

These are the sex cells: sperm and ova. A human gamete usually contains 23 chromosomes, which is the haploid number for humans, and half the number found in somatic cells.

Gamma globulin – see also Immune serum globulin

This is a common term for immunoglobulins fractionated from human blood to produce plasma derivatives of various specificities, used to convey passive, short-term immunity to specific diseases, to susceptible individuals. For example, anti-rabies gamma globulin would be administered to an individual bitten by a dog or other animal suspected of having rabies.

Gel microcolumn technique

These tests may be used in blood grouping and antibody screening and identification. The gel technology utilises cards of microcolumns preloaded with a specific gel, for example a neutral gel, or a gel containing AHG or a gel with specific reagents. Controlled volumes of red cells followed by serum/plasma are added to each

microcolumn/microtube. Following incubation (if required), and centrifugation, any agglutinated red cells are trapped in the gel. Unagglutinated red cells pass through the gel and form a button at the base of the microtube. Gel tests may be automated.

Gene

A gene is the basic unit of inheritance. Genes are segments of DNA that, most commonly, specify the structure of a protein.

Genome

The complete set of genes present in an organism.

Genotype – *see also phenotype*

The genotype is the entire genetic makeup of an individual and not necessarily the outward appearance. Prior to the introduction of molecular techniques for studying blood, the genotype or probable genotype was often based on the interpretation of the phenotype (observed serological results) to deduce which genes were present in an individual.

Gestation

This is the period of time it takes from conception, for the fetus to develop in the uterus (womb). In humans, the normal gestation period is 40 weeks.

Glycerol

This is a viscous, cryoprotective solution, used in the long term frozen storage of red cell samples or rare red cell blood donations, and which prevents the formation of intracellular ice crystals which would cause haemolysis. Red cell donations frozen using appropriate glycerol techniques may be stored for extended periods of time, either in liquid nitrogen vapour (colder than -120°C) or in a low temperature mechanical freezer colder than -65°C . Laboratory samples may be stored in glycerol at -20°C to -30°C .

Glycolipids

Glycolipids are lipids with a carbohydrate molecule attached, that play a role in stabilising the cell membrane.

Glycoprotein

Glycoprotein is the name given to any protein that has carbohydrate groups attached to the polypeptide chain.

Good laboratory practice (GLP) and good manufacturing practice (GMP)

Good laboratory practice (GLP) generally refers to a code of practice designed to reduce to a practical minimum the procedural or instrument variations that could affect the outcome of a process. GLP includes elements of safety, cleanliness, hygiene, equipment maintenance and operation, record keeping and all other aspects of laboratory operations. As with GLP, GMP refers to a code of practice, but in this case aims to minimise all variations within the process of manufacturing that could affect the outcome. Important elements of GMP include personnel, premises, documentation, equipment and materials, and complaint and recall procedures of (defective) products.

Graft versus host disease (GVHD)

Viable lymphocytes within a blood donation, transfused into a recipient with the same haplotype, may implant and proliferate, resulting in rejection of the host (recipient), by the graft. This may occur with transplanted bone marrow or as a result of a blood transfusion. GVHD caused by transplantation may become chronic and debilitating whereas transfusion-associated GvHD (TA-GVHD) is often fatal. To prevent TA-GVHD when transfusing blood from a first degree relative, or into an immunodeficient individual, the cells should first be irradiated to kill any viable white cells.

Granulocytes

These are polymorphonuclear cells with a multilobed nucleus. They are characterised by granules which are visible microscopically in the cytoplasm of the cells, after laboratory staining. Granulocytes fall into three subgroups; neutrophils, whose main function is phagocytosis; basophils which are capable of phagocytosis and contain histamine and heparin, and eosinophils that are also phagocytic and are involved in allergic responses.

Gut

Gut refers to the alimentary canal, or some portion of it.

Haematinic

A medicine that stimulates the production of red blood cells and thus the haemoglobin content of the blood. Examples are iron, vitamin B12, folate and erythropoietin.

Haematocrit (Hct)

This is a term used to describe the percentage of red blood cells in an anticoagulated sample of whole blood subjected to rapid centrifugation in a 'haematocrit' centrifuge. The sample required to carry out the measurement is small and may be taken from a finger-prick. The normal Hct range for adults is approximately 0.4–0.5. It may also be calculated by a laboratory analyser on a venous blood sample.

Haematology

This is the study of blood, such as the Hb testing and full blood counts on blood samples taken from donors who fail the copper sulphate test, to assess whether or not they are anaemic, or if they are just below the acceptance level for blood donation.

Haematoma

This is the term used for a swelling caused by leakage of blood from a blood vessel into the surrounding tissues, as a result of physical trauma. Failure to successfully insert the phlebotomy needle into the vein when commencing a blood donation may cause a haematoma. Within a few weeks, the swelling, pain and bruising caused by a haematoma of this nature, should have resolved.

Haemodialysis

Haemodialysis is the removal of waste products from the blood by means of an artificial kidney machine. Patients with chronic renal failure on haemodialysis frequently develop anaemia that requires correction either by red cell transfusion or by the administration of erythropoietin or related erythropoiesis stimulatory agents (ESAs).

Haemoglobin (Hb)

The red pigment in red blood cells is called haemoglobin. This molecule contains iron as one of its vital components. Hb combines with oxygen in the lungs and transports it in the red blood cells to the tissues of the body. The oxygen carried by the Hb gives arterial blood its characteristic bright red colour.

Haemoglobinaemia

This is the presence of free haemoglobin in the bloodstream. This is not a normal situation and may indicate that an intravascular transfusion reaction has taken place.

Haemoglobinuria

This is the term used for the presence of haemoglobin in the urine, which is not a normal situation. It could be the result of physical trauma. It is also one of the signs of a haemolytic transfusion reaction, when haemoglobin from intravascularly ruptured donor red cells is excreted via the kidneys.

Haemolysin

This is an antibody that in the presence of complement is capable of the rupture of red blood cells, i.e. haemolysis.

Haemolysis

This is the physical rupturing of red blood cells. It may be caused by physical conditions such as freezing, by biochemical agents such as detergents, or by biological agents such as complement. Haemolysis can be seen in laboratory tests or is used to describe a clinical condition in which red blood cells are lysed, either intra- or extravascularly.

Haemolytic

'Haem' refers to red cells and 'lytic' means rupturing. Haemolytic therefore means rupturing of red cells.

Haemolytic disease of the fetus and newborn (HDFN) – see also *erythroblastosis fetalis*

HDFN is caused by maternal IgG antibodies crossing the placenta during pregnancy and sensitising and destroying fetal red cells with the corresponding red cell antigen. At birth, although placental transfer of IgG can no longer occur, maternal IgG circulating in the bloodstream of the newborn is able to continue its red cell destruction, until all maternal antibody molecules are adsorbed. HDFN causes anaemia and hyperbilirubinaemia. The clinical outcome of the disease depends largely on the potency of the maternal antibody and the quality of treatment provided for the newborn (such as exchange transfusion).

Haemolytic transfusion reaction

This is a reaction caused by red cell incompatibility between donor and recipient blood. It results in the *in vivo* destruction of red blood cells of donor origin, with or without the involvement of complement. Intravenous haemolysis is the result of complement

involvement, whereas extravascular destruction of red cells occurs when cells are removed from the bloodstream before being destroyed.

Haemophilia

Haemophilia A refers to the disease where sufferers lack clotting factor VIII. The gene giving rise to this condition is carried by females and manifested in males (X-linked). Haemophilia B or Christmas disease is inherited in the same way, but sufferers lack clotting factor IX. To maintain health, a haemophiliac needs infusions of specific clotting factor concentrates throughout his life.

Haemopoiesis or haematopoiesis

This is the process of formation of the blood cells, from common ancestor cells found in the bone marrow. Mature blood cells differentiate and develop from these precursor cells, so that in the bloodstream there are red cells, white cells (consisting of lymphocytes, monocytes and granulocytes) and platelets.

Haemorrhage

This term describes the loss of blood, either because of traumatic injury, surgery, childbirth or disease. Sudden, severe haemorrhaging can lead to hypovolaemic shock that is fatal if not corrected by rehydration and improvement to oxygen-carrying capacity, either using replacement fluids such as crystalloids or colloids, and oxygen, or by blood transfusion of whole blood, or combinations of blood components, such as red cell concentrates and fresh frozen plasma.

Haemosiderosis

Haemosiderosis is a form of iron overload. Transfusion haemosiderosis results from the accumulation of iron contained in the transfused blood in the body of the patient.

Haemostasis

This is the term used to describe the cessation (stopping) of bleeding. The coagulation cascade involves clotting factors which are activated sequentially and result in the formation of a clot to plug the wound.

Haemovigilance

Haemovigilance is the set of procedures covering the transfusion process from donation through to transfusion

that are designed to monitor, record, investigate and analyse any adverse events that may occur and taking the actions necessary to prevent a recurrence. This may include both donor adverse events as well as recipient adverse events.

Half-life

This is the time taken for a substance to lose half of its activity. For purposes of explanation, one may assume that the half-life of transfused factor VIII is 10 hours. Therefore, 10 hours after transfusion, the available FVIII would have reduced by 50%. Over the second 10 hours, another 50% of the remaining 50% would be lost, and over the third 10 hours, 50% of the remaining 50% would be lost, and so on. Graphically, this means that the reduction does not follow a linear (straight) line but has an extended fall or more gradual reduction over time before becoming depleted.

Haploid

This is a single set of chromosomes that is found in the gametes or sex cells (ova and sperm), and which is half the number found in somatic cells.

Haplotype

The haplotype is the genetic material contributed by either the mother or father to the offspring; one of each pair of the chromosomes found in the nucleus. In humans the haplotype consists of 23 chromosomes. The term haplotype may also be used to describe genes present on one of a pair of homologous chromosomes that usually travel together. For example, no recombination has been observed between D, cc, or Ee in the Rh blood group system and the alleles are inherited as a haplotype such as *DCe*, *dce*, etc. (*d* indicating the deletion of the *RHD* gene or any inactive *RHD* gene).

Hapten

A hapten is a small molecule which is incapable of stimulating antibody production on its own, but when combined with a larger carrier molecule, for example a protein, is able to stimulate the production of antibodies. These antibodies can then bind to the hapten in its free or combined state.

HELLP Syndrome

A group of symptoms that occur in pregnant women, consisting of haemolysis, elevated liver enzymes and low platelet count. The cause is not known.

Hemizygote – see also homozygote and heterozygote

An individual who has only one allele at a given locus instead of two.

Heparin

This is a natural anticoagulant originally of animal origin and may be used to prevent blood samples from clotting.

Hepatitis

The term hepatitis means inflammation of the liver and may be caused by viruses (such as hepatitis B or C virus), certain medicinal drugs or alcohol. The ability of the liver to function correctly is impaired, and as a result, jaundice is evident in severe cases.

Hepatosplenomegaly

This is when both the liver and the spleen are swollen to greater than their normal size.

Heterologous

Heterologous means from a different organism. A heterologous group transfusion means that the blood groups are different, for example Group O transfused into a Group A recipient.

Heterozygote – see also homozygote and hemizygote

An individual who has two different alleles at a particular locus, one on each of the chromosome pair, is said to be a heterozygote (e.g. *A* and *O* alleles forming the ABO genotype).

High-frequency antigen (HFA) – see also low frequency antigen (LFA)

An HFA is one with a frequency of more than 90% in the general population and that has been shown not to belong to a specific blood group system or collection, for example, Lan. Many previous HFAs have now been shown to belong to various blood group systems or collections.

HLA Class I expressing cell

HLA Class I expressing cells present peptides from inside the cell on the surface of the cell. For example, is a cell is

infected by a virus, the HLA Class I system will present fragments of the virus on the surface of the cell so that the cell can be destroyed by the immune system.

HLA Class II expressing cell

HLA Class II expressing cells present antigens from outside the cell to T-lymphocytes. The T-lymphocytes stimulate the B-lymphocytes to produce antibodies.

HLA system

Human leucocyte antigens (HLA) are produced as a result of genes located on the short arm of autosome six (#6). This region is collectively known as the MHC (major histocompatibility complex). The MHC region also contains the genes for some cytokines and various complement components. Gene products occur on the surface membranes of nucleated cells. The class I and class II HLA antigens play a major immunological role in the defence against disease. HLA antigens are found on white blood cells and platelets, and also on tissue cells, which complicates the successful grafting of transplanted organs. HLA antibodies formed as a result of transplantation may lead to graft rejection.

Hold-over

The length of time that a fully loaded storage refrigerator or freezer – when it is kept closed – maintains its temperature without power, is known as its hold-over time. A good hold-over time is particularly useful in areas where electricity supplies are unreliable.

Homologous

Homologous means from the same species. A homologous group transfusion means that the blood groups of the donor and the recipient are the same.

Homozygote – see also heterozygote and hemizygote

An individual who has two identical alleles at a particular locus, one on each of the chromosome pair, is termed a homozygote (e.g. two *A* alleles forming the *A* genotype in the ABO system).

Hormone

A hormone is a regulatory substance transported in the blood or tissue fluids that can stimulate specific cells or

tissues that may be distant from the hormone's site of production.

Host

The host is the organism in which a situation takes place, such as the proliferation of an infection, or the reaction of the immune response to eliminate foreign substances.

Human immunodeficiency virus (HIV) – *see also acquired immune deficiency syndrome (AIDS)*

This is the retrovirus which destroys the immune system over time, and if not adequately treated, causes AIDS. It is of major concern to blood services, as it may be transmitted to recipients from donated blood that is either not tested, or that is non-reactive on testing, for example, if the donor is in the window period.

Humoral immune defence

The word humoral is derived from 'humour' or body fluid. Soluble substances including antibodies, complement and cytokines, are present in body fluids – plasma and lymph, and comprise the humoral immune defence system of the body.

Hybrid

A hybrid is the offspring of a cross between two different species or between two different characteristics within a species. For example, a mule results from a cross between a horse and a donkey. The crossing of homozygous tall and short pea plants gives rise to hybrid offspring that can pass on either tall or short characteristics to the next generation.

Hybridoma

This is a cell which results from the fusion of two unrelated cells; an antibody-producing tumour cell and a plasma cell that has been immunogenically stimulated. These hybrid cells multiply and produce antibodies that are identical in specificity, each being directed against the antigen epitope that was used to stimulate the plasma cell.

Hydrops

This occurs when large amounts of fluid build-up in the baby's tissues. *See Hydrops fetalis.*

Hydrops fetalis

This term means swelling or oedema of the fetus or newborn, a condition that is the result of fluid retention because of disease and could be the result of excessive destruction of fetal red cells by maternal antibody, as occurs in HDFN. This leads to swelling of the liver and spleen.

Hydroxyethyl starch (HES)

Hydroxyethyl starch (HES) is a colloid that may be used clinically as a blood volume expander, to correct hypovolaemia such as would occur as a result of burns or shock.

Hyperbilirubinaemia

This term is used to describe increased bilirubin levels in the bloodstream of the patient.

Hyperimmune globulin

This is a fractionated plasma derivative which contains concentrated, purified antibodies against a given pathogen, such as rabies or Hepatitis B. It may be administered to individuals at risk of infection, e.g. rabies hyperimmune globulin would be given to an individual who had been bitten by a dog or another animal suspected of being rabid.

Hyperkalaemia

Individuals who are hyperkalaemic have raised blood potassium levels, as do patients with impaired renal function.

Hypertension – *see also hypotension*

Hypertension is the term used for abnormally high blood pressure.

Hyperventilation

Fear (e.g. of blood donation) can cause an individual to breathe deeply and consistently over a short period of time, and this can cause an acid-base imbalance in the body, resulting in loss of consciousness (fainting). In severe cases the result may be hyperventilation tetany (convulsions). Getting the individual to breathe into a paper bag so that carbon dioxide is re-inhaled, should rapidly restore acid-base balance.

Hypogammaglobulinaemia – see *agammaglobulinaemia* **Hypotension** – see also *hypertension*

Hypotension

Hypotension is the term used for abnormally low blood pressure.

Hypothermia

Hypothermia refers to body temperature that is below normal, i.e. less than 37°C. It is a condition that may be artificially created during surgery. Although a unit of donor whole blood containing a benign (harmless) cold autoantibody may be compatible with such a patient, it should not be selected for transfusion because the autoantibody may react with the red cells of the patient *in vivo*, as a result of the lowered body temperature.

Hypoxaemia

Hypoxaemia is an abnormally low concentration of oxygen in the blood.

Hypoxia

This is a deficiency in the amount of oxygen reaching the body tissues, shown by the lips turning blue (cyanosis) and rapid breathing, with headache and dizziness. It may be brought about by loss of blood.

Icterus

Jaundiced or icteric plasma or serum, or skin colour, means yellow. The word for jaundice (or yellow colour of the skin) is icterus. Jaundice is the sign of an accumulation in the body of bilirubin, either because of a haemolytic anaemia, HDFN or hepatitis, which is inflammation of the liver – the organ responsible for the digestion and excretion of bilirubin via the urine and faeces.

Idiopathic

A disease which is idiopathic is one in which there is no identifiable cause.

Idiopathic thrombocytopenic purpura (ITP)

When a condition is termed 'idiopathic' it means that it is of unknown cause. Thrombocytopenia is a lack of

platelets, and purpura means bruising. When this condition is caused by platelet autoantibodies, it is referred to as immune thrombocytopenic purpura (also abbreviated as ITP), the two terms are often used interchangeably. The transfusion of platelet concentrates for such conditions may not be beneficial, although they could be needed if thrombocytopenia is severe.

Immune response

This is the body's response to infection or the presence of foreign substances. The immune response is complex and involves many strategies. The objective of the response – whether it is an innate/inborn response or an acquired/learned response – is to react in the best way and as quickly as possible, to provide protection to the body from the health threat of the foreign substance. Because of an immunocompetent response, an infection is contained and eliminated.

Immune response genes

When exposed to foreign immunogens, the immune system usually initiates a response. This response is an inherited ability; and individuals who respond have the appropriate immune response genes. In some instances, individuals do not respond, even though exposed to the same immunogen repeatedly. Such individuals are termed non-responders *to that particular immunogen* and it is theorised that they do not possess the appropriate immune response genes.

Immune serum globulin – see also *gamma globulin*

This is prepared by fractionation of a plasma pool collected specifically from donors known to have immunity to a particular disease (e.g. tetanus, chicken pox or rabies). The specific antibody globulin so produced may be administered to individuals exposed to the specific infection to which they lack immunity, in order to give them temporary (passive) immunity to it.

Immune system

This is the complex system of host defence, whereby the reticuloendothelial system and immunocompetent cells within the body interact with chemicals and enzymes in many ways, so that infection is contained and resolved, and the body is therefore able to restore and maintain health.

Immune thrombocytopenic purpura (ITP) – see idiopathic thrombocytopenic purpura

Immunocompromised

This is the situation when the immune response of the individual is not optimal and the response to an immunogenic stimulus is impaired or not as efficient as it should be; either because of an inherited problem, or a clinical situation, or in the case of an infant whose immune system has not yet become fully developed.

Immunodeficiency

Individuals who are not able to mount a healthy immune response cannot adequately protect themselves from infectious diseases; they are therefore immunodeficient. This condition may be genetic or brought about because of chemotherapy used to treat cancer. It is also seen in AIDS patients or in newborns.

Immunodiffusion – see also precipitation

Immunodiffusion of a soluble antigen and its specific antibody through a gel medium, results in a precipitin line in the gel – where antigen and antibody meet, signifying a reaction.

Immunogen – see also antigen

An immunogen is a substance that is able to provoke an antibody response in an immunocompetent individual. The more immunogenic the substance, the stronger the immune response is likely to be. Sometimes immunogens are referred to as antigens, which can react specifically with antibodies.

Immunoglobulin

This is another name for antibodies. There are several different classes of immunoglobulin and those of most relevance from the perspective of a blood banker are Immunoglobulin G (IgG) and Immunoglobulin M (IgM). Other classes are IgA, IgD and IgE.

Incidence – see also prevalence

Incidence relates to the number of new infections of a particular disease in a population over a defined time span, usually a year. Prevalence relates to the number of individuals living with an infection during the year of analysis. For example, the incidence of HIV positives in a

given population for the year could be 120 per million population, and the prevalence of HIV in that same population could be 900 per million population. Positive infections in a first-time blood donor are usually referred to as prevalent infections, since the donor may have been infected at any time in their life prior to donation. Infections in repeat donors, who previously had negative test results, are referred to as incident infections, since they occurred in the time period between donations.

Incident – see also accident

This may be viewed from the occupational safety angle or from the perspective of transfusion mishaps. An incident may be referred to as a 'near miss' and an example of this would be when a brick falls from the building site, just missing hitting an individual on the head, or when a unit of blood intended for another patient is set up for the wrong patient and just before the infusion is started, the mistake is realised and the infusion aborted.

Incubation

Clinically, the incubation period may be defined as the period of time from when a micro-organism enters the body until it displays its presence by making the host ill. For influenza the incubation period may be 24 hours and for hepatitis B it may take up to 6 months. From the perspective of the laboratory, the incubation period refers to the period of time and temperature that tests are left undisturbed before the results are read.

In line filter

Some blood bag systems are supplied with a filter built into the closed system to ensure sterility and ease of operation during leucocyte-depletion.

Inotrope

An inotrope is a substance that increases the force of muscular contractions. They may be used to increase the strength of the contractions of the heart muscle.

Interleukin – see cytokine

Intra-operative salvage

When the blood lost by a patient during surgery is collected, filtered, anticoagulated and then re-infused, it is termed intra-operative salvage. This may limit the need for allogeneic blood transfusion but may not be used in

cases of intestinal tract operations as a result of the infection risk.

Intravascular – see also extravascular

The term intravascular means within the bloodstream or within the vascular system (blood vessels).

In utero

This is the term used to signify within the uterus (womb).

In vitro and in vivo

In vitro refers to whatever occurs within the laboratory test – the artificial environment. *In vivo* refers to occurrences within the living organism – the natural environment.

Ionic strength

This is a measurement that represents the interaction between water molecules and any other ions present in a particular solution. Isotonic saline has the same tonicity as blood; hypotonic saline has fewer ions than blood, and hypertonic saline has an increased number of ions.

Jaundice – see Icterus

Isoagglutinin

An isoagglutinin is an antibody produced by an individual that will agglutinate the red cells of other individuals belonging to the same species but will generally not agglutinate the cells of the individual producing it. This term is generally used for anti-A, anti-B, and anti-A,B.

Isoimmunisation– see alloimmunisation

Karyotype

This is a photomicrograph of a set of chromosomes arranged in a standard order that shows the number, type and shape of each of the chromosomes, thus facilitating the detection of abnormalities. A karyotype is also used to describe the general characteristics of the chromosomes in a cell, such as size, shape and number.

Kernicterus

This refers to staining of the basal ganglia of the brain with unconjugated bilirubin, which because it has not yet been conjugated by the liver, moves freely in the bloodstream. When it is present in excess in the circulation, it

is able to deposit on brain cells. This leads to brain damage, the severity of which depends on the degree of staining by bilirubin. Kernicterus is a serious complication of hyperbilirubinaemia in neonates and may be prevented by timely treatment using phototherapy and/or exchange transfusion.

Kleihauer-Betke test

This is a test that is used to estimate the volume of Feto-maternal haemorrhage (FMH). The test is useful when determining the amount of Rh immunoglobulin that should be administered to prevent the formation of anti-D antibodies in an unimmunised mother.

Labile

This is the term used to describe a substance that quickly diminishes in efficacy. It usually refers to a substance that is susceptible to time and/or temperature. When it is said that a blood component is labile (such as factor VIII), then it means that without careful preparation and storage of the product, it will lose its potential to treat those conditions for which it was prepared (to correct bleeding in haemophiliacs).

Lactate dehydrogenase (LDH)

The assessment of LDH may be included in the measurement of biochemical markers of haemolysis. LDH is an enzyme that participates in cellular respiration, a process of converting glucose from nutrients into energy. When increased, it provides evidence of tissue damage.

Latent heat of melting

The energy (heat) used to transform a certain mass of frozen ice into a liquid, without changing its temperature, is called the latent heat of melting.

Lectin

A lectin is a plant extract usually prepared from seeds which can agglutinate red cells. Lectins used in blood group serology are specific for certain blood group antigens. Many other lectins react with all red cells tested.

Leucocyte-depleted (leucodepletion), leucocyte-reduced (leucoreduction) – see leucoreduction Leucocytes

White cells are collectively known as leucocytes, which all take part in the body's immune defence system. They consist of monocytes, lymphocytes and

polymorphonuclear granulocytes including basophils, neutrophils and eosinophils.

Leucoreduction

Removal of the buffy coat during processing results in a product with less than 1.2×10^9 leucocytes per bag and is considered to be leucocyte-reduced. Leucoreduction should not be confused with leucodepletion where leucocyte content is further depleted by filtration and has less than 1×10^6 leucocytes per bag.

Lipid

Lipids are oily or waxy molecules comprised of long chains of carbon and hydrogen that are found in living organisms. They include many oils, fats, and waxes.

Lipopolysaccharide

Lipopolysaccharides are complex molecules that contain both lipid (fatty acids) and polysaccharide (a large number of sugar molecules bonded together) parts. They are found in the cell membranes of a variety of cells.

Loci – See 'Locus'

Locus

The locus (plural loci) is the position on a chromosome that is occupied by a specific gene.

Look-back

Blood services should have a recognised 'look-back' procedure/protocol in place so that when a blood donor tests positive for a TTI, there is a process to follow in which the donor is informed and/or counselled and previous donations from the donor can be tracked and traced. Similarly, all services should have a traceback protocol in place if a possible TTI transmission to a patient is reported. All contact with a donor in this regard should be carefully controlled as the information is sensitive. Only trained and authorised counsellors, in collaboration with the medical director, should have access to information and donors.

Low frequency antigen (LFA) – see also high-frequency antigen (HFA)

An antigen which occurs rarely in most populations and has been shown not to belong to a specific blood group system or collection is termed an LFA. To fit this category, the antigen must have a frequency of less than 1%,

for example, REIT antigen. Many previous LFAs have now been shown to belong to various blood group systems or collections.

Low ionic strength saline solution (LISS)

Low ionic strength saline is a solution that may be used in the laboratory to speed up reactions. As a result, the incubation time is shortened, producing faster results. In solutions of low ionic strength, antibody uptake onto red cells with the corresponding antigen is also increased. These two factors ideally suit tests performed in compatibility testing laboratories.

Lymphocyte

Lymphocytes are a type of white cell. There are two types of lymphocytes; T-lymphocytes and B-lymphocytes, responsible for immune defence by cell-mediated immunity and the production of antibodies, respectively.

Macrophage

This is a large cell – either sessile (fixed/unmoving) in the liver, spleen or lymph nodes (reticuloendothelial system) – or mobile like the monocyte, with phagocytic characteristics. As a result of phagocytosis, digested cell debris and/or micro-organisms are presented to lymphocytes and other immunologically competent cells to stimulate their response, either as a nonspecific cell-mediated immunity to eliminate foreign matter, or in the production of specific antibodies.

Major crossmatch – see also minor crossmatch

This involves testing the patient's serum/plasma with the red cells from the donor blood selected, to ensure that there is no reaction between them and that therefore the donor red cells should survive normally in the recipient.

Major histocompatibility complex (MHC) – see HLA system

Malaria – see also plasmodium

This is a parasitic disease that may be transmitted by blood transfusions containing infected red cells. Malaria is contracted as the result of the bite of an infected female *Anopheles* mosquito. The malaria parasite invades and destroys red blood cells and can cause severe disease which may be fatal. Donors who have had malaria or who have visited malaria endemic areas should be deferred from donation according to local guidelines.

Mannans

Mannans are polysaccharides that are polymers of mannose (a type of sugar). They are found in the walls of some micro-organisms,

Meiosis – see also mitosis

Meiosis is haploid cell division in which each daughter cell receives half of the DNA contained in the parent cell, being one of each of the pairs of chromosomes. This occurs during the formation of the sex cells.

Melting point

Melting point is the temperature at which a coolant converts from a solid to a liquid phase.

Membranopathy

This is a disease of a membrane, such as the red cell membrane.

Microaggregates

Platelets and white cells rapidly lose viability when stored as whole blood or within concentrated red cells. After as little as 3–4 days of storage they clump together in small aggregates (microaggregates) capable of passing through a standard blood administration filter at the time of transfusion. In massive transfusions they may cause decreased lung function by blocking lung capillaries. To prevent this, a microaggregate filter may be used when transfusing stored components in which the buffy coat has not been removed.

Microtitre plate

This is a plastic plate or tray, sometimes called a microwell plate, usually with 96 microwells moulded into the surface (8 by 12 wells in the grid). These plates are used for ELISA/EIA tests and many other laboratory techniques. Often the wells are coated with reagent antigen or antibody which adheres to the plastic and forms an invisible coating called the solid phase.

Minor crossmatch – see also major crossmatch

The minor crossmatch is carried out by mixing the red cells of the recipient with the plasma of the selected donor blood, to test for compatibility (which means, no visible laboratory reaction). The minor crossmatch is of

limited value as it is always incompatible when the groups of the donor and recipient are not identical, such as when a group O unit of blood is selected for use in a group A recipient.

Mitosis – see also meiosis

Mitosis is the doubling division of somatic cells in which each daughter cell receives the same amount of DNA contained in the parent cell, and therefore has the same number of chromosomes as the parent cell.

Mixed field

This term is used to describe a type of agglutination in which clumps of strongly agglutinated red cells can be seen against a background of unagglutinated red cells, either macro- or microscopically. Mixed field agglutination may be characteristic of the way in which some irregular antibodies react *in vitro*, such as anti-P₁ or may be seen in a test with anti-A reagent mixed with red cells from a group A patient recently transfused with group O donor blood.

Monoclonal

A group of cells is termed monoclonal when they are all derived from a common ancestral cell and are therefore identical. Monoclonal antibodies are antibodies produced from monoclonal cells and as a result each antibody molecule is of the same type and has the same specificity.

Monocytes

These are large phagocytic white blood cells that are capable of free movement within the bloodstream, to approach, engulf and digest foreign debris or harmful agents encountered in the blood. They work together with other immunologically competent cells to protect the host from infectious diseases.

Monozygotic twins

These are identical twins originating from one zygote.

Morbidity and mortality

A state of morbidity signifies that the individual suffers a degree of disability or disease and is therefore unable to function optimally. The word mortality means susceptible to death. The mortality rate as a result of a particular disease is measured by the number of deaths it causes in a population.

Mosaic

In blood group serology it refers to an antigenic determinant that is composed of a series of different parts or epitopes. Not all parts are necessarily present in every individual when the antigen is expressed, such as the D antigen of the Rh system.

Multiparous

A multiparous female is one who has been pregnant more than once.

Mutation

This is a change in the genetic code of an organism, resulting in a new allele that may result in the appearance of a new characteristic, and which may be inherited by the offspring.

Natural killer cells

Natural killer cells are a type of lymphocyte that plays a role in the destruction of tumours and also of cells infected by viruses.

Neonate

This is a newborn infant, and usually relates to the first 4 weeks of life, regardless of whether or not the infant was born prematurely.

Neutralisation

This is the term used for a chemical reaction during which two solutions react and water forms as a result of this reaction, e.g. an acid solution may be neutralised using an alkaline solution until a neutral pH is reached. On the other hand, neutralisation may refer to the action of a blood group antibody and a soluble antigen (such as in saliva) that react with one another so that the antibody becomes neutralised and incapable of reaction (with its corresponding red cells). For example, reagent anti-A in the presence of soluble A antigen in saliva, becomes neutralised and will not subsequently agglutinate group A red cells.

Non-conformity

On measurement against the standard, it may be found that a process, product or service has not met its defined requirements and that the quality of the process, product

or service may be adversely affected as a result. This is termed non-conformity.

Non-opsonic receptor (*see Opsonic receptor*)

Non-opsonic receptors are cell surface receptors on leucocytes that promote phagocytosis by recognising molecular patterns of carbohydrate subunits on micro-organisms.

Non-secretor – *see also secretor*

A non-secretor is an individual who has the *se* gene (as opposed to the secretor *Se* gene) and therefore does not secrete soluble blood group substances (such as A and B) in their body fluids.

Normal ionic strength saline (NISS)

Normal ionic strength saline (NISS) is saline that is isotonic with blood. NISS is often referred to as normal saline or saline, and not as NISS. It is an isotonic solution of sodium chloride and water containing 8.5–9.0 g/L NaCl that has the same salt concentration as blood and other cells of the body.

Nucleic acid testing (NAT)

Nucleic acid testing (NAT) is a technology that allows for the amplification of very small amounts of genetic material (RNA or DNA) in the laboratory, thus making the detection of the material easier. It may be used for the detection of genetic material specific for a virus, such as HIV, making it a useful tool for the early identification of an infected individual.

Nucleotide

Nucleotides are the building blocks of DNA and RNA. They are composed of a nitrogenous base, a sugar and one or more phosphate groups.

Oedema

This is a term used to describe the retention of fluid in the tissues of the body. For example, pulmonary oedema refers to the retention of fluid in the lungs.

Oligosaccharide

Oligosaccharide is the name given to a carbohydrate that is composed of monosaccharide units, for example glucose, fructose and galactose.

Oliguria

This term describes the reduced output of urine and may simply indicate dehydration. However, it is one of the signs of a haemolytic transfusion reaction, in which the kidneys may be damaged.

On demand filtration

When a unit of blood is stored unfiltered and is later filtered (leucocyte-depleted) on the request of the prescribing clinician, by using an add on or bedside filtration system, it is called on demand filtration.

Open system – see also closed system

If at any stage after being sealed, the blood bag is either deliberately or accidentally opened (i.e. un-sterile air or fluid could enter the bag) it is considered to be an open system. If it is accidentally opened the blood must be discarded; if it was performed intentionally to prepare a special component, then the expiry time is greatly reduced and must be recorded as such on the bag.

Opportunistic infection

This is an infection caused by a micro-organism that does not normally cause disease or debilitation in healthy individuals with a normal immune system, but which uses the 'opportunity' provided by a host with a suppressed immune system, for example in an individual infected with untreated HIV, to proliferate and cause disease.

Opsonic receptor (see *Non-opsonic receptor*)

Opsonic receptors are found on the membrane of phagocytic cells, where they recognise Fc receptors on antibodies and complement receptors. If a micro-organism is coated with opsonising antibodies or the C3b component of complement, this is recognised by the opsonic receptors and the micro-organism is phagocytosed.

Opsonin

Humoral agents include opsonins which are chemicals produced by the body and that promote phagocytosis. Humoral immunity is that which is initiated by antibodies, secreted by B-lymphocytes.

Organelle

The general name given to any of the organised structures within a cell.

Orthopnoea

This is a shortness of breath that occurs in a patient lying down flat and is often treated by propping the patient up or seating them in a chair.

Packed cells – see *red cell concentrates*

Panagglutination – see also *polyagglutination*

This is the term that describes the unexpected agglutination of all normal red cells, which may be caused by several factors, such as the bacterial contamination of the serum/ plasma used in the tests, or by the presence of an antibody that reacts with all red cells within the same species.

Pandemic

This is the term used to describe a disease occurring over an extensive geographical area and affecting a large proportion of the human, animal or plant population.

Panel

A panel or set of reagent red cells may be used to determine the presence or specificity of an antibody, and a panel of antisera may be used to test unknown cells for the presence of an antigen.

Papain

This is a proteolytic enzyme extracted from papaya and used in blood group serology to enhance certain sensitisation/ agglutination reactions.

Paroxysm

A paroxysm is a sudden, acute attack, or the sudden worsening of an existing symptom. For example, a paroxysm of coughing, in contrast with an ongoing or lingering cough.

Paroxysmal cold haemoglobinuria (PCH) – see *Donath-Landsteiner test*

Paroxysmal nocturnal haemoglobinuria

Paroxysmal nocturnal haemoglobinuria is a rare disorder that causes red cells to have a shorter than normal

lifespan, which causes the release of haemoglobin into the urine. The resultant discolouration of the urine is usually seen at night or in the early morning.

Pasteurisation

Pasteurisation is used to sterilise solutions with a high concentration of protein. This is performed by heating them to a temperature that will be hot enough to kill bacteria, but not hot enough to denature the protein. For example, albumin solution is pasteurised at 60°C for 10 consecutive hours.

Pathogen

A pathogen is a bacterium, a virus, a fungus, or any other organism that can cause disease in an infected host.

pH

pH is the measurement of the degree of acidity or alkalinity of a liquid.

Phagocytosis

This is the process whereby monocytes, macrophages and granulocytes approach, ingest and internalise foreign materials with which they come into contact *in vivo*, or in a freshly collected unit of whole blood, with a view to rendering them harmless.

Phenotype – see also genotype

A phenotype is the observable traits or characteristics of an organism, resulting from the interaction between the genotype and the environment. In blood grouping, phenotype refers to a list of antigens which have been shown to be present on the red cells of an individual. Red cells can be phenotyped – they cannot be genotyped.

Phlebotomy

This is the process of inserting a needle into the vein of the blood donor (by venepuncture or venesection), in order to collect a unit of blood. Prior to phlebotomy, thorough cleaning of the venepuncture site is essential to prevent contamination of the donation.

Phosphate buffered saline (PBS)

Phosphate buffered saline (PBS) is normal saline which contains sufficient buffer solution to maintain the desired specific pH during its shelf life. PBS is usually buffered to

pH 7.0 ± 0.2 . It is often one of the recommended suspending media for typing red cells using commercial blood grouping reagents.

Phototherapy

This is a conservative approach to controlling bilirubin level in newborn infants, by exposing the jaundiced newborn to light rays designed to reduce bilirubin. The objective of phototherapy is to try to avoid the need for exchange transfusion and remove the risk of kernicterus. To prevent damage to the retina, the eyes of the newborn are covered during the process.

Placenta

The placenta is the thickened area of lining, rich in blood vessels, that forms inside the uterus from the time of conception, and creates a link between mother and fetus, via the umbilical cord. The placenta is the means whereby soluble substances such as oxygen, nutrients, antibodies and other vital chemicals are transported from the maternal plasma, via the cord, to the fetal bloodstream. It also provides for the passage of waste products from fetal metabolism, back through the cord into the maternal bloodstream for excretion.

Plasma

This is the straw-coloured liquid part of anticoagulated blood remaining after separation from the cellular components. It is the part of blood containing in solution, all the clotting factors and other non-cellular components such as nutrients, wastes, hormones, albumin, enzymes and antibodies. Body plasma transports cellular and non-cellular components to the parts of the body where they are required for metabolism, or in the case of wastes, to sites where they can be excreted.

Plasmacyte or plasma cell

A plasma cell is a B cell which has been activated to produce antibodies of a single specificity.

Plasmapheresis

Plasmapheresis is the process whereby only plasma is taken from a blood donor. Whole blood is drawn and separated by the apheresis machine while it is linked to the donor, into cells and plasma. The red blood cells (and white cells and platelets) are returned to the donor and the plasma is collected in a special bag, so that it may later be converted into various products. Because their

red cells are returned to them, plasmapheresis donors may donate more frequently than whole blood donors.

***Plasmodium* – see also malaria**

This is the general name (genus) given to the malaria parasite. The different strains of malaria include *Plasmodium falciparum*, *P. malariae*, *P. vivax*, *P. ovale* and *P. knowlesi*.

Platelet additive solution (PAS)

Platelet additive solution (PAS) is a solution designed for platelet storage to maintain pH and viability better than plasma. The solution is added to platelet pools instead of plasma and more plasma is therefore available for other therapeutic use.

Platelet concentrate

This is a component obtained by concentrating the platelets from a donation of whole blood into a small volume of plasma or additive solution. Several units are usually pooled together so that a therapeutic dose can be administered to a patient without overloading the circulation. Platelet concentrates can also be collected by apheresis with a therapeutic dose being drawn from a single donor.

Plateletpheresis

This process is also known as platelet apheresis. It is a procedure for obtaining platelet concentrate from a single donor, using a programmed apheresis machine that automatically withdraws whole blood, extracts the platelets and at the same sitting, returns all the other blood constituents (red cells, plasma, white cells) to the donor.

Platelets

Platelets or thrombocytes are small particles found in the blood. They are derived from bone marrow cells called megakaryocytes. Platelets play a major role in blood clotting. They help to stop bleeding from small blood vessels and wounds.

Platelet yield

Platelet yield is the total number of platelets present in the final storage bag and is calculated using the platelet count per litre and adjusting it according to the total volume in which the platelets are suspended, making it a count per bag (e.g. 3×10^{11} per bag).

Polyagglutination – see also panagglutination

Polyagglutinable red cells become agglutinated by most normal adult human sera irrespective of ABO group but are rarely agglutinated by the individual's own serum/plasma.

Polybrene

This is an additive of high molecular mass that may be introduced to the serum/plasma-cell mixture after the incubation period in a test tube. Although it can detect both ABO and IgG incompatibility in crossmatch tests, it may fail to detect clinically significant antibodies within the Kell blood group system. It is also prone to false positives with cells coated with complement.

Polyclonal

The term polyclonal means derived from different cells. Polyclonal antibodies are produced by a variety of cells that are genetically different, and the antibodies may therefore differ in type and in specificity, i.e. not each antibody molecule is directed against precisely the same epitope, as is seen with monoclonal antibodies.

Polycythaemia

This is the abnormally increased production of red cells. The condition is sometimes improved by the therapeutic withdrawal of blood, but blood that is taken for medical reasons should never be used for transfusion into patients.

Polyethylene glycol (PEG)

Polyethylene glycol (PEG) creates a low ionic strength environment in the test and is a potentiator that increases antibody uptake onto cells with the corresponding antigen. It increases the sensitivity of tests involving clinically significant antibodies and decreases the interference of clinically insignificant antibodies. It may be used in both manual and automated systems, but care should be taken when centrifuging tests, as red cells clump tightly and may not be dispersed. Antiglobulin tests in which the serum/plasma-cells-PEG mixture is washed, should not present this problem.

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is used to amplify or duplicate a target section of DNA by enzymatic action so

that the original DNA molecule in a test is initially doubled. PCR has many different applications, including the detection of viral markers in blood donations, genotyping of red cells and identification of hereditary diseases. Specific 'primers' are used to match the target regions on the template DNA and copy and recopy it over many cycles, amplifying the amount of DNA in the test sample a million times and in so doing, making it of sufficient quantity to be detectable.

Polymorphic

Polymorphic means occurring in several (perhaps many) different forms. In genetics, a gene is referred to as polymorphic if more than one allele is found to occupy that gene's locus within a distinct population.

Polyvinyl chloride (PVC)

Polyvinyl chloride (PVC) is the material used to manufacture bags for blood collection. The plastic is treated with plasticisers to make it strong and flexible. PVC bags used for platelet storage differ from collection bags in that they are permeable to gases and allow transfer of oxygen to platelets.

Polyvinylpyrrolidone (PVP)

Polyvinylpyrrolidone (PVP) is a polymer used in red cell antigen-antibody reaction tests to enhance agglutination reactions.

Postnatal

This is the period of time immediately after the birth of an infant. It is also known as the postpartum period.

Postzone *see also Prozone*

If there is a very high number of antigens in a test system compared to the number of antibodies, agglutination may not occur. This is because the ratio of antibody to antigen is not optimal; antigens are present in excess of the number of available antibodies. The antibody-antigen ratio may be corrected by using a less concentrated cell suspension or increasing the serum to cell ratio.

Potentiator

This is a preparation added to laboratory tests, solutions or blood grouping reagents to enhance the reactions.

Precipitation – *see also immunodiffusion*

This is a chemical reaction between two solutions that react with each other to form a solid. When serum antibody is used in precipitation tests, the antigen with which it is mixed is also in solution. Recognition between antibody and antigen results in the formation of particles or aggregates. The rapid plasma reagin test for syphilis is an example of a precipitin test in which the syphilis antibody in the serum causes flocculation of the reagin reagent.

Precursor substance

This refers to the various precursor disaccharides at the nonreducing end of the carbohydrate chain to which specific monosaccharides are attached for the expression of the H, A and B antigens.

Pre-storage filtration

A unit of blood is filtered as soon as possible, preferably within 48 hours of donation, using a closed system which allows for the leucodepleted product to be stored without any reduction in shelf life.

Prevalence – *see also incidence*

Incidence relates to the number of new infections of a disease in a population over a defined time span, usually a year. Prevalence relates to the number of individuals living with an infection during the year of analysis.

Primary bag

In a multiple bag system, the donation is collected into a primary bag which contains anticoagulant.

Primer

Primers are used in polymerase chain reaction (PCR) testing. They consist of nucleic acid strands; commercially synthesised DNA oligosaccharides that are used according to their specific orientation. They are complementary to the target DNA in the test and are used during the PCR process to 'build' new DNA.

Prion– *see also Creutzfeldt–Jacob disease*

Prions are infectious protein particles that may rarely be present in blood. Prions cause untreatable and fatal diseases that affect the brain and other neural tissue, such

as the prions responsible for bovine spongiform encephalopathy in cattle, variant Creutzfeldt-Jakob disease in humans and scrapie in sheep.

Proband

A proband is the person who is the starting point for a genetic study, usually of a family.

Professional phagocytic cells

Professional phagocytic cells are cells of the immune system that have as their main function, phagocytosis. They include neutrophils, monocytes, macrophages and dendritic cells.

Proficiency testing

Proficiency testing is a method of assessing the ability of an individual, or a laboratory, to accurately and competently carry out a specified test. It usually involves the circulation of subsamples of material. The individual, or laboratory, analyses the material for a specific component, for example testing a blood sample for the presence of irregular antibodies. The performance of the individual, or laboratory, is usually established by comparison with the results from other individuals and laboratories that have tested an identical sub-sample for the same component, using the same or comparable methods.

Prophylactic – see also therapeutic

This term is used to refer to preventative/preventive medicine. An example is the use of anti-D immunoglobulin in the prevention of Rh HDFN. This product is administered to an unimmunised D negative mother with a D positive fetus or infant, to prevent the maternal development of anti-D antibodies.

Propositus

In genetics, the name propositus is given to an individual participant in a family study who provided the reason for the family study being initiated. An alternative term is proband; proposito referring to a male proband, and proposita to a female proband.

Protocol – see also standard operating procedure (SOP)

The term protocol is often used interchangeably with standard operating procedure but may also be used to include other written instructions or guidelines such as

equipment operating instructions, maintenance instructions and general quality policies.

Prozone see Postzone

On rare occasions, serum containing very high titre antibodies is unable to form a latticework of agglutination with red cells containing the corresponding antigen. Agglutination therefore does not occur. This is because the ratio of antibody to antigen is not optimal; antibodies are present in excess of the number of available antigen sites. The antibody-antigen ratio may be corrected by diluting the serum or using a stronger cell suspension. Once the ratio is corrected reliable results may be obtained.

Pruritis

Pruritis is a severe itching of the skin, which may be associated with allergic transfusion reactions.

Purpura

Purpura means bruising. This may be the result of trauma, such as when a blow to an area of the body causes blood to escape from the vascular system into the peripheral tissues, or it may be the sign of a disease, such as immune thrombocytopenic purpura (ITP).

Pus

Pus is the thick liquid produced in infected tissue, consisting of dead white cells, bacteria, and serum.

Pyrogens

These are endotoxins produced as a result of the metabolism of gram-negative bacteria. Alternatively, pyrogens may be exogenous in origin, being the debris of micro-organisms from the environment. Pyrogens are heat stable and are accidental and rare contaminants of intravenous fluids. Pyrogenic fluids produce fever in the recipient, and this may be life-threatening in its severity.

Qualitative – see also quantitative

A qualitative measurement refers to the characteristics of the item being described, rather than to any exact measurements. Whether a result is Positive or Negative is a qualitative result as it gives no indication of the strength of the Positive or the level of component being detected.

Quality assurance (QA)

This is a programme of procedures, controls, audits and corrective actions implemented to provide adequate confidence that a procedure, structure, component or system will function as expected and deliver results that meet or exceed predefined specifications.

Quality control (QC)

Quality control (QC) involves the procedures intended to ensure that a process, product or service meets or exceeds defined quality criteria.

Quality manual

The quality manual is the document that describes the quality management system to be used by the organisation.

Quantitative – see also qualitative

A quantitative measurement refers to the measurement of quantity or amount. A haemoglobin level of 125 g/l is a quantitative measure.

Random donor platelets – see also single donor platelets

Random donor platelets refer to platelet concentrates prepared (or recovered) from donations of whole blood or apheresis that are not specifically matched for a given patient (for example, not HLA matched).

Random sample

A random sample is a sample drawn from the total population, and that is representative of the total population; drawn in such a way that each member of the population has the same chance of being included in the sample (the smallest sample size to be considered relevant to analysis, is usually a minimum of 30 individuals, i.e. $n = 30$). The larger the sample, the more relevant the data obtained.

Recessive – see also dominant

The term recessive refers to an allele that expresses its product only when present in a double dose (e.g. short pea plants).

Recipient

The recipient in the context of this publication refers to the individual who receives a blood transfusion.

Red cell – see erythrocyte**Red cell additive solution – see additive solution****Red cell concentrates**

The blood cells containing the red pigment, haemoglobin, are called red blood cells (RBCs) or erythrocytes. RBCs remain as packed cells after the plasma from donated whole blood has been separated off during blood processing. This is one of the most useful blood components provided by a blood service and is used to correct anaemia and blood loss.

Renal function

This refers to the ability of the kidneys to eliminate waste products from the blood.

Reticulocyte

This is an immature red cell that contains remnants of its nucleus and is released into the bloodstream from the bone marrow prematurely, particularly when an individual is anaemic. A reticulocyte count that is higher than normal is therefore indicative of the body compensating for an anaemic condition.

Reticulocytosis

This is an increase in the number of circulating reticulocytes and is often seen as a consequence of anaemia as the body attempts to restore red cells to normal levels.

Reticuloendothelial system

The liver, spleen and lymph nodes together constitute what is known as the reticuloendothelial system and are important components of the body's system of immune defence.

Retrovirus

This is a virus that stores its genetic material in the form of single stranded RNA rather than the more common double stranded DNA. Retroviruses make use of the enzyme reverse transcriptase to form DNA copies of their RNA, and this DNA can then be integrated into the chromosomes of the infected cell. HIV is an example of a retrovirus.

Reverse (serum) grouping – see also forward (cell) grouping

This involves the testing of unknown serum/plasma against reagent red cells to determine the presence or absence of the corresponding antibody as related to the ABO blood group system. For example, if agglutination is absent when the patient's serum/plasma is tested against group A reagent red cells then it may be concluded that the serum/plasma does not contain anti-A.

Rh factor

This is an older term that was used to describe the D antigen of the Rh system.

Rh immunoglobulin

This is gamma globulin prepared by fractionation of a plasma pool collected specifically from donors known to have anti-D. As a plasma derivative, it is administered to unimmunised D negative mothers to prevent alloimmunisation to the fetal D antigen which could lead to HDFN in future pregnancies.

Ribonucleic acid (RNA)

Ribonucleic acid (RNA) is a nucleic acid molecule found in the nucleus of the cell. RNA is similar to DNA, but differs slightly in structure, containing ribose in place of deoxyribose.

Rituximab

Rituximab is a monoclonal antibody against the CD20 molecule which is found on the surface of B cells. The antibody brings about the destruction of the B cells, which is an effective treatment for certain autoimmune conditions and types of cancer.

Rouleaux

This phenomenon may be observed microscopically. The red blood cells stick together by their flat surfaces and strong rouleaux could be mistaken for agglutination. Unlike agglutination, the aggregation of red cells resembles a stack of coins.

Score

In an antibody titration against red cells with the corresponding antigen, the score is obtained by the addition of

numerical values allocated to the degree of agglutination obtained with each dilution of a titration. It takes into account both the avidity and the titre of the test sample. For example, agglutination strength of '4' relates to a score of '12'.

Screening cells

These are group O reagent red cells that have been fully tested for blood group antigens and are used to screen unknown serum/plasma samples for irregular antibodies.

Secretor – see also non-secretor

Individuals who carry the Se gene are called secretors because they secrete soluble substances (such as A and B) in their body fluids.

Sensitivity – see also specificity

Sensitivity is a measure of the minimum detection level of an assay. The more sensitive an assay is, the less likely it is to produce a negative result on a sample that is actually positive. Infectious disease tests used for blood donors are extremely sensitive, so infected donors are not missed.

Sensitisation

This type of red cell antigen-antibody reaction is not visible to the naked eye. It may occur when IgG antibodies are involved in laboratory testing. In order for the reaction to become visible a potentiator such as an enzyme, or AHG is used to bring about the agglutination of the sensitised red cells.

Sepsis/septicaemia

This is a serious clinical condition in which the body is infected by micro-organisms. When this occurs in the bloodstream it is known as septicaemia. It is usually accompanied by fever and leucocytosis and may be rapidly fatal if not treated.

Seroconversion

If an individual is exposed to an infection (such as HIV) defensive antibodies are produced. This process takes time. When antibodies become detectable in laboratory tests, the individual is said to have seroconverted.

Serology

This is the study of antigen and antibody reactions. For blood bankers, it usually involves the interpretation of reaction tests between red cells and serum/plasma.

Seropositive

An individual is said to have become seropositive to a virus, bacteria or some other foreign substance when antibodies are developed against that micro-organism or substance, and these are detectable in laboratory tests.

Serum

This is the liquid residue of whole blood, collected into a plain tube or a container that does not include anticoagulant. The blood is able to clot and as the clot retracts, serum is exuded. During the clotting process, coagulation factors are consumed, so therefore it may be said that serum is plasma minus the clotting factors.

Serum grouping

This involves the testing of serum with unknown characteristics, against red cells containing known antigens, with a view to identifying the presence or absence of antibodies in the serum. For example, if the serum is tested with red cells that contain the X antigen and agglutination occurs, the serum may contain anti-X. If there is no agglutination the serum lacks anti-X. If the serum agglutinates cells that lack X antigen, an antibody other than anti-X is present in the serum but it remains unidentified.

Sessile

Sessile is a term used to describe an organism that is immobile and fixed in one place.

Sex chromosome

The X and Y chromosomes in humans determine the sex of the individual. Females have two X chromosomes and males have one X and one Y.

Sex-linked

This refers to an allele that is located on one of the sex chromosomes, and the transmission of which is therefore linked to the sex of the offspring. Sex-linked characteristics are usually carried on the X chromosome.

Shelf life

This refers to the length of time that a product or blood component remains efficacious provided that it is stored appropriately. The storage period varies according to the blood component, product or plasma derivative. It is important that efficacy is maintained throughout shelf life.

Shock

A physical state of shock may be caused by sudden haemorrhage. Other causes are cardiogenic shock, septic shock and anaphylactic shock. Any type of shock can become irreversible and fatal if not controlled, usually by appropriate and timely medical intervention.

Sialic acid

Sialic acid is associated particularly with the glycoproteins of the MNS blood group system.

Sickle cell anaemia

This is an inherited disorder caused by a mutation in the β -globin gene. Individuals who inherit a double dose of the defective gene, have red cells that change into a sickle or crescent shape during a sickle cell crisis (period of abnormality). Sickle cells can become lodged in the capillaries; they also have a decreased lifespan.

Signs and symptoms

Signs are those manifestations of a medical condition that the clinician is able to observe, such as inflammation, swelling and pallor. Symptoms are those aspects which the patient is able to describe, such as headache, nausea and tightness of the chest.

Single donor platelets

Single donor platelets refer to platelet concentrates prepared by apheresis (plateletpheresis) of a donor dedicated to donating only platelets. A therapeutic adult dose of platelets is harvested from a single donor.

Solid phase

This is a term used in ELISA/EIA techniques to describe an antigen or an antibody that is immobilised onto a solid surface such as the inner lining of the wells of a plastic microtitre plate, or on beads.

Somatic cell

A somatic cell is any cell in the body other than the gametes.

Specificity – see also sensitivity

Specificity is a measure of the likelihood that an assay will produce a negative result when the component being measured is not present. In the laboratory the identification of a red cell antibody may be referred to as the specificity of the antibody.

Spherocytosis

This is the presence in the blood of spherical red cells, called spherocytes. This is seen in autoimmune haemolytic anaemia and may be seen following a haemolytic transfusion reaction.

Standard operating procedure (SOP) – see also protocol

An SOP is an authorised document that describes, in detail, the only approved method that is to be followed to complete a process or task.

Stem cell

This is a cell from which other types of cells develop. In the bone marrow the blood stem cells differentiate into specific blood cells (red cells, white cells, platelets), depending on the requirements of the body at the time.

Stridor

Stridor is a harsh grating sound during breathing and is usually the result of an obstruction of the larynx. Stridor may be associated with an anaphylactic transfusion reaction.

Storage lesion

This is the term used to describe the cumulative deterioration of a cellular blood component, such as platelet or red cell concentrates during storage.

Subgroup

The term subgroup refers to phenotypes with weak expression of an antigen, e.g. subgroups of A.

Surrogate test

A surrogate test is one that is used to indicate a related condition. For example, syphilis may be considered a surrogate test for other sexually transmitted infections. Although it is unlikely for syphilis to be transmitted by transfusion (because *T. pallidum* is susceptible to time and temperature), in many countries the test for syphilis is a good surrogate test because it indicates a high risk lifestyle, and reactive donations are therefore more likely to be carrying other sexually transmitted infections such as hepatitis and HIV.

Svedberg unit

The Svedberg unit is a unit for measuring the sedimentation coefficient. It provides a measure of a particle's size and mass based on how fast it settles at the bottom of the solution in which it is suspended. It is a measure of time and is equal to 10^{-13} seconds.

Syncope

Syncope is a temporary loss of consciousness that is caused by a drop in blood pressure.

Syphilis

Syphilis is a sexually transmitted disease, caused by a spirochaete (*Treponema pallidum*). It may be transmitted by very fresh blood, so blood donations should be tested for syphilis and discarded if reactive.

Systolic – see also diastolic

The systolic blood pressure refers to the measurement taken when the heart is at work and is contracting in order to pump the blood around the body. This is the time when the arteries are under maximum pressure.

Tachycardia

This is the term used to describe an increase in the rate of heartbeats, usually to more than 100 beats a minute. Amongst other causes, it can be triggered by stress or a medical condition. It could also be one of the signs of a transfusion reaction.

Tachypnea

This is abnormally rapid breathing and may be a symptom of an adverse reaction during a transfusion.

T-activation – see also *Arachis hypogaea*

Red cells which are T-activated have had their surface membranes eroded by the action of bacteria. This may occur *in vivo* as the result of infection or disease, or *in vitro* as a result of bacterial contamination of the blood specimen. Most fresh serum contains anti-T, so T-activated red cells become polyagglutinable and react with most serum when tested.

Teichoic acid

A polymeric compound that is present in the cell membranes of some bacteria.

Tetany – see hyperventilation**Thalassaemia**

This is an inherited disorder caused by quantitative defects in α or β globin chain synthesis leading to a shortened red cell lifespan. The disease may be mild to severe. Thalassaemia major is the more serious form of the disease which results in severe anaemia. Such patients are transfusion dependent. The disease also results in enlargement of the liver and spleen, and bone expansion, all of which occur as the body tries to compensate by expanding haemopoietic tissue into these areas of the body.

Therapeutic – see also *prophylactic*

A therapeutic agent is administered to relieve a medical condition, such as the administration of an analgesic to relieve pain. In the case of blood transfusion technology, a good example of a therapeutic agent is the transfusion of red cell concentrate to correct blood loss. This could be referred to as transfusion therapy.

Thermal amplitude

In blood bank laboratory terms, this is the temperature range over which an antibody will react.

Thrombin

Thrombin is activated coagulation factor II and is involved in the coagulation cascade. It converts soluble fibrinogen into insoluble strands of fibrin, as well as catalysing many other coagulation-related reactions. Thrombin is produced from prothrombin during one or other of the coagulation pathways; the contact of blood

with a foreign surface (extrinsic) or connective tissue (intrinsic).

Thrombocytes – see *platelets***Thrombocytopenia**

This is the term used to describe the medical condition of having too few platelets. Thrombocytopenia caused by the presence of autoantibodies (ITP) does not respond well to platelet transfusions, whereas thrombocytopenia caused by chemotherapy responds well.

Thrombosis

A blood clot *in vivo* is termed a thrombosis. It occurs inside a blood vessel. If of sufficient size, a thrombosis may have serious implications for health.

Thrombotic thrombocytopenic purpura (TTP)

Thrombotic thrombocytopenic purpura (TTP) is a rare disorder, often caused by an autoimmune condition in which the inhibition or absence of the von Willebrand factor (vWF) cleaving enzyme ADAMTS13 leads to the accumulation of high molecular weight vWF multimers. As vWF plays an important role in coagulation, this causes platelet activation and platelet-rich clots in small blood vessels (microvasculature), followed by haemolysis and organ damage. Causes of secondary TTP are not clearly defined. The disorder has been noted in individuals with cancer, in bone marrow transplant recipients, during pregnancy, and in patients on certain medications. Therapeutic plasmapheresis is used to treat TTP, removing the inhibitor and large vWF multimers and replacing the deficient enzyme.

Titration

This is the process whereby increasing dilutions of antibody containing serum/plasma are prepared and tested against the corresponding antigen to determine the strength or titre of the antibody.

Titre

Titre may be defined as the reciprocal of the greatest dilution of serum/plasma in which enough antibody remains to react with the corresponding antigen in a detectable manner. For example, if the dilution at which the last evidence of reactivity is seen is 1 in 256 (equivalent to 1 drop of serum and 255 drops of diluent) then the titre is said to be 256.

Toll-like receptors

Toll-like receptors are receptors found on the cell membranes of phagocytic cells such as macrophages and dendritic cells. The receptors recognise structurally conserved molecules on many micro-organisms. This ability gives them a key role in the innate immune system.

Trait

A trait is a feature of an organism. It is not a synonym for phenotype although the two words are sometimes used interchangeably. As an example, eye colour is a trait in which the phenotypes include blue, brown and hazel.

Transcription factors

Transcription factors are proteins that control the rate of transcription of genetic information from DNA to messenger RNA.

Transfusion

This is the administration of a blood component or product, or plasma derivative, into a recipient. The blood is originally taken from the vein of the donor and after processing and testing, is finally infused into the vein of the recipient.

Transfusion-associated acute lung injury (TRALI)

Transfusion-associated acute lung injury (TRALI) is a clinical syndrome of acute lung injury, usually arising during or shortly after transfusion, and resolving within 96 hours. Symptoms and signs include difficulty breathing (dyspnoea), fever, chills, hypotension and bilateral pulmonary oedema. TRALI may occur after transfusion of any component that contains plasma. The major mechanism is infusion of plasma containing anti-HLA or anti-granulocyte antibodies. Such antibodies could have been present in female donors as a result of previous pregnancies. When transfused, plasma containing these antibodies may activate primed neutrophils in the pulmonary microvasculature and cause lung injury in the recipient.

Transfusion reaction

This term describes the adverse effect of a blood transfusion. The majority of reactions reported are allergic, but reactions may occur as a result of failure to detect an incompatibility during crossmatching, or if blood was

accidentally administered to the wrong patient. Other rare causes of transfusion reaction include bacterial contamination of the donation.

Treponema pallidum – see syphilis

Trypsin

This is a proteolytic enzyme extracted from hog's stomach and used in blood group serology to enhance certain sensitisation/ agglutination reactions.

Tumour necrosis factor

Tumour necrosis factor is a cytokine of the acute phase reaction and is involved in the inflammation process.

Type and screen

The clinician may request a 'type and screen' from the crossmatching laboratory in case the patient needs a transfusion during a planned medical procedure. The blood bank groups the specimen and screens the serum/plasma for irregular antibodies that could cause delays in the provision of compatible blood if required. If a request for blood is later received, it is much quicker to process the request. A 'type and screen' facility does not reserve blood units unnecessarily and therefore helps the blood bank to manage blood in available stock.

Ulex europaeus

This is a lectin derived from *Ulex europaeus* (common gorse) and has anti-H specificity. It is very useful in the determination of secretor status in group O.

Ultrasound see also Doppler ultrasound

A procedure during which high-frequency soundwaves are bounced off tissue using special equipment, and the echoes converted into a picture, or sonogram. Often used to examine a fetus during pregnancy.

Universal recipient/universal donor

These are historical terms. Universal recipients can theoretically be transfused with blood from all other individuals. They are group AB and therefore have A and B antigens. Universal donors can theoretically donate blood for all other individuals. They are group O and therefore lack A and B antigens. They should also lack ABO haemolysins.

Urticaria

This is a raised, reddish skin rash and is one of the signs of an allergic transfusion reaction.

Vaccine

Effective vaccination involves a regime of administration of attenuated organisms to non-immune individuals with the aim of stimulating an immune response and providing lifelong immunity. Vaccines may be preparations of bacteria or viruses which have been modified (attenuated) so that they stimulate the production of antibodies without causing illness, they may also be totally synthetic

Validation – see also evaluation

This is the process of confirming that a process, product or service meets or exceeds a predefined set of criteria.

Van der Waals forces

This is a term that relates to agglutination strength/affinity between red cells and serum/plasma antibodies. Covalent compounds demonstrate van der Waals intermolecular forces that form bonds of various strengths with other covalent compounds. There are three grades of van der Waals forces; dispersion (weak), dipole-dipole (medium), and hydrogen (strong).

Variable region

The variable region is that part of the structure of an antibody that differs between antibodies that display different specificities.

Varicella zoster (VZ)

This is the organism that causes chicken pox in children and shingles in adults who have had chicken pox. Individuals who have recently had shingles infection often develop high titre antibodies. Their plasma is therefore useful as a source of hyperimmune globulin, used in the treatment of immunocompromised children suffering from chicken pox. These children may be leukaemic patients on chemotherapy, and therefore immunodeficient and unable to combat the chicken pox infection effectively.

Vasoconstriction and vasodilatation

Vasoconstriction is the contracting of the blood vessels, especially peripheral vessels, so that the blood (and therefore oxygen) only continues to be adequately

supplied to the brain and vital organs. It may be initiated by conditions such as shock, fear or a particularly cold environment (to limit loss of body heat). Vasodilatation or vasodilation may be medically induced or seen when the surrounding environment is particularly hot, and the blood is encouraged to reach the peripheral areas of the body where it can more rapidly be cooled down.

Vasovagal attack or reaction

This happens around the time of blood donation when the involuntary nervous system causes the heart rate to slow, the blood vessels in the extremities to dilate and the blood pressure to drop. The result is that the brain is temporarily deprived of oxygen and the individual faints as a result. Vasovagal attacks are nearly always self-limiting. An attack may be psychologically initiated as a result of fear at the thought of donating blood.

Venepuncture/venesection – see phlebotomy

Viability

An example to explain viability would be the ability of a red blood cell to transport oxygen. A viable red cell would be able to do this; a non-viable red cell would not be able to transport oxygen.

Vicia graminea

This is a lectin derived from seeds of the Brazilian plant *Vicia graminea* that can be used for N typing in the MNS blood group system. An extract from the leaves of *Vicia unijuga* is also used.

Viral markers

These are substances in the bloodstream, either antibodies or antigens or the products of viral infection, which are detectable in laboratory tests and which indicate that the individual is infected with the particular virus. Examples of viral markers used to test for the presence of HIV in a specimen include anti-HIV, p24 antigen and viral RNA.

Viral-safe

Blood products treated in some way to inactivate or remove viruses, are termed viral-safe products. This means that they will not transmit those viral diseases that are inactivated or removed by the treatment, to the recipients of these products. For example, factor IX complex and factor VIII, isolated from plasma, may be treated using solvent/detergents to make them viral-safe for HIV, hepatitis B and hepatitis C.

von Willebrand's disease (VWD)

This is a bleeding disorder caused by a defect or deficiency of a blood clotting protein, called von Willebrand factor (vWF) which is a protein critical to the initial stages of blood clotting. The factor interacts with platelets to form a plug which prevents the blood from flowing at the site of injury. Individuals with VWD are unable to make this plug, either because of insufficient or dysfunctional vWF.

Washed red cells

This is a blood bank term to describe the washing of donated red cells repeatedly in sterile saline to remove all traces of plasma. When a patient with anti-IgA antibodies who has had previous anaphylactic transfusion reactions requires red cells, it is necessary to wash the donor cells free of plasma prior to transfusion. Normal plasma contains IgA protein, and routine transfusion of red cells – which contain residual plasma from the donor – could cause a repeat anaphylactic reaction. It is also the term used in the laboratory to indicate that a red cell suspension has been washed in saline prior to resuspension and testing.

Western blot

The Western blot or immunoblot is a technique used for various studies including molecular biology and immunogenetics. Western blotting uses gel electrophoresis to separate proteins so that later each protein can be specifically identified. After the separating process, individual proteins are transferred to a cellulose membrane and exposed to antibodies. If there is a reaction between protein and antibody, then the presence of that particular protein in the test sample is confirmed.

West Nile Virus (WNV)

A virus that is spread by the bite of infected mosquitoes. Although many infected individuals display no symptoms of the infection, it can result in a wide variety of symptoms, including fever, disorientation, tremors, coma, loss of vision and paralysis.

'Wet' cryoprecipitate – see also cryoprecipitate

This refers to cryoprecipitate that is produced by a freeze/thaw method and that is stored frozen until required. Treatment of the haemophilic patient can be

by use of wet cryoprecipitate (often shortened to 'wet cryo').

Wharton's jelly

This is the name given to the jelly-like substance that may rarely contaminate a cord blood sample taken from a newborn infant. This jelly-like substance is found within the umbilical cord and may make testing of the blood difficult and likely to lead to false results.

White cells – see leucocytes

Whole blood

This is an unprocessed unit of blood collected from a single donor. The donation may be retained and stored as whole blood, and transfused as such, although many blood services today have the facilities to process whole blood into its various components. It is preferred that recipients receive only that part of the blood that they need. Whole blood is generally considered to be the starting material for component production.

Window period (or window phase)

The window period refers to the time between contracting an infection and the appearance of detectable antibodies or other markers in the bloodstream. It is a latent period of silent infection (immunosilence), as laboratory tests for markers of the infection are non-reactive. A blood donation given during the window period cannot be detected, is classified safe for use, placed in available stock and when transfused, may transmit infection to the recipient. The donor interview carried out when assessing the suitability of an individual to donate blood is critical to the detection and deferral or exclusion of individuals who appear at risk of TTIs. *A common misunderstanding amongst students is that if blood donated during the window period is stored for a period of time equal to the window period in days, then it could be retested, and a true result obtained. This is not correct! The status of the donation is like a snapshot in time, and that time relates to the time the donation was given. Viruses do not proliferate in a unit of donated blood and neither do antibodies develop.*

ZZAP solution

ZZAP is a solution which contains papain and dithiothreitol. It was originally used to treat the red cells of patients with warm autoimmune haemolytic anaemia to remove the antibodies from the red cells. The red cells could then

be used in auto adsorption tests. ZZAP destroys the M, N, Fya, and Kell system antigens. ZZAP solution is useful in a reference laboratory for preparing artificial Ko cells for laboratory use as an additional tool in antibody identification.

Zeta potential

All red blood cells have a negative charge (and a cloud of positively charged ions that surround them). This negative charge causes the red blood cells to repel each other; thus, they do not clump freely. The distance created between red blood cells because of this negative charge is called zeta potential.

Zika virus

A virus spread by mosquitoes of the *Aedes* family and first isolated in Uganda in 1947. Although many infected individuals display no symptoms, infection can result in fever, joint pain and rash. Infection during pregnancy has been found to cause microencephaly (abnormal smallness of the head and incomplete brain development) in the fetus.

Zygote

This is the cell formed as a result of the union between a male sex cell (sperm) and a female sex cell (ovum).

Units of measurement

Abbreviations	Unit	Description
Å	Angstrom	Unit used for the measurement of very small distances or lengths. Equal to 10^{-10} metres.
°C	Degree Centigrade	Unit of temperature.
cm	Centimetre	Unit of length or distance. Equal to 10 millimetres.
Da	Dalton	Unit used for the measurement the mass of very small particles such as molecules.
g	Gram	Unit of mass.
<i>g</i>	G force	Unit of gravitational force. In centrifugation it is dependent on the radius of the rotor and speed of revolution.
Gy	Gray	Unit of ionising radiation.
h	Hour	Unit of time. Equal to 60 minutes.
kg	Kilogram	Unit of mass. Equal to 1000 grams.
km	Kilometre	Unit of length of distance. Equal to 1,000 metres.
l	Litre	Unit of volume. Equal to 1,000 millilitres.
m	Metre	Unit of length or distance. Equal to 100 centimetres.
min	Minute	Unit of time. Equal to 60 seconds.
M	Molarity	The number of moles of a solute in one litre of solution. For example, normal saline contains 9 g of NaCl per litre and has a molarity of 0.154 moles per litre (One mole of NaCl = 58.4 g. and $9 / 58.4 = 0.154$)
M	Mole	Unit of mass. A mole of a substance is its molecular mass expressed in grams. For example, one mole of carbon is 12.0 grams and one mole of sodium chloride is 58.4 grams.
µl	Microlitre	Unit of volume. Equal to 10^{-6} l
ml	Millilitre	Unit of volume. Equal to 10^{-3} l
mm	Millimetre	Unit of length or distance.
pH	pH	Expressed the acidity or alkalinity of a solution. Values between 0 and 7 are acid, 7 is neutral, values between 7 and 14 are alkaline.
rcf	Relative centrifugal force	Unit of centrifugal force, dependent on the radius of the rotor and speed of revolution.
sec	Second	Unit of time
S	Svedberg unit	Unit for measuring sedimentation coefficient

SECTION 1

Haematology

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Introduction

Haematology is the study of blood. An introduction to the subject helps to form a firm foundation on which to build an understanding of the science of blood transfusion technology. Being aware of the nature of blood, its composition and its role in the maintenance of health are of value in better addressing the transfusion needs of patients.

Learning objectives

By the end of this section, the student should be able to describe the following in simple terms:

- Haemopoiesis or haematopoiesis
- Circulatory system
- Composition and functions of blood
 - plasma
 - red blood cells (erythrocytes)
 - white blood cells (leucocytes)
 - platelets (thrombocytes)
- Red cell abnormalities leading to chronic anaemia
- Haemostasis
- Clotting factors
- Coagulation defects
- Haemorrhage and shock

Haemopoiesis (Haematopoiesis)

Haemopoiesis or haematopoiesis is the production of blood cells, which in normal healthy adults occurs in the bone marrow and the lymphatic system. (During fetal life, haemopoietic tissue is found in the liver and spleen.) Because all blood cells have a limited lifespan, ongoing replacement of cells that are no longer viable is needed. This happens by division and maturation of precursor cells, mainly in the bone marrow. Self-renewing stem cells can develop into either of two types of cells, one giving rise to erythrocytes and platelets and the other giving rise to lymphocytes and natural killer cells. Mature cells are released into the bloodstream where they circulate and continue to age until they are no longer functional, at which time they are withdrawn from the bloodstream, broken down and the elements either reused or excreted. Figure 1 shows a simplified process of haemopoiesis. Arrows indicate cell development in the direction of maturation.

The specific blood cells that are produced and eventually released into the bloodstream are the red cells or erythrocytes, the various types of white cells or leucocytes and the platelets or thrombocytes.

All these formed elements, as the cells are called, originate from common ancestor stem cells that after many cell divisions in the bone marrow result in the mature blood cells that are found in the circulation. The precursor cells are known as blast cells, e.g. the precursor lymphocyte is called a lymphoblast.

Normal peripheral blood contains mature cells that do not undergo further division. Mature erythrocytes and platelets are non-nucleated.

Erythropoiesis describes the production of red blood cells. Red cells contain haemoglobin, which is needed for the transportation of oxygen in the bloodstream to all parts of the body. Throughout fetal life, fetal haemoglobin F (HbF) is produced by maturing red cells. It is only after birth that adult haemoglobin A (HbA) predominates.

Anaemia is the word used to describe in broad terms, a state of red cell deficiency, either in quantity or quality. An erythropoietic stimulus or trigger is needed for the body to initiate the production of new red cells, to maintain the balance between red cell production and

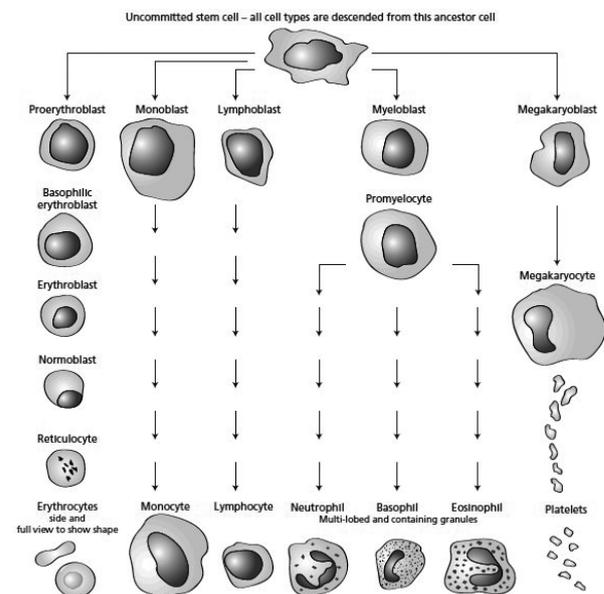


Fig. 1 Haemopoiesis.

red cell loss. Erythropoiesis is dramatically increased when there is a sudden substantial loss of blood. However, a very gradual yet regular loss of red cells over a long period of time may not result in adequate compensation and an individual could gradually become anaemic without obvious signs or symptoms until the haemoglobin level is dangerously low.

The major factor controlling the rate of red cell production is the oxygen content of the blood. A low blood-oxygen level leads to low tissue oxygen, a condition called hypoxia. Hypoxia is the strongest stimulus for erythropoiesis, as seen in the compensated erythropoiesis occurring in individuals living at high altitudes. There is less oxygen in the air at higher altitudes so inhabitants in such areas need more red cells to attach sufficient oxygen than individuals living at sea level, where oxygen in the air is in greater supply.

Hypoxia does not have direct control on the bone marrow but causes the release of a hormone called erythropoietin, produced in the kidneys, which then stimulates the bone marrow, causing the erythrocyte-forming cells to differentiate and divide and eventually form erythrocytes. Erythropoietin also causes the release of reticulocytes (immature red cells) into the circulation. The increased red cell mass then increases the amount of haemoglobin available to deliver oxygen and so the tissue oxygen level is increased.

The sudden loss of a moderate number of red cells through accident, or by donating a unit of blood, for example, is sufficient to stimulate the production of red cells to replace those lost.

Circulatory system

The heart pumps the blood around the body in the cardiovascular circulatory system, through the pulmonary (lungs) and systemic (body) circuits. The pumping of blood from the heart to the lungs allows the red cells to become oxygenated, and this oxygenated blood is then pumped to the brain and around the body via the arteries so that all parts of the body are reached. The veins transport carbon dioxide from the tissues back to the lungs, where it is released. The circulatory system transports nutrients to all parts of the body for metabolism, and waste products to the kidneys and other organs for excretion. Figure 2 depicts a simplified circulatory system showing the major blood vessels and organs. The human body is far too complex to depict accurately in such a simplified form. This figure is intended to illustrate the positioning of major arteries and veins in relation to vital organs – all of which are reached by both arteries and veins.

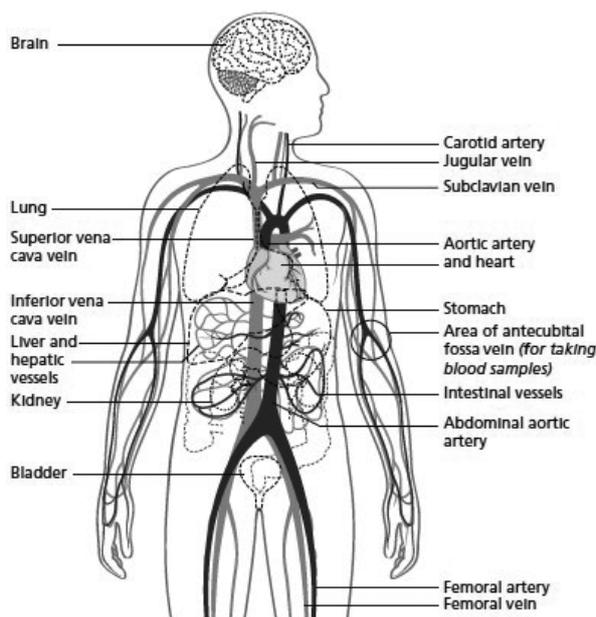


Fig. 2 Simplified circulatory system showing major vessels and organs.

The lymphatic system is part of the immune system and is also a circulatory system because it transports fluids to the tissues of the body. It consists of a series of glands and vessels that collect interstitial fluid from tissue spaces and then transport it back again to the circulatory system.

Arteries take oxygenated blood to the tissues; veins take deoxygenated blood back to the lungs. Where arteries meet veins there is an anastomosis (a cross connection between adjacent blood vessels). Figure 3 shows the direction of blood flow from artery to arteriole, arteriole to capillary and then capillary to venule and venule to vein. The anastomosis is found in the tissues, where the capillaries meet.

Composition and functions of blood

Blood is a suspension of various types of cells in a highly complex, aqueous (watery) fluid known as plasma.

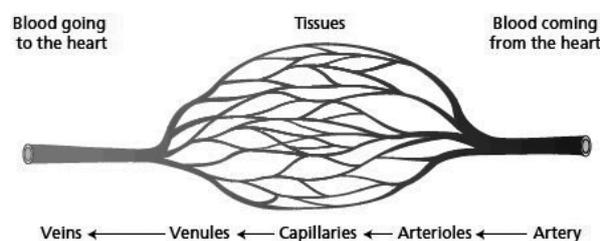


Fig. 3 Anastomosis between artery and vein.

The volume of blood in the average healthy adult is about five litres. Circulating blood volumes per kg of body weight differ between children and adults as follows:

- Neonates: 85–90 ml per kg
- Infants: 75–80 ml per kg
- Children: 70–75 ml per kg
- Adults: 65–70 ml per kg

The pH is 7.3–7.4 which means that the degree of acidity-alkalinity is nearly neutral. Acidic fluids have a pH of less than 7.0 and alkaline fluids have a pH of more than 7.0.

The specific gravity (SG) of blood is 1.050–1.065, which indicates that it is slightly heavier than pure water, which has an SG of 1.000.

The two major portions of blood are plasma, which, in healthy individuals, is approximately 60% of the total volume, and cellular elements or blood cells, which account for the remaining 40%.

About 90% of plasma consists of water.

Blood plasma

Plasma is a light yellow or straw-coloured fluid. Many substances are dissolved in the plasma, including oxygen, carbon dioxide, nitrogen, electrolytes (such as sodium, chloride and potassium), proteins, hormones, lipids, carbohydrates, amino acids, vitamins and nitrogenous wastes such as urea and uric acid. Water is the solvent in which these substances are dissolved.

There are many different proteins in plasma. These may be divided into two groups: albumin and globulin (clotting factors, enzymes and immunoglobulins).

Total serum protein is 60–80 g/l and about half of this is composed of albumin, which is synthesised in the liver. As protein does not freely diffuse through intact vascular endothelium (blood vessel lining), it provides osmotic pressure that regulates the passage of water and diffusible solutes through the capillary walls. This means that fluid does not readily diffuse out of the vascular system into the tissues but is retained in the circulation where it is needed. Albumin also serves as a carrier protein for various substances such as unconjugated bilirubin (the waste product of haemoglobin).

After albumin, the most plentiful plasma proteins are the immunoglobulins (antibodies) of which five main classes have been recognised: immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin D (IgD) and immunoglobulin E (IgE). These all serve in one way or another to protect the host from infection (see *Section 2: Immunology* for more details).

Plasma is a transportation system for the following:

- Cellular elements – red cells, white cells and platelets.

- Oxygen is carried by the red cells from the lungs to the tissues.
- Carbon dioxide is carried, mainly by the red cells, from the tissues back to the lungs.
- Hormones, antibodies, coagulation factors and other substances are taken from the site of their production to the site where they are needed.
- Nutrients are absorbed into the bloodstream from the digestive system and taken to the tissues.
- Waste products from tissue metabolism are transported to the organs of excretion (e.g. kidneys).

Main constituents of plasma (remembering that 90% of plasma is water):

(1) Solids

- Plasma proteins: albumin, globulin, fibrinogen.
- Regulatory proteins: enzymes, hormones, antibodies.

(2) Inorganic substances include the following:

- Calcium.
- Bicarbonate.
- Sodium.

- Chloride.
- Iodine.
- Iron.

(3) Organic substances

- Nutrients: sugars, amino acids and fats.
- Waste products: urea, uric acid and creatinine.

(4) Gases

- Oxygen.
- Carbon dioxide.

Blood cells

The blood cells, or formed elements, constitute approximately 40% of total blood volume in healthy individuals. They are divided into three main groups:

- Red cells (erythrocytes)
- White cells (leucocytes)
- Platelets (thrombocytes)

Figure 4 shows a stained blood smear on a slide, viewed microscopically.

Blood counts are done in the laboratory to estimate the numbers of cells in a patient, to determine whether these numbers fall within normal limits. For example, there are approximately 5 million red cells per cubic millimetre in normal adults. This result may be written in many ways, all with the same interpretation:

- Five million cells per cubic millimetre = 5 million cells/mm³
- Five million cells per cubic millimetre = 5 × 10⁶/mm³
- Five million cells per microlitre = 5 million cells/μl

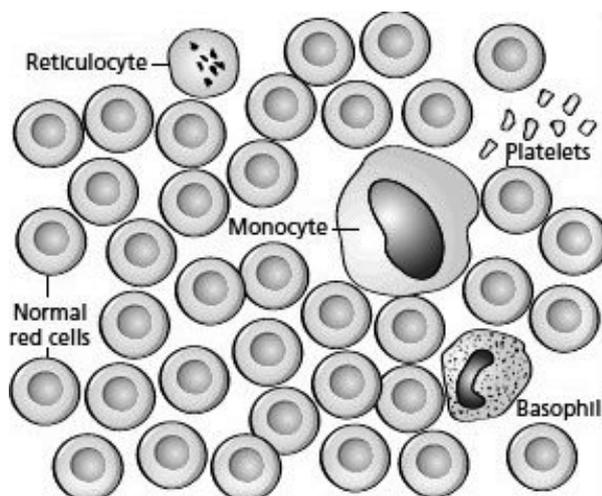


Fig. 4 Stained blood film showing red cells, white cells and platelets.

The white cells and platelets form the 'buffy' coat layer, which is seen between the red cells and the plasma in anti-coagulated blood samples that have been centrifuged. Figure 5 shows a centrifuged test tube of normal, anticoagulated blood.

Red cells (erythrocytes)

Red cells are described as non-nucleated, biconcave discs with a characteristic red colour as a result of a pigment contained in their haemoglobin. Oxygenated blood is bright red; deoxygenated blood loses its bright red colour and becomes a dull, dark red. Haemoglobin consists of four closely linked polypeptide chains (globin), each of which is attached to an iron-containing complex (haem).

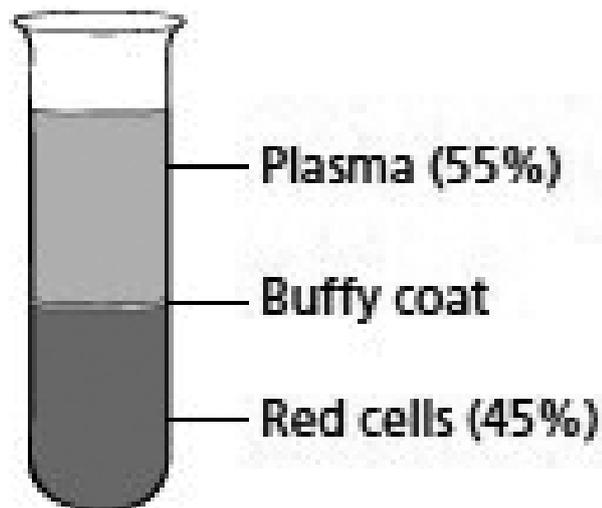


Fig. 5 Centrifuged test tube of anticoagulated blood.

The molecular mass of haemoglobin is about 68 000 Da. The haem is the part of the molecule responsible for its oxygen carrying capability.

The major function of red cells is to transport oxygen from the lungs to the tissues for use in cellular respiration. The red cells also remove carbon dioxide from the tissues for excretion via the lungs during exhalation. The normal *in vivo* survival of red cells is 100–120 days. *In vitro* survival depends on storage conditions and the type of anticoagulant and preserving fluid used. For a unit of donated blood, there should be sufficient viable cells at the end of the shelf life of that donation, for approximately 75% of the red cells still to be detectable in the bloodstream of the recipient, 24 hours after transfusion.

White cells (leucocytes)

White cells are responsible for host defence and for protecting the body from infection. There are many kinds of white cells. They are nucleated cells, some of which are capable of independent movement and can change their shape to surround and then ingest or 'swallow' foreign matter such as bacteria. The process of removing potentially harmful agents in this manner is called phagocytosis. See Fig. 6 for an illustration of the process of phagocytosis. Other white cells produce immunoglobulins (antibodies) that react with the foreign matter, also with the intention to inactivate or make harmless, and remove from the circulation.

Leucocytes are classified into three major groups:

- Monocytes
- Lymphocytes
- Granulocytes

Each type of leucocyte has a distinct function and morphology.

Monocytes

Monocytes are capable of ingesting bacteria and tissue debris or fragments by phagocytosis. They act as scavenger cells, clearing the body of foreign particles and the remains of cells, at the site of infection.



Fig. 6 Phagocytosis.

Lymphocytes

Lymphocytes vary in size from small lymphocytes, which are slightly larger than red cells, to large lymphocytes, which are larger than monocytes. Lymphocytes are either T- or B-lymphocytes (also known as T cells or B cells).

- T-lymphocytes are involved in cellular immunity and have a regulatory role in humoral immunity.
- B-lymphocytes are responsible for humoral immunity that involves antibody production.

Granulocytes

Granulocytes are polymorphonuclear cells. They contain a nucleus which has three lobes. They are characterised by granules that are visible microscopically in the cytoplasm of the cells, after laboratory staining. Granulocytes fall into three subgroups:

- Neutrophils – main function is phagocytosis.
- Basophils – capable of phagocytosis and contain histamine and heparin.
- Eosinophils – capable of phagocytosis and are involved in allergic responses.

Platelets (thrombocytes)

Platelets are small particles that have no nucleus and come from the cytoplasm of bone marrow cells called megakaryocytes. They participate in blood coagulation. Platelets form a plug to seal damaged blood vessels and thus have a crucial role in haemostasis or the control of bleeding.

Table 1 Normal blood measurements in SI units

Measurement	Men	Women
Red cell count	4.5–6.5 × 10 ¹² /l	4.0–5.5 × 10 ¹² /l
White cell count	4.0–11.0 × 10 ⁹ /l	4.0–11.0 × 10 ⁹ /l
Haemoglobin	130–180 g/l	120–160 g/l
Haematocrit	0.40–0.54	0.38–0.47
Platelets	150–400 × 10 ⁹ /l	150–400 × 10 ⁹ /l

Table 2 Lifespan of formed elements *in vivo* and *in vitro*

Blood cells	Lifespan <i>in vivo</i>	Approximate lifespan <i>in vitro</i> (in storage)
Erythrocytes (red cells)	100–120 days	Up to 6 weeks storage as red cell concentrate
Leucocytes (white cells)	13–20 days	A few days only, except for lymphocytes
Lymphocytes	Up to 4 years	Lymphocytes can proliferate when transfused
Thrombocytes (platelets)	5–10 days	About 5–7 days storage as platelet concentrate

Table 3 Terms used to describe blood cells with abnormal counts

Blood cells	Excessive number of cells	Depleted number of cells
Erythrocytes (red cells)	Polycythaemia	Anaemia
Leucocytes (white cells)	Leucocytosis	Leucopaenia
Thrombocytes (platelets)	Thrombocytosis	Thrombocytopenia

Table 1 lists the normal blood measurements in adult men and women, Table 2 gives the normal lifespan of the formed elements *in vivo* and *in vitro*, and Table 3 notes the terms used to describe blood cells with abnormal counts.

Red cell abnormalities leading to chronic anaemia

When the blood of an anaemic individual is examined, it is frequently found to have a raised number of immature red cells called reticulocytes. They are identifiable under the microscope by their remnants of cell nucleus. Although reticulocytes are also found in the bloodstream of healthy individuals, the number is raised in an anaemic state.

Examples of red cell abnormalities leading to chronic anaemia, and which are of concern within the field of blood transfusion, include thalassaemia and sickle cell anaemia.

Thalassaemia

This is an inherited disorder seen mostly in individuals from areas of Asia and the Eastern Mediterranean. It is caused by a quantitative abnormality in the α or β globin chain in the haemoglobin molecule that leads to the red cells having a shortened lifespan. The disease may be mild to severe. Thalassaemia major is a more serious form of the disease than thalassaemia minor. Patients with thalassaemia major are severely anaemic and are often transfusion dependent. The disease causes enlargement of the liver and spleen, and bone expansion, as the body tries to compensate by expanding haemopoietic tissue into these areas.

Sickle cell anaemia

This is an inherited disorder seen in the Arabian Peninsula, and parts of Asia and Africa. It is caused by a structural defect within the β globin chain of the haemoglobin molecule, which give rise to formation of HbS and haemolysis. Individuals who inherit a double dose of the defective gene have red cells that change into a sickle or crescent shape during a sickle cell crisis. Sickle cells can become lodged in the capillaries; they also have a decreased lifespan. Some patients are asymptomatic most of the time and do not require routine transfusion. However, in other cases, transfusion may be necessary to lower the concentration of HbS. Figure 7 shows a blood

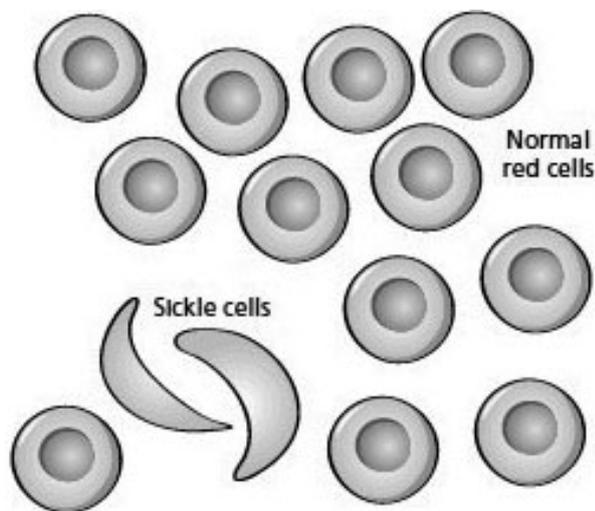


Fig. 7 Diagram showing sickle cells and normal red blood cells.

smear on a slide, with normal and sickle-shaped red blood cells as they appear under the microscope.

Haemostasis

Haemostasis is the term used to describe the control of bleeding, the formation of a clot, and in the time of healing, the resolution of the clot and a return to normal. The process has four major components:

Vasoconstriction. Vascular constriction occurs in a damaged blood vessel with a muscular wall. This means that the blood vessel contracts or tightens to minimise further loss of blood.

Platelet plug. Blood does not clot in the body because there is insufficient free thromboplastin. However, as a result of injury, thromboplastin is released and in the presence of calcium ions (Ca^{++}), combines with prothrombin to form thrombin, which in turn activates platelets. Activated platelets amplify the response by activating more platelets, and fibrinogen stimulates their sticking together as a soft plug that loosely blocks the wound, dramatically slowing down the bleeding.

Clot formation. Each clotting factor has in the meanwhile been participating in the process, which is why it is called the coagulation cascade. A mesh of insoluble fibrin is finally formed, resulting in a platelet plug and so reaching the endpoint of clot formation. As the clot shrinks a clear fluid called serum is exuded.

Dissolving the clot. The last component of the process is carried out by the enzyme plasmin, which is responsible for the gradual absorption of the clot by the body, so that a return to normal is eventually achieved.

Clotting factors

The coagulation of blood is a complex process initiated typically when the skin is breached. When normal levels of coagulation factors are present in the body, they act in a cascade, where one factor when activated, activates the next, and so on, until a clot is formed, and bleeding is stopped (i.e. haemostasis is achieved). Each clotting factor has been assigned an individual Roman numeral as shown in Table 4.

Blood coagulation is the culmination of a series of complex reactions that bring about a fibrin clot. The clotting cascade has two pathways; (1) the tissue factor pathway or (2) the contact activation pathway. This is followed by a common pathway, in which clotting factor X, thrombin and fibrin are activated. Clotting factors circulate in the bloodstream in the form of inactive precursors. Table 5 shows a simplified coagulation cascade. The Roman numerals indicate the number of the clotting factor. The letter 'a' after a Roman numeral signifies that the clotting factor has been activated.

Except for FV, FVIII and platelets, clotting factors are relatively stable in stored blood. Factor V and FVIII levels in donated blood plasma are stable only when frozen soon after collection to temperatures lower than -25°C . Platelet concentrates prepared from donated blood remain viable for 5 to 7 days under the correct conditions of storage: they need to be kept constantly in motion within a temperature range of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

The process of coagulation is illustrated as a cascade in Fig. 8.

Coagulation defects

Life-threatening bleeding can occur as a result of defective haemostasis, which may be acquired or congenital.

Table 4 Listing of clotting factors

Factor No.	Abbreviation	Description of factor
Factor I	FI	Fibrinogen
Factor II	FII	Prothrombin
Factor III	FIII	Tissue factor (thromboplastin or thrombokinase)
Factor IV	FIV (Ca^{++})	Calcium
Factor V	FV	Pro-accelerin
Factor VI	FVI	Not assigned
Factor VII	FVII	Proconvertin
Factor VIII	FVIII	Anti-haemophilic factor A
Factor IX	FIX	Anti-haemophilic factor B
Factor X	FX	Stuart-Prower factor
Factor XI	FXI	Plasma thromboplastin
Factor XII	FXII	Hageman factor
Factor XIII	FXIII	Fibrin stabilising

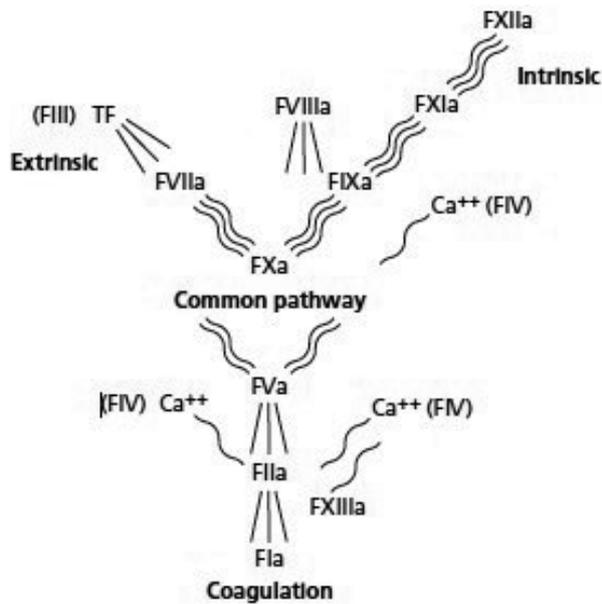


Fig. 8 Coagulation cascade.

Acquired defects

Acquired defects are those that develop in an otherwise normal individual, and include:

- Quantitative thrombocytopenia (low number of platelets in the body) as a result of massive transfusions of stored blood (which lack functional platelets).
- Drug-induced qualitative platelet deficiency (poor quality of platelets, that do not function normally, due to the influence of circulating drugs).
- Deficiencies of FII, FVII, FIX and FX due to vitamin K deficiency, anticoagulant medications such as warfarin, and hepatic dysfunction.
- Disseminated intravascular coagulation (DIC) when the balance between bleeding and thrombosis is disturbed. This condition typically affects critically ill patients.

Congenital defects

Congenital defects are those that are inherited and therefore present from birth, and include:

- Classic haemophilia (haemophilia A) is inherited as a sex-linked recessive trait (characteristic) in which there is a lack of functional FVIII. Females are carriers of the trait, whereas males are clinically affected and have a bleeding tendency, the severity of which varies from one individual to another.
- Christmas disease (haemophilia B) is inherited as a sex-linked recessive trait in which there is a lack of FIX. The clinical signs and symptoms are very similar to haemophilia A and laboratory testing may be required to distinguish between the two.
- von Willebrand's disease (VWD) is seen in both males and females and its commonest form is inherited as an autosomal dominant trait. This means that it is not sex-linked, and because it is a dominant gene, can be expressed even if inherited in a single dose (from one parent). VWD is caused by abnormalities of von Willebrand's factor (vWF), a glycoprotein that is important both for platelet adhesion at the site of blood vessel injury and the coagulation cascade.

Haemorrhage and shock

Haemorrhage is the sudden, abnormal loss of large amounts of blood as a result of injury or medical emergency, such as a heavy loss of blood after delivery of an infant (postpartum haemorrhage). When an individual loses more than 30% of circulating blood volume, normal compensatory mechanisms for correction of the situation, such as vasoconstriction, will not be enough and the individual will go into shock. In general, shock means that tissues and organs are not receiving an adequate supply of blood. Haemorrhage is the most common cause of

Table 5 Simplified coagulation cascade

Tissue damage (extrinsic pathway)		
FVII	FVIIa	+ Tissue factor (FIIIa) (in presence of injury)
FX	FXa	
Contact activation (intrinsic pathway)		
FXII	FXIIa	(exposure to non-vascular surface)
FXI	FXIa (in presence of calcium – FIV)	
FIX	FIXa	+ FVIII → FVIIIa (in presence of calcium – FIV)
FX	FXa (in presence of calcium – FIV)	
Common pathway		
FXa (starting point)	FV	→ FVa (in presence of calcium – FIV)
	FII	→ FIIa (prothrombin → thrombin + FXII → FXIIIa)
	FIII	→ Fla (fibrinogen → cross-linked fibrin)

hypotensive shock, which is characterised by the following:

- Peripheral vasoconstriction: the patient appears pale because the blood supply to the surface is restricted in order to supply the vital organs.
- Hypotension: there is a drop in blood pressure.
- Tachycardia: the heart beats much faster than usual.
- Oliguria: there is a reduction in the excretion of urine.

Haemorrhage is not the only condition that is able to cause a state of shock. Other causes are listed below. Note that shock of any kind can be rapidly fatal if not controlled before reaching an irreversible stage.

- Cardiogenic shock occurs when there is a reduction of cardiac output as a result of coronary occlusion (blockage of the blood vessels that supply the heart with oxygen).
- Distributive shock is characterised by inappropriate vasodilatation of the peripheral blood circulation secondary to sepsis or anaphylaxis (severe allergy).
- Obstructive shock: caused by obstruction of the heart or great blood vessels. This may be caused by the presence of fluid or air around the heart or lungs (tension pneumothorax, cardiac tamponade).

Management of shock is different according to its type.

Key Points

- Haemopoiesis is the term used to describe the production of blood cells in the bone marrow.
- Erythropoiesis is the process of red cell production. Erythropoietin is a hormone that influences erythropoiesis.
- Because blood is transported in a system, it circulates throughout the body, carrying oxygen from the lungs to the tissues, nutrients from the digestive system to the tissues where they are needed, wastes from tissue metabolism to the organs of excretion, organic and inorganic substances needed to sustain a healthy body, and hormones and enzymes to regulate body functions.
- Blood plasma forms approximately 60% of the blood in healthy individuals, and 90% of this plasma is water. The remainder consists of all the soluble elements of the blood including albumin and globulins.
- The formed elements are the blood cells of the body, which include red cells, white cells and platelets. They form approximately 40% of the blood in healthy individuals.
- Red cells are responsible for transporting oxygen to the tissues and carbon dioxide back to the lungs for exhalation.
- There are several types of inherited red cell abnormalities; these include thalassaemia and sickle cell anaemia.
- White cells are responsible for body defence. Monocytes are phagocytic; lymphocytes are either T or B cells that are responsible for cellular immunity or produce antibodies (respectively), and the granulocytes or polymorphonuclear cells have several functions including phagocytosis. They are made up of neutrophils, basophils and eosinophils, the latter being involved in allergic responses.
- Platelets play a vital role in blood coagulation.
- Haemostasis is the term used to describe coagulation or the stopping of bleeding.
- A series of many different coagulation or clotting factors are activated sequentially to stop bleeding. Most of them are stable in donated blood, but those that are labile (short-lived) include FV and FVIII.
- Haemophilia A is an inherited disease caused by lack of factor VIII.
- Haemophilia B is an inherited disease caused by lack of factor IX.
- von Willebrand's disease is caused by abnormalities of von Willebrand's factor, affecting both coagulation and platelet function.
- Shock may be initiated by hypovolaemia, sepsis, cardiogenic factors, anaphylaxis or obstruction. This condition can be rapidly fatal if prompt medical treatment is lacking.

SECTION 2

Immunology

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Introduction

Immunology is the study of the immune system and how the body protects itself from infection using a variety of methods. Failure of the immune system to function correctly can result in disease, such as autoimmunity, allergy and cancer.

Immunological defence is important to confer protection of an organism against invasion by foreign micro-organisms and to facilitate tissue repair. The body recognises 'self' and targets the foreign 'non-self' as potentially harmful and to be destroyed. Differentiation between self and non-self, and the body's reaction to non-self, forms the basis of the immune response.

Allogeneic blood transfusion essentially introduces a foreign substance into the body. It is therefore crucial that the blood product to be infused is selected to be as immunologically compatible as practically possible, i.e. it should match 'self' as closely as possible to prevent undue immune reaction in the host, to the blood being transfused. An immune reaction can result in reduced clinical efficacy or have an adverse effect on the recipient. Without a clear understanding of immunology, it is difficult to interpret the clinical rationale and consequences of blood transfusion as well as to interpret immunohaematological tests in the laboratory.

Learning objectives

By the end of this section, the student should be able to define and describe the following:

- Role of the immune system
- Innate immunity
- Cells of the immune system
- Inflammation
- Phagocytosis and antigen presentation
- Complement
- Adaptive immunity
- Antigens and antibodies
- Nature of immunogenicity
- Primary and secondary response
- Active and passive immunity
- Characteristics of immunoglobulins
- Immune paralysis and immune tolerance
- Autoimmunity
- Immunodeficiency

- Viruses and the immune response
- Cancer and the immune system

Role of the immune system

The role of the immune system is to defend the body against pathogens and other foreign substances (i.e. foreign agents) that may be harmful, by identifying and destroying them. Figure 1 shows a simplified version of the location of the main components of the immune system. Table 1 lists the main cellular components of the immune system.

The natural barriers of the body i.e. the skin and mucous membranes, guard against the entry of foreign agents and serve as the first line of defence, i.e. the interface between the body and the environment.

- Intact skin prevents pathogens and other foreign agents from entering the body.
- Mucous membranes protect vulnerable parts of the body that are exposed to air. Examples are the nostrils and eyes, and internally, the gut and lungs. The mucous secreted by these membranes forms a

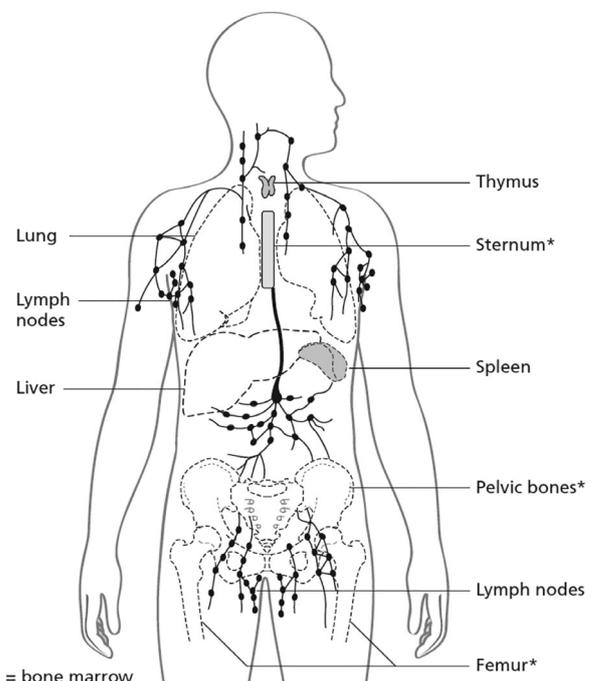


Fig. 1 Sketch of immune system components.

Table 1 Cells of the immune system

B lymphocytes	Lymphocytes (B cells) responsible for the production of antibodies
Dendritic cells	The antigen-presenting cells of the immune system
Granulocytes	These are polymorphonuclear cells with a multilobed nucleus whose main function is phagocytosis
Lymphocytes	White cells responsible for immune defense by cell-mediated immunity and the production of antibodies
Macrophages	Large white cells whose main function is phagocytosis
Monocytes	Large white cells whose main function is phagocytosis
Natural-killer (NK) cells	A type of lymphocyte that play an important role in the destruction of virally infected cells
T lymphocytes	Lymphocytes (T cells) responsible for identifying foreign antigens and initiating the adaptive immune response
Neutrophils	White blood cells that mediate innate immunity
Eosinophils	White blood cells that help promote inflammation

Table 2 Comparison between innate and adaptive immunity

Innate immunity	Adaptive immunity
The response is inborn	The response is learned
The response time is the same every time and is usually rapid	There is a delay between first exposure and peak of response
The response actions are the same regardless of the nature of immunogens encountered	The type of response depends on the nature of the immunogens encountered
There is no immunologic memory following repeated exposure to an immunogen and response actions are repeated as before	Immunologic memory follows first exposure, so when the same immunogen is encountered again, it is recognised, and response is faster and better as a result

protective barrier, slowing down and hindering the entry of foreign agents into the body.

When foreign agents breach the skin or penetrate mucous membranes, other defence mechanisms in the body are activated. These include cellular and non-cellular components of the immune system which then start to protect the body from potential damage by the invading agent(s). The immune system is broadly classified into two subsystems: innate and adaptive. The innate immune system is evolutionarily primitive (i.e. it was developed very early in evolutionary history and has not changed very much since) but plays an important role as the first line of defence and is activated early in the immune response. Innate immune response is broad-based and non-specific. Adaptive immunity is evolutionarily recent (i.e. has developed relatively recently in evolutionary history) and is confined to vertebrates. Its response is slower but more flexible, specifically targeting foreign agents and conferring memory, so that re-exposure to the same agents in future is met with a more rapid, effective and specifically charged response. Table 2 provides a summary comparing innate and adaptive immunity. Despite differing functions, the two arms work in harmony (synergistically) to mount an effective response to infection and tissue injury.

Innate immunity

Innate immunity includes various components of natural (inborn) host defence that range from non-specific

epithelial barriers to various effector cells and chemicals. Although rapidly mobilised, the innate immune response tends to be generalized. External defence is maintained by means of intact skin, by mucous, which traps and eliminates micro-organisms, and by body secretions such as saliva and perspiration, which have disinfectant properties.

Effector cells and chemical mediators recognise foreign agents that enter the body, thereby initiating an immune response against them. Pathogens contain structures that exhibit specific motifs (identifying markers) that are not present in mammals. These molecular motifs or patterns are present in many classes of pathogen and are termed pathogen-associated molecular patterns (PAMP). Examples of PAMP include lipopolysaccharides (LPS), mannans and teichoic acids that are present on bacterial cell walls. The innate immune system responds to the presence of PAMP through a group of proteins termed pathogen-recognition receptors (PRR). PRR are expressed on many of the cells mediating the innate immune response as well as within secreted and locally produced molecules that are involved in inflammatory processes. Cells mediating innate immunity include dendritic cells, monocytes, macrophages, granulocytes and natural-killer (NK) cells.

An important group of PRR are the Toll-like receptors (TLR) that are expressed on the innate immune cells and on the cells of various tissues including epithelial cells, endothelial cells and fibroblasts. TLR play an important role in the initiation of the innate immune response by detecting pathogens. Various classes of TLR bind to

different molecular structures on viruses, bacteria, fungi and other pathogens, leading to phagocytosis and the killing of the pathogen as well as release of pro-inflammatory cytokines (signalling molecules that promote inflammation) and anti-microbial substances that assist in the destruction of the pathogen.

Inflammation

Inflammation is an early defence process designed to protect the body from potentially harmful foreign agents and to facilitate the eventual process of tissue repair. Its primary function is to rapidly destroy or isolate the underlying source of danger, remove damaged tissue, and then restore tissue integrity. The first step in the inflammatory process is the recognition of the offending pathogen or agent. In the case of pathogens, this is achieved by detection of PAMP through the various PRR such as the TLR. This leads to the upregulation (activation) of certain transcription factors e.g. Nuclear Factor kappa B (NF- κ B) leading to an increased production of proinflammatory cytokines such as interleukin-1- β (IL-1 β), IL-6 and tumour necrosis factor- α (TNF- α). Chemokines further recruit (i.e. enlist or engage) inflammatory cells such as neutrophils and monocytes to the site of damage. The gathering together of this immune reaction is helped by changes in the local vascular system as indicated in the list that follows. The consequences of inflammation and its major signs and symptoms include:

- Increased blood flow (feeling of heat)
- Pain
- Swelling
- Increased blood supply to nearby capillaries (redness)
- Loss of function in the inflamed area, particularly if a joint is affected
- Attraction of phagocytic cells, with evidence of pus as a result of phagocytic activity.

Phagocytosis and antigen presentation

Phagocytosis occurs as a result of recognition of particles and microbes and their subsequent ingestion by cells which eliminate them. These 'professional' phagocytic cells include monocytes, macrophages, neutrophils, dendritic cells and eosinophils. They recognise pathogens or particles (i.e. foreign agents) and engulf them through a variety of receptors that can be divided into opsonic and non-opsonic. Non-opsonic receptors can directly recognise molecular motifs, or distinct molecular patterns, on the surface of the phagocytic target such as microbial lectins. Opsonic receptors meanwhile detect host-derived opsonins that attach to the foreign particles and target

them for ingestion. Important opsonic phagocytic receptors include Fc receptors (FcR) and complement receptors (CR), both of which are described in detail later in this section. FcR bind to IgG bound particles while CR bind to complement molecules deposited on cells and particles following complement activation. Phagocytic cells are also important for processing ingested extracellular proteins into peptides that then become attached to MHC-I class molecules, to be taken to and presented to T cells for destruction. T cells are part of the adaptive immune process.

Complement

Complement (C) consists of a group of more than 20 soluble proteins that play a pivotal (i.e. key, vital) role in the immune response. These proteins are present in the plasma in an inactive form until stimulated. It usually takes one immunoglobulin M (IgM) antibody molecule or two immunoglobulin G (IgG) antibody molecules in juxtaposition (close together) on a target cell, to initiate the complement response. Each complement protein then acts in sequential fashion, one activating the next like a cascade. The result is that complement either becomes attached to the target cell membrane, making it more susceptible to phagocytosis, or continues in a chain reaction to its end, which results in the lysis (breaking) of the cell membrane. The consequence of cell lysis is loss of cell contents, and cell death. Complement activity is therefore a very important immunological process to address threats to the host.

The complement cascade is a complicated sequence that in simple terms consists of nine major protein components, C1 to C9. When a component is activated, it is written with a line on top. The sequence of activation is C1, C4, C2, C3, C5, C6, C7, C8, C9. The activation stage involves C1, C4 and C2. The activation is amplified when hundreds of molecules of C3 are then activated and become target cell bound, first as C3b and later as C3d. The cascade sometimes stops at this point. If it continues to the point of cell lysis, then the remaining components from C5 onwards play a role, with the membrane attack unit consisting of C7, C8 and C9. The complement cascade is discussed in more detail in *Section 3: Antigen-antibody reactions*, from the perspective of its involvement in antigen-antibody reactions in the laboratory.

The major actions of complement are:

- Participation in acute inflammatory processes.
- Assisting in opsonisation that helps the process of phagocytosis.
- Modification of cell membranes that results in lysis.

Complement may become activated in one of three ways, each of which causes the activation of C3, splitting it into a large fragment (C3b) that attaches to the

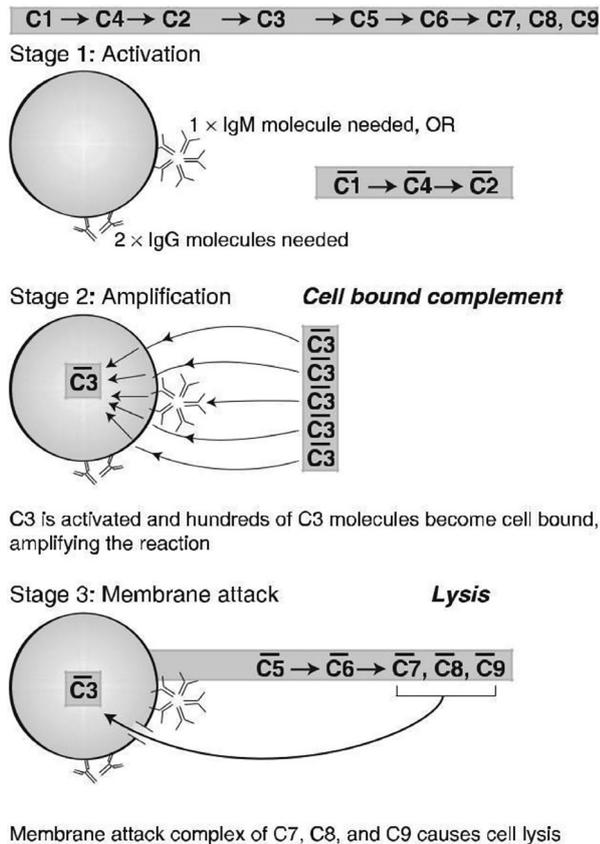


Fig. 2 Sequential activation of complement – classical pathway.

pathogen causing the activation, and a small fragment (C3a) that is released into the plasma and promotes inflammation:

- Classical pathway (1): initiated as a result of some antigen-antibody reactions and the activation of the C1-complex. Fig. 2 shows the sequential activation of complement via the classical pathway.
- Alternative pathway (2): triggered by the presence of polysaccharides and lipopolysaccharides found on the surfaces of micro-organisms and tumour cells. An antigen-antibody reaction is not required as activated C3 (specifically C3b) binds directly to the surface of the pathogen, thus by-passing the activation Stage 1 shown in Fig. 2.
- Lectin pathway (3): triggered by the binding of mannan-binding lectin (found in the plasma) to mannan residues on the pathogen. This results in the cleaving of C3 and the production of C3b and C3a.

Adaptive immunity

The adaptive immune response is brought about or mediated by T and B lymphocytes. Unlike innate immunity

where only a limited number of foreign molecules or foreign molecular patterns are recognised by its cellular and chemical mediators, adaptive immunity has the capacity to recognise an infinite number of peptide or carbohydrate molecules. This is achieved through the generation of highly diverse T cell receptors (TCR) and B cell receptors (BCR) that can recognise and bind to foreign molecules. As will be seen in the section on autoimmunity below, the immune system may sometimes fail to recognise 'self', resulting in the host's own cells being treated as foreign.

T lymphocytes: cell-mediated immunity

Thymus dependent lymphocytes, also known as T lymphocytes or T cells, are responsible for cellular defence - lymphocytes circulate throughout the body as small lymphocytes. T lymphocytes recognise foreign molecules that are presented by HLA Class-I and Class-II expressing cells. All nucleated cells as well as platelets, express HLA Class-I molecules. HLA Class-II molecules are restricted to professional (specialised) antigen-presenting cells such as macrophages and dendritic cells. Professional antigen-presenting cells are those cells that specialise in presenting an immunogen (i.e. antigen) to a T cell. When encountering an antigen, these cells can attach, process and present the antigen molecule on its cell surface, bound to the HLA molecule. T lymphocytes interact with the antigen complex via its TCR. This results in the activation of the T lymphocyte and release of various cytokines that facilitate cell-mediated immunity and humoral immunity. For more information on HLA, see *Section 6: Blood Group Systems*.

The results of this contact are:

- T lymphocytes with a specific reactivity site are produced, which can then react directly with the particular foreign immunogen that was presented to them by the antigen-presenting cell in the first place. These activated T cells also release lymphokines, which instruct uncommitted lymphocytes (developing lymphocytes that are not yet committed to a specific function) on the nature of these immunogens, so that they too can react with the immunogens and destroy the cells on which they are situated. This greatly amplifies the intensity of the immune response.
- Circulating memory cells are produced. These are responsible for the anamnestic or 'memory' response, which occurs on subsequent contact with the same immunogen. This facility allows for the recognition and rapid elimination of micro-organisms containing immunogens that were encountered before.

It is cell-mediated immunity that causes delayed hypersensitivity reactions. This is an inflammatory reaction that occurs a few days after exposure to an immunogen. Such a response is caused by T lymphocytes and macrophages and not by antibodies. One of the unfortunate consequences of delayed-type hypersensitivity is the rejection of a graft such as a transplanted organ.

B lymphocytes: humoral immunity

The humoral immune response occurs when antibodies are produced. This is initiated when soluble immunogens entering the lymphatic system (usually via lymph nodes), are taken up by antigen-presenting cells such as macrophages and dendritic cells. The antigens are processed and presented in complex with HLA Class-II molecules to T lymphocytes that contain CD4 receptors. These are known as 'helper cells'. The activated helper cells then secrete cytokines such as IL-4 that stimulate B lymphocytes. The B cells become differentiated into plasmacytes (plasma cells), and memory B cells. The plasmacytes initially produce IgM antibodies. Interaction with other receptors present on the B lymphocyte such as CD40 with the T cell receptor induces immunoglobulin switching and production of IgG antibodies. The strength of the response is regulated by T helper and T suppressor lymphocytes (T lymphocytes that carry CD8 receptors). Helper cells boost the immune response; suppressor cells stop or regulate the production of antibody. Plasmacytes usually continue to secrete antibodies for several weeks after activation. Memory B cells remain in the body for many years, retaining a high affinity for the antigen that stimulated their activation.

'Naturally occurring' antibodies found in the ABO blood group system are formed as a result of humoral immune response to environmental A and B antigens, that are similar in immunogenic nature to human red cell antigens. For example, A and B antigens may be present on ingested particles, or in medical vaccinations or on bacteria. Newborns do not have their own ABO alloantibodies; it takes time and exposure to the environment, before they develop.

A note on CD terminology

The cluster of differentiation (abbreviated to CD) is a protocol for the identification of cell surface molecules that provide targets for the phenotyping of cells. CD molecules often act as receptors for various other molecules, and some play a role in cell signaling. Some examples are CD8 found on cytotoxic T cells and NK cells, and CD4 found on T helper cells. In order to distinguish these cells, they may be referred to as CD8 cells and CD4 cells. To date more than 370 unique CD clusters and sub clusters have been identified.

Antigens and antibodies

Antigens

Although the use of the term antigen is always correct when discussing laboratory testing, when an antibody is first stimulated to be produced, the foreign substance that elicits the immune response is correctly called an immunogen. Because of the common usage of the term's 'antigen' and 'immunogen', they are viewed as synonyms, and are often used interchangeably. Although antigens can stimulate the production of antibodies, they must be immunogenic to do so.

Most antigens are of biologic origin. These are usually proteins, but they may also be polysaccharides, lipids or nucleic acids. The actual antigenic determinant (or epitope) is usually small, with a molecular mass of at least 10 000 Daltons (Da). This determinant is often coupled to a carrier such as a red blood cell (which has on its surface many different antigenic determinants or epitopes).

Haptens are substances with a molecular mass of less than 10 000 Da, which when coupled with larger carrier proteins become immunogenic (capable of provoking antibody response) but are not immunogenic on their own. Despite their small size, haptens are however, capable of reacting with antibodies.

When considering blood transfusion, one should note the following. To avoid life-threatening reactions, compatibility tests between recipient, and the products intended for infusion, are performed prior to giving a blood transfusion. Nevertheless, a blood transfusion inevitably involves the introduction of foreign matter into the recipient, as no two individuals are alike. Antigens are expressed on red cells and within soluble factors in plasma. These antigens can induce the formation of new antibodies in the recipient or provoke an antigen-antibody reaction if the corresponding antibody to antigen introduced during transfusion is already present in the recipient. Conversely, antibodies present in the transfused plasma can react with corresponding host antigens.

Definition of immunogen. An immunogen is a foreign substance, that when it enters the body of an immunocompetent vertebrate animal that lacks that substance, is capable of provoking the formation of antibodies that will react specifically with it. The term antigen is sometimes used as a synonym for immunogen and although antigens can react with specific antibodies, they may not necessarily be able to provoke an antibody response. Immunogens and antigens are widespread in the environment; such as in the surrounding air, in foods and in vaccines. They are also found on micro-organisms and on blood and other cells.

Definition of antigen. An antigen (Ag) is a substance that when introduced into the circulation of a subject

lacking that antigen, can stimulate the production of a specific antibody.

In blood banking, the term antigen is used as the point of reference because the blood group systems are defined by antigens on red blood cells.

Antibodies

When an immunogen enters the body, an immune response may occur. This means that the immunogen is recognised as foreign by the host lymphocytes, which then start to manufacture specific antibodies. This process occurs in the lymph nodes, liver and spleen, which together form the reticuloendothelial system. Antibodies are usually released into the plasma. However, certain antibodies do not appear to circulate but are fixed to body tissues or cells and are termed 'sessile'.

An important characteristic of antibodies is that they are specific. An antibody will only bind to an antigen identical to, or very similar to, the antigen that initially stimulated its production. As each plasma cell can produce antibody with only one specificity and the body is faced with a vast number of different pathogens bearing differing antigens, the immune system is required to generate large numbers of plasma cells, each producing antibodies of differing specificities.

Definition of antibody. An antibody (Ab) is a specifically reactive immunoglobulin produced in response to immunogenic stimulus, the objective of the antibody being to react with and destroy the immunogen that stimulated its production.

The specificity of an antibody is determined by the shape of the variable region of the light chains (see Fig. 7) i.e. an antibody will bind to an antigen that has a complementary structure to the antibody's variable region.

The antigen-antibody recognition may be compared with a lock and key, the concept of which is depicted in Fig. 3. Antigen and antibody must match each other for a reaction to take place. Figuratively speaking, the key needs to fit the lock for the recognition to occur. The 'match' may also be likened to the way in which one jigsaw puzzle piece fits the next.

Nature of immunogenicity

For a substance to be immunogenic, and stimulate a response, it must appear to be foreign to the host and be of adequate size (more than 10 000 Da). It can be of protein origin e.g. Rh, or a carbohydrate or sugar e.g. ABO. Even lipids and nucleic acids can be immunogenic. All immunogens are antigens but not all antigens are immunogens. By definition, the immunogen is the

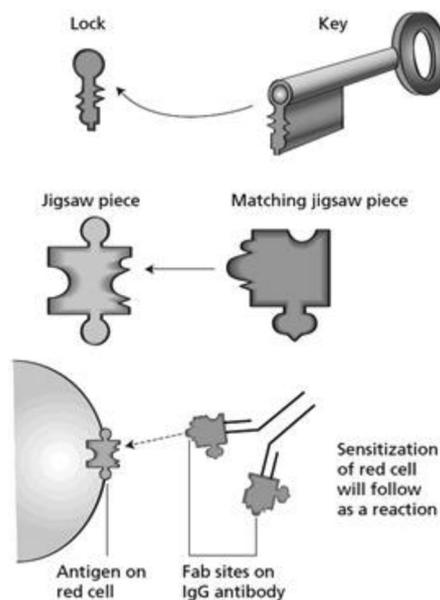


Fig. 3 Lock and key principle of antigen-antibody reaction.

molecule that stimulates the adaptive immune response while the antigen is the molecule that binds to the antibody or T cell receptor. Some very small molecules can bind to antibodies, but they cannot initiate an immune response by themselves. Such small molecules are called haptens. For example, drugs such as penicillin are usually haptens or incomplete antigens. They require a carrier, such as a protein, before they can induce an immune response.

The strength and quality of the immune response depends on many variables. Factors that may play a role include:

- Age and physiological state of the individual (host) being immunised.
- Capacity of the individual to respond (individuals vary greatly from one another).
- Chemical nature and molecular size of the immunogen.
- Volume and frequency of each inoculum (dose of immunogen administered, and timing).
- Route of administration (e.g. subcutaneous, intravascular).
- Presence or absence of adjuvants.

Adjuvants are inert substances injected together with immunogen when deliberately provoking an antibody response, as in immunisation programmes. They amplify antibody response and their action is largely threefold:

- There is a better response to a smaller dose of immunogen.
- There is a more sustained or longer lasting response.
- There is greater production of antibody.

Primary and secondary response

Primary response

When the individual is first exposed to an immunogen, then immunogen presenting cells such as macrophages and dendritic cells process and present it in such a way that it can be recognised by T helper cells. Activation of the T helper cells facilitates and induces the development of clonal B lymphocytes that can produce antibodies specific to that immunogen. If the immunogen is encountered by the host for the first time, the antibody may become detectable in the plasma between five and 180 days afterwards. The antibody level rises gradually, remains fairly stable for a while and then gradually declines. The class of antibody produced first is usually IgM. The antibody undergoes switching to IgG at a later phase. An important step in the primary immune response is the generation of a clone of memory B cells that retain the capacity to recognise the antigen on re-exposure, after which they clonally proliferate. These memory B cells are long-lived and the ability to recognise the same antigen may be retained for many years. Circulating memory B cells facilitate an immediate and brisk IgG immune response if the individual is exposed again to the same antigen. This is called the secondary response.

Secondary response

When a second or subsequent exposure to the same antigen occurs, the antibody response is usually much more rapid. Higher levels of antibody are produced, and the response is much more sustained. This is because retained memory B cells in the body can recognise the antigen that was encountered during primary response and react more efficiently and effectively. Secondary response is therefore also referred to as the recall or anamnestic response. The antibodies produced in the secondary response are usually IgG.

Figure 4 is a graph showing the time lag, log (increase in strength), plateau and decline phases of primary (IgM) and secondary (IgG) immunoglobulin response to immunogenic stimulation.

Active and passive immunity

Active immunity

Active immunity is known as such because the host is actively involved in antibody production as a result of coming into contact with an immunogen. Because the immune response is initiated in the host, the immunity that results is long lasting. The immunity may be regarded as natural or artificial, based on the method by which the immunogen is introduced into the host.

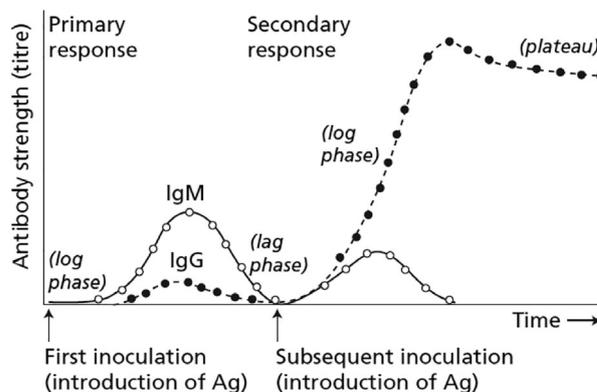


Fig. 4 Graph showing immunoglobulin response.

Natural active immunity. When an individual contracts measles or mumps or some other infectious disease, the body responds by producing antibodies against the infectious agent and overcoming it. The individual usually recovers and does not suffer the same infection again. This is because if the same infectious agent enters the body in the future, it is recognised by the memory cells and eliminated. Similarly, in blood transfusion, organ transplantation and during pregnancy, foreign antigens are introduced into the circulation of the host, and this may provoke antibody development.

Artificial active immunity. Lifelong immunity may be provoked artificially by medical immunisation, such as hepatitis B vaccination. In this case, controlled doses of attenuated organisms (organisms that have been rendered harmless), or non-infectious laboratory-created immunogens, are administered, so that the individual does not become ill yet mounts an antibody response and becomes immune and protected against the infection in the future.

Passive immunity

Passive immunity does not involve the immune system of the individual concerned. Immunity is conferred by introduction of antibodies (immunoglobulins) into the individual from some other source. Because the immune response of the host is not involved, there will be no memory or recall regarding the infectious agent and therefore no immunity beyond the lifespan of the introduced immunoglobulin. The immunity may be regarded as natural or artificial, based on the method by which the antibodies are introduced.

Natural passive immunity. Placental transfer of maternal antibodies to the fetus (mainly IgG), or transfer of maternal antibodies to the newborn during breastfeeding (mainly IgA), ensures that the infant will have protection

against those infections to which its mother is immune. The protection afforded by the mother is limited to the lifespan of the transferred antibodies, and the infant is just as susceptible as any other non-immune individual when the maternal antibodies reach the end of their lifespan. In addition to antibodies, complement is transferred from mother to child and this assists in providing further protection.

Artificial passive immunity. Administration of specific immunoglobulins to non-immune individuals at the time of exposure to infectious agents may prevent illness in the short term. Concentrated immunoglobulins are obtained by fractionation of specially selected donations of plasma. For example, plasma from blood donations with a high level of hepatitis B antibodies may be fractionated to extract the hepatitis B immunoglobulin. This concentrate, if given to a susceptible, non-immune individual, would help prevent infection with hepatitis B on that occasion. The state of immunity is only effective for as long as the immunoglobulin from the donated plasma remains in the circulation of the individual receiving the concentrate.

Limitations of the immune system

Some viruses persist even after antibody production has occurred as they are of a nature where they cannot be totally cleared by the antibodies of the host. In such cases, the host becomes a carrier, and can infect others. Such viruses, an example of which is the human immunodeficiency virus (HIV), may eventually destroy the host. Other viral diseases, such as influenza, may infect the same individual many times. This is because there are many different forms of viruses that cause influenza, and these are immunologically distinct. Each time a new influenza virus invades the host, it is seen as being encountered for the first time and therefore a primary immune response is initiated.

Characteristics of immunoglobulins

In humans, five distinct classes of immunoglobulins or antibodies are found. These are IgM, IgG, IgA, IgD and IgE.

Immunoglobulin M (IgM)

IgM is usually the first immunoglobulin produced in the primary response. IgM constitutes 5–10% of circulating antibodies. It is a large molecule (900 000 Da) and is mainly confined to the bloodstream. The molecule consists of five monomers joined in a cyclic manner, and although there are theoretically 10 antigen-combining sites, only five sites are readily available to combine with

antigen. IgM is therefore referred to as a pentavalent antibody or a cyclic pentamer. When IgM red cell antibodies are mixed with a suspension of red cells bearing the corresponding antigens, each IgM molecule is large enough to simultaneously bind to red cell antigen sites on adjacent red cells. Figure 5 is a diagrammatic representation of IgM. This activity results in a latticework of haemagglutination and is readily visible as a clumping reaction in the laboratory. An important result of some IgM activity is the activation of complement. However, IgM antibodies cannot cross the placenta from mother to fetus, so they play no role in causing haemolytic disease of the fetus and newborn. Figure 6 is a diagrammatic representation of haemagglutination, depicting the antigenic determinants on the red cells as dots.

Immunoglobulin G (IgG)

About 80% of circulating antibodies are IgG, and this immunoglobulin is small enough (160 000 Da) to infiltrate the tissues of the body. IgG molecules are equally distributed in both intra- and extracellular space. Although each IgG molecule has two antigen-combining

Immunoglobulin M consists of 5 groups
– each group the same as IgG

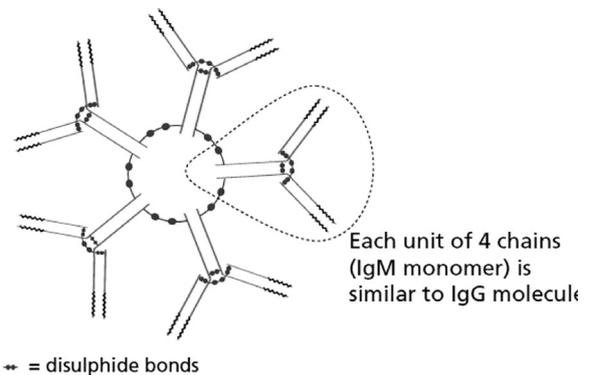


Fig. 5 IgM molecule.

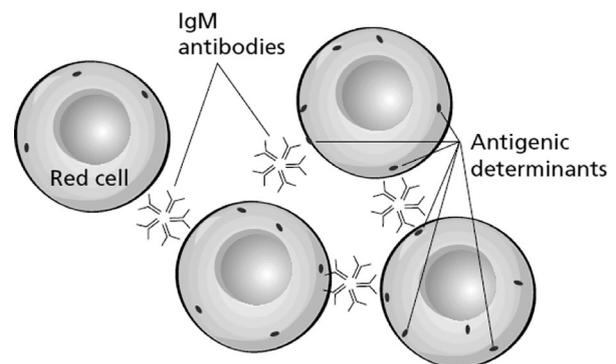


Fig. 6 Diagrammatic representation of haemagglutination.

sites, it acts as a monomer or monovalent antibody that coats or sensitises a single antigen on a single cell. Sensitisation is not a visible reaction, even with the use of a microscope, and requires the addition of other agents in order to be detected in the laboratory. The *in vivo* coating of cells by IgG antibodies is a catalyst or trigger for the activation of neutrophils and macrophages. IgG is found in various subtypes: IgG1, IgG2, IgG3 and IgG4. All IgG subtypes except IgG2 cross the placenta and may therefore be implicated in haemolytic disease of the fetus and newborn, and all subtypes except IgG4 can activate complement. Each subtype has a highly specific role to play in the immune response.

Figure 7 is a diagrammatic representation of IgG. It illustrates the two fragment antigen binding (Fab) portions, which are responsible for the attachment to antigen at the time of reaction, and the fragment crystallizable portion that initiates the involvement of complement and to which antihuman globulin is specifically directed (Fig. 8). The role of complement and of antihuman globulin in antigen-antibody reactions *in vitro* is described in detail in Section 3: *Antigen-antibody reactions*.

IgM and IgG are of primary importance in the field of blood transfusion. A brief account of the other three types of immunoglobulins follows:

Immunoglobulin A (IgA)

IgA is found mainly in mucosal secretions and in the saliva, although it is also present in the bloodstream, particularly after immunisation. It is secreted into the intestines in large amounts and accounts for approximately 15% of

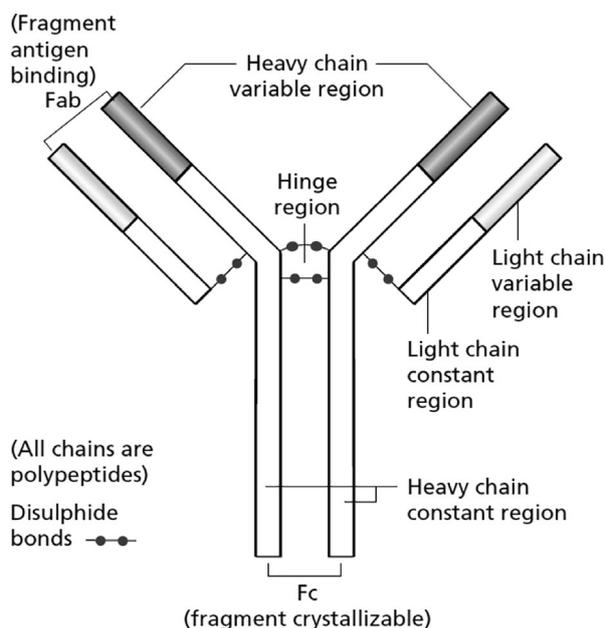


Fig. 7 IgG molecule.

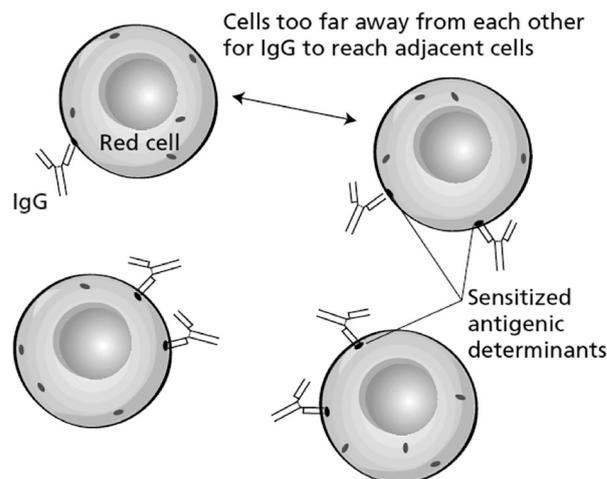


Fig. 8 Diagrammatic representation of red cell sensitization by IgG antibodies.

the total immunoglobulin produced each day. It is a weak opsonin and is a poor activator of complement.

Immunoglobulin D (IgD)

IgD is present in only minute amounts in the bloodstream and it is usually fixed to the plasma membranes of immature B lymphocytes, where it plays a role in the activation of B cells.

Immunoglobulin E (IgE)

IgE plays a major role in allergy, allergic reactions and conditions such as asthma and anaphylaxis. It is present in trace amounts, even in severely allergic individuals, comprising approximately 0.05% of the immunoglobulins present.

General terminology of immunoglobulins

- Immunoglobulins are commonly referred to as antibodies.
- Alloantibodies (sometimes called isoantibodies) or isoagglutinins are terms used to describe antibodies against antigens found in members of the same species (e.g. antibodies against the red cells of humans who are group A are found in the plasma of humans who are group B).
- Heteroagglutinins or heterophile antibodies are directed against different interspecies-specific antigens (e.g. Forssman antigen).
- Autoantibodies or autoagglutinins describe antibodies that are directed towards antigens within the same individual (e.g. antibodies found in the plasma of an individual and that react specifically with that individual's own cells). Table 3 outlines the general properties of IgM and IgG antibodies.

Table 3 General properties of IgM and IgG

Property	IgM	IgG
Immune response	Primary	Secondary
Time lapse for response to antigen	Prolonged: 5–180 days	Rapid: 2–3 days
Pattern of response	Antibody level rises, reaches a plateau and may then decline	Antibody level increases, reaches a plateau and then declines very slowly over time
Total percentage in bloodstream	5–10%	80%
Shape of antibody molecule	Cyclic pentamer	Monomer
Antigen-combining sites	Five (theoretically 10)	One (theoretically two)
Length of molecule in Angstrom units	300 Å	120 Å
Type of reaction against red cells with the corresponding antigen, in saline	Haemagglutination is the typical result of a reaction, and is observable	Sensitization is the typical result of reaction, and is not usually observable
Mass (measured in Daltons)	900 000 Da	160 000 Da
Sedimentation coefficient: (measured in Svedberg units)	19S	7S
Complement fixation	Yes	Yes
Optimum reaction temperature	2°C to 24°C	37°C
Ability to cross the placenta	No	Yes
Neutralized by dithiothreitol (DTT) or 2-mercaptoethanol (2-ME):	Yes	No
Half-life:	5 days	18–23 days

Immune paralysis and immune tolerance

The immune system of the host may fail to respond when large amounts of immunogens are presented to it. This may be caused by the immune system being overwhelmed with immunogens, and therefore failing to act (becoming transiently or temporarily paralysed), or by the immune system becoming 'confused' by the simultaneous presence of several different immunogens. The result is immune paralysis; there is no response, and antibodies are not developed.

On the other hand, immune tolerance can occur when the host 'tolerates' a foreign immunogen such as a group O chimera with tolerance for A antigen, lacking the expected anti-A antibody in the serum/plasma. A genetic

chimera is an individual whose cells originate from more than one zygote. The one zygote develops into the individual, whereas the other implants in the host. Although the implant contains different antigens, they are tolerated by the host as 'self'. This is a rare occurrence but aptly describes immune tolerance.

Immune tolerance is also used to describe the fact that an individual does not usually produce antibodies against his/ her own antigens. A breakdown in this immune tolerance - or recognition of self - results in the production of autoantibodies.

Expected antibodies may also be absent in hypogammaglobulinaemia (lack of immunoglobulin), after transplantation, and in old age.

Autoimmunity

An autoantibody is an antibody that the body directs against its own healthy cells. When this situation results in illness, the individual is said to have an autoimmune disease. Examples of such diseases are:

- Systemic lupus erythematosus (SLE) results in inflammation and tissue damage and can affect many parts of the body. The signs and symptoms of SLE occur intermittently as episodes or 'flares' of illness alternating with periods of absence of disease. SLE does not target specific parts of the body but is widespread.
- Immune thrombocytopenic purpura (ITP) targets only platelets, causing thrombocytopenia.
- Autoimmune haemolytic anaemia (AIHA) is the result of the body producing antibodies against its own red cells.

The reason why the body starts producing autoantibodies is not clearly understood, but results from a loss of immunological tolerance, which is the ability of the immune system to ignore 'self' and to react only with 'non-self'. It may be that some individuals are genetically prone to do so, or it may be that the condition is triggered by the presence of harmful substances such as viruses or toxic contaminants in the environment.

Immunodeficiency

Immunodeficiency describes a condition in which the host is unable to react effectively with and overcome harmful agents, such as viruses, bacteria or abnormal (tumour) cells. The immune system malfunctions and the individual is said to be immunocompromised. This state is either inherited, in which case the individual is born with the problem, or it is acquired as the result of some trigger. Triggers are known to include viruses, immunosuppressant drugs, malnutrition and stress. Immunodeficiency leads to the individual becoming vulnerable to opportunistic infections; those that do not normally cause disease in healthy individuals. Examples of why an individual would be immunocompromised include the following:

- Inherited primary immunodeficiency is a rare state in which the individual is unable to produce antibodies as a result of a genetic disorder/ abnormality.
- Acquired secondary immunodeficiency may be caused by a variety of factors such as drugs e.g. chemotherapy, as the drugs administered (in this case to control cancer) adversely affect the cells of the immune system, malnutrition, aging and

environmental toxins such as mercury and some pesticides. Some infections can cause immunodeficiency e.g. infection with HIV impairs the individual's immune response to infection by microorganisms.

Viruses and the immune response

The role of cytotoxic cells

After a virus has infected a host it enters the target cells of the host where it can survive and replicate. Different viruses will target different cells, e.g. poliomyelitis viruses target nerve cells and the hepatitis viruses target liver cells. When they are inside the targeted cells, the cells of the host's immune system are unable to detect the virus directly and employ an indirect method to determine its presence in the cell. Molecules of the major histocompatibility complex (MHC) display fragments of the proteins made by the virus on the surface of the cell. Circulating cytotoxic T cells have specialised proteins on their surface (called T cell receptors) which enable them to recognise these viral protein fragments. Following this recognition, cytotoxic factors are released by the T cell which kill the infected cell and thus also the infecting virus.

Some viruses are able to prevent the MHC molecules from displaying the viral proteins on the surface of the infected cell, but this reduction in the number of MHC molecules on the surface of the cell is recognised by another of the cells of the immune system – the NK cells. If an NK cell identifies a cell displaying fewer than expected MHC molecules, it releases cytotoxic factors that kill the virally infected cell.

The cytotoxic factors are stored within granules in the cytotoxic T cells and NK cells until their release is triggered by contact with an infected cell. The cytotoxic factors include those that make holes in the membrane of the infected cell and those that initiate a process called apoptosis or programmed cell death. In both examples the end result is the death of the infected cell and the death of the virus.

The role of interferons

Cells which are invaded by viruses are able to produce small protein molecules called interferons. These interferons directly interfere with the virus's ability to replicate and alert nearby cells of the presence of the virus. This alerting signal causes the nearby cells to increase the number of MHC molecules on their surfaces where they are recognised by circulating T cells. In this way the process of eliminating foreign agents is accomplished.

The role of antibodies

Viruses can be bound by antibodies in an infected host before they are able to enter the target cells. This binding reaction neutralizes the virus and prevents it from entering the target cells, activated circulating phagocytes that promote phagocytosis and can activate the complement system, resulting in increased phagocytosis or cell lysis (of the invading virus).

Human Immunodeficiency Virus and the immune response

The human immunodeficiency virus (HIV) is a retrovirus that is the cause of acquired immunodeficiency syndrome (AIDS) in humans. It differs from most other viral infections in that the virus specifically targets some of the cells of the immune system, thus inhibiting the immune response.

HIV infects T cells which carry the CD4 molecule, the 'helper' cells of the immune system which play an important role in mobilizing the NK (natural killer) cells when an infection occurs. Infection of the CD4 cells results in a decline in the number of CD4 T cells and a reduction in the level of cell-mediated immunity. The host becomes progressively more susceptible to infection and to cancer.

The role of the innate immune response. Cells of the immune system, such as macrophages, and NK cells are the first line of defence against viruses, including HIV. However, macrophages are one of the target cells for HIV and the infected cells lose their ability to kill the invading viruses and present the viral antigens to the T cells in the usual way. This failure plays a significant role in the overall failure of the immune system to deal effectively with HIV.

NK cells can destroy cells that have reduced expression of MHC molecules on their surface and in this way, play an important part in the defence against HIV.

The role of antibodies. >Antibodies against HIV are produced after a significant delay following infection (this delay in the appearance of antibodies is often referred to as the 'window period'). Antibodies may only be present at a low level during the acute phase of the infection. Neutralising antibodies that target proteins involved in the viral entry into the host's cells play an important role in the control of HIV infection, and in some infected hosts delay the reduction in the number of CD4 lymphocytes. However, by infecting and replicating within CD4 T cells, HIV is able to effectively disable one of the major components of the adaptive immune response. Within the CD4 cells, HIV is able to

escape detection by NK cells and replicate relatively unhindered.

Cancer and the Immune System

Introduction

Cancer cells differ from normal host cells in several ways:

- Normal cells divide and grow as part of the individual's normal growth during childhood, or if there is a need to repair damaged tissue due to injury. Cancer cells divide and grow when no further growth is required. The signals that halt cell replication of normal cells are ignored and the tumour increases in size.
- Normal cells respect boundaries and do not encroach on neighbouring cells or organs. Cancer cells have no such respect and often invade adjacent tissue. This is one reason why it is sometimes difficult to remove a tumour surgically.
- Normal cells (other than blood cells) remain in the positions in which they were created. For example, kidney cells do not wander off to the brain. Cancer cells, by contrast, may break free from their original positions and float in the bloodstream to other parts of the body. The cancer cells metastasize to create tumours in other organs.
- Normal cells have a finite lifespan, after which they die and are replaced by new cells. This is because the structure found at the end of each chromosome, called the telomere, becomes shorter each time a cell divides, and when it becomes too short the cell dies. Cancer cells are able to restore their telomeres so that they do not shorten with each cell division, with the result that the cell does not die and is 'immortal'.
- Normal cells can become cancerous when mutations result in the cell continuing to grow and divide when further growth is not required; essentially their growth is out of control. In addition, these mutations may give cancer cells the ability to invade tissues that are both nearby and also those located in distant parts of the body.
- The mutations that give rise to cancer cells may be inherited but are more often caused by carcinogens in the environment. Some individuals have a genetic predisposition to cancer, in that some mutations already exist, making it more likely that the cells will become cancerous. Because several mutations are usually required for cancer to develop, if some are inherited, it is more likely that cancer will occur. The need for several mutations to take place, and the fact that mutations take place over time, is one

explanation for the fact that cancer is more common in older individuals.

Types of cancer

Broadly, cancers are named for the type of cells in which the cancer first arose.

- Carcinomas start in the epithelial cells that line body cavities.
- Sarcomas start in the mesenchymal cells in bones, muscles and other tissues.
- Leukaemias and lymphomas are blood cancers that affect the white cells. Leukaemia affects the bone marrow and results in too many white blood cells; lymphomas affect the lymph nodes and the lymphocytes.

The role of the immune system

As has been described earlier in this Section, the immune system works fundamentally on the principle of discriminating between 'self' and 'non-self' and mounting an immune response to rid the host of the latter. This enables the host to remove parasites and thus prevent, or at least limit, infection. Non-self is identified by differences in biochemistry, such as the recognition of microbial carbohydrate residues on invading micro-organisms. This recognition triggers the events that lead to the destruction of the pathogen.

Cancer cells, though, start out as 'self' and although they go through many changes that may be recognised by the immune system, leading to the death of the cancerous cell, some of the less immunogenic cancerous cells may escape this detection. By the time a tumour is detected, the tumour will have evaded the host's system of immunosurveillance and is essentially unaffected by the expected immune response.

The DNA in cancer cells often directs the mutated cell to produce abnormal molecules, called tumour antigens, on the cell membrane. These tumour antigens are recognised by the cells of the immune system, mainly the NK cells, as non-self and the cells are destroyed. It seems likely that this happens regularly even in healthy hosts.

However, cancer cells possess complex mechanisms that allow them to escape recognition as non-self, and the immune responses that would otherwise prevent the development of a malignant tumour.

Cancer can reduce the immune response by spreading to the bone marrow, most often in leukaemia and lymphoma, but also in other types of cancer.

Cancer treatment can also weaken the immune response, in part because the number of white cells produced by the bone marrow is reduced. Examples of the treatment most likely to have this effect are chemotherapy and radiotherapy.

Key points

- The role of the immune system is to defend the body against pathogens and other foreign substances.
- The major components of the immune system are the bone marrow and thymus.
- The secondary lymphatic tissue includes the spleen, tonsils, lymph vessels, lymph nodes, adenoids and liver.
- The liver, spleen and lymph nodes are collectively known as the reticuloendothelial system.
- There are two strategies for body defence; innate and adaptive.
- Innate or natural defence does not vary, even following multiple exposures to the same foreign substance, whereas the adaptive response becomes stronger with repetitive stimulation.
- Adaptive immunity is seen only in vertebrates.
- Immune defence is multifaceted – it has many options including phagocytosis, chemical agents, complement and the production of antibodies for removing harmful agents.
- Opsonins are chemicals produced by the body that promote phagocytosis.
- Once phagocytes are activated, they release soluble substances called cytokines, which regulate the strength of the immune response.
- Complement consists of a group of soluble proteins that are present in the plasma in an inactive form until stimulated by an antigen-antibody reaction. They then act in a sequential fashion, each activating the next.
- Complement either becomes fixed to target cells that are then more easily subjected to phagocytosis, or continues its chain reaction to its end, which results in cell lysis.
- Lymphocytes are the white cells critical to adaptive immunity. T lymphocytes are responsible for cellular defence and the production of lymphokines, whereas B lymphocytes are responsible for the development of antibodies.
- An antigen is capable of specific combination with antibody. It is frequently used as a synonym for immunogen, although some antigens that react with antibodies are not capable of eliciting an immune response.
- An immunogen is capable of provoking an immune response when introduced into an immunocompetent vertebrate in which it is foreign.
- To be immunogenic, the substance must appear foreign to the host.
- A hapten is a substance with a molecular mass of less than 10 000 Da, which when coupled with a larger carrier protein can become immunogenic.

- During primary response, when the host encounters an immunogen for the first time, IgM antibodies are formed.
- After secondary (learned) response, most circulating antibodies are IgG.
- An adjuvant is a substance combined with an immunogen to make it more immunogenic to the host. Adjuvants are usually inert substances of a molecular mass large enough to attract immunologically competent cells, which then 'find' the antigen on the surface of the adjuvant, and the process of antibody production starts. This tactic is sometimes used to increase the effectiveness of vaccination.
- Active immunity signifies that the immune system of the host is activated, so the response will be long lasting.
- Passive immunity is the term used when the immune system is not activated, and defence is carried out by the antibodies of another – such as maternal antibodies crossing the placenta and protecting the newborn – for only a limited period of time.
- In certain circumstances the body does not respond to the presence of foreign immunogens, either because of immune paralysis or because of immune tolerance.
- An alternative name for an antibody is an immunoglobulin. They fall into five major classes: the two of most practical importance in blood transfusion practice are IgM and IgG; the others are IgA, IgD and IgE.
- There are many characteristics that differentiate IgM and IgG antibodies, such as the ability to cross the placenta and activate complement.
- An autoantibody is an antibody that the body directs against its own healthy cells, often resulting in disease.
- The immune system protects the host against viruses through the actions of cytotoxic cells, interferons and antibodies.
- Although there are several differences between normal cells and cancer cells, normal cells can become cancerous following a series of mutations.
- The immune system plays an important role in protecting the host against cancer.

Antigen–antibody reactions

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Introduction

In Section 2: Immunology the role of antibodies in the prevention of infection was dealt with in some detail. Although this is the prime function of antibodies, and the reason they are produced as part of the immune response to foreign immunogens, antibodies have been extensively used by laboratory scientists for *in vitro* investigations. The fact that antibodies are by their nature specific makes them a useful tool in the detection and identification of antigens in the laboratory.

Blood bankers are often concerned with *in vivo* reactions that take place between antigens on red blood cells and antibodies in plasma. Antibodies against red cell antigens transfused to a recipient, can cause transfusion reactions, ranging in severity from the shortened survival of incompatible transfused red cells without any clinical symptoms, to a fatal reaction and the death of the recipient.

In the laboratory, though, we are concerned with *in vitro* reactions.

In vitro antigen–antibody reactions can occur in different ways, influenced by the volume and strength of reactants and methods used. Antigen–antibody reactions are enhanced intentionally in the laboratory to make very weak reactions observable, enabling the laboratory technologist to draw practical conclusions and report on clinical conditions with accuracy. Provided that the correct methods are followed, it is usually not difficult to determine blood groups and the nature and specificity of circulating blood group antibodies. Occasionally, however, this can be more challenging, and it sometimes takes considerable knowledge, skill and experience to accurately interpret anomalous test results, determine what tests should be performed next, carry out further tests appropriately and then correctly interpret the results.

In addition to antibodies obtained from the plasma of blood donors, serologists often make use of reagent antibodies that are produced synthetically explicitly for the purpose of antigen detection and identification.

In this section the focus is on serum/plasma antibody reactions with antigens on red cells and how to visualize these. *Section 4: Principles of laboratory techniques* describes the principles of methodologies currently used in the laboratory.

Learning objectives

By the end of this section, the student should be able to describe the common types of antigen–antibody reactions, discuss the factors that influence them and use this knowledge to carry out laboratory tests in such a way as to obtain the best results. The student should have a good understanding of the following topics:

- Binding of antibodies to red cell antigens
- Common types of antigen–antibody reactions
 - haemagglutination
 - sensitisation
 - haemolysis
 - neutralisation
 - precipitation
- Factors that influence antigen–antibody reactions
- Use of proteolytic enzymes
- Use of high molecular mass substances
- Use of low ionic strength saline solution
- Use of labelled antibodies
- Role of complement
 - factors that influence the action of complement in laboratory tests
- Role of antihuman globulin
 - application of antiglobulin tests in the laboratory
- Polyclonal and monoclonal antibodies
- Recent developments

Binding of antibodies to red cell antigens

When a blood sample is drawn, it is taken either into a dry test tube, leading to clot formation, or into a test tube containing an anticoagulant to prevent clotting and keep both the cellular and plasma component of the blood available for analysis. If blood is taken into a dry tube, and given sufficient time to coagulate, the fluid part of blood, which is called serum, can be collected when clotting is complete. In respect to the presence of antibodies, serum has similar properties to plasma, but the advantage is that serum retains the viability of complement factors for a limited period of time, and these can therefore be activated to participate in the reaction. On the other hand, complement is generally inactivated by anticoagulants and plasma

samples are therefore unsuitable for use in tests that require the demonstration of complement activity in a reaction.

Another difference between serum and plasma is that coagulation factors are utilised in the clotting process and are therefore absent from serum samples. To prevent coagulation, an anticoagulant, such as acid citrate dextrose (ACD), ethylenediaminetetraacetic acid (EDTA) or heparin is added to the blood drawing tubes prior to collection of the sample, but as previously stated, anticoagulants prohibit the participation of complement in tests.

Red cell antigen-antibody reactions

When red cells carrying the target antigen are mixed with serum, plasma or a reagent in which the corresponding antibody is present, an antigen-antibody reaction occurs. The antibodies make random contact with the red cells and can then bind to the corresponding red cell antigens. The speed and strength of the reaction is variable and dependent on many factors that will be discussed later in this section. The initial binding of antibodies to red cells occurs rapidly but is not necessarily visible. To obtain a demonstrable effect of attachment of antibodies to red cell antigens, the reaction may need to be enhanced in the laboratory. However, many red cell antigen-antibody reactions are visible from the outset due to haemagglutination – the term used for the clumping of red cells that occurs when they come into contact with the corresponding/ specific antibodies. The prefix 'haem' refers specifically to red cells, while the term agglutination is used to refer to the clumping of particles in general. The most widely used strategy in the laboratory to hasten agglutination is centrifugation. After allowing enough time for the antibody to recognise and react with its corresponding antigens, which may occur within seconds or may take much longer, even up to hours, the test mixture can be centrifuged under conditions of time and speed of rotation such that the centrifugal force will force the red cells closer together. In this way the agglutination of cells to which antibody has become bound is enhanced, whereas cells lacking the corresponding antigen, remain unagglutinated.

Common types of antigen-antibody reactions

When an antigen-antibody reaction takes place *in vitro*, the outcome that can be observed is dependent on many factors, including:

- The class of immunoglobulin to which the antibodies belong (e.g. IgG, IgM).
- The location of the antigens (e.g. on red cells, or as soluble antigens in body fluid).
- The test environment (e.g. test tubes, slides, or gel).
- The presence of additional or supplementary reagents (e.g. enzymes, or high molecular mass reagents).

- The presence of complement.

There are five types of antigen-antibody reaction, each with a different observable outcome, that are commonly used in the serology (blood bank) laboratory:

- (1) Haemagglutination.
- (2) Sensitisation.
- (3) Haemolysis.
- (4) Neutralisation.
- (5) Precipitation.

Haemagglutination

Every red cell carries an electrical charge that is dependent on the characteristics of its membrane-bound proteins and the solution in which the red cell is suspended. The negative charge on red cells keeps the cells apart. This is the natural repulsive force that exists between molecules with the same electrical charge and is called zeta potential.

Red cells suspended in normal saline are kept 50–100 Angstrom units (Å) apart by zeta potential. The negatively charged red cells in saline or suspending medium attract a cloud of positively charged ions around them. These positive ions may hinder (interfere with or hamper) the ability of the antibody to bind with the antigen, a phenomenon called steric hindrance, and tests may be designed to reduce the strength of this ionic layer around the cells to promote or improve antibody binding.

IgM antibodies, being 300 Å long, are able to span the distance between adjacent red cells and as a result, bring about haemagglutination of cells with the corresponding antigen. This is what typically happens in ABO blood grouping tests.

Haemagglutination takes place when serum/plasma antibody (e.g. anti-A) is mixed with red cells carrying the corresponding antigen (i.e. A antigen). This reaction may occur in a test tube, on a microscope slide, or in a microwell. The result is the development of a three-dimensional latticework of red cells held together by antibodies and visible as clumping.

Figure 1 shows a range of haemagglutination reaction strengths, from strong agglutination that is easily visible, to very weak agglutination that is difficult to discern.

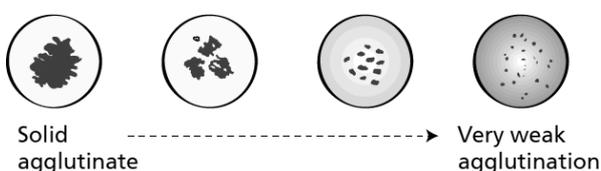


Fig. 1 Range of reaction strength – haemagglutination.

Sensitisation

Sensitisation occurs when an antibody binds to the corresponding antigen on the red cell, but agglutination does not occur, and the reaction remains invisible. Sensitising antibodies are, in the majority of cases, IgG antibodies that are about 120 Å in length. Although they are able to sensitise red cells with the corresponding antigens, zeta potential must be reduced or altered for the red cells to move closer together, so that the smaller IgG antibodies are able to reach an antigen on an adjacent red cell and achieve haemagglutination. Laboratory tests have therefore to be modified in a prescribed way to enable sensitisation to become observable *in vitro*. Additives such as bovine serum albumin or proteolytic enzymes like papain or bromelain are able to reduce zeta potential, and thereby change the environment surrounding the red cells so as to allow sensitised cells to become agglutinated. The sensitisation reaction, although just as significant as haemagglutination, is not observable. There is a notable exception to this rule, and that is agglutinating IgG anti-A and anti-B, mentioned in *Section 4: Principles of laboratory techniques*.

Figure 2 illustrates red cell sensitisation and the ionic cloud of positively charged ions surrounding the negatively charged red cells, kept apart by zeta potential.

There are two commonly used ways to cause sensitised cells to become agglutinated, and these are described in detail later in this section. In brief, the principles of these methods are:

- Reduction of zeta potential – substances such as proteolytic enzymes or bovine serum albumin may be used to reduce the repelling force between red cells, bringing them closer together so that if sensitised, they are able to become agglutinated.
- Bridging the gap between sensitised cells – antihuman globulin (AHG) is an antibody to human globulin and is used in the laboratory to react with

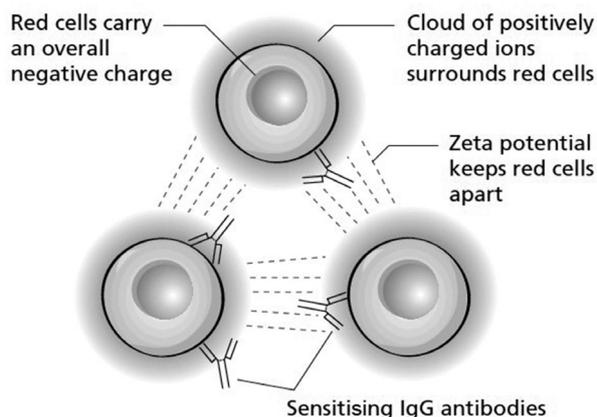


Fig. 2 Red cell sensitisation showing ionic cloud.

sensitising globulins (antibodies), bridging the gap between sensitised cells and bringing about their agglutination.

Apart from antibodies of IgG class, antibodies of IgA class can also be formed against red cell antigens and when binding with these antigens will cause sensitisation. These serum/plasma antibodies are monomeric IgA, differing in structure from the dimeric IgA found in saliva and other mucosal fluids. Furthermore, not all IgM class antibodies induce haemagglutination, but rather sensitise the cells.

Antibody affinity and avidity.

- The strength of the actual bond between a single antibody combining site and a single epitope is known as the affinity of the antibody and relates to its goodness of fit with the corresponding antigen.
- The combined strength of multivalent antibody binding to many epitopes on the same carriers (such as red blood cells) is known as the avidity of the antibody. In blood banking terms, this condition could apply to IgM or IgG antibodies, as both have more than one binding site per molecule.

The prozone effect. On rare occasions, an undiluted antibody with high avidity, when mixed with a suspension of red cells containing the corresponding antigen, will fail to show any demonstrable reaction *in vitro*, but will do so when diluted and mixed with these same cells. Testing this undiluted sample will therefore result in a false negative outcome. The prozone effect (also called the hook effect) is the result of antibody excess in the neat (undiluted) serum/plasma that prevents the development of a regular latticework resulting in visible agglutination. When suitably diluted, the number of antibody molecules is reduced sufficiently to allow normal agglutination of the cells to take place. This is discussed in more detail under the causes of false results in *Section 4: Principles of laboratory techniques*.

Qualitative and quantitative agglutination tests.

- Qualitative tests determine the presence or absence of an antigen or antibody. Blood grouping tests that are performed to determine the presence or absence of an antigen using an antibody of known specificity are qualitative tests. For example, ABO blood grouping may be carried out by cell (or forward) grouping using reagent anti-A and anti-B with patient or donor red cells of unknown group to determine which of the antigens is present, and reverse grouping of unknown serum/plasma with reagent red cells with known ABO groups, usually A cells and B cells, to determine which of the antibodies is present.

- Quantitative tests determine the highest dilution at which an antibody can react with its corresponding antigen. This endpoint is the titre of the antibody, expressed as a reciprocal (or inverse) of the highest dilution at which agglutination was observed. For example, if the highest dilution at which a reaction is observable is 1 in 32, then the titre is expressed as 32. Quantitative tests may include comparing the strength of the antibody with an International Standard and presenting the results in International Units (IU) per ml.

Haemolysis

Sometimes antigen-antibody reactions result in lysis, which is the breakdown or rupture of the cell membrane on which the epitopes or antigenic determinants are situated, resulting in the release of the cell contents into the surrounding fluid. When the cells lysed are red blood cells, this is called haemolysis, and causes the release of haemoglobin. This is an observable reaction in laboratory tests and must be noticed and recorded as a positive antigen-antibody result. For haemolysis to occur *in vitro*, the antibody involved in the reaction must be able to utilise complement, present in fresh serum. As will be discussed later in this section, complement is a group of proteins which when triggered by antibody adherence (attachment or sensitisation) to the cell, act in a chain reaction to attack and break or rupture the cell membrane. Haemolysis of this nature is therefore a demonstrable endpoint of certain antigen-antibody reactions. Figure 3 illustrates the appearance of haemagglutination and haemolysis in test tubes, compared with an unagglutinated test.

Neutralisation (agglutination inhibition)

Most individuals (approximately 80% in most populations) secrete water soluble ABO blood group antigens in their body fluids such as saliva. For example, should

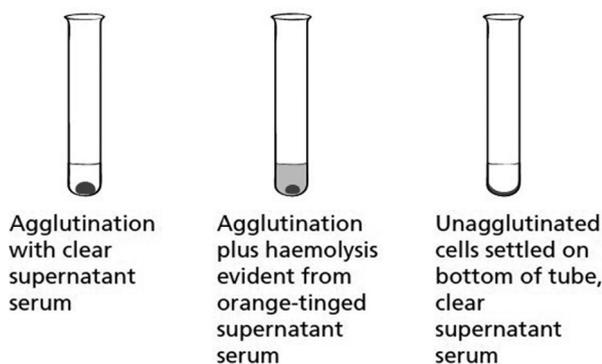


Fig. 3 Haemagglutination and haemolysis in test tubes, and an unagglutinated test.

saliva containing A antigen be mixed with anti-A in the laboratory, then this anti-A reagent will become neutralised. The antibody binds to the soluble group A antigen in the saliva and in this way the antigen-binding site of the antibody becomes occupied. This is called neutralisation or inhibition of the antibody. Such a reaction is usually not observable and can only be deduced later in the second stage of the process. The neutralised anti-A reagent, when mixed with the corresponding antigen expressed by the group A red cells does not lead to the expected agglutination. Figure 4 is a diagrammatic representation of the process of neutralisation or inhibition of anti-A by soluble A antigen so that it can no longer agglutinate red cells containing A antigen.

Precipitation

Precipitation is commonly seen as a precipitin line, such as in the process of immunodiffusion, when antibody and antigen are added to different wells cut into a semi-solid medium such as agar gel set onto a microscope slide. For example, anti-Z may be added to a central well cut into the gel, and several unknown samples that are being tested for the presence of the corresponding soluble Z antigen added to different wells surrounding it. After allowing time under the correct conditions for diffusion of all the solutions into the gel, it is examined for the presence of precipitin lines. If the antibody diffusing out from the central well comes into contact with the corresponding antigen that has diffused out from one of the peripheral wells, then a white line of reaction may be seen in the gel between them – where they have come into contact with each other. This line is formed by the precipitation of insoluble antigen-antibody complexes.

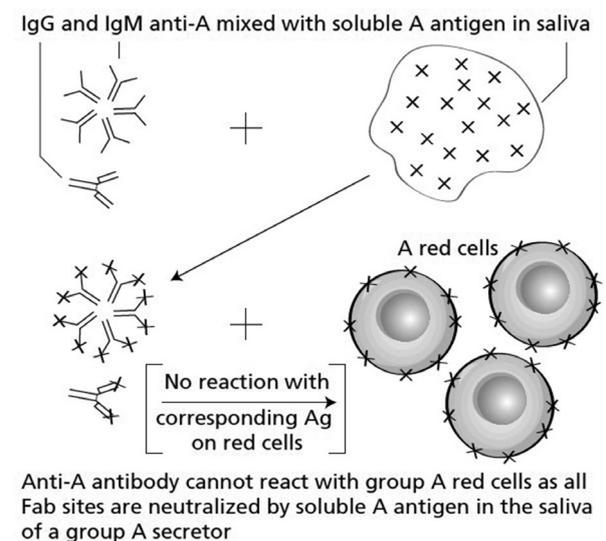


Fig. 4 Neutralisation of anti-A by soluble A antigen.

Precipitin lines in an immunodiffusion gel are shown in Fig. 5.

Factors that influence antigen–antibody reactions

Distance between reactive sites on antibodies

The distance between adjacent red blood cells is an important factor in the outcome of an antigen–antibody reaction. Red cells suspended in saline are generally 50–100 Å apart, and this distance must be bridged by antibodies if agglutination is to occur. If the distance cannot be bridged, the result will not be agglutination, but will be sensitisation, which is not visible.

IgM antibody molecules are 300 Å long and are able to react observably by haemagglutination of red cells in saline. IgG antibodies are 120 Å long and usually sensitise cells in saline.

Monomeric IgA is also 120 Å long and can also sensitise cells in saline.

Electric repulsion between red cells – zeta potential

The repelling force between red cells that carry the same negative electrical charge is called zeta potential, which prevents the agglutination of sensitised red cells in saline. Zeta potential must therefore be reduced or altered in some way for the shorter IgG antibodies to achieve agglutination.

Site of the antigenic determinants

It is thought that some antigens (such as the A and B antigens) protrude from the red cell surface further than others (such as the Rh antigens). Because of this, the actual distance between antigens on adjacent cells may vary to a certain extent, affecting the nature of the reaction or the ability of corresponding antibodies to react with them.

Number of antigenic determinants

It is easier for antibodies to react with antigens that are more abundant on each red cell, than to react with

antigens that are located only sparsely on the cells. Cells that are homozygous for a particular antigen may carry more antigen sites (or epitopes) than cells which are heterozygous. This is termed the gene dosage effect. For example, Jk^a -positive red cells from donors who are genetically Jk^a/Jk^a (thus with two Jk^a -encoding alleles, which are equally well transcribed and translated, resulting in a double dose of Jk^a) may react more strongly with anti- Jk^a than cells which are heterozygous Jk^a/Jk^b , depending on the anti- Jk^a used in the tests.

Goodness of fit

Antigens and antibodies react in a ‘lock-and-key’ way. When the combination between lock and key is precise, then the goodness of fit is high, and the reaction will be stronger; a weak fit results in a weaker reaction. The strength of the bond between antigen and antibody is also known as antibody affinity and is dependent on non-covalent bonds such as hydrogen bonds, electrostatic forces and Van der Waals forces.

Effects of time

Reactants should be incubated for the optimum time for a good antigen–antibody reaction to develop. Too short an incubation period means that the antigen and the antibody may not have had sufficient time to form a good bond. On the other hand, prolonged incubation may cause antigen–antibody complexes to dissociate. The optimal time should be determined, documented and followed each time tests are performed.

Effects of temperature

Cold antibodies, which are usually IgM, react well at 2°C to 10°C, agglutinating or sensitising red cells in the cold. These antibodies will usually dissociate from the cells when the temperature of the tests is raised. Thus, cold antibodies may be eluted (removed or forced to be released) from red cells by raising the temperature from 2°C to 37°C.

Most IgG antibodies react best with the corresponding antigens at 37°C. At this temperature, the speed of their reaction is also increased. In order to dissociate antigen–antibody complexes formed by antibodies with an optimum reaction temperature of 37°C, one would have to raise the temperature to about 56°C. At this temperature, antibody would be eluted from the cells and could then be isolated and further tested. Red cells however, become denatured at temperatures in excess of 50°C and would have to be discarded.

Effects of pH

pH is the measure of alkalinity or acidity of a solution, with 7.0 being neutral. A lower pH number indicates

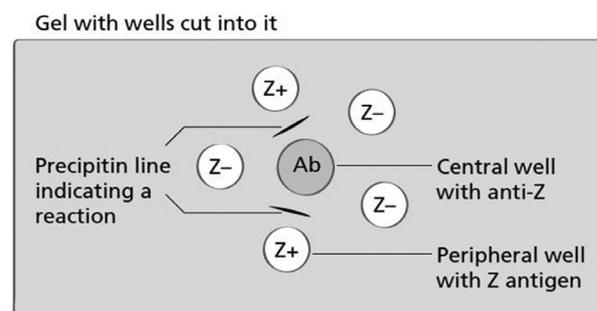


Fig. 5 Precipitation by immunodiffusion.

acidity and a higher pH number indicates alkalinity. The optimal pH range for red cell antigen-antibody reactions to occur is between pH 6.5 and pH 7.0, with an acceptable range of pH 6.0-8.0. Outside this range, results become unreliable.

Effects of ionic strength

Negatively charged red blood cells attract a 'cloud' of positive ions from the surrounding medium, which is usually saline (sodium chloride dissolved in water). What is commonly known as normal ionic strength saline solution is isotonic with blood; it has the same tonicity as blood. It is a solution of about 0.85% to 0.9% weight to volume of sodium chloride in water. Low ionic strength saline solutions are commonly used to increase the sensitivity of antigen-antibody reactions, and details on this are to be found later in this section.

Concentration of antigen and antibody

Although most antigen-antibody reactions provide observable results at various concentrations of either antigen or antibody, the best results are obtained when a large number of antibody molecules are bound to each cell.

Number of fragment antigen binding sites

IgM antibodies have 10 fragment antigen binding (Fab) sites, whereas IgG antibodies are monomers with a maximum of two Fab sites. To bring about the agglutination of two adjacent red cells, an IgM antibody could bind with several antigens on one cell and several on the second cell and form a fairly strong bond. An IgG molecule, or monomeric IgA molecule, though, could bind to only one antigen on one cell and one antigen on another cell, and unless it is an avid antibody, may form a weaker bond. In both cases, many molecules of antibody are required to result in a demonstrable reaction, but the principle remains the same.

Use of proteolytic enzymes

Proteolytic enzymes are sometimes added to antigen-antibody mixtures or are used to pre-treat reagent red cells for enhanced sensitivity of agglutination reactions. Proteolytic enzymes (proteases) act by reducing the amount of sialic acid on the red cell membrane, which effectively reduces the zeta potential; and allows the red cells to move closer together, assisting in their agglutination by specific antibodies. Bromelain, for example, lowers the zeta potential by over 50% and papain by over 35%.

The addition of enzyme to unsensitised cells has no visible effect. It is important to take into consideration, however, that enzymes are also able to modify or alter the expression of certain red cell antigens, so that the

corresponding antibodies are no longer able to recognise these antigens. As a result of this, certain antibodies will not be demonstrable using enzyme methods, and thus enzyme methods should supplement but not replace other techniques. Antigens that are not recognisable after the addition of enzyme include M, N, S, Fya and Fyb, and to a certain extent, K. On the other hand, some antigen-antibody reactions are enhanced by the addition of enzyme, and this includes antibody reactions to the Lea, Leb, I, P1 and Rh antigens. More information is to be found in *Section 6: Blood group systems*.

There are several methods for using proteolytic enzymes or proteases in blood grouping work.

One-stage technique

One-stage methods are simple and fast. One volume each of serum/plasma, cells and enzyme are added in quick succession and in this order. Modification of the cells takes place during incubation.

Two-stage technique

This is the more sensitive method but is more laborious. Red cells are pre-treated with enzyme and then washed in saline before being mixed with the serum/plasma. One part of serum/plasma is mixed with one part of treated cells and incubated. There is no dilution of the serum/plasma under test by the addition of enzyme separately, as for the one-stage method.

Figure 6 illustrates the action of enzymes on sensitised red cells, and how agglutination is achieved by the reduction of zeta potential.

The choice of enzyme to be used depends largely on the preferences within the laboratory, although papain is most often used. Common enzymes used in serology may be obtained from the following sources:

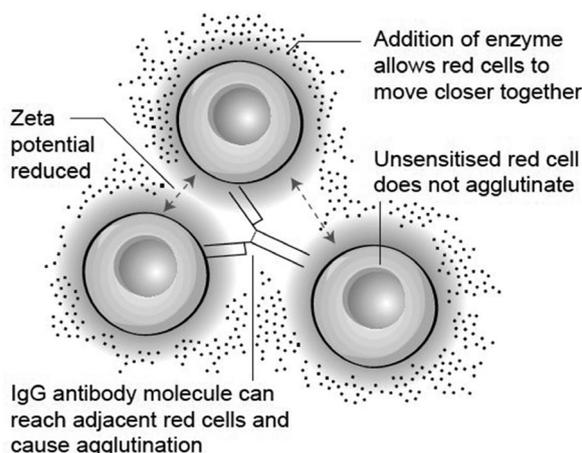


Fig. 6 Action of enzymes on sensitised red cells.

- Pineapple stem: source of bromelin.
- Dried latex of fig tree: source of ficin.
- Latex of papaya fruit: source of papain.
- Extract of pig stomach: source of trypsin.

Raw or concentrated enzymes are potent chemicals and care should be taken when working with them. For example, powdered ficin may cause a skin rash and may lead to conjunctivitis if accidentally rubbed into the eyes. The enzymes listed, as well as pre-treated red cells, are available commercially.

Use of high molecular mass substances

There is a range of substances of high molecular mass that can enhance (potentiate) or demonstrate that a reaction has occurred between cells and sensitising antibodies. These include the following:

- Bovine serum albumin
- Polyethylene glycol
- Polybrene (a polymer of hexadimethrine bromide)
- Polyvinylpyrrolidone
- Gelatin
- Gum acacia

Note: Potentiators may also be added to reagents to enhance their reactivity.

The addition of 22% bovine serum albumin, particularly in low ionic strength saline (LISS), has been found to increase the sensitivity of some antigen-antibody tests, but this may be in part due to the low ionic strength medium, in which it is diluted. Albumin also enhances reactions when used in normal ionic strength saline (NISS), which was routinely used before LISS became available. One theory is that albumin increases the dielectric constant, a measure that relates to stored electrical energy.

Being a dipolar molecule, both positively and negatively charged, albumin is forced to rotate in the serum/plasma-cell mixture, as it is alternatively attracted and repulsed by the negatively charged red cells. In this way zeta potential is reduced and as a result the cells are able to move closer together, so that should these cells already be sensitised, they are then able to become agglutinated.

Polyethylene glycol (PEG) in a low ionic strength environment is one of the best potentiators to increase antibody uptake onto cells with the corresponding antigen. It increases the sensitivity of tests involving clinically significant antibodies and decreases the interference of clinically insignificant antibodies. It may be used in both manual and automated systems, but care should be taken when centrifuging, as red cells clump tightly and may not be dispersed. Antiglobulin tests that require the

serum/plasma-cells-PEG mixture to be washed, should not present this problem.

Polybrene is an additive of high molecular mass that is introduced to the serum/plasma-cell mixture after the incubation period. Although it is capable of detecting both ABO and IgG incompatibility in crossmatch tests, it may fail to detect clinically significant antibodies within the Kell blood group system. It is also prone to false positives with cells coated with complement.

Use of low ionic strength saline solution (LISS)

Low ionic strength saline solution is commonly used for red cell suspensions and is commercially available. LISS has reduced ionic strength of about 0.03 M as compared to 0.17 M for normal saline. The low ionic strength enhances antigen-antibody reactions by reducing the ionic cloud around the red cells, thus increasing the rate of association between antigen and antibody and enabling a reduction in the incubation time. It is important that the manufacturer's instructions are carefully followed, otherwise false results may occur. LISS has two major benefits:

- Reduces the incubation time required for tests.
- Increases the amount of antibody uptake onto red cells expressing the antigen.

Low ionic strength saline solution should not be used when the quantity of serum/plasma is changed in the test, either by adding excess serum/plasma, or when serum/plasma is diluted in saline, such as for titration studies, where the proportion of saline diluent to serum/plasma antibody increases from test tube to test tube. It is important that the ratio (and strength) of cell suspension to the volume of serum/plasma remains within the limits stipulated by the manufacturer of the LISS. Failure to ensure that tests are carried out according to these concentrations could lead to false results.

Use of labelled antibodies

Another way to demonstrate antigen-antibody reactions is to put a label or tag onto a known antibody, and then use this label or tag to give demonstrable evidence of reactions.

Tests using labelled antibodies are designed in such a way that the antibody is "trapped" in the test system by the antigen, while unbound antibody is removed, usually by washing. If the presence of the label is confirmed at the end of the test, it can therefore be concluded that an antigen-antibody reaction took place.

Techniques using a label as an indicator include the following:

- Enzyme linked immunosorbent assay (ELISA) – the antibody is labelled with an enzyme. Presence of the antibody is demonstrated by a colour change when an enzymatic substrate is added.
- Radioimmunoassay (RIA) – before the development of ELISA tests, the antibody was labelled with a radioisotope. Presence of the antibody is shown by measuring the signal on completion of the test. Nowadays, this method is rarely used in the diagnostic laboratory
- Immunofluorescence (IFA) – the antibody is labelled with a fluorescent dye. Presence of the antibody is shown by measuring the reflection of light from the dye.

The commonly used ELISA methods are described in *Section 4: Principles of laboratory techniques*.

Role of complement

The role of complement was introduced in Section 2: Immunology. Complement may participate in red cell antigen-antibody reactions *in vitro* in two ways; either by red cell adherence, or by causing haemolysis. The nature of the antigen-antibody reaction determines which way it becomes involved.

Complement (C') is a large group of more than twenty proteins present in abundance in the body, and in freshly drawn serum samples. Complement from one species is effective in antigen-antibody reactions in many other species. When complement is activated, a cascade of reactions starts, and is amplified as it progresses. One molecule of C1 attached to the cell wall, results in hundreds of molecules of C3 being activated.

Summary of classical pathway of complement cascade

When complement-binding antibodies react with their corresponding antigens, the fragment crystallizable (Fc) portions undergo a change that attracts the C1 component of complement. C1 is a large molecule consisting of C1q, C1r and C1s that when combined with Fc, react and cause further changes and cleavage of C1. The complex C1 component is stabilized in the presence of calcium ions (Ca^{++}), without which C1 dissociates and becomes inactive. (This explains why anticoagulants, such as EDTA, that chelate calcium are anti-complementary.)

Activated C1s cleaves C4 into C4a and C4b. Some C4b also becomes cell bound and forms the binding site for C2. C1s also cleaves C2 into C2a and C2b. The larger fragment, C2b, becomes cell bound and complexes with C4b forming the activated complex C4b2b, also called C3 convertase. This happens in the presence of magnesium

(Mg^{++}). C3 convertase cleaves hundreds of molecules of C3 into C3a (which dissociates from the cell) and C3b, which becomes cell bound.

C5 convertase (C4b2a3b) cleaves C5 into C5a and C5b. C5b then binds C6, C7, C8 and C9, which results in the cell membrane being pierced. When C8 is cell bound, lysis is slight; when C9 is cell bound, lysis is pronounced.

C3 convertase can progress to the membrane attack stage, or inhibitors can stop the process, which then ends with C3b coating the cell wall. Cells coated with complement *in vivo* are removed from the circulation for destruction in the liver and/or spleen. Figure 7 shows the chain reaction of the complement cascade in detail. C3b can also be degraded by inhibitory factor I, resulting in conversion of C3b to iC3b. iC3b is capable of engaging complement receptors as an opsonin. However, it is inactive and unable to further amplify the complement cascade. Additional cleavage of iC3b by factor I results in the formation of C3d, which loses both opsonic and complement fixing activity. C3d is often used as a marker of antibody mediated complement activation in the laboratory.

In the laboratory, complement plays several roles:

- Haemolysin test – used to demonstrate the presence of immune ABO antibodies that utilize complement

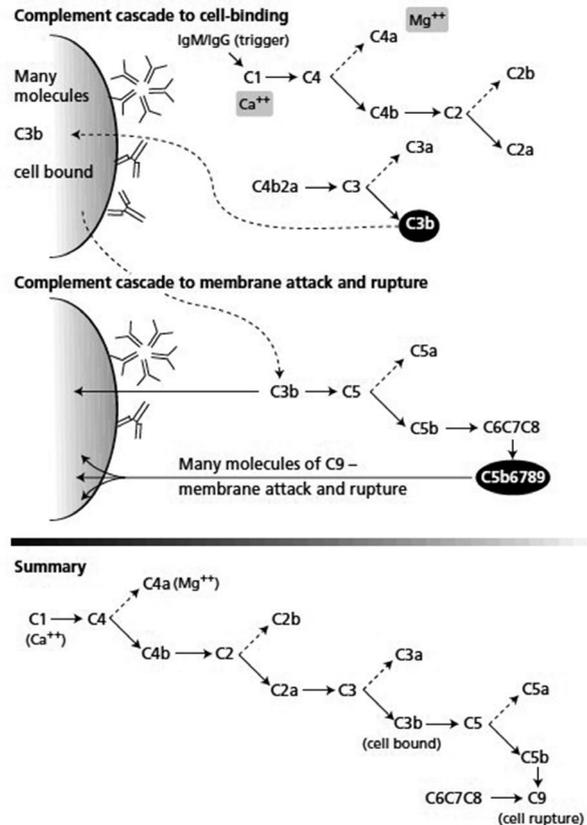


Fig. 7 Detail of complement cascade.

when they react with red cells expressing the corresponding ABO antigens by causing activation all the way to membrane attack and subsequent haemolysis.

- Direct or indirect antiglobulin test: used to demonstrate the presence of complement-binding antibodies attached to red cells with the corresponding antigens, causing the activation of complement, but not all the way to its membrane attack stage. Instead, the complement cascade stops when it becomes cell bound. This is referred to as complement fixation, which is detectable using an antiglobulin reagent that contains anti-complement antibodies and will therefore agglutinate cells with complement coating their surfaces.

Factors that influence the participation of complement in laboratory tests

Chemicals

- Clotted samples are ideal for tests involving complement. However, most blood samples today are taken into sample tubes containing anticoagulant. Anticoagulants such as citrate, EDTA and oxalate are anti-complementary, as they chelate or bind calcium. This means that they block complement participation in antigen-antibody reactions. The complement cascade needs calcium to proceed, and if calcium is chelated or bound by the anticoagulant it will prevent the complement cascade from proceeding. Therefore, anticoagulated plasma samples should not be used for haemolysis tests or to screen for complement-binding antibodies in antiglobulin tests or to carry out complement utilization studies.
- Heparin is an example of an anticoagulant that does not chelate calcium, so heparinised samples may be used for tests requiring the participation of complement.
- Reagent red cells are often suspended in preservation fluid rather than normal saline, so that they may be used over a longer period of time (up to 6 weeks). However, this preserving fluid may also be anti-complementary, so if the reagent cells are required for tests dependent on the presence of complement, they should be washed and then resuspended in saline just before use.

Time and temperature

- When a clotted blood sample is taken, it contains complement, but this deteriorates steadily. Its properties are also affected by the surrounding temperature; the higher the temperature, the quicker the deterioration. Complement is said to be 'labile' because it easily loses its active characteristics.

- Serum samples stored in the refrigerator at 2°C to 10°C lose their complement activity after 24 hours. Serum samples stored frozen at -18°C or colder will not lose their complement activity for several weeks, provided that the sample is frozen while fresh (within a few hours of being taken). Complement activity may be deliberately inactivated in serum by placing it at a temperature of 56°C for 30 minutes.
- If the test to be performed requires complement, either to demonstrate haemolysis or complement-binding, and the serum to be tested is aged, then complement from an external source may be added. In daily practice one uses freshly drawn or immediately frozen and one-time thawed serum from a Group AB donor who is a non-secretor (lacks A and B antigen in solution in the serum).

Role of antihuman globulin

In 1945 Robert Coombs, Robert Race and Arthur Mourant introduced a test that had been developed many years earlier by Carlo Moreschi, for detecting weak, incomplete (sensitising, IgG) antibodies. Previously known as the Coombs' test, it is now referred to as the antiglobulin test. Historically, AHG was prepared by injecting animals of another species, for example rabbits or goats, with human proteins. The two types of human proteins that were introduced as antigens into the animals were:

- Immunoglobulin G (IgG)
- Complement

Several animals were used; the one batch injected with IgG and the other with the C3 component of complement. Because these human globulins/proteins are foreign to the animals, their immune system was stimulated to respond, not only to human species proteins, but specifically to the IgG and complement proteins, producing antihuman species (heterophile) antibodies and also antibodies specific to IgG and C3.

After an appropriate response time, samples of blood were drawn from the animals, and after adsorption of the anti-species antibodies, their serum/plasma examined for the presence of anti-IgG and/or anti-C3. To prepare a broad-spectrum reagent, a blend or pool of the best anti-IgG and anti-C3 was processed and standardised for routine use in the laboratory.

The purpose of a broad spectrum or polyspecific AHG reagent is to detect and agglutinate cells which have been sensitised by human IgG or C3 or both. (In order to simplify the anticomplement activity, in this publication the term anti-C3 is used to signify predominantly anti-C3d, with or without the presence of anti-C3b). By introducing AHG into the test, an antigen-antibody reaction will occur between the cell bound globulin/protein and the

antiglobulin reagent. The AHG is able to bridge the gap between adjacent sensitised cells, agglutinating them and making the original reaction (between red cell antigens and specific antibodies, with or without the involvement of complement) visible.

Most AHG used in laboratories today is commercially manufactured monoclonal AHG and is readily available as a blend of anti-IgG and anti-complement or as a mono-specific reagent for either IgG or complement.

Washing the cells after their incubation with serum/plasma, and prior to the addition of the antiglobulin reagent, is crucial to the success of any AHG test. It is important to remove all unbound globulin from the cells to be tested prior to the addition of AHG. If the cells are not adequately washed, unbound globulins or complement components are able to react with AHG and in so doing neutralise it, rendering it inactive and unable to combine with cell bound material. This leads to false negative results and demonstrates the importance of controlling AHG tests that are found to be negative. For additional information, refer to *Section 4: Principles of laboratory techniques*.

The reaction between globulin and antiglobulin is specific in that the AHG recognises human globulin, or complement, or both. It does not recognise the actual specificity of the antibodies, which originally reacted with the antigens on the red cells.

Unlike proteolytic enzymes and high protein media, AHG does not reduce zeta potential, but bridges the gap between sensitised cells.

Figure 8 describes and depicts the action of AHG on IgG and complement sensitised red cells. The last picture in the figure shows the involvement of both components of broad spectrum AHG.

Application of antiglobulin tests

Direct antiglobulin test (DAT)

The DAT is performed on red cells to determine whether they were sensitised *in vivo*. The serum/plasma in the blood sample does not play a role in this test, and there is no incubation stage. The test involves immediate testing of patient's red cells, by the addition of AHG. If tested in tubes, patient's cells need to be washed, which is not always necessary if the column technique is used (see *Section 4: Principles of laboratory techniques*). A positive result indicates that the patient's cells are sensitised in the body and this has the potential to cause *in vivo* haemolysis. A positive DAT can indicate the presence of haemolytic anaemia caused by autoantibodies or induced by drugs, or the sensitisation of transfused red cells, as the result of an incompatible blood transfusion, or various diseases. A positive DAT result when testing cord

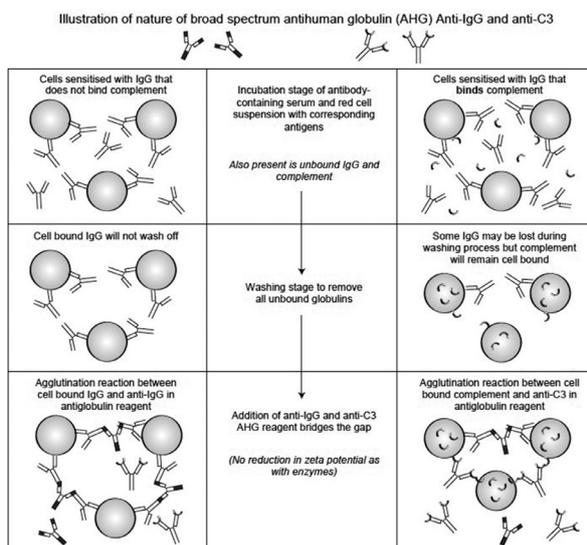


Fig. 8 Action of broad spectrum AHG on IgG and complement coated red cells.

blood cells from a newborn infant indicates haemolytic disease of the fetus and newborn,

If the DAT is performed with AHG containing antibodies against both IgG and complement and is found positive, one can further specify with monospecific reagents if IgG or complement is cell bound. If autoimmune haemolytic anaemia is suspected one can also test with monospecific reagents which are able to detect cell bound IgA or IgM.

There are numerous other *in vitro* uses for the DAT, such as to confirm that laboratory prepared red cells for use in tests to detect IgG antibodies are unsensitised prior to use.

A DAT performed on a refrigerated clotted blood sample may give false positive results should broad spectrum AHG be used. This is because complement can become bound to the red cells in the refrigerator, as a result of the presence of cold antibodies, not necessarily of clinical significance. For this reason, many laboratories prefer to use an anticoagulated sample (i.e. a sample in which the complement was inactivated at the time of collection) for performing the DAT.

Indirect antiglobulin test (IAT)

In the case of the IAT, serum/plasma and cells are incubated in the laboratory to allow sensitisation to take place. Only then are the cells washed to remove unbound globulins, before the addition of AHG. Agglutination indicates that an antigen-antibody reaction took place during the incubation phase.

When the IAT is carried out, usually the cells or the serum/plasma has known characteristics. For example,

one may use an IgG typing reagent in order to establish whether unknown cells have the corresponding antigen or not. This is known as red cell typing. On the other hand, one may use reagent red cells of known antigen type to detect unexpected antibodies in serum/plasma with unknown properties. This is known as antibody screening or antibody identification.

It is preferable to use fresh serum for the detection of complement-binding antibodies in the IAT. If an antibody of this nature is very weak (i.e. if it has low affinity), then it may be dislodged from the cells after incubation during the washing process. However, if complement became cell bound, it will not wash off the cells. Use of AHG reagent containing anti-C3 will cause these complement bound cells to agglutinate, indirectly indicating that an antibody was present.

Polyclonal and monoclonal antibodies

Polyclonal antibodies

Polyclonal antibodies are usually of human origin. Polyclonal is a term used to describe antibodies that have the same specificity but are produced by different clones of antibody producing cells (plasma cells) in the body, and each antibody molecule is therefore not exactly the same, although they react with the same epitope. One may liken polyclonal antibodies to several keys that have very similar structure and are therefore all able to fit and operate the same lock. In some cases, such as anti-D, a polyclonal is a mixture of antibodies directed against different epitopes of the Rh(D) antigen.

Antibodies produced in one individual are not exactly the same as those produced in another individual. Because of this individual variation, batches of anti-A, for example, (or anti-B, etc.) prepared from humans, must be standardised every time that a new batch is produced, to ensure continuity of reaction behaviour and strength from batch to batch and to identify any possible contaminating antibodies of other specificities. Because of the widespread use of monoclonal antibodies as grouping reagents in the laboratory today, variation of this nature is seldom a challenge.

Monoclonal antibodies

Monoclonal antibodies (mAbs) are produced *in vitro*, using tissue culture techniques involving the production of hybridomas. They are produced from a single immune cell line i.e. a clone of cells arising from a single plasma cell, therefore all the antibody molecules produced are exactly the same. There is no variation from batch to batch prepared from the same antibody producing clone under identical conditions. The starting cell line may be of murine (mouse) or human origin.

Outline of process to produce murine monoclonal antibody.

- (1) Mice are immunised with human red cells containing the antigen corresponding to the specificity of antibodies to be produced (e.g. A cells to produce anti-A).
- (2) After immunisation, B lymphocyte cells are removed from the mouse's spleen and fused with human myeloma cells (plasma cells that have become malignant and can be continuously cultured *in vitro*).
- (3) After fusion, some of the cells produced are a mixture of mouse and myeloma cells – these are called hybridomas.
- (4) The cells are placed in a selective medium in which any unfused B lymphocytes and unfused myeloma cells die off. Only hybridoma cells survive.
- (5) Those hybridoma cells that are good antibody producers (the antibodies are secreted into the culture medium) are selected and cloned (clones are reproduced from a single cell and are identical in their inherited characteristics).
- (6) In time, clones grow into colonies of monoclonal cells (all the same).
- (7) Antibody content is measured again, and colonies producing good antibodies are selected and stored frozen.
- (8) These colonies can later be retrieved to produce large-scale tissue cultures, secreting monoclonal antibodies of exactly the same specificity.
- (9) This allows for the production of vast quantities of high titre monospecific antibody for ongoing laboratory use.

Advantages of monoclonal antibodies.

- Availability of large quantities of monospecific reagent antibody that reacts in a reproducible way.
- No variation from batch to batch. Antibodies are produced in pure form, whereas antibodies of human origin are polyclonal.

Disadvantages of monoclonal antibodies.

- The generation and successful recovery of stable, cloned hybridomas is time-consuming and expensive.
- The process requires special laboratory equipment and storage facilities.
- Murine antibodies (e.g. anti-D) cannot be used in an AHG test.

Figure 9 is a diagram that shows the production of murine monoclonal antibodies by hybridoma.

Laboratory reagents such as anti-A and anti-B were originally of human origin. Antiglobulin was originally produced in animals. These reagents, being polyclonal and subject to batch variation, had to be carefully

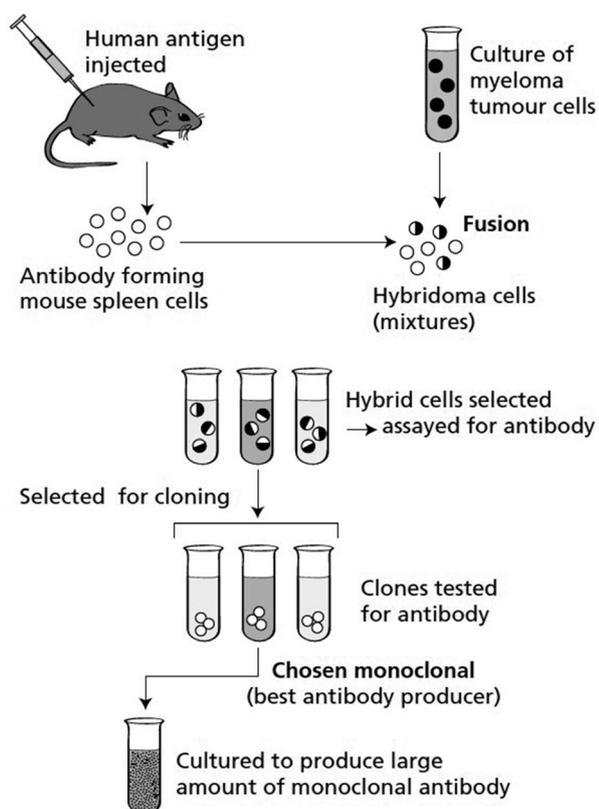


Fig. 9 Production of mouse monoclonal antibodies by hybridoma.

standardised every time a batch was produced. With the development of monoclonal antibodies that lack variation, it is not surprising that they are now the reagents of choice. Unlike human polyclonal antibodies, monoclonal antibodies are highly specific and of high titre. They are not subject to interference with non-specific cross-reactions like their polyspecific counterparts. Because of their high affinity for antigen, they are able to react strongly even with weakly expressed antigens. Commercially prepared type IgM, IgG and various blends of monoclonal antibodies are now commonly used in blood grouping and antiglobulin tests.

Recent developments

A promising technology developed in recent years to produce inexpensive mAbs is the use of genetically engineered plants (these antibodies are called “plantibodies”). In this process DNA encoding a specific antibody is introduced into the plant’s cells and these transgenic plants then produce antibodies similar to those that would be produced by immunised humans. Several harvesting and purification steps are required before the antibodies are rendered useful. Production of antibodies

by this method is expected to be less expensive than conventional culture methods, and for antibodies against infectious diseases has the advantage of not requiring the use of an antigen.

During the 2014–2015 Ebola outbreak in Liberia two American medical workers were treated with plantibodies produced by incorporating the genes for the Ebola antibody into tobacco plants with promising results.

Key Points

- Red cell antigen-antibody reactions occur in two stages; the first is rapid and the second takes time for the reaction to become demonstrable.
- Antibodies bound to red cells can be detected in different ways for IgM or IgG (and IgA) antibodies.
- Complement fixing antibodies can be detected by the presence of C3 on the red cell surface.
- Centrifugation is the most widely used way to enhance antigen-antibody reactions.
- Haemagglutination or the clumping of red cells occurs when IgM antibodies react with their corresponding red cell antigens.
- Sensitisation occurs when IgG antibodies adhere to their corresponding red cell antigens. However, this reaction is not observable.
- To investigate if red cells are sensitised with antibodies, potentiators may be used to enable sensitised cells to become agglutinated.
- Haemolysis is the result of antigen-antibody reactions utilising the complement cascade all the way to cell membrane attack and rupture.
- Neutralisation of antibody occurs in the presence of the corresponding antigen in soluble form. An antibody that has been neutralised cannot thereafter react with red cells containing the corresponding antigen.
- Immunodiffusion allows for the development of a precipitin line between soluble antigen and antibody, in an appropriate gel medium.
- Many factors influence antigen-antibody reactions; these include the number and site of antigenic determinants on cells, the distance between epitopes, the electric repulsion between red cells, the goodness of fit between antibody and antigen, the immunoglobulin class, the concentration of antibody, as well as the effects of time, temperature, pH and ionic strength of the surrounding test environment.
- Proteolytic enzymes are able to reduce zeta potential, causing sensitised cells to agglutinate.
- Enzymes may be used in one- or two-stage techniques; it is important to note, however, that some antigens are modified by enzymes and that their

corresponding antibodies will therefore not be detectable in an enzyme medium.

- The proteolytic enzymes in laboratory use include bromelin, ficin, papain and trypsin.
- High molecular mass substances such as albumin, polyethylene glycol, and polybrene, are also able to affect zeta potential and cause the agglutination of sensitised cells.
- Instead of normal ionic strength saline (NISS), low ionic strength saline (LISS) is commonly used in antibody detection tests because incubation time is reduced, and antibody uptake is increased. Therefore, the sensitivity of antigen–antibody reactions is enhanced.
- Complement is a serum/plasma protein complex that amplifies antigen–antibody reactions. When involved in antigen–antibody reactions, it either leads to lysis, or complement fixation (binding to cell walls).
- In vitro, complement is labile and adversely affected by time and temperature. Its action is also prevented by anticoagulants, which block calcium, an ingredient required in the complement cascade.
- Antihuman globulin (AHG) causes the agglutination of sensitised cells by bridging the gap between them. AHG may be monospecific, or broad spectrum, containing antibodies to both IgG and complement.
- The direct antiglobulin test (DAT) will detect red cell sensitisation in vivo.
- If the DAT is positive with broad spectrum AHG, monospecific AHG should be used to detect if IgG or complement or both are bound to the red cells.
- The indirect antiglobulin test (IAT) is used to determine whether an unknown serum/plasma sample contains IgG anti-bodies or to determine, when using an IgG grouping reagent, whether a red cell sample contains a particular antigen.
- Monoclonal antibodies are produced using hybridoma technology. Each monoclonal antibody is produced from an ‘immortal’ single cell line and produces antibody of exactly the same specificity every time. There is therefore no need for comprehensive standardisation from batch to batch in the blood bank, as for human polyclonal antibodies.

Principles of laboratory techniques

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Introduction

Laboratory techniques relate to many other sections in this publication. The information to be found in *Section 16: Quality* is central to all laboratory methodologies. Some laboratory methods are best studied by cross-checking with other sections to which the technique relates.

General aspects and guidelines that are common to many test methods are addressed first, followed by more specific details on widely used methods, such as the determination of blood groups and the testing for transmissible infection markers. This section also includes various other tests, not necessarily carried out by the blood service, but that students in this field should be aware of and understand in principle. Detailed test methods are not described, as these differ from laboratory to laboratory.

The recommended approach to be used in understanding this section is as follows:

- Read *Section 1: Haematology* and *Section 2: Immunology*, as well as *Section 3: Antigen-antibody reactions* prior to reading this section.
 - Techniques that relate specifically to other areas in this publication, such as to *Section 7: Haemolytic diseases*, *Section 10: Donation testing* and *Section 13: Compatibility testing*, should be read in context where necessary.
- Relative proportion of reactants (Serum: Cell ratio)
 - Incubation time and temperature
 - Centrifugation
 - Use of enzymes
 - Use of controls
 - Post analytical considerations
 - Recording of test results
 - Grading and scoring of agglutination reaction
 - Mixed field reaction
 - General Techniques
 - Saline phase method
 - ABO blood grouping
 - D grouping
 - Antiglobulin test
 - Erythro-magnetic technology
 - Microcolumn techniques
 - Red cell antibody screening and identification
 - Antibody titration and quantification
 - Antibody neutralisation/inhibition
 - Elution
 - Adsorption
 - Causes of false results in serological tests
 - Techniques in the Transfusion Transmissible Infections (TTI) Laboratory
 - Rapid diagnostic tests
 - Enzyme linked immunosorbent assay (ELISA)
 - Chemiluminescence
 - Nucleic acid test (NAT)
 - Quality issues in the TTI laboratory
 - Automation in the blood bank laboratory
 - Molecular technology
 - Genome amplification technique

Learning objectives

By the end of this section, the student should be able to describe the principles related to the laboratory techniques listed below and the causes of false positive and false negative results in serological tests; how these false results may be recognised, and how they may be avoided or overcome.

- Pre-analytical considerations
 - Blood sample
 - Type of sample
 - Order of draw
 - Labelling of sample
 - Storage of sample
- Analytical considerations
 - Red cell suspension
 - Preparation of red cell suspension
 - Strength of red cell suspension
 - Red cell suspending media

Pre-analytical considerations in laboratory techniques

Blood sample requirement

Blood sample

Most blood samples today are drawn into plastic tubes that are discarded after a single use. Certain types of plastic tubes (or dropper bottles), not susceptible to cracking, are also suitable for the long-term frozen storage of samples. Plastic dropper bottles can be used for the refrigerated storage of reagent red cells suspended in a

preservation fluid. When samples are no longer required, they should be discarded in an appropriate manner, in compliance with local health regulations for the safe disposal of potentially biohazardous materials.

Blood samples collected into gel tubes cannot be used for red cell typing tests. After centrifugation the gel forms a layer between the serum/plasma and red cells. Cells collected by plunging the pipette through the gel layer may become contaminated with the gel and this may lead to false results.

If cold agglutinins are suspected, it is best to collect blood into a previously warmed syringe and then to deliver the blood into containers that have been kept warm at 37°C. When filled, the containers should be promptly replaced in the 37°C waterbath/incubator. The blood sample must be kept at 37°C from the point of collection until the sample has been processed.

Accurate patient identification and sample labelling at the time of collection are key factors in reducing the incidence of collecting the wrong blood into the tube and problems related to sample misidentification.

Haemolysed samples

Haemolysed blood samples can create problems in the interpretation of test results, especially when using gel cards. Moreover, antibody mediated haemolysis may be masked if the blood sample is haemolysed. Therefore, wherever possible, haemolysed blood samples should not be used for laboratory techniques.

Type of sample

Clotted samples are suitable for most manual tests, especially those in which the action of complement is to be measured. For example, serum (not plasma) is required for ABO blood group haemolysin tests and to demonstrate

complement-binding IgG antibodies using broad spectrum antiglobulin reagent.

When plasma is needed, many different anticoagulants can be used to prevent blood samples from clotting. Nowadays, most automated machines require a plasma sample for testing. It is important that the most suitable anticoagulant is used for the tests to be carried out on the sample. Examples of anticoagulants and some of their uses are shown in Table 1.

If automated equipment is used, all samples need to be anticoagulated, as the presence of clots prevents aspiration of contents. The volume must be within the specifications of the analyser.

Depending on local laboratory requirements, ACD or EDTA are usually the anticoagulants of choice for immunohaematology studies.

If detection of haemolysis is important to the test end point serum samples (red top nonanticoagulated tube) must be used in order to ensure complement activation is possible.

Order of sample draw

Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw for plastic vacutainer tubes is:

- First – blood culture bottle or tube (yellow or yellow-black top, i.e. cap/bung/stopper).
- Second – coagulation tube (light blue top).
- Third – non-additive tube (red top).
- Last draw – additive tubes in this order:
 - SST (red-gray or gold top). Contains a gel separator and clot activator.
 - Sodium heparin (dark green top).
 - PST (light green top). Contains lithium heparin anticoagulant and a gel separator.
 - EDTA (lavender top).

Table 1 Commonly used anticoagulants

Anticoagulant	Colour code of stopper tube	Mechanism of action	Tests typically performed
None	Red	For serum so that the action of complement in tests is not compromised	Serum grouping Crossmatching Antibody screening and antibody identification
EDTA	Lavender	Binds calcium and this prevents clotting as well as the action of complement	Blood grouping Direct antiglobulin test (DAT) Cross-matching Red cell antigen typing Antibody screening and antibody identification Blood group genotyping
Citrate	Light Blue	Anticoagulant binds calcium	Coagulation studies
Acid Citrate Dextrose (ACD)	Yellow	Citrate binds calcium	HLA/HPA antigen typing Monocyte monolayer assays

The timing of the sample for pre-transfusion testing should be taken in accordance with the guidelines given in Table 2.

Labelling of sample

Misidentification at blood sampling may lead to fatal transfusion reactions, especially if the patient has not previously had their blood group documented, which allows for checking the blood group in laboratory records. Inadequately or mislabelled samples carry a significantly increased risk of containing blood from the wrong patient. Risk of misidentification may be reduced by electronic systems, but all sampling should be carried out in line with the following principles by trained and competent staff:

- Positive identification of blood donor or recipient.
- Drawing and labelling of the sample tubes must be performed as one uninterrupted process.
- Pre-labelled tubes must never be used.
- Label on the sample must contain the minimum patient identifiers (surname/family name, hospital registration number) date and time of sampling and identity of individual taking the sample.
- Laboratories should have a policy for rejecting samples that do not meet the above minimum requirements.

Storage of sample

Whenever possible, samples should remain as they were received, and the serum/plasma should not be separated routinely. This is to avoid the chance of separation errors and serum/plasma misidentification later.

- Depending on the requirements of the blood service, clotted samples should not be used after 1 week but may be kept for up to 2 weeks for reference purposes in the refrigerator at $4 \pm 2^\circ\text{C}$, whereas anticoagulated samples may be kept for seven to 10 days.
- Serum/plasma may be kept frozen for years. The lower the temperature of storage, the longer the samples may be stored. The temperature for frozen storage should be as low as possible, or at least -15°C .
- When frozen samples are to be used over a long period of time, the serum/plasma should be divided into

small aliquots so that the same samples are not thawed and frozen repeatedly, which could reduce reactivity.

- Serum samples stored in the refrigerator at $4 \pm 2^\circ\text{C}$ lose their complement activity after 24 h. Serum samples stored frozen at -18°C or colder will not lose their complement activity for several weeks, provided that the sample is frozen while fresh (within a few hours of being taken). Complement activity may be deliberately removed from serum by placing it at a temperature of 56°C for 30 min.

Analytical considerations in laboratory techniques

Red cell suspensions

Red cell suspensions prepared from clotted samples should be free of clots, and suspensions prepared from freshly anticoagulated samples should be free of fibrin, both of which could lead to false results. Depending on the suspending medium, cell suspensions may be stored for 8 h or more, at $4 \pm 2^\circ\text{C}$.

Preparation of red cell suspensions

Washing of cell suspensions depends on the testing to be carried out, the number of samples being tested, and whether the testing is manual or automated. It is not necessary to wash cell suspensions prior to routine testing, except when suspensions are prepared from blood bag segments, in which case they are washed once in normal saline. Cell suspensions prepared for special investigations, e.g. for the resolution of anomalous results obtained during routine testing, are also washed at least once in normal saline.

Strength of cell suspensions

Cell suspensions should be between 2% and 5% for standard tube agglutination tests, with an ideal strength of about 3% (Table 3). Much weaker suspensions are used for microcolumn techniques, such as gel card techniques (1% or less), and much stronger suspensions (25–50%) for tests performed on slides. For the weaker suspensions required for gel card and similar tests, suspensions should

Table 2 Timing of pre-transfusion samples

Patient transfused within the last:	Samples to be taken not more than
3–14 days	24 h before transfusion
15–28 days	72 h before transfusion
29 days to 3 months	1 week before transfusion

Table 3 Strength of red cell suspensions for various tests

Agglutination test performed	Strength of suspension
Conventional test tube	3–5%
Micro-column technique	$\leq 1\%$
Solid phase technique	$\leq 1\%$
Slide	25–50%

be prepared using specific measured volumes and specific solutions according to the type of card/column in use – following manufacturer's instructions. Microcolumn card techniques are widely used for many blood grouping tests including crossmatching, both manual and automated (Table 4).

Samples usually require centrifugation prior to testing. This should be carried out preferably with the bungs (i.e. stoppers) on, but it depends on the type of centrifuge being used. Centrifugation with the bungs on avoids aerosols and prevents the possibility of cross-contamination of samples. After the removal of the bung, a sample should not be re-bunged using the same bung, but a clean stopper/cap should be applied to the tube if required. When tests are centrifuged, speeds producing approximately 1000 *g* or the comparable relative centrifugal force (rcf) is used. Centrifugation for laboratory tests is not as critical as for blood components, but protocols should state centrifuge speed in the appropriate G-force (*g*), and if this cannot be done, then 'centrifuge' or 'gentle centrifugation' as the case may be, could be used. The time of centrifugation is also important and must be included in laboratory protocols. Standard operating procedures (SOPs) should be in place and should always be followed. Most laboratories use commercial reagents, in which case the instructions provided by the manufacturer must be followed. The terms 'spin' or 'spun' are sometimes used when referring to the centrifuging of tests.

Use of enzymes

Various proteolytic enzymes are used in blood group serology, such as ficin (isolated from fig), papain (from papaya), trypsin (from pig stomach), and bromelain (from pineapple). Treatment of RBCs with enzymes results in the release of sialic acid from the membrane with a subsequent decrease in the negative charges or zeta potential of the RBCs. Enzyme treatment also removes hydrophilic glycoproteins from RBC membranes, causing them to become more hydrophobic, which facilitates the agglutination of sensitised cells.

Enzyme tests are generally considered to be redundant for routine manual tests but may be useful when

Table 4 Preparation of red cell suspension

% Cell Suspension	Volume of normal saline (μl)	Volume of washed packed red cells (μl)
1%	99	1
3%	97	3
5%	95	5
40%	60	40

identifying red cell antibodies and are used in many automated techniques. Enzyme tests may be carried out at $22 \pm 2^\circ\text{C}$ or $37 \pm 1^\circ\text{C}$. The time of incubation depends on the enzyme used; with the average incubation time of 15–30 min. Over-incubation with enzymes may lead to false results. Enzymes are also able to alter some red cell antigens and in so doing, prevent the detection of the corresponding antibodies (See *Section 3, Antigen-Antibody reactions* and *Section 6, Blood Group Systems*, for more information.)

One-stage enzyme method

Serum/plasma is added to the test tubes followed by red cell suspension and then enzyme; the contents mixed and incubated for the appropriate time and at the appropriate temperature, after which the tests are centrifuged and read.

Two-stage enzyme method

Red cells are pre-treated with enzyme, washed and re-suspended in saline before use. Serum/plasma is added to the test tubes followed by the enzyme-treated red cells. This is the more sensitive of the two enzyme methods as the mixture is not diluted by the addition of enzyme as a third reactant. Tests are incubated as required, and then centrifuged and read.

Enzyme techniques detect both IgM and some IgG antibodies. Although enzyme methods enhance some antigen-antibody reactions, it is important to note that some antigens are denatured by enzymes, and false negative results will be obtained if the corresponding antibodies are present in the serum/plasma.

Use of controls

Appropriate controls should be set up with every batch of tests, to confirm that reagent antisera, reagent red cells and all other reactants used, such as enzymes or AHG, are working as expected. The use of good quality reagents is important to ensure the effective and correct functioning of the laboratory. In most developing countries, reagents used are from a commercial source and have been standardised by the manufacturer. Quality control should be done daily to check that in use reagents comply with established requirements.

Post-analytical considerations

Recording of test results

It is important to note that there are two stages to reading tests:

- Reading and recording the results.

- Interpreting the results.

Centrifuged test tubes should be gently agitated to resuspend test contents and results then read by holding the tube at an angle, rather than upright. All results should be read in good light, either over a light background or by use of a light source. In the interests of quality and to avoid errors, the individual responsible for reading the results should not be interrupted. It is important that the *actual* results are recorded, and not the *expected* results – a common error when a technologist lacks concentration during this task. A good technologist is one who concentrates on what is actually observed in the test being viewed, and then recalls the principles of the test, being aware of the factors that influence antigen–antibody reactions, and the causes of false results. The record of the test must always reflect what is observed and NOT what SHOULD be observed. In this way results will be recorded correctly, and anomalies detected and investigated.

Test results should be recorded immediately, checking the number on the test tube with the laboratory protocol to ensure that each set of results is entered in its correct place. Control results should be read and recorded first, and only if the expected results are obtained, should the tests then be read.

Grading and scoring of agglutination reaction

The degree of red cell agglutination observed is significant and should be recorded; it gives an indication of the amount of antigen or antibody present. When reading tests, an additional source of light such as a light box, or viewing results over a light background, is advisable. The technique used in the resuspension of the cells is critical for accurate results. Tests should be gently agitated to resuspend the cell button, and the results read by holding the tube at an angle (tip-and-roll method), rather than upright. The tip-and-roll method involves gentle shaking of the test tube to free the cell button from the bottom of the tube. Gently ‘tip’ the tube to allow the contents to ‘roll’ along the tube, and do this a few times over a good light, to better see the strength of the agglutination. A grading chart may then be used to note the strength of agglutination observed. The designations used for the degree of agglutination strength and its corresponding score are shown in Table 5, where grade 4 indicates the maximum strength of agglutination observed.

Some laboratories use the plus () sign instead of a number to record the degree of agglutination, with the number of plus signs indicating the degree of strength of the reaction, e.g. , or . Plus signs may also be used to signify the presence of an antigen, such as in interpretation of red cell panel results during antibody detection and

identification. The score is a numeric value assigned according to the degree of agglutination observed.

For recording a negative result, it is preferable to use a zero, as a minus sign or dash may be interpreted as the test not having been carried out. Other reactions which may occur are the mixed-field reaction, in which mixtures of agglutinated and unagglutinated red cells are present; and haemolysis, in which red cells are haemolysed by the antibody. Both of these are considered positive reactions.

Mixed field reaction

Mixed field agglutination in serology usually describes the presence of two populations of red cells: agglutinated cells admixed with many unagglutinated cells. Mixed-field agglutination is an important cause of ABO typing discrepancies. The cause of mixed field agglutinations should be sought prior to setting up blood for transfusion. Often the cause of the mixed field reactions is easily ascertained on checking the patient’s history, when the patient is found to have had recent non-group specific transfusion or stem cell transplantation from a non-group identical donor. Certain ABO subgroups and pathologic or physiologic conditions may also lead to mixed field reactions in ABO typing; however, they should rarely affect Rh(D) typing. One of the potential causes of mixed field reactions on ABO and Rh(D) typing is the presence within an individual of a chimeric state. A chimera is present when two or more distinct cell populations containing genetic material from more than one zygote co-exist within one individual. Although descriptions highlighting the concept of chimerism as a cause of blood group typing discrepancies have existed for decades, chimerism is uncommon and presents challenges when mixed field agglutination is encountered during forward or reverse ABO typing. Both serologic and molecular methodologies may be useful in determining the cause of mixed field reactions. In addition, a highly sensitive flow cytometric

Table 5 Observed agglutination strengths and designated interpretation and score

Findings	Designation	Score
One solid agglutinate	4	12
Several large agglutinates	3	10
Medium large agglutinates in a clear background	2	8
Small agglutinates in a turbid background	1	5
Very small agglutinates in a turbid background	1 ^W	4
Barely visible agglutinates in a turbid background	W or /-	2
No agglutination	0	0
Mixed field agglutination	mf	
Complete haemolysis	H	
Partial haemolysis	PH	

method may assist in characterising ABO subgroups through the detection of low levels of A and B antigen on RBCs or on a small number of RBCs in a sample.

Mixed field reactions are typically seen when Group O donor blood has been transfused into a Group A, B or AB recipient (heterologous group transfusion). Table 6 illustrates mixed field reaction during ABO grouping of a Group A recipient who was transfused with Group O blood. The mixture of red cell populations is demonstrated by the recipient Group A red cells agglutinating with the anti-A reagent, while the donor Group O red cells remain unagglutinated in the same test. Figure 1 demonstrates mixed field agglutination in test tubes and on slides. In Fig. 2, mixed field agglutination is observed using a column agglutination technique.

It is very important to detect mixed field reactions in forward ABO grouping when investigating a transfusion reaction, as this may be a sign that blood of the incorrect and incompatible ABO group was transfused in error.

General techniques

The basic techniques described in succeeding discussions have many applications, such as the grouping and typing of red cell samples, crossmatching, antenatal and postnatal testing, and antibody detection and identification. Whenever tests are carried out, they should be performed according to the laboratory SOP or the manufacturer's instructions.

Saline phase method

The saline technique is used for ABO grouping, other red cell typing and for the detection of type IgM antibodies. Only "cold reacting antibodies" are detectable using saline technique. Serum/plasma or known reagent is added to the test tubes followed by an equal volume of red cells suspended in saline or other appropriate medium and then the contents are mixed. The tests can be centrifuged immediately and read (immediate or rapid spin technique) or can be incubated at the appropriate temperature for 10–60 min before reading. With a long incubation time, there may be no need for centrifugation of tests prior to reading.

Table 6 Mixed field reaction during ABO grouping

Forward ABO Grouping			
Anti-A	Anti-B	Anti-A,B	Interpretation
2mf	0	2mf	Group A recipient transfused with group O red cells

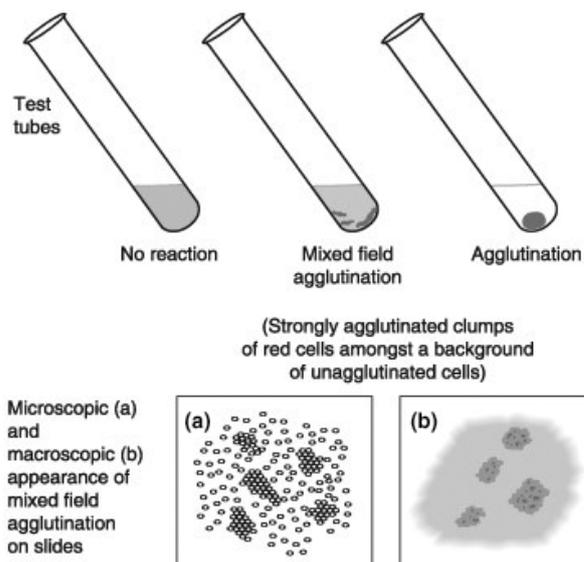


Fig. 1 Mixed field agglutination in test tubes and on slides.

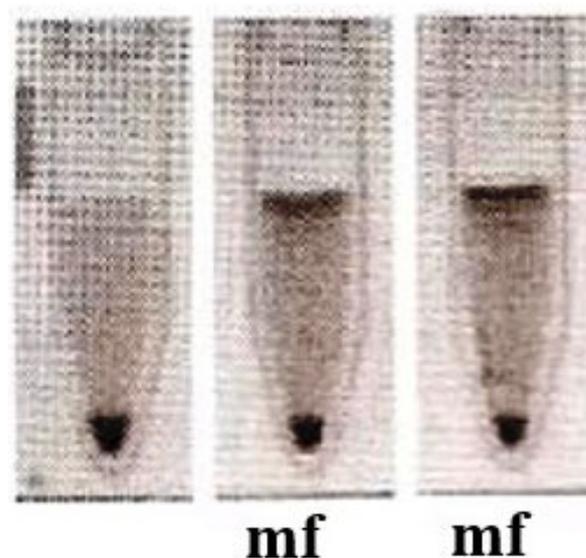


Fig. 2 Mixed field (mf) reactions in column agglutination techniques (CAT).

ABO grouping

ABO grouping involves the testing of red cell suspensions of unknown group to determine the presence or absence of A and/or B antigens and testing the unknown serum/plasma to determine the presence or absence of antibodies corresponding to the antigens lacking on the red cells. More information on ABO groups is to be found in *Section 6: Blood group systems* and *Section 10: Donation testing*.

ABO grouping involves *forward* and *reverse* grouping. In forward grouping the unknown red cells are tested

against known ABO grouping reagents, to determine the identity of antigens on the red cells. When red cells are tested with reagent known to contain a specific antibody and no interaction occurs between the red cells and the grouping reagent, it can be assumed that the red cells lack the corresponding antigen. Conversely any interaction implies that the cells possess that antigen. Depending on the reaction of red cells with anti-A and anti-B reagents, red cells can thus be typed as A, B, AB, or O.

Table 7 denotes ABO group according to ABO antigens on red cells and ABO isohaemagglutinins in serum/plasma. (When referring to antibodies in general, the term alloantibodies is commonly used. However, when referring to the antibodies in the ABO system, the term isohaemagglutinins is sometimes used.)

Individuals exposed to an antigen that they lack may produce an antibody specific for that antigen. Since most blood group antigens are restricted to red cells, production of blood group antibody usually requires introduction of foreign red cells by transfusion or pregnancy. However, A and B antigens have close structural similarity with antigens of bacteria and plants to which there is constant, ongoing exposure. As a result, all normal individuals eventually produce isohaemagglutinins (i.e. anti-A and/or anti-B) depending on blood group (antibodies are produced against the A and/or B immunogens detected in nature). Testing for anti-A or anti-B in the serum/plasma of individuals (using red cells with known antigen specificity) is known as reverse grouping.

Reverse ABO grouping confirms the results obtained in forward grouping. If it does not, then further tests need to be performed to determine the ABO group of the individual. Carrying out both forward and reverse grouping at the outset confirms the ABO type of an individual. Table 8 summarises the interpretation of ABO groups using both forward and reverse grouping.

Additional considerations during ABO grouping

- Anti-A and Anti-B react optimally with saline suspended cells at about 4°C and the strength of reaction decreases as the temperature of testing is raised. ABO grouping is routinely performed at room

Table 7 Identification of ABO group

ABO Group	Antigens on red cells	Isohaemagglutinins in serum/plasma
A	A	Anti-B
B	B	Anti-A
O	None	Anti-A,B
AB	A and B	None

Table 8 Interpretation of ABO group using forward and reverse grouping

Forward grouping		Reverse grouping		Interpretation ABO group
Anti-A	Anti-B	A cells	B cells	
4	0	0	4	A
0	4	4	0	B
0	0	4	4	O
4	4	0	0	AB

temperature ($22 \pm 2^\circ\text{C}$). At lower temperatures non-specific agglutination by cold agglutinins in samples may interfere with reverse grouping.

- During forward grouping, unknown red cells may also be tested against Group O serum that contains anti-A,B in an inseparable form. Anti-A,B has the ability to detect weak forms of A and B antigens. Anti-A,B also serves as a control; whenever there is agglutination in either the anti-A or anti-B test the anti-A,B test should also be positive.
- If monoclonal anti-A,B is used, it is a blend of anti-A and anti-B from different clones and reacts with the majority of Group A cells, including Group A_x cells. More information on weak forms of A are to be found in *Section 6: Blood group systems*.
- During reverse grouping, unknown serum/plasma may also be tested against Group O reagent red cells that contain neither A nor B antigens. Including this test during ABO grouping provides an additional negative control. A positive test result with Group O RBCs needs further identification to determine the cause of the unexpected agglutination. The presence of anti-A and/or anti-B in the serum/plasma when the corresponding antigens are detected on the red cells is an abnormal finding and further testing is indicated to determine the true ABO type of that individual.
- In general, anti-A titres are higher than anti-B and the titre of anti-A in Group O individuals is usually higher than the titre in group B individuals.
- Occasionally the expected isohaemagglutinins are only weakly demonstrated or are undetectable in the serum/plasma, especially if the serum/plasma is from an elderly individual or a neonate. Absence of agglutination in a young individual may indicate conditions such as hypo- or agammaglobulinemia and leukaemia.
- If there is a discrepancy between the forward and the reverse grouping, further testing may be required to establish the true group. It is important to follow the SOP for ABO grouping, and especially in cases where the outcome is not straightforward.

Table 9 Interpretation of ABO groups, including use of anti-A,B reagent and group O red cells

Forward grouping			Reverse grouping			Interpretation ABO Group
Anti-A	Anti-B	Anti-A,B	A cells	B cells	O Cells	
4	0	4	0	4	0	A
0	4	4	4	0	0	B
0	0	0	4	4	0	O
4	4	4	0	0	0	AB

Table 9 gives the interpretation of ABO groups including the use of anti-A,B for the forward grouping and group O cells for the reverse grouping.

Quality control

Controls should be performed on all reagents (including reagent red cells) to ensure that they are working correctly. This means that when reagents are tested with known materials, they should give results as expected.

Controls validate the test results that were obtained using those reagents. Controls should be set up in parallel with test samples so that the batch of tests can be validated. In some laboratories, controls on certain reagents may be set up once daily. In these cases, the reagents should be kept in a separate area indicating that they are the 'controlled' reagents for testing. All new vials/bottles of reagents drawn from stock (when controlled aliquots are depleted) should also be controlled before use. The results of all controls carried out should be documented. Suggested ways of carrying out quality control for the major blood banking reagents are given in Table 10.

Subgroups of A and B

Serum/plasma of Group B individuals contains two separable antibodies, anti-A and anti-A₁. Cells of Group A that react with anti-A₁ are said to be of subgroup A₁. Cells that react with anti-A but do not react with anti-A₁ have a weaker expression of A antigen; the majority being classified as A₂. The cells of about 80% of the Group A population are A₁ while the remaining 20% are A₂ or weaker subgroups. Similarly, based on the results with anti-A, anti-A,B and anti-A₁, Group A red cells can

be subdivided into various subgroups (A₁, A₂, A₃, A_x, A_y and others). Subgroups of B are relatively rare; they are usually recognised by variations in strength of reaction with anti-B.

The antigens in different subgroup individuals may be so weak that they are not recognised, and the cells are mistyped as Group O. Transfusion of mistyped red cells may have serious consequences in the recipient. Refer to *Section 6: Blood group systems* for more information.

Neonates

Although ABO antigen development usually occurs early in fetal life, in some cases ABO antigens may not be fully developed at birth. Use of monoclonal antibodies, which have a higher potency compared to polyclonal antibodies used in the past, lead to agglutination of the corresponding red cells from most newborns. The production of anti-A and anti-B begins at about 3 months of age, reaches its highest level during adolescence and decreases with advancing age. ABO antibodies detected in neonatal blood samples are usually agglutinating IgG antibodies of maternal origin. The ABO group of an infant up to 6 months of age is therefore frequently determined on the basis of forward grouping only.

H Antigen

A and B antigens develop on a precursor H antigen produced by the action of the *H* gene. H antigen is found in greatest concentration in Group O individuals. Individuals who lack H antigen (e.g. Bombay phenotype) are extremely rare. The inheritance of *H* is independent of ABO, but A, B and H antigens are all formed from the same basic material, having a protein or lipid backbone to which sugars are attached. Addition of another sugar in the chain creates the A and B antigens. Refer to *Section 6: Blood group systems* for more information.

Anti-H

Individuals lacking H antigen (such as those with the Bombay phenotype) contain clinically significant anti-H antibody in their serum/plasma. Anti-H in non-Bombay individuals is usually benign and reacts most strongly

Table 10 Controls for ABO and D blood grouping

Reagent	Positive control	Negative control
Anti-A	A cells	B cells
Anti-B	B cells	A cells
Anti-D	D cells	D- cells
A ₁ grouping cells	Anti-A ₁	Anti-B
B grouping cells	Anti-B	Anti-A

with the red cells of Group O, Group A₂ and weaker subgroups, but reacts only very weakly with Group A₁ and A₁B cells. The reactions of a lectin derived from the plant *Ulex europaeus* closely parallels the reactions of human anti-H and is used for the resolution of blood grouping discrepancies. See *Section 6: Blood group systems* for more information.

ABH antigens in saliva

Most individuals (approximately 80%) secrete in their saliva, soluble substances with the same specificity as antigens on their red cells. Tests for ABH substances in saliva may help in establishing the ABO genotype of individuals whose red cell antigens are poorly developed or absent. See *Section 6: Blood group systems* for more information.

D grouping

The Rh blood group system is regarded as the second most important blood group system, after the ABO system. This is due to its involvement in severe haemolytic transfusion reactions and haemolytic disease of the fetus and newborn (HDFN). See *Section 6: Blood group systems* for more information.

Reagents for Rh typing

Monoclonal antibodies are now becoming more readily available. These reagents are derived from single clones of antibody-producing cells. Because the D antigen is composed of many epitopes and monoclonal Rh antibodies have a narrow specificity, monoclonal anti-D reagents are usually a combination of monoclonal anti-D reagents from several different clones to ensure reactivity with a broad spectrum of Rh-positive RBCs. Some reagents are a blend of IgM and IgG anti-D to maximise visualisation of reactions at immediate spin testing and to allow indirect antiglobulin testing for weak D antigen with the same reagent. Monoclonal blends can be used for slide, tube, and microwell techniques, and in most automated Rh testing.

As with all commercial typing reagents, Rh antigen typing must be performed with strict adherence to manufacturer's directions, use of proper controls, and accurate interpretation of test and control results. Table 11 provides Rh typing results when testing samples including those with weak D antigens, using monoclonal anti-D. The term 'NT' denotes 'not tested'.

Variation in D antigen expression

When D samples are typed for the D antigen, they are expected to show strong positive reactivity with anti-D

Table 11 Rh typing results using monoclonal anti-D reagents

Rh type	Immediate spin anti-D (IgM)	2nd phase anti-D IAT	DAT control
D	4	NT	NT
Previously typed as weak D using polyclonal reagents	3 or 4	NT	NT
Weak D	0 or 1	1 or 2	0
Partial D	3 or 4	NT	NT
D negative	0	0	0

reagents. However, some individuals have RBCs that possess a weaker expression of D antigen that requires an indirect antiglobulin test (IAT) to detect its presence. For many years, all individuals with altered D antigen were referred to as having weak D. Now these individuals with altered D antigen are categorised into different phenotypes defined as weakened D due to C in *trans* position genetically, to *RHD*, weak D, partial D, and DEL. Figure 3 is a schematic representation of normal D, partial D and weak D phenotypes representing the variable characteristics of the D antigen. Numbered bars protruding from the cell surfaces of each phenotype are used for illustrative purposes only, to show the commonalities and differences between inherited types of D phenotype.

Rh phenotyping

In routine Rh phenotyping, the unknown red cells are tested with anti-D, anti-C, anti-E, anti-c, and anti-e reagents. There are several reasons why an Rh phenotype may be performed. It is useful to phenotype a patient with Rh antibodies to ensure that he/she is not transfused with Rh incompatible donor blood. It may sometimes be of assistance to phenotype the red cells of a father to determine the likelihood of a fetus inheriting a paternally derived antigen, to which the mother has the corresponding antibodies. On interpretation of the results, the phenotype can be determined, and the probable genotype calculated. Table 12 shows some typical Rh phenotyping results.

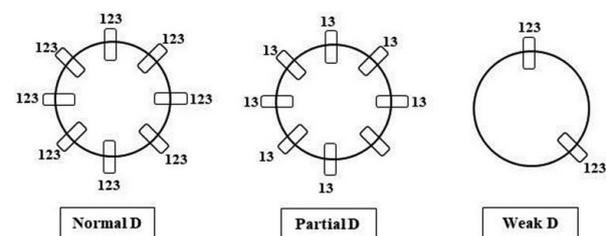


Fig. 3 Diagrams to illustrate the differences between normal, partial and weak D phenotypes.

Table 12 Rh phenotyping results

Rh phenotype	Anti-D	Anti-C	Anti-E	Anti-c	Anti-e
R ₁ R ₂	4	4	4	4	4
R ₂ R ₂	4	0	4	4	0
R ₀ r	4	0	0	4	4
R ₁ R ₁	4	4	0	0	4
R ₁ r	4	4	0	4	4
r'r	0	4	0	4	4
R ₂ r	4	0	4	4	4
r''r''	0	0	4	4	0
rr	0	0	0	4	4

Antiglobulin test

The antiglobulin test is based on the principle that anti-human globulins (AHG) obtained from immunised non-human species bind to human globulins such as IgG or complement, either free in serum or attached to antigens on RBCs. The AHG test is used to bring about the agglutination of red cells sensitised with IgG alloantibodies, IgG autoantibodies or complement components.

Principle

Red cells coated with IgG or complement components C3b or C3d, or both, will show agglutination with broad spectrum AHG reagent. The coating (sensitisation) of red cells can occur either *in vivo* or *in vitro* following incubation at 37°C with serum which contains the corresponding antibody.

The IgG antibodies attach to the red cell membrane by the Fab portion of the immunoglobulin molecule (IgG). The IgG molecules attached to the red cells are unable to bridge the gap between sensitised red cells which are kept separated from each other by the negative charge on their surface and the sensitised red cells do not agglutinate. When AHG is added to the washed sensitised cells, the Fab portion of the AHG molecule (anti-IgG) attaches with the Fc portions of two adjacent IgG molecules which are already attached to red cells. This bridges the gap between sensitised red cells and causes agglutination.

Reagents

AHG reagents may be made by injecting rabbits (or other suitable animals) with purified human IgG or C3, then harvesting the antibodies produced by the animal. Monoclonal technology may be used to make monoclonal antiglobulin reagent. There are mainly two types of AHG reagents in use.

- Polyspecific reagent: blend of anti-IgG and anti-C3b, -C3d.
- Monospecific reagents: anti-IgG alone or anti-C3b, -C3d alone.

Table 13 illustrates the various AHG reagents available for use. Note that in this table, polyclonal reagents are made in rabbits, and as such, show batch variations as do human antibodies. Therefore, a rabbit polyclonal anti-IgG may be specific for immunoglobulin G only but is termed polyclonal because of its source; i.e. being subject to batch variation.

Types of AHG test

Direct antiglobulin test (DAT)

The DAT detects *in vivo* coating of patient's red cells either with IgG antibodies, complement or both. In the patient's bloodstream, antibodies attach to specific antigens on the patient's RBCs, with or without the involvement of complement. This happens in various clinical conditions such as HDFN, and auto immune haemolytic anaemia (AIHA). Certain drugs can also activate complement and coat the red cells *in vivo*. The DAT does not require an *in vitro* incubation phase because antigen-antibody complexes are formed *in vivo*. When the DAT is positive, it indicates that the patient has antibody and/or complement coating the cells *in vivo*. If IgG or other complement binding antibodies are involved, a DAT performed in the laboratory, using a broad spectrum AHG (i.e. containing anti-IgG and anti-C3) will be positive.

Indirect antiglobulin test (IAT)

The aim of the IAT is to detect *in vitro* sensitisation of red cells. It is used in the following situations:

- During crossmatching, for the detection of IgG antibodies present in the serum/plasma of the patient, specific for the RBCs of a potential donor (compatibility testing) or to detect irregular or clinically significant IgG antibodies in the serum/plasma of the patient by testing with reagent Group O screening cells.
- Determination of RBC phenotype (e.g. Kell, weak D) using specific antisera.
- Titration of IgG antibodies.

Significance of washing red cells in AHG test

- While performing both the DAT and IAT, red cells used for the test must be washed in normal saline a minimum of three times before the AHG reagent is added to the cell suspension.

Table 13 AHG reagents and their contents

Type of AHG reagent	Contents
Polyspecific	
Rabbit polyclonal	Contains anti-IgG, anti-C3b and anti-C3d (may also contain other anti-complement antibodies e.g. anti-C4d)
Rabbit murine monoclonal	Contains rabbit polyclonal anti-IgG and a blend of murine monoclonals, i.e. anti-C3b and anti-C3d
Murine monoclonal	Contains a mixture of murine monoclonal antibodies, i.e. anti-IgG, anti-C3b and anti-C3d
Monospecific anti-IgG	
Rabbit polyclonal	Contains anti-IgG with no anti-complement activity
Monoclonal anti-IgG	Contains anti-IgG only
Anti-complement	
Rabbit polyclonal AHG	Contains antibodies against designated anti-C3d and anti-C3b complement components, no anti-IgG
Murine monoclonal anti-C3d	Contains antibodies against C3d
Murine anti-C3b and C3d	Contains antibodies against C3b and C3d

- Washing the RBCs removes free unbound serum globulins which would otherwise lead to neutralisation of the AHG reagent. Inadequate washing may result in a false negative results.
- The washing should be performed in as short a time as possible to minimise the elution of low-affinity antibodies.
- The cell pellet should be completely re-suspended before adding the next saline wash to ensure that no unbound globulins remain trapped amongst the cells, which may then neutralise the AHG.
- All saline should be discarded completely after the final wash because residual saline dilutes the AHG reagent and therefore decreases the sensitivity of the test.
- The speed and time of centrifugation after each wash should be optimised to minimise the loss of cells with each discard of saline.
- The tubes should not be overfilled with saline, as it leads to a loss of red cells and risks cross-contamination between tubes.
- Control IgG sensitised cells are added to negative tests after reading to ensure that the washing technique removed all unbound globulin, and the AHG reagent was active. See *Quality control in AHG Test* below.

Cell washers

Automated and semi-automated cell washers significantly reduce the time taken to do antiglobulin tests. Cell washers should be tested once a week to ensure that they are working correctly. This is carried out by placing one drop of inert serum and one drop of IgG sensitised cells into the required number of tubes to fill all cell washer tube positions. After washing, addition of AHG to all tests should give the same strength of reaction. Only if results are satisfactory may use of the cell washer be continued.

pH of saline for washing

Saline used for washing should be fresh and have a pH of 7.2–7.4. If the saline is stored for long periods in plastic containers, the pH of the saline decreases, which increases the rate of antibody elution during the washing process. Stored saline may cause significant levels of bacterial contamination which may contribute to false positive results.

IAT Method

Serum/plasma is added to the test tubes followed by red cells suspended in the appropriate medium; the contents mixed and allowed to incubate for 30 min (minimum) to 2 h (maximum) at 37°C. After incubation, the tests are washed three times in normal saline to remove any unbound globulins. After the last wash, AHG is added, and the tube contents are mixed, centrifuged and read, and re-read after the addition of sensitised cells to the negative tests.

The use of LISS in the IAT

LISS enhances antibody uptake and allows incubation time to be decreased. A LISS medium may be achieved by either suspending RBCs in LISS in place of normal saline, or by using a LISS additive reagent. Serum/plasma is added to the test tubes followed by red cells suspended in LISS for increased sensitivity and a shorter reaction time, thus reducing incubation time to a minimum of 10 min.

The use of albumin in the IAT

The macromolecules of albumin allow antibody coated cells to come into closer contact with each other so that aggregation occurs. If albumin was incorporated into the reaction medium, it has shown to provide the same sensitivity at 30 min of incubation as a 60-min saline test. Albumin does not provide any additional advantage over LISS.

The use of polyethylene glycol (PEG) in the IAT

PEG is a water-soluble linear polymer and is used as an additive to increase antibody uptake, thus allowing a reduction in the incubation time required when compared to an IAT carried out without additives.

The use of polybrene in the IAT

Polybrene is a potent rouleaux-forming reagent that allows the red blood cells to approach each other more closely and thus agglutination of sensitised cells can occur, in which case the use of AHG is not required. If the test is carried through to the AHG step, a monospecific anti-IgG reagent must be used because polybrene causes considerable amounts of the complement components C4 and C3 to coat the cells and this will cause false-positive reactions if a polyspecific AHG reagent containing anti-C3 is used.

Automation in AHG

Solid phase red cell adherence AHG

This technique is used in various commercial systems. Red cell ghosts from lysed red cells are attached to U-shaped microwell plates. The patient's serum/plasma is added to the wells together with a low ionic strength solution. After incubation, the plates are washed either by using an automated plate washer, or manually. Indicator red cells that have been coated with AHG reagents are then added. This will detect whether antibodies reacted with the red cell ghosts on the plate, because the AHG coated cells will detect and adhere to the antibodies. After centrifugation, a positive result shows as a 'carpet' of cells, whereas a negative result shows as a button of cells in the bottom of the well.

Microplates can be supplied where the wells have been 'pre-activated' for this technique so that laboratory personnel can fix red cell ghosts of their choice to the surface of the wells.

Quality control in AHG test

Controls should be performed on all reagents (including reagent red cells) to ensure that they are working correctly. Negative antiglobulin tests should be controlled by adding washed sensitised cells to the test tubes after reading and recording of the initial negative results. The sensitised cells should become agglutinated, indicating that the antiglobulin reagent was added and was able to agglutinate sensitised cells. This confirms that the original negative test results were correct and not the result of AHG neutralisation. Both positive and negative controls are also included with each batch of tests. Failure of these

controls will require all the tests in the batch to be repeated. Tables 14 and 15 illustrate these two sets of controls; to confirm that sensitised cells will become agglutinated using AHG, and that negative results are not due to accidental neutralisation of AHG.

Errors during AHG testing

An anticoagulant such as EDTA should be used to collect blood samples for the DAT in order to avoid *in vitro* complement attachment associated with refrigerated clotted samples.

False positive results in antiglobulin tests

Sample related

- *Refrigerated samples:* False positive results may be the result of adsorption of cold autoantibody onto red cells during refrigeration of the sample, with the result that complement in the sample becomes bound to the cells.
- *Bacterial contamination:* Use of bacterially contaminated cells, or serum/plasma, may give false positive results.
- *Using red cells with a positive DAT:* It may be that red cells used in an IAT test are sensitised prior to testing. For example, the conclusion that is drawn when a crossmatch test is positive is that the recipient has an antibody to an antigen on the red cells of the donor. However, it could be that the red cells from the pilot tube of a unit selected for crossmatch were sensitised prior to testing, causing a positive result.

Table 14 Control cells for AHG test

Control Cells for AHG Tests	
Positive control cells	IgG sensitised O D cells
Negative control cells	Unsensitised O D cells

Table 15 Pattern of expected reaction of AHG control cells

Reagent red cells used for controls	Expected result with AHG reagent
Positive control: Weakly sensitised reagent red cells	2 weakly positive
Negative control: Unsensitised reagent red cells	0

Technique related

- *Over-centrifugation:* An increase in speed and/or time of final centrifugation of tube tests causes excessive packing of unagglutinated cells, which appear agglutinated.
- *Over-zealous reading:* Inexperienced personnel may be over-anxious to find positive results or be afraid to find tests negative and therefore misread tests that are in fact negative, as weakly positive.
- *Cross-contamination from test to test:* If tests are over-filled with saline during washing, tube contents may become cross-contaminated.

Instruments and reagents related

- *Dirty glassware:* Particles of dirt in tubes or saline used for suspending and washing cells could cause red cells to adhere to each other and result in the recording of these aggregates as agglutinates.
- *Colloidal silica:* Colloidal silica leaches out of poor-quality glassware, and if this glassware is used to store saline it may lead to false positive results by causing red cell aggregation.

False negative results in antiglobulin tests

Technique related

- *Failure to add AHG:* After completing antiglobulin tests, to prove that AHG was present and active, washed sensitised cells should be added to each negative test result. AHG reagent may also be coloured for visual confirmation, although this does not test activity.
- *Failure to detect a positive result before washing:* Before washing, tests should be examined for haemolysis or agglutination, and if present, this should be documented, and the test taken no further. If such tests are washed, the few remaining red cells could be unagglutinated at the conclusion of the test, resulting in a false negative result.
- *Inadequate red cell washing:* AHG may be neutralised by traces of serum/plasma remaining after inadequate washing.
- *Loss of red cells during washing:* Insufficient centrifugation time results in red cell loss and a reduction in the number of red cells available to react with AHG. Over-filling tubes during washing may also result in cells being lost as they spill out of the tubes.
- *Incorrect strength of cell suspension:* If the red cell suspension is too strong and the antibody very weak a good latticework will not be formed when the AHG is added and false negative results could be obtained. If the cell suspension is too weak, the test may be misread as too few red cells are present.

- *Over-manipulation:* Excessive centrifugation after the addition of AHG would cause the red cells to pack firmly at the bottom of the tube. Such tests would then need to be over-agitated to resuspend them before reading, in an effort to dislodge the cell button. This could cause weak agglutinates to disperse, resulting in false negatives.
- *Under-centrifugation:* Although tests should be centrifuged gently after the addition of AHG, weak agglutination may pass unnoticed if cells are not sufficiently centrifuged to enhance the agglutination.

Reagents related

- *Neutralisation of AHG:* Extraneous globulin from a variety of sources could neutralise AHG. For example, traces of protein on inadequately cleaned tubes, or contamination of the AHG vial by using the lid of another reagent vial to close it, may neutralise AHG.
- *Lack of broad-spectrum reactivity:* It is important in some laboratory tests, for AHG to be broad spectrum (anti-IgG and anti-C3) so that it is able to detect the presence of IgG and/or C3 on the red cell surface.
- *Prolonged or inadequate storage of AHG:* Storage of AHG at the incorrect temperature will lead to its deterioration. Any reagent stored for longer than its shelf life, should no longer be used. Such reagents may still appear to react well with strong antigen-antibody associations but may not react when the serum/plasma contains weak antibodies or when the cells have a weak expression of antigen.
- *Bacterial contamination:* Use of bacterially contaminated reagents may give false positive results.

Dissociation of antibody

Antibody may become dissociated leading to false negative results, for several reasons including:

- Prolonged period of incubation of serum/plasma and red cells.
- Delays between additions of saline to tests while washing.
- Prolonged centrifugation of tests while washing.
- Delays between mandatory washing steps, such as filling tubes with saline and leaving them to stand.
- Delays after the addition of AHG reagent and the centrifugation of tests.
- Delays after centrifugation of tests and the reading of results.

Erythro-magnetic technology (EMT)

This technology is based on the magnetisation of RBCs. Magnetic particles are adsorbed onto the surface of RBCs.

The RBCs are incubated together with the serum/plasma being tested in a pre-prepared microtitre plate. The microplates are coated with anti-IgG antibody and also contain a high-density solution that prevents contact between patient's plasma and the surface of the microtitre plate. Following the incubation, a magnetic force is applied at the bottom of the microplate using a magnetic plate. This causes the RBCs to be pulled toward the bottom of the microplate. In this technology, the magnetic force replaces the centrifugation step. On re-suspension of the red cells the reactions may be deciphered.

Under the influence of the magnet, the red cells migrate through the high density solution and, if sensitised with IgG, react with the anti-IgG coating the bottom of the wells. A positive reaction appears as a cellular layer or 'carpet' coating the surface of the well, while a negative reaction is seen as a dot of red cells at the very bottom of the well. In contrast with solid phase RBC adherence tests, EMT removes the need for washing since unbound antibodies stay in the upper layer of the well, above the high density solution, and do not interact with anti-IgG fixed to the microwell.

Microcolumn techniques

Cards of microcolumns containing a matrix of gel or glass microbeads are available commercially for microcolumn techniques. Dedicated equipment is required, and it is important that laboratory personnel are appropriately trained, and that the package inserts/manufacturer's instructions are followed.

The systems can be manual, semi-automated or automated. The gel technology uses cards of microcolumns preloaded with a neutral gel, or a gel containing AHG or a gel containing specific reagent antibodies. For example, when testing for red cells for the D antigen, the gel contains reagent anti-D and a controlled volume of the red cells to be tested is added to the microcolumn/microtube. After incubation and centrifugation, agglutinated red cells are trapped on the upper surface of the matrix or within the column. Unagglutinated red cells pass through the gel and form a button at the base.

If AHG reagent is incorporated into the gel, sensitised RBCs come in contact with AHG in the upper part of the gel and are trapped, while unsensitised cells will migrate to the bottom of the column. The gel test provides an advantage over the conventional tube technique as washing of red cells is not required. Cell mixtures are readily observable. The cards can be retained as records, for quality control purposes.

Red cell antibody screening and antibody identification

Naturally occurring anti-A and anti-B are the most commonly found antibodies in human blood. Any other antibody found in the serum or plasma is an unexpected, irregular antibody which may be clinically significant i.e. capable of causing harm. Unexpected antibodies can be alloantibodies or autoantibodies. The major purpose of antibody screening is to detect clinically significant, unexpected antibodies, which are reactive at 37°C or in the AHG test and with characteristics that are known to have caused HDFN, transfusion reactions or the shortened survival of transfused RBCs. Antibody identification tests should be carried out by suitably trained technologists as a follow-up procedure to identify antibodies detected during antibody screening.

Indications for red cell antibody screening

- As a part of pre-transfusion testing.
- Antenatal antibody screening.
- Screening of the blood donor.
- As a part of transfusion reaction investigation.
- To investigate a case of HDFN.

Sample requirement

Serum is preferred over plasma for testing as plasma is not suitable for detecting complement-activating antibodies.

Medical history

It is important to know the patient's clinical diagnosis, history of transfusions or pregnancies, and recent drug therapy as this information will help in interpretation of the results.

Reagent red cells used for the testing

Screening cells are available commercially or may be prepared locally in the laboratory. They should include the following characteristics:

- Reagent red cells for antibody screening must be Group O. Sets of two or three single donor cells are commercially available for antibody screening and pooled cells may be used for antibody screening of blood donor samples. Table 16 shows typical red cell reagent antigens on a two-cell set of Group O antibody screening cells.

Table 16 Typical antigens on a two-cell set of reagent Group O antibody screening cells

Screening cells	Rh phenotype	K	k	Kp ^b	M	N	S	s	Jk ^a	Jk ^b	P ₁	Le ^a	Le ^b	Fy ^a	Fy ^b	Antigens probably
																homozygous
Screening cells no. 1	DcE/DcE (R ₂ R ₂)							0		0		0			0	D, c, E, S, Jk ^a , Fy ^a
Screening cells no. 2	DCe/DCe (R ₁ R ₁)						0		0				0	0		D, C, e, s, Jk ^b , Fy ^b

- Reagent red cells are selected to express antigens associated with commonly encountered antibodies.
- Panel cells used to identify antibodies must (as a panel) together express the following antigens: D, C, E, c, e, M, N, S, s, P₁, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b.
- One of the panel cells must have homozygous expression of Rh, Duffy, MNS, and Kidd system antigens, so that antibodies known to show dosage effect are not missed.
- Antibody identification panels are selected so that, taking all the results into account, a distinctive pattern of positive and negative reactions is shown for a variety of irregular antibodies. Table 17 provides an example of a decode sheet showing the presence and absence of antigens on a panel of Group O cells used for antibody identification.

Method

- Tube, CAT or solid phase assays can be used for antibody screening and identification.
- Testing may need to be done in three batches, at three different temperature phases, i.e. $4 \pm 2^\circ\text{C}$, $22 \pm 2^\circ\text{C}$ and $37 \pm 1^\circ\text{C}$ for the AHG phase (IAT) along with an auto control.
- Both positive and negative reactions are important in antibody identification. The phase and strength of reaction shall be noted on the antigram (the decode sheet) of screening and identification cell panels.

Interpretation of results

- Exclusion of antibody specificities that do not match the pattern of reactions observed, is commonly used for interpretation of results.
- The antigen pattern of non-reactive cells on the panel indicate that the corresponding antibody can be excluded (especially if the antigen on the panel cell is present in homozygous state).
- Panel cells which react with the serum under test, are evaluated. If there is a pattern that matches exactly, that is most likely the specificity of the antibody in the serum.
- If there are remaining specificities that have not been excluded, additional testing may be needed to eliminate remaining possibilities and to confirm the specificity identified.
- Phenotyping of red cells of the patient, to confirm that the corresponding antigen is absent, is done to confirm the finding. However, this is not possible if the patient has recently had a blood transfusion.
- Each panel cell batch/lot or set should have a unique reference number, and each panel set should be used as one complete set. It is critical when interpreting antibody identification results, that the reference number of the panel cells and the decode sheet are correlated with the actual set of panel cells used for the tests. This ensures that the correct decode sheet is used for the interpretation of the tests and that the previous decode sheet or a new batch's decode sheet

Table 17 Example of panel decode sheet showing typical panel of Group O cells for antibody identification

Panel cells	Rh					Kell			Duffy		Kidd		MNS				Lewis		P P ₁
	D	C	E	c	e	K	k	Kp ^a	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	Le ^a	Le ^b	
1 R ₁ R ₁			0	0				0	0			0			0		0	0	
2 R ₁ R ₁			0	0				0							0			0	
3 R ₂ R ₂		0			0	0		0	0		0		0		0			0	
4 R ₀ r		0	0			0		0	0		0		0		0			0	0
5 r'r	0		0					0		0		0	0		0			0	0
6 r''r	0	0				0		0			0	0	0		0			0	
7 rr	0	0	0					0	0		0			0				0	
8 rr	0	0	0			0		0		0								0	0
9 rr	0	0	0			0					0			0		0		0	
10 R ₁ R ₁			0	0		0		0			0							0	0

Table 18 Examples of reaction patterns in antibody identification

Panel cells		Sample 1			Sample 2			Sample 3		
Technique		Saline	Enzyme	IAT	Saline	Enzyme	IAT	Saline	Enzyme	IAT
1	R ₁ R ₁	0	3	3	2	0	0	0	3	3
2	R ₁ R ₁	0	3	3	2	0	0	0	3	3
3	R ₂ R ₂	0	3	3	0	4	2	0	3	0
4	R ₀ r	0	3	3	2	2	2	0	3	0
5	r'r	0	0	0	0	2	2	0	3	3
6	r''r	0	0	0	0	4	2	0	3	0
7	rr	0	0	0	2	2	2	0	3	3
8	rr	0	0	0	2	2	2	0	3	0
9	rr	0	0	0	2	2	2	0	3	0
10	R ₁ R ₁	0	3	3	2	0	0	0	3	0
Auto control		0	0	0	0	0	0	3	0	

is not used in error. Using the incorrect decode sheet can result in incorrect antibody identification.

Table 18 shows the results obtained when irregular antibodies in three different sera were tested, using saline, enzyme and antiglobulin techniques, with the panel cell types shown in Table 17. Table 18 shows the reaction patterns that would be expected when identifying a single specificity antibody in a sample, followed by two examples of antibody mixtures. It is important to consider, when identifying irregular antibodies, that a negative result may not be a true negative when the panel cells have a single rather than a double dose of antigen and the antibody being identified is weak. The panel cell decode (Table 17) was used to determine the specificities of these antibodies and the outcomes are noted in Table 19.

Should haemolysis be detected, either during antibody screening or identification, this is an important observation that must be noted when recording the results. Clinically significant antibodies that cause lysis are usually more potent and capable of more harm than non-haemolyzing antibodies.

Table 19 Interpretation of antibody identification results shown in Table 18

Antibody sample number	Interpretation of results obtained
Sample 1	Anti-D reacting by enzyme and IAT methods only, therefore IgG
Sample 2	Anti-M reacting by saline, anti-E by enzyme, anti-c by enzyme and IAT
Sample 3	Cold antibody evident using enzyme method; anti-K by IAT

To confirm the specificity of an antibody, the red cells of the sample being tested may be phenotyped for the relevant antigen, as the cells should be negative for the antigen that would react with the antibody detected. For example, if the antibody detected was anti-K then the red cells from the sample should type K negative.

Antibody titration and quantification

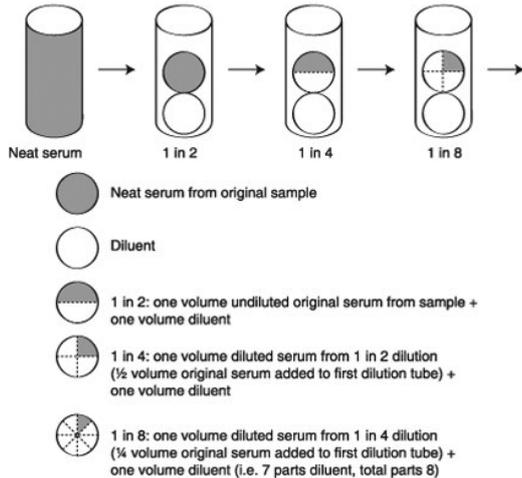
Antibody titrations may be carried out to determine the titre (strength) of the antibody present in the serum/plasma. A titre may be defined as the reciprocal (inverse) of the highest dilution at which an antibody can react observably *in vitro* (for example, if the dilution at which an observable reaction is last visible is 1 in 32, then the titre is 32). Titrations may be performed manually by making doubling dilutions as shown in Fig. 4, or by semi- or fully automated techniques.

Antibody titration

The conventional test tube titration is performed by labelling tubes according to the sample dilution. The first tube will contain a volume of undiluted sample and the second and subsequent tubes will contain an equal volume of saline. An equal volume of sample is added to the second tube, which is mixed, and one volume of the mixture is transferred to the next tube. The same process is continued for all dilutions with a clean pipette tip to mix and transfer each dilution. After the dilutions, two drops of the 5% red cell suspension possessing the antigen are added to the tubes. Some laboratories add an enhancement medium, such as low ionic saline solution, and some do not. The tubes are incubated for times and temperatures appropriate for the antibody. For

Explanation

- Neat (undiluted) serum is in the first tube (depicted as grey in colour)
- A fixed volume of diluent is added to each of the following dilution tubes
- An equal volume of serum is added to the diluent in the first dilution tube (1 in 2)
- The contents are mixed thoroughly (at each dilution step)
- One volume is then transferred to the next dilution tube

**Technique**

- First step: Making dilutions along the row from left to right
- Second step: Transferring dilutions from greatest dilution on right, to lowest on left

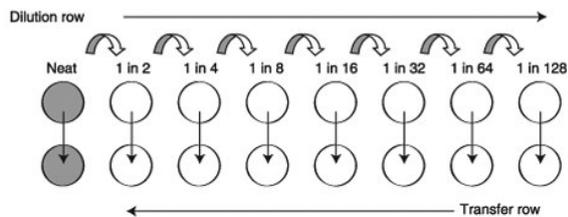


Fig. 4 Explanation of doubling dilution method for performing a manual titration.

instance, for titration of IgM antibodies (e.g. ABO antibodies) tubes are incubated at room temperature while for IgG antibodies (e.g. anti-D), tubes are incubated at 37°C. The tubes may simply be centrifuged and read for reactivity (for saline reacting IgM antibodies) or washed and AHG added before centrifugation and interpretation of results (for clinically significant IgG antibodies) (see Table 20).

Indications for antibody titration

Antibody titration is clinically indicated in the following cases:

- Determining the strength of clinically significant antibodies in antenatal cases.
- Resolution of high titre low avidity antibodies.
- Distinguishing between the strengths of multiple antibodies in a single sample.

Table 20 Summary of procedural steps for titration

Procedural steps	IgM antibodies e.g. anti-A/anti-B	IgG antibodies e.g. anti-D, anti-K
Enhancement media	None	None or LISS
Red cell suspension	2–5%	2–5%
Incubation	Room Temperature × 30 min	37°C × 60 min
Wash	Not required	At least 3 times with saline
Testing phase	Saline phase	AHG phase
End point	Visible macroscopic agglutination	Visible macroscopic agglutination

- Performing ABO isohaemagglutinin titrations on apheresis platelet units.
- Performing ABO isohaemagglutinin titrations on recipients due to receive organs that are to be transplanted across the ABO barrier (i.e. where the organ to be transplanted is ABO group incompatible with the recipient).

When a clinically significant antibody capable of causing HDFN is detected in maternal serum/plasma, it is important to determine the antibody titre, when the antigen is present on the paternal red cells. Throughout pregnancy, the mother's antibody titre is repeated to determine if there is a rise in titre or concentration of the antibody. A rise in antibody titre by two dilutions is considered clinically significant. Once the titre reaches a critical threshold and the fetus is at least 18 weeks gestational age, additional investigations are performed to monitor the level of anaemia in the fetus and decide the necessity of intrauterine transfusion or other interventions. When monitoring the antibody levels of a patient there is often insufficient serum/plasma to perform comparative titrations over a period of time. There can be a considerable variation in titration results even when the titrations are performed using the same methodology. Therefore, only a two-dilution tube difference or greater between one sample and the next in a particular patient should be considered significant. Titres may not correlate well with the severity of HDFN. It is recommended that the same technologist perform the titrations on a particular patient (to limit variability) and that the starting point titre is used on each successive testing, to determine whether or not the titre has increased.

Some institutions determine the titres of anti-A and anti-B antibodies, also known as isohaemagglutinin titres, on Group O apheresis donor platelets before issuing to non-Group O recipients. Apheresis platelets from Group O donors contain large plasma volumes and may contain unusually high titres of anti-A or anti-B antibodies,

which have the potential to induce a haemolytic transfusion reaction in a non-Group O recipient.

With better understanding of immunological mechanisms and various regimens for effectively controlling it, ABO-incompatible renal & liver transplantation is now being done with increasing frequency. Performing ABO titres in potential recipients is critical for determining both the effectiveness of pre-treatment regimens and when titres are low enough to permit transplant.

Antibody quantification

Antibody quantification using an automated technique is a more reliable and reproducible way of estimating the amount of anti-D or anti-c antibodies present in a sample, than an antibody titration. Studies have shown that the quantification results correlate better than a titration with the severity of HDFN. For specificities other than anti-D (as well as anti-D together with anti-C or anti-E antibodies) and anti-c, antibody titrations should be used as reliable antibody standards are not usually available for quantification.

A single channel auto analyser is used for the quantification. As with all procedures, this should be carefully standardised and be proved to be sensitive and reliable. Automated dilutions of serum/plasma are prepared. The reagent red cells are treated with an enzyme such as bromelain and a red cell aggregating agent (methyl cellulose) is used to increase the reaction speed. The endpoint is read in the spectrophotometer. For anti-D, the test results are compared with a known standard (in µg/ml; of IgG) and the results are reported in International Units (IU)/ml based on the value of the standard, e.g. 1 µg anti-D may equal 5 IU/ml. Values below 5 IU/ml anti-D indicate that HDFN is unlikely, whereas values greater than 15 IU/ml indicate a high risk of HDFN. As with titrations, the trend is important and an increase of 50% or more in IU/ml in a subsequent sample is considered significant.

Antibody neutralisation/inhibition

It may be helpful in the identification of several antibodies suspected to be present in a single sample of serum/plasma, to disable one of the antibody specificities, making identification of other antibodies easier.

Neutralisation/inhibition techniques may be used to identify antibodies or to determine ABO secretor status by the detection of soluble antigens found in body fluids like saliva. Inhibition of blood group antibodies by soluble substances can aid in the identification of specific antibodies. Antibody activity of known specificity can be selectively “removed” by using the inhibition method, thus leaving behind other antibodies to be identified.

Other indications for inhibition are to determine ABH secretor status and immunoglobulin class of anti-A and/or anti-B.

Some antibodies can be inhibited by soluble substances such as sugars, proteins, and peptides; examples include ABH, Lewis, P₁, Sd^a, and Chido/Rodgers. Human saliva, hydatid cyst fluid, pigeon egg white, human or guinea pig urine, human serum, and human milk have been used as soluble substances to inhibit RBC antibodies. Table 21. lists some of the antibodies that can be neutralised and the source of corresponding neutralising substance.

The procedure starts with labelling two test tubes: one for the sample containing antibody (e.g. anti-P₁) and one for the dilution control. Combine test sample and soluble substance (e.g. pigeon egg white) into the tube labelled “sample.” To the tube labelled “dilution control,” combine test sample and inert substance such as saline. Incubate both tubes for a specific time and temperature determined by the known “ideal” for the target specificity (e.g. 22 ± 2°C for 30 min). After incubation, test the samples with previously reactive RBCs selected by phenotype (i.e. P₁). Inhibition has occurred when the “sample” is non-reactive and the “dilution control” is still reactive. These results confirm that inhibition of anti-P₁ antibody took place and the lack of reactivity was not caused by dilution.

Use of controls

The dilution control containing the sample plus inert substance should result in a positive reaction when tested against an RBC positive for the corresponding antigen to the antibody under investigation. The lack of reactivity in the dilution control indicates dilution of weakly reactive low-titre antibody and invalidates the test.

Elution

Elution is the process of removing antibodies (usually IgG) from the surface of red blood cells. An elution may be indicated to aid in the:

Table 21 Sources of substances for neutralisation of certain antibodies

Antibody	Source of neutralising substance
Anti-ABO	Human saliva (secretor)
Anti-H	Human saliva (secretor)
Anti-P ₁	Hydatid cyst fluid, pigeon egg whites or droppings
Anti-Lewis	Human plasma or serum, saliva
Anti-Chido & anti-Rodgers	Human serum containing complement
Anti-Sd ^a	Urine (guinea pig or human)
Anti-I	Human breast milk

- Diagnosis of autoimmune haemolytic anaemia (AIHA).
- Diagnosis of ABO haemolytic disease of the fetus and newborn (HDFN).
- Identification of specificity when multiple antibodies exist in a patient's serum or plasma.
- Phenotyping of red cells in patients with a positive DAT.

An eluate is a fluid medium containing the antibodies that have been deliberately removed from RBCs, allowing for further investigations such as antibody identification or titration. Prior to performing an elution, a DAT should be performed on the patient's red blood cells. If the DAT is negative or positive with anti-complement only, the likelihood of retrieving an antibody from the red cells is unlikely. When the DAT is positive with IgG antibody or IgG antibody and complement, an elution may provide meaningful results.

Types of elution:

- Total Elution: A total elution is one in which the red blood cells are destroyed. The cells cannot then be used for phenotyping or autoadsorption.
- Methods for total elution include: 56°C heat, Lui freeze and Acid.
- A partial elution removes the antibody while leaving the red blood cell membrane intact. The treated RBCs may then be used for phenotyping or autoadsorption.

Mention is made of methods that are available for partial elution, which include: Gentle heat at 45°C, ZZAP and Chloroquine.

Methods of elution

Elution removes antibody molecules from the red cell membrane either by disrupting the antigen or changing conditions that lead to dissociation of antibody from antigen. Many techniques are available, and no single method is best in all situations. If an eluate prepared by one

technique is unsatisfactory, it may be helpful to prepare another eluate utilising a different technique. Eluates may be prepared from red cells using various methods as shown in Table 22.

Heat elution method

The eluting medium is the medium into which the sensitising antibody may be dissociated from the cells. For all antibodies other than ABO and Lewis, a suitable eluting medium is antibody-free group AB serum. In the case of ABO and Lewis antibodies, bovine albumin or saline should be used instead, so that antibodies in the eluate do not become neutralised by soluble ABH or Lewis antigens that may be present in group AB serum. This is an important point to remember, as heat elution could be considered as an aid in the diagnosis of ABO HDFN.

- Wash the sensitised red cells six times in cold normal saline.
- Place 1 volume of washed cells in a test tube with 3 volumes of eluting solution (e.g. 2 ml of red cells and 6 ml of eluting solution).
- Mix well and incubate for five to 15 min at $56 \pm 2^\circ\text{C}$. Mix frequently during incubation.
- Centrifuge the test tube in a warmed centrifuge bucket at approximately 1000 g for 2 min.
- Transfer the supernatant eluate to a clean test tube.

The eluate may be tested to determine the specificity of antibodies that caused the sensitisation, and indirectly the identity of the antigens targeted. However, red cells subjected to heat elution are denatured and are therefore no longer suitable for further testing.

Glycine-HCl/EDTA elution method

- Wash the sensitised red cells six times in cold normal saline.
- Place 1 volume of washed cells in a test tube with 2 volumes of glycine-HCl /EDTA eluting solution (e.g. 2 ml of red cells and 4 ml of eluting solution).
- Mix well and incubate for one to 2 min at $22 \pm 2^\circ\text{C}$.
- Add 1 volume of TRIS-NaCl (e.g. 2 ml) and mix well.

Table 22 Methods of elution

Method	Mechanism of action	Advantages	Disadvantages
Heat 56°C	Heat causes thermal dissociation of Ag/Ab complexes and denatures the red cell membrane	Simple to perform and ideal for diagnosis of ABO HDFN	Poor recovery of other blood group allo- and autoantibodies
Lui freeze-thaw	Rapid extremes in temperature cause the dissociation by a structural configuration change to the red cell membrane	Simple to perform and ideal for diagnosis of ABO HDFN. Only a small volume of RBC is needed.	Poor recovery of other blood group allo- and autoantibodies
Cold acid	Acid alters the charge of proteins causing a change in structural configuration	Simple to perform	Less sensitive for warm allo- and autoantibodies
Glycine-HCl and EDTA	Acid alters the charge of proteins causing a change in structural configuration	Good for preparing an eluate with intact red cells	Denatures antigens of the Kell system

- Centrifuge the test tube at approximately 1000 g for 2 min.

Transfer the supernatant eluate to a clean test tube. The eluate may be tested to determine the specificity of antibodies that caused the sensitisation, and indirectly the identity of the antigens targeted.

Several technical factors affect the success of the elution procedure:

- The greater the volume of the suspending medium in proportion to the volume of packed red cells, the greater the dilution of eluted antibodies, so the volume of the suspending medium used should be similar to that of packed red cells so there is some standardisation. If a commercial kit is used, however, then the manufacturer's instructions regarding volumes must be followed, otherwise the proportions may be incorrect.
- Before starting an elution, RBCs with a positive DAT must be thoroughly washed to free them of unbound IgG, and the last wash must prove to be negative for antibody reactivity.
- Washed RBCs must be placed in a clean test tube prior to elution to avoid contamination of the eluate with antibody bound to the test tube wall during the previous phase.
- A unreactive eluate may be due to antibodies already having dissociated during washing. This is particularly possible if the DAT is due to anti-A or anti-M and can be avoided by washing the cells with cold saline.
- If organic solvents are used for elution or if the tonicity or pH of the eluate is not corrected prior to testing, the eluate can cause haemolysis or nonspecific clumping of the reagent RBCs.
- Because eluates are not stable, they should be tested immediately after preparation. If that is not possible, the eluate may be frozen in 6% weight/volume bovine albumin.
- As soon as the elution is completed, the supernatant fluid must be removed and placed into a clean tube to avoid reattachment of antibody to cell stroma which could lead to a false negative eluate result.

Interpretation of elution test results

Once the eluate is prepared, it should be tested with reagent red cell panels to identify the specificity of antibody. Table 23 explains interpretation of eluate results.

Adsorption

Adsorption is a method designed to remove antibody from serum/plasma by incubating it with red cells

Table 23 Interpretation of eluate results

Eluate results	Explanation
Eluate reacts with all cells in the panel	- Alloantibody or Autoantibody
Eluate reacts with a few cells in the panel	- Alloantibody. Use the panel decode sheet to identify the antibody.
Eluate does not react with any panel cells	- Low concentration of eluted antibody - Drug dependent autoantibody - Anti-A or Anti-B antibody - Antibody dissociated prior to elution - Long delay between elution and testing
Haemolysis of RBCs occurs after incubation with eluate	- Improper method of elution (e.g. wrong pH)

positive for the corresponding antigen. The temperature of incubation depends on the optimum temperature of reactivity of the antibody, i.e. 4°C for cold antibodies and 37°C for warm antibodies. If RBCs show agglutination during the incubation period, it may be assumed that antigen sites have been saturated, but excess antibody may still be free in the serum/plasma. Therefore, the serum/plasma is harvested and placed on a fresh aliquot of cells to continue the absorption. RBCs are often pre-treated using ZZAP (DTT papain) to enhance reactivity. The absorbed plasma is tested to detect any remaining antibodies, which had previously been masked by the antibody that was absorbed out of the serum/plasma, i.e. adsorbed onto the RBCs.

Applications

- Removing autoantibody activity to permit detection of coexisting alloantibodies.
- Reagent preparation.
- Separating multiple antibodies to aid in identification.
- Confirmation of the presence of a weak antigen on red cells.
- Confirmation of antibody specificity.

Types of adsorption

Autoadsorption

Autoadsorption is performed by using the patient's own RBCs to remove autoantibodies. It is used most often in the case of warm autoantibodies, to remove autoantibodies in order that underlying ("masked") alloantibodies may be identified.

Alloadsorption

Alloadsorption (sometimes called "differential adsorption") is a technique designed to remove certain

antibodies from the serum/ plasma of the patient under test, when it is suspected that the serum/plasma contains antibodies of multiple specificities.

Adsorption and elution combined

Adsorption/elution techniques may be useful in separating suspected mixtures of antibodies in a patient's serum/plasma. For example, if a sample is suspected to contain anti-e and anti-Fy^a, the serum/plasma can be adsorbed with e, Fy(a-) cells. The serum/plasma should contain only anti-Fy^a post adsorption. The eluate prepared from the cells used for the adsorption should contain only anti-e.

Adsorption/elution studies are useful for detecting weak red cell antigen variants. For example, an apparent Group O sample with missing anti-A isoagglutinins may be a very weak A type such as the rare A_{el} if anti-A can be adsorbed onto, and eluted from, the cells under test.

Causes of false results in serological tests

False positive or false negative results may occur in serological tests and lead to misinterpretation of results, which could harm the patient. Most often this occurs as a result of poor technique or lack of knowledge and can be avoided if technologists are suitably trained to recognise and take corrective action to obtain a valid result. In order to maintain suitable standards of quality, the technologist should follow all testing procedures carefully and be aware of any unusual results. The best approach is to view every result objectively, and each anomalous (unexpected or unusual) result with suspicion. Think through the steps that were taken to reach the results:

- Was each step of the procedure carried out according to quality standards?
- Were all tests controlled in the correct manner?
- Was any reagent changed or modified? ... and so on.

Only with an enquiring and open mind and observing every test result without presuming the result, can the technologist reach the standard of excellence that the laboratory is expected to achieve – that of zero errors.

Most serological tests in blood banking laboratories are performed on venous blood samples, taken either into plain test tubes or into test tubes containing suitable anticoagulant. After blood samples have been drawn, their temperature changes from 37°C to ambient (environmental/room) temperature. In hot climates, there is a danger of samples warming to temperatures above 37°C and in cold climates, samples may be in danger of freezing.

In order to preserve blood samples so that they retain their properties and give the correct results on testing,

they should be cooled to about 4°C in the laboratory refrigerator as soon as possible after being taken, and kept refrigerated both before and after testing, with a total storage time of about 2 weeks for reference purposes. The actual testing of the sample should take place within 3 days of collection for serological testing, to avoid the danger of antigens or antibodies deteriorating, or the contents becoming bacterially contaminated. Samples for viral marker testing may have more stringent requirements; kit/equipment manufacturer's instructions must be followed.

Samples need to be removed from the refrigerator for testing. Without air conditioning, room temperature will depend on whether the laboratory is situated in a hot or a cold environment. It is important that the technologist takes steps to avoid sample deterioration when ambient temperatures are extreme.

Many of the causes of false results have been addressed in preceding text. However, it is important that students are able to perceive false results as a separate entity. The causes of false results are therefore listed together and kept brief where appropriate to avoid unnecessary repetition of what has already been explained.

Causes of false positive results

Cold autoantibodies

Cold antibodies are sometimes demonstrable in samples that have been refrigerated. Although these antibodies are usually not of clinical significance, they may interfere in laboratory tests. For this reason, some laboratories only test at 37°C for antibody screening and identification.

Cold IgM autoantibodies in anticoagulated samples may be observed as clumps of agglutinated cells if the sample is tilted after removal from the refrigerator. These clumps disperse once the sample is warmed. Cold IgG autoantibodies sensitise red cells in the cold and are often demonstrable at 22 ± 2°C if an enzyme technique (such as bromelain) is used. Cold autoantibodies may also cause complement to become cell bound, causing agglutination when using an AHG reagent containing anticomplement antibodies.

Although cold antibodies often dissociate from cells on warming to 22 ± 2°C, some do not, even when warmed to 37°C; and cell bound complement remains cell bound, complicating testing. Red cells from such samples will be DAT positive using a broad spectrum AHG reagent. Washing such cells in warm saline may be helpful in resolving the problem. When using reagents, the tests should be carried out at the temperature recommended by the supplier, e.g. room temperature tests should not be warmed to resolve a grouping problem; the red cell

suspension should rather be washed and retested and the antibody in the serum/plasma should be identified.

Warm autoantibodies

These autoantibodies have an optimum reaction temperature of 37°C and are often clinically significant and cause diseases such as autoimmune haemolytic anaemia. Warm autoantibodies that react with most cells may mask other irregular antibodies and prevent their detection.

Cells that have been sensitised with warm IgG autoantibodies will be difficult to type by IAT as the DAT will be positive. An advantage of monoclonal reagents is that they can be used to type sensitised cells, but the tests must be appropriately controlled with a neutral control/typing control.

Bacterial contamination

Bacterial contamination may occur during collection of blood samples as a result of poor technique or with repeated use of the sample. This becomes more evident as the sample ages and the bacteria in the sample have time to multiply. Reliable results cannot then be obtained.

Bacterial erosion of red cell membranes results in antigens that are normally found beneath the membrane, becoming exposed. This is known as T-activation. It makes cells polyagglutinable, which means that they become agglutinated with all fresh serum/plasma.

Serum/plasma which has become bacterially contaminated may cause pan agglutination of all red cells, and this can be seen microscopically as aggregates of red cells, as opposed to the appearance of typical agglutination.

Discoloration of blood samples, foul smell and cloudiness of serum/plasma are indications that a sample is bacterially contaminated.

Chemical contamination

If glassware is not properly washed, traces of the chemicals used in the washing procedure may influence test results. Detergents should therefore be carefully selected and thoroughly rinsed from the glassware before drying.

Rouleaux

When red cells appear under the microscope as a stack or pile of coins, this is called rouleaux formation. Most cells are stuck by their flat surfaces, whereas agglutinated cells are stuck together in a disorderly manner. Agglutinated cells shine when examined under the microscope, but rouleaux have a more translucent appearance.

Rouleaux may be observed when the plasma protein concentration is high, such as in the plasma of patients who have been given volume expanders such as hydroxethyl starch or gelatine, or in patients suffering from

infections, inflammatory diseases, connective tissue disorders and cancers.

Rouleaux may also occur when cell suspensions are left to dry on laboratory slides. Rouleaux formation may not always be that simple to recognise microscopically, as clumps may become irregular in shape and be difficult to distinguish from agglutination. To distinguish it from agglutination, strong rouleaux may be dispersed by dilution with sterile saline, at the stage of reading the test. Over-manipulation of rouleaux may lead to false negative results as agglutination present in the same test tube may also disintegrate. Figure 5 illustrates the appearance of rouleaux when viewed microscopically.

Wharton's jelly

Wharton's jelly is the stringy, jelly-like material lying between the blood vessels in the umbilical cord. It is sometimes an accidental contaminant of cord blood samples. Washing the cord cells with saline sometimes frees the red cells of this jelly. However, badly contaminated samples will be impossible to use.

Particles

Serum/plasma may contain particles of dust or other debris or may develop particles of fibrin or denatured protein after prolonged storage. Red cells tend to stick to such particles, and this can resemble agglutination leading to false positive results. This may be resolved by centrifugation or filtration of the serum/plasma to either settle or remove the particles prior to testing.

Clots in the cell suspension

Samples of clotted blood often contain small fragments of clots broken off when the red cell suspension is prepared, and these small clots may be mistaken for agglutinates in tests. Clots should be allowed to settle in the suspension tube and cells then drawn from the clot-free upper layer for testing.

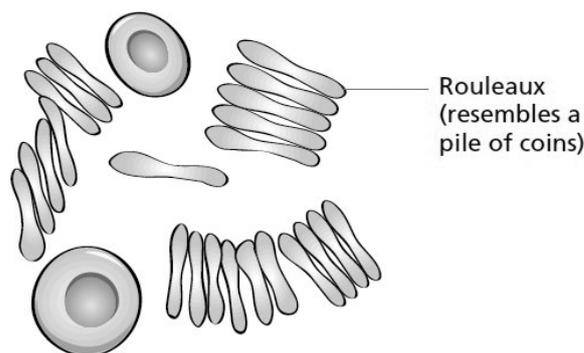


Fig. 5 Microscopic view of rouleaux formation.

Ionic strength

Incubation of red cells and serum/plasma in a low ionic strength saline medium (i.e. LISS, which has a low concentration of dissolved salts), increases the amount of antibody that becomes bound to cells with the corresponding antigen, and increases the speed with which the antibody binds to the cells. However, to avoid false results, it is important to ensure that the correct method is used for the LISS technique.

Causes of false negative results

Age of samples

Red cell antigens may deteriorate with storage so samples should always be tested while still fresh. When red cells are suspended in saline, these suspensions should only be used for one batch of tests. Red cells suspended in preserving fluid will last for many weeks at $4 \pm 2^\circ\text{C}$. Red cells suspended in LISS are usually suitable for up to 24 h, but usage time must comply with manufacturer's instructions.

In time, serum loses complement activity. This may cause false negative results when tests are performed either to assess the haemolysing potential of antibodies, or the ability of broad spectrum antiglobulin reagent to detect complement involvement in antigen-antibody reactions. When serum samples are more than 24 h old, fresh complement from an external source should be added before performing these types of tests.

Use of plasma instead of serum

Ethylene-diamine-tetra-acetic acid binds calcium ions so that clotting cannot occur. Calcium is required for the complement cascade, so when calcium is bound by EDTA the complement cascade is inhibited and some complement-binding antibodies will not be detected.

Prozoning

On rare occasions, serum/plasma containing very high titre antibodies will be unable to form a normal lattice-work of agglutination with red cells containing the corresponding antigen. This is because the ratio of antibody to antigen is not optimal; antibodies are present in excess of the number of available antigen sites and this leads to weak or even negative results. The antibody-antigen ratio

may be corrected by diluting the serum/plasma. Once the ratio is corrected reliable results will be obtained (Table 24).

Storage temperature

Reagent red cells should not be left at ambient temperature for an extended period of time. Commercial reagents should be stored according to the manufacturer's instructions. They should be refrigerated as soon as possible after use to avoid deterioration. The higher the ambient temperature, the quicker the reagents will deteriorate.

Samples should also not be left at ambient temperature or incubated with tests at 37°C unless appropriate.

Storage time

Antigen reactivity is well preserved for years in frozen cells maintained at -30°C or below. This also applies to antibody activity in serum/plasma. Sample volumes for frozen storage should be kept to a minimum so that thawing and refreezing, with resultant loss in reactivity, is avoided.

pH

The normal pH of blood is 7.3–7.4. This is close to neutral (pH 7.0) and in most cases blood grouping should be performed at values near this. At pH values above 8.0 or below 6.0, however, there is a loss of reactivity.

Treatment with enzymes

Proteolytic enzymes modify some blood group antigens so that the cells are no longer able to react with their corresponding anti-bodies, giving rise to false negative results. Examples of blood group antigens that are affected include M, N, S, Fy^a , Fy^b and K.

False results in column agglutination tests (CAT)

There are several reasons why false results could occur in CAT, including the following:

- Expired cards.
- Incorrectly stored cards.
- Cards that have dried out.
- Using the incorrect diluent for a specific card.
- Sample contamination or switching samples would be the most likely cause of false results in manual

Table 24 Doubling dilution titration of an antibody with and without prozone

Antibody dilution factor	Neat	2	4	8	16	32	64	128	256	512	1024
Prozoning antibody	0	0	2	3	4	4	3	2	1	0	0
Non-prozoning antibody	4	4	4	3	3	3	2	2	1	0	0

gel tests. These may be prevented if the correct technique is practiced and concentration on the work being done, is maintained.

Provided that the correct cells or serum/plasma are added to the correct tubes, false results should not occur.

Techniques in the TTI (Transfusion Transmissible Infections) Laboratory

Screening of donated blood for TTIs is one of the most important strategies for blood safety. The microbial agents of importance to blood services are those that are transmissible by blood transfusion and can cause morbidity and mortality in recipients. The major TTIs are listed below:

- Human immunodeficiency virus (HIV).
- Hepatitis B virus (HBV).
- Hepatitis C virus (HCV).
- Syphilis.

See *Section 10: Donation testing and transmissible infections* for more details about pathogens that may be transmitted by transfusion.

Rapid diagnostic tests for TTI

Developed in the 1980s, rapid assays have proved to be almost as sensitive and specific as ELISA and take <30 min to do. However, despite their simplicity, technical errors are more common because tests are done manually, and sometimes by inadequately trained staff. Rapid tests may be best suited to developing countries, as they are relatively easy to perform and require no electrical power as they are manual tests.

Rapid/simple single-use assays are discrete, individual, disposable assays: i.e. they are used once only and discarded. These assays exist in several different presentations. Many rapid tests are based on a form of immunochromatography in which the added sample flows down an inert strip and reacts with previously immobilised reagents. The sample can be serum, plasma or even whole blood in some cases. Any positive reaction is visualised as a dot or a band appearing on the device strip. Most of the assays also include a control dot or band that is used to validate the results of each individual device, irrespective of the specific test result. These are divided into three main groups-

- Immuno-chromatographic method.
- Immuno-filtration method (e.g. dot blot assay for HCV).
- Conventional enzyme immunoassay (EIA).

Rapid tests are provided in simple-to-use formats that generally require no reagents except those supplied in the

test kit. They are read visually and give a simple qualitative result within minutes, i.e. reactive or non-reactive.

The use of rapid assays is generally not recommended for blood screening as they are designed for the immediate and rapid testing of small numbers of samples. These assays are performed using manual techniques; the results therefore must be transcribed by staff and there is a lack of permanent records and traceability. As a result, they may have limited use in laboratories where through-put is medium to high. They may, however, be considered for use in small blood centres with limited resources and where only a small number of tests are done daily. They provide flexibility and no major items of equipment are needed. They may also be appropriate when a laboratory needs to screen specific donations on an emergency basis for immediate release of products due to a critically low blood inventory or when rare blood is required urgently.

Enzyme linked immunosorbent assay (ELISA)

ELISA may also be referred to as enzyme immunoassay (EIA). The ELISA methodology is often referred to as a sandwich technique.

ELISA is a microwell plate-based technique used to detect TTIs in donated blood units. For example, an antibody may be fixed to the plate, and then captures the corresponding antigen if present in the test sample which is added. An enzyme conjugated with another (free) antibody of the same specificity reacts with a colourless chromogenic substrate to generate a coloured product, indicating a reactive result. Several enzymes have been used for ELISA such as alkaline phosphatase, horseradish peroxidase and beta galactosidase. A substrate such as ortho-phenyldiamine dihydrochloride (for peroxidase), or paranitrophenyl phosphate (for alkaline phosphatase) is used and this is hydrolysed by the enzyme to give a coloured end product.

An ELISA assay is typically performed in a microwell or multi-well plate (96- or 384-wells). The multi-well plate provides the solid surface to immobilise the antigen (if an antibody is the target to be captured) or antibody (if an antigen is the target). Immobilisation of the analytes (chemical constituents of substances being identified and measured) facilitates separation of the antibody (or antigen) from the rest of the components in the sample. This characteristic makes ELISA one of the easiest assays to perform on multiple samples simultaneously.

General principle of ELISA

- The solid phase antigen or antibody, depending on the type of test, is attached to the surface of a well (this is the first 'slice of bread' for the 'sandwich').

This step is carried out by the manufacturer before the ELISA test kit is purchased by the user.

- Up to 94 unknown test samples can be tested on one 96-well microtitre plate. The two remaining wells are used for positive and negative controls. However, the number of tests that can be performed on a plate depends on the number of controls stipulated by the kit manufacturer. Often these controls are used to determine the cut-off level at which samples are considered reactive and may often require three negative and two positive controls as well as a blank (empty) well.
- The sample plasma/serum is added, one sample per well, and the plate is incubated according to the time and temperature prescribed by the manufacturer. Should a test sample contain the target marker, which is the antibody or antigen specific for the solid phase agent, then it will be captured by the solid phase agent in that microwell (this may be likened to the 'filling in the sandwich').
- After incubation, the microtitre plate is washed to remove any unbound substances in the wells.
- An antibody specific for the captured target, and which has been tagged (conjugated) with a 'flag' such as an enzyme, is then added to each well. This antibody is called a conjugate. In tests where the target was captured, the conjugate becomes specifically attached and completes the 'sandwich' (the second 'slice of bread').
- The tests are washed a second time to remove any unbound conjugate. There is no evidence of any reaction in the wells up to this stage. In fact, visually, all wells appear empty.
- A clear chromogenic substrate (chemical dye) is added to the wells and if the conjugate (enzyme 'flag') is present, it triggers a demonstrable colour change, which is measured in a spectrophotometer or some other optical mechanism. The enzyme acts as an amplifier of the reaction and the intensity of the colour change produced by the substrate is relative to the degree of reactivity of the test being measured.
- ELISA results may be either qualitative or quantitative. Qualitative assays give either a positive (reactive) or negative (non-reactive) result for a sample with the cut-off statistically determined to distinguish between a reactive and a non-reactive. Quantitative assays measure optical density of each sample against a known standard and interpret the degree or strength of each result.
- ELISA may be used in manual, semi-automated or fully automated systems. It may be used to test for the presence of antigens, such as HBsAg, or

antibodies, such as anti-HIV. Microtitre plates with 96 wells are usually used although economical test 'strips' are also available in plates that are able to be broken into strips, depending on testing requirements. The strips are generally four to 12 wells long but may even be broken into single wells.

Types of ELISA

Indirect ELISA. The indirect ELISA, as shown in Fig. 6, detects the presence of antibody in a sample such as when testing samples for the presence of anti-HIV in an infection with the human immunodeficiency virus. The HIV antigen is fixed (by the manufacturer) to the wells of the microwell (or microtitre) plate. After addition of the sample to the well, should it contain anti-HIV, the anti-HIV will bind specifically to the solid phase HIV antigen. The well is washed to remove unbound antibodies and then enzyme conjugated anti-IgG is added. If anti-HIV has been captured by the solid phase, the anti-IgG will be bound to the captured antibody, effectively fixing the enzyme to the solid phase. The well is washed to remove any unbound material and the substrate for enzyme is

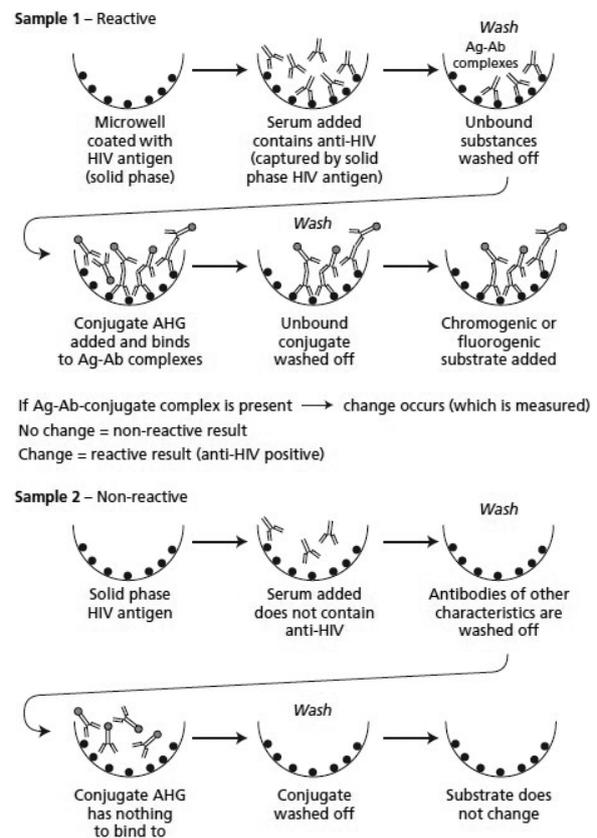


Fig. 6 Diagrammatic representation of indirect ELISA.

added. The action of the enzyme on the substrate results in a colour change, and for the first time the reaction becomes visible. The strength of this primary antibody present in the serum under test, will correlate directly with the intensity of the colour change.

Sandwich ELISA. The sandwich ELISA, as shown in Fig. 7, is used to identify a specific sample antigen such as when testing samples for the presence of hepatitis B surface antigen (HBsAg) in Hepatitis B. The wells of the microtitre plate are coated with antibodies such as anti-HBs. The samples to be tested are added to the wells. If the sample contains the antigen, such as HBsAg, the antigen will be bound to the surface of the well by the antibody. The wells are washed to remove any unbound material. Enzyme linked antibody with the same specificity as the antibody that was fixed to the well is added. If the antigen was trapped by the fixed antibody, the enzyme linked antibody will also bind to the antigen, effectively trapping the enzyme in the well. The well is washed to remove any unbound material and the substrate for enzyme is added. The action of the enzyme on the substrate results in a colour change, and for the first time the reaction becomes visible. The strength of this primary antigen present in the serum under test, will correlate directly with the intensity of the colour change

Competitive ELISA. Competitive ELISA, as shown in Fig. 8, depends on competition between the antigen in the sample under test, and the antigen already bound to the wells of microtitre plate.

In the competitive ELISA for anti-HIV, for example, the samples to be tested are added to HIV antigen coated wells at the same time as enzyme labelled anti-HIV conjugate. During incubation any anti-HIV in the test sample competes with the labelled reagent anti-HIV for the trapped antigen sites. The presence of antibody in the test sample therefore reduces the amount of labelled antibody that is bound to the well surface. The well is washed to remove any unbound material and the substrate for enzyme is added. The action of the enzyme on the substrate results in a colour change, and for the first time the reaction becomes visible. The strength of this primary

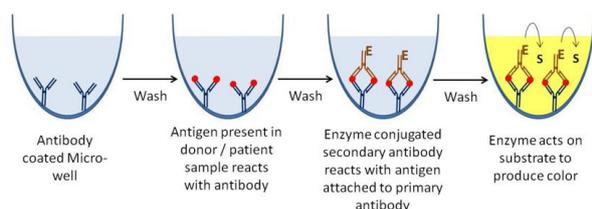


Fig. 7 Schematics of sandwich ELISA.

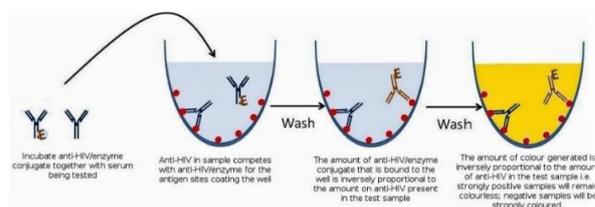


Fig. 8 Schematics of competitive ELISA.

antigen present in the serum under test, will correlate inversely with the intensity of the colour change i.e. colour is strongest in samples negative for anti-HIV, while there is little or no colour change in samples positive for anti-HIV.

Fourth generation ELISA

Fourth generation EIA was developed to reduce the diagnostic window period (time when the transfusion may be infectious, but testing is negative), due to increased sensitivity. In the case of HIV screening, this means that both antigen (p24) and antibody (anti-HIV – both IgM and IgG) are able to be detected in the same test. HIV p24 antigen is detectable about 2–18 days in HIV infected patients before seroconversion.

Recently, fourth generation assays have also been developed for HCV infection.

Chemiluminescence technology

Chemiluminescence (CL) is defined as the emission of electromagnetic radiation caused by a chemical reaction to produce light. Chemiluminescence immunoassay (CLIA) utilises chemical probes which are able to emit light through chemical reaction to label the antibody in place of the enzymes that are used in ELISA tests.

Chemiluminescence immunoassays differ from ELISA only in the mode of detection of immune complexes formed – colour generation in ELISA and measuring light produced by a chemical reaction in CLIAs. Instead of a chromogenic substrate that changes colour in the presence of conjugate, a fluorescent dye is added to the wells. Fluorogenic substrates have better sensitivity than chromogenic substrates and are now more commonly used. The strength of the result produced by the fluorogenic substrate is measured using a spectrofluorometer, or other electro-chemical mechanism.

Nucleic acid testing of blood donations

Nucleic acid amplification testing (NAT) is a molecular technique for screening blood donations to reduce the

risk of TTIs to a minimum by shortening the window period.

NAT is highly sensitive and specific for viral nucleic acids. It is based on amplification of targeted regions of viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) and detects these earlier than the other screening methods thus narrowing the window period for HIV, HBV and HCV infections. NAT also adds the benefit of resolving false reactive tests found during serological methods which impacts on donor notification and counselling. Donations may be tested singly or by using a pool.

Quality issues in the TTI laboratory

Implementation of a Quality Management System (QMS) is an important requirement for blood safety in the TTI laboratory. A QMS directs all activities to focus on the prevention of transmission of infections to recipients and also to minimise wastage of blood donations because of false positive results. Essential quality elements in the TTI laboratory include the following:

- Quality of the kits used for testing.
- Quality and calibration of equipment used in testing.
- Selection of high quality controls (internal kit controls and external controls).
- Interpretation of results, proper calculation of cut off.
- Documentation of results.
- Use of Standard Operating Procedures.
- Establishment and implementation of protocols for troubleshooting and corrective action.
- Application of statistical process.
- Training of staff.

For more information see *Section 16: Quality*.

Automation in the blood bank laboratory

Before introducing automated techniques, it is important to consider the impact on the laboratory. This should be investigated and carefully planned before the decision to implement is made.

All steps in the testing process can be automated, including the generation of reports. Multiple tests may be performed at the same time on each sample. Automated techniques allow samples to be tracked through the testing process; at any point during testing the user may locate the position of a sample in the testing procedure. Each sample is usually barcoded with a unique number allowing for positive identification throughout the process.

Most laboratory tests may be automated such as ABO grouping, Rh typing, antibody screening, TTI testing and crossmatching. Automation is particularly useful in

laboratories that perform large numbers of tests on donor and patient samples. For antenatal testing, smaller automated and partially automated systems are available for the efficient handling of approximately 100 samples a day or samples that do not have to be tested urgently may be batched for two days. When the number of tests is very low, automation may not be helpful.

Advantages

The advantages listed are extremely useful for total quality assurance and audit purposes.

- Faster result output or turnaround time is achieved as multiple tests can be set up in each batch. The results are supplied as each batch is completed.
- Results are more reliable than when tests are carried out manually, as the possibility of human error (e.g. pipetting, sample identification, transcription errors, sample cross-contamination) is reduced.
- Results are accurate, precise and reproducible.
- Automated equipment is usually serviced ongoing by the supplier and continues to be updated based on new techniques and technology, assisting laboratory personnel in remaining up to date.
- The system can be fully automated or various parts of the process can be divided into individual modules.
- The most important advantages include:
 - Positive sample identification.
 - Accurate reproducible results.
 - Automatic capture and link to an appropriate computer system.
 - Avoidance of human transcription errors.
 - Excellent tracking systems for quality control purposes as each step is validated and controlled.

Disadvantages

- Automation is expensive, especially the start-up cost of equipment, although various financing options may be available from the supplier.
- Reagents are more expensive, but the smaller volumes go much further as they are extensively diluted according to manufacturer's instructions.
- Equipment may need daily, weekly as well as monthly maintenance. i.e. it demands high maintenance, and also requires regular calibration.
- Personnel who operate the instruments must be initially trained by the supplier of the automation.
- The skill to carry out manual techniques may be somewhat reduced as a result of lack of practice, and when the machine fails, technologists may find difficulty in testing samples manually. However,

most laboratories still test certain repeat samples manually, so personnel do not lose their skill.

- When calibration tests fail, equipment cannot be used or if a part breaks or needs servicing, testing cannot be carried out, causing loss of working time until the problem is corrected.
- Adequate back-up systems must be in place, and this may mean duplicating all aspects of the equipment, from robotics to computers, at considerable cost, or having adequate spares available. Good supplier back-up service is critical to the success of the automation.
- Most systems require an uninterrupted power supply.

Molecular technology

The introduction of molecular techniques that can be used routinely in diverse laboratories has had an important impact on laboratory testing in general. Of significance to the field of blood transfusion is the development of genomic amplification techniques, which can be applied to the testing of donor blood.

By amplifying the viral nucleic acid to detect the actual virus, genomic amplification (i.e. NAT) considerably reduces the window period of infectious agents. Infected blood donations are therefore detected at an earlier stage, when they may not have been detected previously using antigen or antibody detection assays. Infected donations are therefore much less likely to enter the blood supply, thus greatly improving patient safety. The introduction of NAT on blood donations, has become a requirement in many services, organisations and countries.

The development and introduction of molecular genetic techniques has impacted greatly on the study of human blood groups, and this has resulted in most of the genes controlling the many blood group systems being cloned and sequenced. This has led to a much greater understanding of the blood groups and explains many of the serological complexities that have been observed over time.

Genomic amplification techniques

There are several nucleic acid amplification techniques including:

- Polymerase chain reaction.
- Ligase chain reaction.
- Nucleic acid sequence-based amplification.
- Transcription-mediated amplification.

All these techniques are considerably sophisticated and require specialised reagents, equipment, laboratories and expertise to perform the assays. Various commercial kits

and test systems are available, ranging from manual to fully automated systems. Because of the sensitivity of NAT, great care must be taken to prevent contamination of tests with traces of material from other sources. The various stages in the process should take place in dedicated areas, and unauthorised personnel should not be permitted entry into the work areas.

Polymerase chain reaction

Polymerase chain reaction (PCR) has become a basic molecular technique used for many applications and will therefore be described in some detail.

Principle of PCR

The method is used to amplify or duplicate a targeted section of DNA by enzymatic action so that the original (template) DNA molecule is increased to a number that can be detected. Specific 'primers' are used to target the specific regions on the template DNA and copy and recopy it. The number of copies is doubled with each cycle of the reaction, and the procedure is performed over many cycles. The amount of DNA in the test sample can be amplified up to a million times or more and in so doing, sufficient quantity to be detected is produced.

PCR was primarily designed to detect specific DNA rather than RNA. However, detection of RNA is also possible when the reverse transcriptase enzyme is added to convert RNA to DNA during testing.

Components of PCR

The PCR reaction requires the following components:

- *DNA template*: The double stranded DNA (dsDNA) of interest, separated from the sample. This is the target region of DNA within the original double helix (double stranded DNA).
- *DNA polymerase*: Usually a thermostable enzyme called Taq polymerase (after the bacteria *Thermus aquaticus* from which it was first isolated) that does not rapidly denature at high temperatures (98°C), and can function at a temperature optimum of approx. 70°C. It acts as a catalyst to add nucleotides to the reaction, and new DNA strands are formed so that the number of DNA copies is amplified, according to the number of cycles.
- *Oligonucleotide primers*: These are nucleic acid strands or 'primers' (short pieces of single stranded DNA, often 20–30 base pairs); commercially synthesised to correspond to the 'start' and 'end' of the DNA segment to be amplified.
- *Nucleotides and their corresponding enzymes*: Single units of the bases A, T, G, and C and the enzymes dATP, dTTP, dGTP, dCTP provide the building blocks and the enzymes for DNA synthesis.

- **Buffer system:** Includes magnesium and potassium to provide the optimal conditions for DNA denaturation and renaturation; also important for polymerase activity, stability and precision.
- **Thermal cycler:** this is a specially designed waterbath in which the PCR cycles take place and is able to control the temperature for precise periods of time, as required for each cycle of the process. These temperature controls are automated. The process of denaturing at 94°C, annealing at 54°C and extending at 72°C is repeated over and over again.
- **Agarose gel:** once the product of the test has been produced, it needs to be visualised, and this may be performed using agarose gel electrophoresis. Agarose is a polysaccharide extract of seaweed. It creates a neutral environment for molecule migration. It is therefore suited to the separation of nucleic acids by electrophoresis, gel chromatography or immunodiffusion, in order to interpret the results.

Procedure of PCR

All the PCR components are mixed together and are taken through series of three major cyclic reactions conducted in an automated, self-contained thermocycler machine.

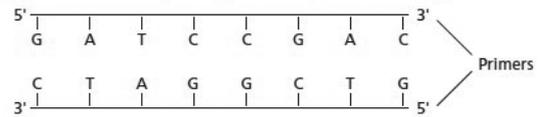
- **Denaturation:** This step involves heating the reaction mixture to 94°C for 15–30 sec. During this step, the double stranded DNA in the test sample is denatured to two single strands due to breakage in weak hydrogen bonds.
- **Annealing:** The reaction temperature is rapidly lowered to 54°C for 20–40 s. This allows the primers to bind (anneal) to their complementary sequence in the template DNA i.e. the section of DNA in the test sample (e.g. the section of DNA that is the HIV) that is to be copied.
- **Elongation:** Also known as extension, this step usually occurs at 72°C. In this step, the polymerase enzyme sequentially adds bases complementary to the template, recreating the double stranded DNA molecule. As each strand of DNA (from the Denaturation step) is a template, two double strands of DNA are made from each single strand initially in the assay.

With one cycle, a single segment of double-stranded DNA template is amplified into two separate pieces of double-stranded DNA. These two pieces are then available for amplification in the next cycle, in which four copies will be created. As the cycles are repeated, more and more copies are generated and the number of copies of the template is increased exponentially. This process is illustrated in Fig. 9.

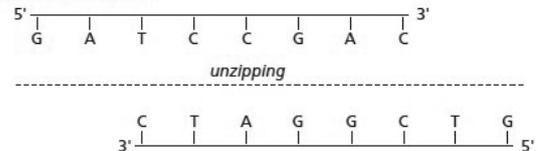
Decode of nucleotides:

A = Adenine, T = Thymine, G = Guanine, C = Cytosine

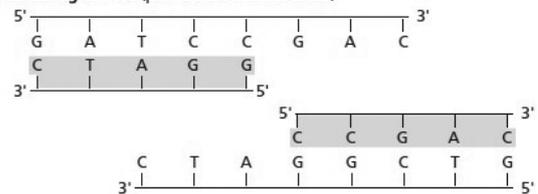
One segment of DNA in sample: template of target DNA to be amplified



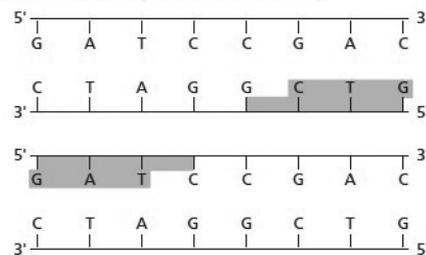
Step 1: denaturation at 94°C



Step 2: annealing at 54°C (primers are shaded areas)



Step 3: extension at 72°C (extension areas shaded)



Now there are two segments of DNA

This is amplified using the same three step cycle as above, over and over again

Fig. 9 Amplification of DNA fragments in PCR assay.

Gel electrophoresis

At the end of the PCR, the PCR product should be loaded into an agarose gel for electrophoresis. This will allow one to confirm the correct size of the amplicon, and if required, to purify it for further molecular experiments such as cloning. The PCR procedure can be useful for DNA cloning, DNA sequencing, phylogenetic studies, forensic analysis, and more. Gel electrophoresis may be used to visualise PCR results by causing DNA (or RNA) molecules to separate according to their size. Negatively charged molecules of nucleic acid are propelled through the gel by an electric current. Smaller molecules move faster and further than the longer molecules. This technology may be used to determine if the target DNA fragment was generated. A molecular mass marker solution, called a DNA 'ladder', is run in parallel with the test DNA/RNA. The ladder contains a mixture of DNA or

known molecular mass and is used to compare with the test to estimate the size of DNA/RNA fragments in the test sample and therefore their identity.

Variations in methods for performing PCR

- Multiplex PCR is a technique that enables simultaneous amplification of many targets of interest in one reaction by using more than one set of primers.
- Kinetic PCR measures the amplification in real time at the end of each cycle to quantify the amount of starting nucleic acid. Two common methods of quantification use either fluorescent dyes, or oligonucleotide probes, which fluoresce when they hybridise with the complementary DNA. The data are analysed using computer software and the results are shown graphically.

Examples of application of PCR in transfusion medicine

- Blood group typing in multiply transfused patients.
- Blood group typing for which antisera is not available (e.g. Dombrock).
- Resolution of weak expression of D antigens.
- Determination of zygosity for D.
- Fetal blood group typing.
- Human platelet antigen/Granulocyte antigen typing.
- Screening for transfusion transmissible infections.

Key points

- It is important that the correct sample type, i.e. clotted or anticoagulated, is used when performing assays.
- To avoid contamination, samples must be drawn in the prescribed order and stored correctly prior to testing.
- Red cell suspensions must be prepared correctly.
- Control results should be read and recorded and only if results are as expected, should test results be interpreted and documented.
- Test results should be recorded as per laboratory protocol immediately after reading. Interpretation of recorded test results should be done without a preconceived idea of what the result should be, with every precaution taken to detect and investigate anomalous results.
- It is important to record the grade/strength of red cell agglutination reactions, to highlight weak or anomalous reactions. Where applicable, it may also be necessary to record agglutination score, and this would be included in the SOP.
- For negative results, it is preferable to use zero '0' instead of a dash, i.e. a minus (-) sign.

- Mixed field reaction (presence of agglutinated as well as unagglutinated cells) indicates the presence of two red cell populations and is important for identification of ABO typing anomalies.
- Presence of mixed field reaction in an ABO/D forward grouping of a recently transfused patient may indicate group mismatched transfusion.
- Strength of red cell suspension, suspension media used, serum:cell ratio, incubation time and temperature, speed of centrifugation and use of proteolytic enzymes are important analytical variables that may affect the result in red cell serological tests.
- Washing of red cells before routine testing is not necessary. However, it is advisable to wash red cells with saline if the sample is taken from a donation bag segment (which means that the cells are in anticoagulant), or for the resolution of an anomalous result.
- Serum/plasma and red cell suspensions are usually added to tests in equal proportions to encourage standardisation and to avoid a prozone or a postzone phenomenon.
- Centrifugation enhances agglutination by bringing the reactants closer together.
- Proteolytic enzymes (ficin, papain, trypsin, bromelin) facilitate agglutination reactions by changing (reducing) the zeta potential and are useful in identification of some red cell antibodies.
- Appropriate controls should be set up with every batch of tests, to confirm that reagent antisera, reagent red cells and all other reactants used such as enzymes or AHG are working as expected.
- Test results should be recorded as per laboratory protocol immediately after the test is read.
- Control results should be recorded first, and only if validated, should tests performed at the same time, be read.
- It is important to record reaction strength, and if the SOP states as such, then also the score of red cell agglutination, to be able to identify weak or anomalous reactions.
- Mixed field reaction (presence of agglutinated as well as unagglutinated cells) in a test indicates presence of two red cell populations and is important for the detection of ABO typing anomalies.
- ABO grouping involves forward and reverse grouping. In forward grouping the unknown red cells are tested against known ABO grouping reagents.
- Testing for the presence of anti-A or anti-B antibody in the serum/plasma, using Group A and B red cells, is known as reverse grouping.
- When group O cells are included in the reverse grouping, tests are normally negative unless the serum/plasma contains irregular antibodies,

- Individuals lacking H antigen (Bombay phenotype) contain anti-H antibody in their serum/plasma, as well as anti-A and anti-B.
- Most individuals secrete soluble ABO substances in their saliva (secretors) and testing for secreted substance may help in establishing ABO type.
- Rh phenotyping may be performed to determine the presence or absence of the five major Rh antigens (C, D, E, c and e). Such red cell phenotyping may be beneficial in finding suitable blood for transfusion in Rh incompatibility problems, or in determining paternal phenotype (and therefore the likelihood of a fetus being affected by HDFN), when the maternal blood contains Rh antibodies.
- Tests which remain unagglutinated or react only weakly in D typing need to be further tested for D variants such as weak D, partial D.
- D antigen is highly polymorphic with many epitopes, therefore, selection of antisera for D typing is critical.
- The AHG test is used to detect clinically significant antibodies such as anti-D.
- The DAT detects *in-vivo* sensitisation of red cells while the IAT is used *in-vitro* to detect whether or not red cell sensitisation took place during incubation with serum/plasma during testing in the laboratory.
- Antibody screening involves testing unknown serum/plasma against a set of group O reagent red cells that together contain antigens corresponding to specificities of most clinically significant antibodies.
- Antibody screening does not indicate the specificity of an irregular antibody detected.
- Antibody identification is performed to determine the specificity of irregular antibodies. This involves testing the serum/plasma against a panel of group O cells that have been extensively typed to determine the presence or absence of antigens that correspond to all major antibody specificities.
- Doubling dilution titrations may be performed to determine the titre of clinically significant antibodies. Titre may be defined as the reciprocal of the highest dilution at which an antibody gives a demonstrable reaction against red cells with the corresponding antigen.
- Titration of ABO antibodies is routinely performed for ABO incompatible organ transplantation and Group O apheresis platelets transfused to Group A or B recipients.
- Neutralisation/inhibition is used to identify antibodies or to determine ABO secretor status by the detection of soluble antigens found in body fluids like saliva.
- Antibody elution techniques may be used to remove sensitising antibodies and determine their specificity. In so doing, the specificity of antigens on the red cells that were sensitised, may be deduced.
- Antibody elution procedures may be performed by different techniques such as heat for ABO antibodies, and glycine-HCl/EDTA for other antibodies.
- Adsorption is done to remove antibody from serum by incubating it with red cells with the corresponding antigen.
- Adsorption/elution studies are useful for detecting weak red cell antigen variants.
- There are many causes of false positive and negative results in laboratory testing. In broad terms, false results may be caused by materials, equipment or may be the result of human error. Faulty centrifuges, reagent red cells or antisera, blood samples, chemicals, time and temperature of incubation, as well as poor technique, negligence or lack of knowledge, may all contribute to false results.
- ELISA methodology may be used to test for TTIs. ELISA uses solid phase technology.
- By amplifying the viral nucleic acid and thus detecting the physical presence of the virus, nucleic acid amplification techniques reduces the window period of infectious agents considerably. Infected blood donations are therefore detected at an earlier stage than detected previously using antigen or antibody detection assays.
- The introduction of NAT testing has significantly reduced the window period of infectious disease markers in blood donations.
- NAT systems are available for the detection of individual viruses or for a combination of viruses such as HIV, HBV and HCV in the same test. Donations may be tested using a pool system or a single donation testing system.
- Automation may be used for blood grouping, antibody screening, antibody identification, crossmatching and TTI testing.
- The implementation of automation should be carefully evaluated before introducing automated techniques into a laboratory.
- The development of molecular genetic techniques has impacted greatly on the study of human blood groups, and this has resulted in most of the genes controlling the many blood group systems being cloned and sequenced.
- PCR is used to detect and amplify target DNA sequences. PCR involves three steps: denaturation, annealing and extension.
- PCR is useful in resolving serological discrepancies.

Genetics

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Introduction

Genetics is the study of heredity or how living organisms receive common traits, i.e. observable or detectable characteristics, from previous generations. Genes are the units of inheritance and provide the mechanism for the passing on of these traits. For example, children will generally look like their parents because they have inherited genes from their parents, 50% of their father's genes and 50% of their mother's genes. Genetics is the science of identifying the various traits that are passed from one generation to the next, and to explain the mechanisms for this transmission.

Genetics is of importance regarding the expression of the blood groups as the varieties of blood group antigens that we recognize on the surface of red blood cells result from variations in the genetic code. It is of value to understand the way in which blood group characteristics are passed on from generation to generation and the genetic events associated with their expression; this knowledge helps in the classification and understanding of the blood groups, gives insight into the management of haemolytic disease of the fetus and newborn and provides input into parentage testing and personalised transfusion medicine.

Learning objectives

Describe and define the following genetic concepts and be able to use the blood groups in examples to assist in explanations:

- Mendelian genetics
 - law of segregation
 - law of independent assortment
- Key components of inheritance
 - ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)
 - chromosomes and genes
- Mitosis and meiosis
 - crossing over
 - mutation
 - X-linked characteristics
- Molecular genetics
 - DNA basic rules
 - gene
 - genotype vs phenotype
 - homozygous, hemizygous, heterozygous

- dominant gene, recessive gene
- mutation
- From DNA to blood groups
 - chromosome locations of genes encoding blood group systems
 - molecular mechanisms associated with the expression of blood groups
 - examples of inheritance and gene rearrangements

Mendelian genetics

Much of the progress in the field of blood group serology, as well as the classification of blood groups into systems, has been possible because of genetic studies. Work in the field of genetics began with discoveries made in the mid-19th century by Gregor Mendel, an Augustinian monk in St Thomas's Abbey in Brno, in the present-day Czech Republic.

Mendel, who is known as the 'father of modern genetics', planted a small strip of garden to experiment on hybridization in plants (hybridization is the process of breeding individuals or plants from genetically different populations, thus producing a hybrid). He used his own living quarters to hybridize mice as part of his investigations into heredity but stopped this line of investigation as it was not considered proper for a monk to be investigating the breeding of mice. He also experimented with bees, but his work with garden peas is the most widely known and will be used here to present a sample of his findings.

Mendel compared seven different characteristics in peas that appeared to be inherited independently of each other. Between 1856 and 1863 he cultivated and carried out measurements on close to 30 000 plants. One of the characteristics that he measured was the height of the plant. In the parental generation he mixed or 'crossed' tall (T) pea plants with short pea plants (t). The first generation resulting from this cross, called the first filial, or F1, generation were all found to be tall. He then crossed the tall plants of the F1 generation with each other and in the second filial generation, F2, tall and short plants were produced in a ratio of 3:1.

Mendel reasoned that all the F1 generation plants were tall because the factor determining tallness in plants is dominant over the factor for determining shortness. When the two characteristics were inherited together, plants therefore grew tall. Table 1 shows a Punnett square

diagram to illustrate the F1 crossing of tall and short pea plants. The Punnett square shows the probabilities of offspring inheriting a particular genotype, by pairing the four possible combinations of alleles for that characteristic, from maternal and paternal origin. In its simplest form the diagram consists of four squares, each showing one of the possible genetic combination, with a probability of 25%.

A unique word for the 'factor' of inheritance described by Mendel was not introduced until 1909, when the Danish botanist Wilhelm Johannsen first used the word 'gene'. He was also the first researcher to clearly make the distinction between the appearance of an individual (the phenotype) and that individual's genetic traits (the genotype). 'Allelomorph', meaning 'other form' and now shortened to 'allele' was the name given for the alternative forms of a gene that are found at the same location, or locus, on a chromosome. In the example of Mendel's pea plants, the gene 'T' (for tall), and 't' (for short) are alleles.

It is customary to depict dominant alleles with upper case letters, and recessive alleles with lower case letters, and in italics, as shown in the diagrams. All offspring of the F1 generation are tall plants because *T* (tallness) is dominant over *t* (shortness). When these hybrid tall plants (F1) were 'selfed' (crossed amongst themselves), a quarter of the F2 generation plants were short. Table 2 shows the F2 crossing of hybrid offspring of F1. Mendel therefore reasoned further that a hidden 'recessive' factor for shortness was manifested only if two recessive characteristics were inherited, one from each parent. In F2, 75% of offspring are tall pea plants and 25% are short pea plants (ratio of 3:1). This is because any plant that inherits a *T* allele for tallness, either in single or double

dose, grows to be a tall plant because *T* is the dominant allele.

As a result of his studies, Mendel was able to establish two laws that are still considered the basis of genetics:

Law of segregation

- The variation seen in different members of the same species is the result of genetic variety within each single characteristic (such as height of pea plants). This part of the law relates to the fact that there are alternative forms of the gene (alleles), that determine different outcomes for the same characteristic.
- For every characteristic inherited, such as from a pair of pea plants, each parent contributes equally to make the seed. This part of the law relates to the fact that for each characteristic, two alleles are inherited, one from each parent.
- Alleles are either dominant or recessive. If two dominant alleles are inherited, the offspring develops as a dominant; if the allele from one parent is dominant and that from the other parent recessive, the offspring develops as a dominant but can transmit to the next generation, either a dominant or a recessive allele; and lastly, the offspring only manifests a recessive characteristic when the recessive allele was inherited from both parents. This part of the law relates to the fact that if the two alleles differ, then the one that encodes for the dominant trait is fully expressed in the appearance of the offspring, whereas the allele encoding for the recessive trait has no noticeable effect on appearance.

Table 1 Punnett diagram: F1 crossing of tall and short pea plants

	Tall plant	
Filial 1 (F1)	<i>T</i>	<i>T</i>
Short plant <i>t</i>	<i>Tt</i>	<i>Tt</i>
<i>t</i>	<i>Tt</i>	<i>Tt</i>

Table 2 Punnett diagram: F2 crossing of offspring of F1

	F1 plant	
Filial 2 (F2)	<i>T</i>	<i>t</i>
F1 plant <i>T</i>	<i>TT</i>	<i>Tt</i>
<i>t</i>	<i>Tt</i>	<i>tt</i>

Law of independent assortment

This law states that different traits are inherited independently of each other. In other words, the manifestation of one trait will not affect the manifestation of another. For example, the inheritance of eye colour is independent of the inheritance of body height.

Note: It is now known that independent assortment only applies to characteristics that are not genetically linked. Genetically linked genes from unrelated characteristics, such as male sex and red-green colour blindness are inherited together, meaning that a male with red-green colour blindness will pass the colour blindness gene on to his daughters, who will carry the colour blindness gene as a recessive. If this recessive gene is passed on, by the carrier mother, to a male child, that individual will have red-green colour blindness.

Key components of inheritance

Inheritance of observable or detectable characteristics (traits) takes place from one generation to the next. The genetic material responsible for every trait is found in the nucleus of every nucleated cell in the body. This genetic material is called chromatin and consists of deoxyribonucleic acid (DNA), a long molecule that is made up of simple units called nucleotides that are arranged in a specific order. The order of the nucleotides carries the genetic information of the individual, much as the arrangement of letters on a page make up the words and hence the content. The 'language' used by DNA is called the genetic code and contains all the instructions necessary for the construction and operation of the organism.

When it is necessary for cells to reproduce themselves as a result of growth, tissue repair or cell death, the chromatin undergoes changes; rod-shaped organelles called chromosomes are formed within the cell nucleus as a result. Figure 1 depicts a body cell showing the cytoplasm, nucleus and chromosomes (23 pairs per nucleated cells), with an enlargement of one of the pairs of chromosomes to show the gene loci (the positions of the genes on the chromosomes).

Chromosomes consist of two strands of DNA, like the upright supports of a ladder, linked together by strips that resemble the rungs or steps of the ladder, and twisted into a spiral. Each 'rung' consists of two nucleotide bases linked together in a strictly regulated way, and it is the order of these bases that provides the genetic code, or the identity stamp of the organism (such as a human). Each section of DNA and its nucleotide bases that contains the code for a specific characteristic is referred to as a 'gene', and each chromosome contains many different genes distributed along its length. The chromosomes are arranged

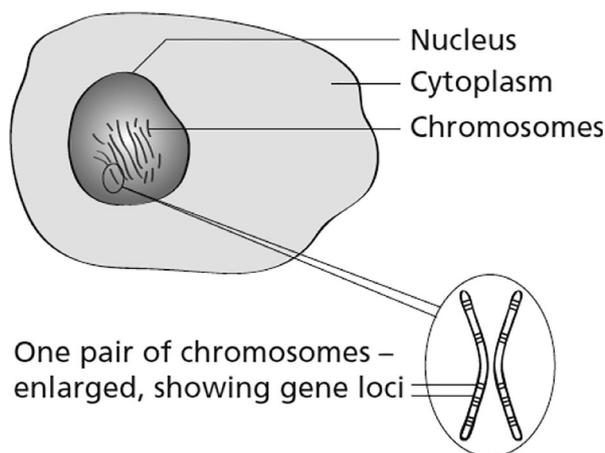


Fig. 1 Body cell showing cytoplasm, nucleus and chromosomes, with enlargement of one pair of chromosomes to show gene loci.

in pairs inside the nucleus of body cells and these pairs are called homologous chromosomes, one passed on from each parent. Cells with two sets of chromosomes, which are most of the cells in the body, are called diploid (or body) cells, while the sex cells (ova or egg cells and sperm cells) have only one set of chromosomes and are called haploid cells.

DNA has two major functions. One is to reproduce itself, in the process of mitosis, and the other is to provide the recipe or code to produce the proteins which are necessary for ongoing life.

In order to reproduce itself, i.e. in the duplication of the chromosomes, DNA is unzipped into two complementary DNA strands when the two nucleotides making up each 'rung' separate. Each strand of the original molecule then acts as a template for the synthesis of a new complementary DNA strand, resulting in two new chromosomes, each identical in every way to the original.

In the production of proteins, the DNA is unzipped into two complementary strands as before, by the separation of the two nucleotides making up each 'rung'. One strand of the DNA is used as a template by an enzyme called ribonucleic acid (RNA) polymerase to synthesize a short length of messenger RNA (mRNA) through a process known as transcription. Although chemically slightly different from DNA, this mRNA contains the same information that was encoded by the DNA in a similar sequence of nucleotides, i.e. the recipe, encoded by the gene, to produce a specific protein. Following the assembly of the mRNA, the two strands of DNA reunite, reforming the unaltered chromosome. The mRNA migrates from the nucleus to the ribosomes in the cytoplasm of the cell where the sequence of nucleotides in the mRNA (i.e. the recipe for the protein) is translated into a sequence of amino acids. Each sequence of three nucleotides codes for one amino acid, and these amino acids are linked together to form the protein. In summary, the protein encoded by the gene located on the chromosome is thus, through the process of transcription followed by the process of translation, produced (in most cases) faultlessly in the ribosomes of the cell. See 'Molecular genetics' later in this section for more details on this process.

Humans can produce over 100 000 different proteins, which gives some measure of the amount and complexity of information encoded in the DNA. Figure 2 is a simplified diagrammatic representation of DNA and RNA.

Chromosome typing may be carried out in the laboratory for diagnostic purposes, and to identify chromosomal abnormalities. This process is called karyotyping and is achieved by stimulating a sample of human cells to duplicate themselves in the laboratory. When the duplication is fairly advanced the process can be halted, the chromosomes stained with dye and analysed using the

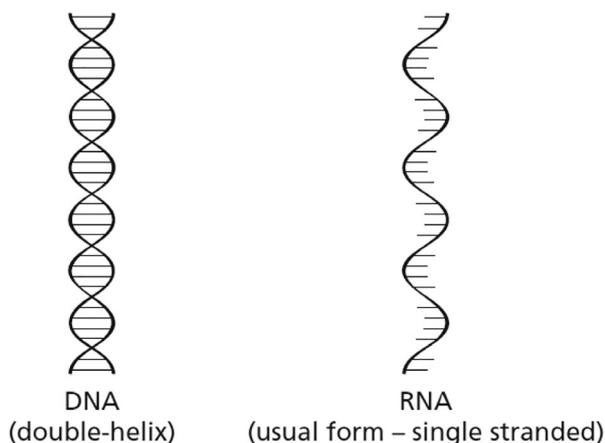


Fig. 2 Simplified diagrammatic representation of DNA and RNA.

appropriate computer software. This digital method has largely replaced the former method whereby the chromosomes were photographed through a microscope and the photographs analysed. The human genome consists of 46 chromosomes: 22 pairs of autosomes, and one pair of sex chromosomes, called X and Y. The X chromosome is more than five times larger than the Y chromosome and carries approximately ten times as many genes. Human females typically have two X chromosomes, and males typically have one X and one Y chromosome.

Mitosis and meiosis

Most cells within the body can reproduce themselves. There are two types of cells; body or somatic cells, and sex cells or gametes. When body cells replicate themselves, the result is two daughter cells that are identical to the parent cell, each with a full complement of chromosomes. When sex cells are produced the result is four daughter cells, each of which has half the genetic material that was present in the parent cell i.e. one of each of the pairs of chromosomes. The duplication process carried out by body cells is called mitosis, and the reduction division carried out by the gametes is called meiosis. Both processes go through several stages at the end of which, so-called daughter cells are produced. Both processes are complex and therefore only the outline of the stages during cell division is described. In the description, each stage is identified by N, being the number of chromosomes within the nucleus of the cell during that stage ($1N = 23$, $2N = 46$, i.e. the total number of chromosomes in humans, and $4N$ means double the full complement).

Stages of mitosis (somatic cell duplication)

Mitosis is a form of cell division that results in two daughter cells, each with the same genetic component at

the parent cell. Each daughter cell has a copy of every chromosome that was present in the parent cell. The process typically takes approximately 1 h.

- (1) Interphase: state of rest (holding phase) of body cells between the processes of duplication ($2N$).
- (2) Prophase: chromatin loses its granular appearance and become the shorter and thicker chromosomes. The genetic material has been duplicated; hence there are two identical copies of each chromosome in the cell ($4N$).
- (3) Metaphase: chromosomes migrate to the centre of the cell and split lengthwise into two equal (homologous) sets, each set composed of 23 matching pairs ($4N$).
- (4) Anaphase: homologous pairs of chromosomes are split and migrate to opposite ends of the nucleus ($4N$).
- (5) Telophase: nucleus divides, with each half taking a full set of chromosomes, which revert to chromatin ($2 \times 2N$).

Mitosis must ensure that one copy of each chromosome – and only one copy – ends up in each daughter cell after cell division. Each diploid daughter cell therefore contains the full genetic complement of the original parent cell Fig. 3.

Stages of meiosis (sex cell production)

Meiosis is the form of cell division that produces four haploid sex cells, or gametes, that each contain a single copy of each chromosome carried by the parent cell.

The sex cells multiply initially by mitosis and then start a process of growth. (When describing the meiotic process in writing, stages are identified by using Roman numerals.) The arrangement of chromosomes is maintained and not temporarily doubled, as in mitosis. The

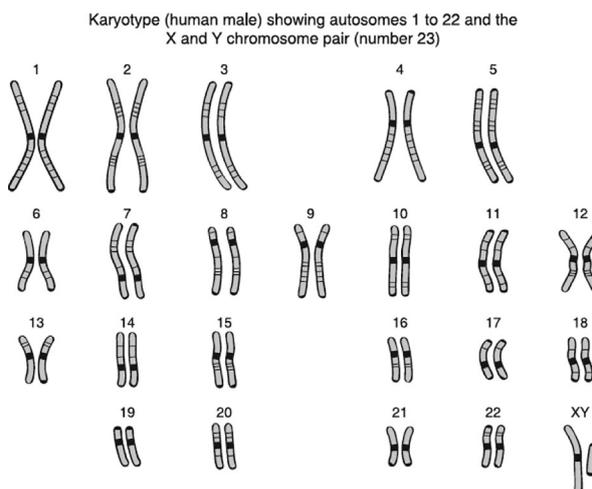


Fig. 3 Human male karyotype showing autosomes and the X and Y chromosomes.

new cells therefore receive only half the number of chromosomes – one of each pair instead of complete pairs.

- (1) Prophase I: as for mitosis (4N).
- (2) Metaphase I: as for mitosis (4N).
- (3) Anaphase I: as for mitosis (4N).
- (4) Telophase I and cell division: diploid daughter cells divide, but do not temporarily double, as is the case for mitosis ($2 \times 2N$).
- (5) Metaphase II ($2 \times 2N$).
- (6) Anaphase II, telophase II and cell division: reduction division ($4 \times 1N$).

Four haploid (i.e. cells with half the quota, or 23 chromosomes instead of 23 pairs of chromosomes) daughter cells (or germ cells as they are also called) are produced. Each germ cell contains half the genetic complement, drawn randomly from the genetic material of both parents. One of the four germ cells produced by a human female matures into an ovum, i.e. egg, whereas all four of the germ cells produced by a human male mature into sperm.

Figure 4 illustrate the mitotic process in a simplistic form and compares it with the process of meiosis.

Crossing over

Chromosomal crossing over refers to the process during which a pair of chromosomes, which are aligned during the first phase of meiosis, exchange a segment of their

Mitosis			Meiosis		
	2N	Interphase		2N	Interphase I
	4N	Prophase		4N	Prophase I
	4N	Metaphase		4N	Metaphase I
	4N	Anaphase		4N	Anaphase I
	2 x 2N	Telophase and cell division → 2 x diploid daughter cells		2 x 2N	Telophase I and cell division
				2 x 2N	Metaphase II Gametes
				4 x 1N	Anaphase II, Telophase II and cell division → 4 x haploid germ cells

Fig. 4 Simplified process of mitosis and meiosis.

DNA. Crossing over most commonly occurs when similar regions on matching chromosomes separate and then re-join to the other chromosome. This results in an exchange of genes between the chromosomes, that is, a new assortment of genes on each chromosome, but no new genes. Figure 5 shows the process of crossing over.

Recombination during meiosis allows more random selection between the two alleles that occupy the same position, as it serves to shuffle the alleles between the paired chromosomes.

In practice it is found that the frequency of recombination is not the same for all genes. Recombination is influenced greatly by the physical distance between one gene and another. If two genes are located close together, the likelihood that they will be separated is less than if they are located further apart. This is termed 'genetic linkage'.

Although crossing over usually occurs between the same regions of paired chromosomes, this is not always the case. Sometimes dissimilar lengths of chromosome can be exchanged, resulting in an unbalanced recombination. This means a duplication of genes on one chromosome and a deletion of the same genes on the other chromosome, which can have serious consequences when it occurs during meiosis, and the affected cell becomes part of a gamete.

X-linked characteristics

When a characteristic is always inherited with another unrelated characteristic, it is called genetic linkage. Sex-linked characteristics are easier to identify than linkage between characteristics on autosomes, as they travel with gender. Several well-known characteristics are inherited

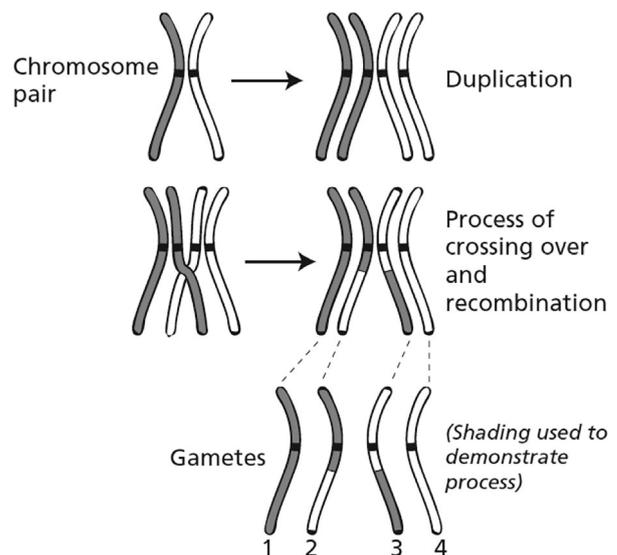


Fig. 5 Process of crossing over.

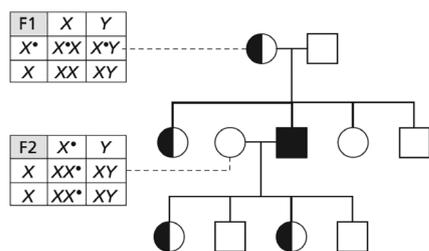
on the X chromosome, such as haemophilia A and red-green colour blindness. Most sex-linked characteristics are found on the X chromosome (rather than the Y chromosome) because it is much longer and carries many more gene loci. Recessive X-linked characteristics are manifested in male offspring (who have only one copy of the X chromosome, and hence one copy of the allele) whereas female offspring (who have two copies of the X chromosome) are the carriers, as Fig. 6 shows, using the sex-linked inheritance of haemophilia A as an example.

Molecular genetics

All human genetic information is encoded in DNA molecules consisting of two complementary twisted strands of millions of nucleotides arranged into a double helix (like a spiral ladder) through non-covalent interactions. These are the chromosomes. Each nucleotide is composed of a pentose (a sugar molecule containing five carbon atoms), with a phosphate group attached to the fifth carbon atom (C5) of the pentose and a base attached to the first carbon atom (C1) of the pentose. The sugar molecules form the uprights of the ladder-like structure and are linked together by the phosphate molecules, while the 'rungs' of the ladder are the complementary bases, linked together by hydrogen bonds.

Variations in the chemical composition of the bases give rise to four different nucleotides: two purines (adenine - A, and guanine - G) and two pyrimidines (cytosine - C, and thymidine - T). The four nucleotides bind to each other in DNA in a strict way - adenosine is always paired with thymidine (A-T) and cytosine is always paired with guanine (C-G) to form the rungs of the ladder, shown in Fig. 7.

This complementary binding between the bases accounts for two important properties of DNA. First, using chemical treatments or high temperature, DNA can be opened from the double helix form to a single-stranded form by



F1 offspring of carrier female (X*X) and unaffected male (XY)
50% of female offspring are unaffected, 50% are carriers
50% of male offspring are unaffected, 50% are haemophiliac

F2 offspring of unaffected female (XX) and haemophiliac male (X*Y)
100% of female offspring are carriers
100% of male offspring are unaffected

Fig. 6 Pedigree showing sex-linked inheritance of haemophilia A.

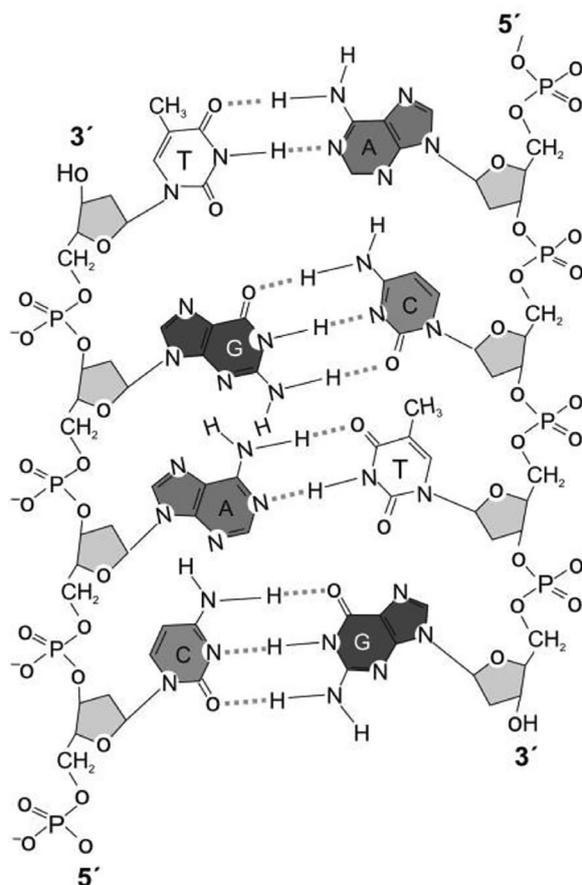


Fig. 7 DNA molecule with the two complementary strands of nucleotides arranged into a double helix.

breaking the hydrogen bonds between the bases. Second, DNA molecules have both a head end and a tail end, determined by the carbon atom on deoxyribose sugar, which is free to join with another deoxyribose sugar to extend the length of the chain. The carbon atoms of deoxyribose are numbered 1, i.e. #1, through 5, i.e. #5, and as they join to form the DNA ladder, carbon #5 of one deoxyribose sugar joins to carbon #3 of the next deoxyribose. Therefore, each DNA strand has a 5' (5 prime) end, i.e. the chain ends with an unbound 5' carbon, and a 3' (3 prime) end i.e. the chain ends with an unbound 3' carbon. Nucleotide sequences are commonly written in the 5'-3' direction.

When single strands of DNA pair together to form double-stranded DNA, the two ladder sides run in antiparallel directions i.e. they are parallel but oriented in opposite directions. The 5'-3' direction in one sequence runs opposite to 5'-3' direction of the other sequence. Figure 7 shows the double helix form of a DNA molecule.

Basically, all methods of DNA-based testing for blood group polymorphisms are founded on two basic rules,

namely, that A-T and C-G are complementary, and that 5'- 3' direction of binding is in opposite directions on the two strands of double-stranded DNA.

There are billions of variations in the order these nucleotides can appear on the strands. The sequence in which these four nucleotides appear determines the genetic code that guides the production of all proteins, including red cell antigens. Slight variations in the sequence of nucleotides in a gene can lead to an individual having different features, for example eye colour or blood type.

Gene

A gene is a segment of DNA that encodes a protein and each gene occupies a specific location known as the locus. A locus can be occupied by one of multiple alleles. A gene is composed of regions that encode amino acids (called exons) and regions which do not encode amino acids (called introns). Introns are removed by a process called splicing to generate the mRNA.

Genotype and phenotype

The genotype corresponds to the total set of genes of an individual. An individual's genotype is a set of genes inherited from the parents and the term is often used to refer to the pair of alleles at a specific locus e.g. the ABO genotype. The DNA encoding a given gene is transcribed into mRNA. The structure of RNA is similar to that of DNA with the following differences:

- Ribonucleotides have an additional hydroxyl group at C2.
- Uracil (U) replaces T.
- RNA encoding gene products are typically single stranded.

The mRNA is essentially a copy of DNA. After its synthesis in the nucleus, the mRNA is processed and exported to the cytoplasm where ribosomes transcribe it into proteins that give origin to the antigens and, consequently, to the phenotype. Each sequence of three bases (called a codon) on the mRNA strand encodes one amino acid (e.g. the codon AAG is the code for lysine while AAC is the code for asparagine) or provides a stop signal (UAA, UAG, UGA) indicating the end of the translation. There are only 64 possible codons ($4 \times 4 \times 4$) of which 61 encode the 20 amino acids and three are stop codons. There are more codons than there are amino acids because some amino acids are encoded by more than one codon for example CCC, CCA, CCT and CCG all code for the amino acid glycine.

Transcription of DNA always begins at the ATG, or 'start', transcription codon. Figure 8 illustrates the process

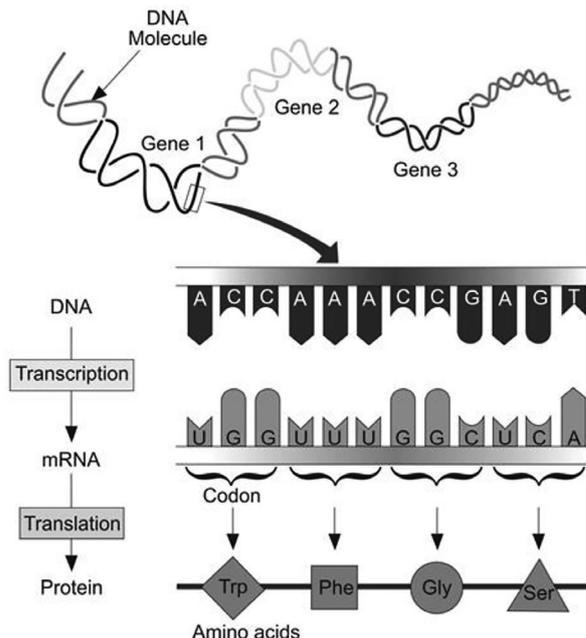


Fig. 8 DNA transcription and translation (from mRNA) to a protein.

of DNA transcription and translation (from mRNA) to a protein.

The phenotype is the observable outcome of gene expression and reflects the biological activity of its genes. Thus, the presence or absence of antigens in the erythrocytes, which may be determined by serological tests, represents the phenotype.

It is possible for the phenotype to be altered by environmental factors, such as the change in hair coloration in Himalayan rabbits as a function of ambient temperature. At low temperature (5°C), rabbits show black and white coloration, while raising the temperature to 20°C, the rabbits are only white in colour. Many possible genotypes, and resulting phenotypes, are possible from the pairing of genes from mother and father.

Homozygous, hemizygous and heterozygous

If the same alleles for a characteristic are present at a given locus on both chromosomes constituting the pair, the individual is called homozygous, and is considered pure for a character. This is represented by two identical letters, for example, AA or aa. However, when the alleles on the two chromosomes are different, the individual is called heterozygous, and is considered a hybrid for the character. This is represented by two different letters – using the former example, Aa. An individual is termed hemizygous when there is only one copy of the allele e.g. a D positive individual who has a deleted *RHD* allele on one chromosome. XY males are hemizygous for the genes

that are found on the X chromosome, as the corresponding genes are not present on the Y chromosome. XX females may be either homozygous or heterozygous for these genes.

Antigens that are encoded by alleles at the same locus are called antithetical, so C and c of the Rh blood group system are a pair of antithetical antigens. Thus, an individual with the C⁺ c⁻ phenotype is homozygous for C and the red cells have a double dose of C antigen.

Dominant and recessive genes

A dominant gene is a gene that determines the same phenotype, both in the homozygous (AA) and in heterozygous (Aa) state. In blood grouping, if the gene is dominant, the antigen is always expressed when the relevant allele is present, regardless of whether the individual is homozygous or heterozygous for that allele. For example, in the ABO blood group system the A gene is dominant (as is the B gene) and individuals who are homozygous (AA) as well as those who are heterozygous (AO) express the A antigen. Co-dominant alleles are those that express the product of both alleles when two different alleles are present, e.g. in the Kell system if the allele for the K antigen is present as well as the allele for the k antigen, the red cells will carry both these antigens, K⁺ k⁺.

A recessive gene is expressed only in the homozygous state and has no effect if another allele is also present. Therefore, only when inherited from both parents, can a recessive gene express itself. Figure 9 shows the genetic inheritance with AA representing a homozygote of the dominant allele; aa – a homozygote of the recessive allele; and Aa – a heterozygote with both dominant and recessive alleles.

Mutation

Although the replication of DNA during mitosis and meiosis is very precise, it is not always perfect.

Gene: Inheritance unit

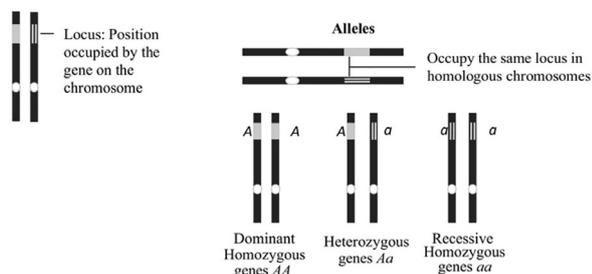


Fig. 9 Genetic inheritance showing dominant homozygous, heterozygous and recessive homozygous states.

Sometimes, albeit very rarely, mistakes take place during replication and the newly created DNA differs from the original. This 'mistake' is called a mutation and can occur within any part of the DNA, resulting in a change to the genetic code. This may result in a change in the characteristics of the protein for which the gene provides the code. During mitosis this could lead to the production of tumour cells.

When a mutation takes place at the time of meiosis, the change in the protein can be passed on to the offspring. This mutation may have no effect whatsoever, or the effect may have important consequences. For example, sickle cell haemoglobin and normal haemoglobin differ by only a single amino acid, brought about by a mutation of the normal haemoglobin gene. This is a negative consequence. On the other hand, a mutation could result in a benefit to the organism and could increase the chance of survival in the offspring.

From DNA to blood groups

Chromosomal locations of genes encoding blood group systems

The specific positioning, i.e. assignment and sub-localisation to a specific human chromosome, of the genes encoding for blood group system expression are known and illustrated in Fig. 10.

Encoding of genes or gene families that have been ratified to particular blood group systems, by the International Society of Blood Transfusion, have been localized to single cytogenetic bands on 16 of the 22 autosomes (genes for 38 systems) or on the X chromosome (genes for 2 systems). The individual chromosomal banding patterns are based on the nomenclature established by the Committee of the International Society for Cytogenetic Nomenclature.

The internal reference point for each chromosome is the centromere (the 'pinched' region in the middle); with the short arm (p) depicted above and the long arm (q)

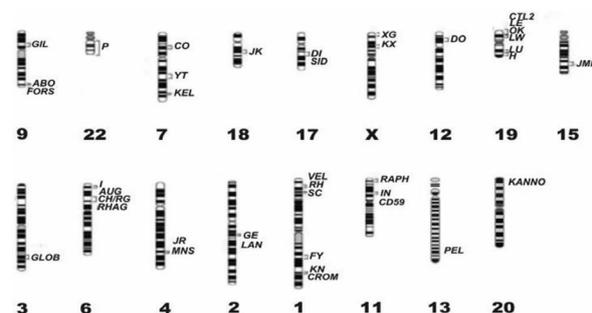


Fig. 10 Chromosomal locations of genes encoding blood group systems.

depicted below it. Moving from the centromere toward each telomere (the structure at the end of the chromosome) each band is numbered consecutively, with the bands closest to the centromere numbered as, respectively, p1 and q1, the next, respectively, p2 and q2, and so on. At higher resolution, each of these major bands can contain sub-bands (e.g., p11, p12, q11, q12,) and sub-sub-bands (e.g., p11.1, p11.2, p11.3 if p11 had three sub-sub-bands). Using this system, it is possible to describe very precisely the position of a gene on a specific chromosome. For example, the gene coding for the antigens of the ABO system is located on chromosome number 9 at 9q34.1 to 9q34.2.

Figure adapted from Castilho L, Pellegrino Jr J, Reid M. *Fundamentos de Imuno-hematologia*, Ed. Athenei, 1st ed., 2015 with permission.

Molecular mechanisms associated with the expression of blood groups

Several genetic events that generated new blood group antigens and phenotypes have been identified. Events can occur at the level of the chromosome (deletion or translocation of part of the chromosome), the gene (deletion, conversion or rearrangement), or the exon (deletion or duplication or nucleotide deletion, substitution or insertion). Examples of events associated with blood group antigen diversity are single nucleotide polymorphisms (SNPs); deletion; insertion; altered splicing mechanisms; intragenic crossing over; intergenic crossing over; gene conversion or other gene rearrangements.

SNPs: Substitution of a single nucleotide in the coding region of the gene, i.e. a G to an A, may be used as an example. This substitution can have one of three effects: 1) no amino acid change determined by the codon (silent mutation); 2) a change in the identity of the encoded amino acid (missense mutation); or 3) conversion of a codon that specifies an amino acid into a terminating codon (nonsense transcriptional sense mutation). Most antigens of antithetical blood groups arise from 'missense' variations. For example, a single nucleotide change in the gene coding for the C antigen of the Rh system resulted in the c antigen, and likewise a single nucleotide change in the gene coding for the E antigen resulted in the e antigen. Examples of 'nonsense' variations are seen in the Duffy and Colton systems.

Deletion: This is the loss of a single nucleotide or DNA segment. A deletion of nucleotides in multiples of three in the in-frame DNA sequence will alter the translation codon and result in the absence of one or more amino acids and probably encode a protein with different characteristics. Deletion of three non-sequential nucleotides in the DNA structure (out of frame), or in multiples other

than three (1, 2, 4, etc.) will result in alteration of the entire mRNA sequence, a new amino acid sequence and a new stop codon and, consequently, will determine the production of an inactive mutant protein. Some null blood group phenotypes arise as a result of deletion of nucleotides, exons or genes. Examples: Jk (a-b-), U- and D--.

Insertion: which is the addition of a single nucleotide or DNA segment. Like a deletion, insertion of a nucleotide into a given DNA sequence may cause a change in the DNA translation codon. The null phenotype in the Colton blood group system, i.e. Co (a-b-) is an example of a change caused by a nucleotide insertion.

Splicing: Intron removal is a well-defined process in which introns (non-coding sections of the DNA) are cut away by RNA processing and the exons are spliced together to produce translatable mRNA. A single nucleotide variation at either the donor site or the receptor site of the DNA may alter the splicing and generate removal of the exon before or after the site during the production of the mRNA, resulting in the code for the protein being missing. If this code was the template for a blood group antigen, the antigen would not be produced. The Jk (a-b-) phenotype of the Kidd blood group system is an example of this mechanism in action.

Chromosomal translocation: is the transfer of a segment from one chromosome to another non-homologous chromosome (i.e. a chromosome that is not from the same pair). This can occur in certain diseases (leukaemia, lymphoma, and myeloproliferative fibrosis), where parts of one chromosome are relocated to another chromosome. Examples of blood group antigens that may be altered by this mechanism are A, B, H, D, M and N.

Crossing over: is the reciprocal exchange of parts of a gene between homologous chromosomes (i.e. chromosomes from the same pair). This may occur during meiosis if the same gene has homologous areas aligned (intragenic crossover) or if two highly homologous genes misalign (intergenic crossover).

Intergenic crossing over occurs in the MNS, Rh and Ch/Rg blood group systems that are encoded by two adjacent homologous genes on the same chromosome.

Example of inheritance

The genes for the ABO blood group system have three major alleles: *A*, *B* and *O*. Only one of these can occupy the locus on a particular chromosome. The *A* and *B* alleles are co-dominant, and *O* is an amorph that does not produce any detectable characteristics. Therefore, when either *A* or *B* is inherited, it is always expressed. *O* is only detected in the absence of *A* or *B* when inherited in double dose (one from each parent); such individuals are

homozygous, and their genotype is *OO*. On the other hand, one cannot tell whether a group A (or B) individual is homozygous *AA* (or *BB*), or heterozygous *AO* (or *BO*) in routine laboratory testing. This may be established using molecular techniques or by doing a pedigree (family study).

Table 3 shows ABO blood group phenotypes and indicates the possible genotypes. To simplify explanation, group A has not been differentiated into subtypes in the table.

Table 4 shows that it is possible for parents who type in the laboratory as group A and group B (but who are genetically *AO* and *BO*, respectively) to have offspring of group A, B, AB or O.

Example of gene rearrangements

The Rh loci on chromosome 1 consists of two homologous genes: *RHD* and *RHCE*: the former encodes D and the latter encodes C/c and E/e antigens. The *RHD* gene arose through duplication of the *RHCE* gene at some point in primate evolution. The genes are close together ('tightly linked'), in opposite orientation and separated by an apparently unrelated small gene. Because of the homology and opposite orientation of the two Rh genes, many rearrangements between these two genes have occurred and resulted in hybrid genes. These arrangements are thought to allow 'hairpin' formation and exchange between them. Parts of the *RHCE* gene can join the *RHD* gene and parts of *RHD* gene can join the *RHCE* gene, generating new hybrid genes. That leads to hybrid proteins and new antigens and RH variants. This replacement of parts of CE explains how many 'partial D'

antigens are generated, i.e. D antigens that are missing some epitopes. Partial D individuals type as D positive and are able to make anti-D that is directed against the missing segment of the D antigen.

Key points

- Genetics is the study of heredity. Gregor Mendel is known as the father of genetics; his experiments provided proof of how characteristics are inherited.
- Genetic material is found in the cells of the body. Each cell contains a nucleus surrounded by cytoplasm, a substance that surrounds the nucleus and forms the remainder of the cell.
- The nucleus is the inside sac of the cell and contains chromatin.
- Chromatin is comprised of DNA, the basic molecules or code of genetic material.
- When the cell starts to divide, the chromatin becomes thickened and forms chromosomes.
- Chromosomes are rod-like structures found in the nucleus of the cell, which carry the factors of inheritance. Humans have 23 pairs or 46 chromosomes altogether, in every somatic (body) cell.
- A gene is a segment of DNA that encodes for a specific protein.
- Genes are the factors of inheritance on the chromosomes. There are many hundreds of these genes, inherited in pairs, one from each parent.
- Various forms of the same gene are called alleles. It is alleles that are either dominant or recessive, and not the genes.
- The locus is the specific position of a gene on a chromosome. The loci (plural of locus) of many genes have been mapped.
- Alleles (or allelomorphs) are the alternative forms of a gene determining a single characteristic.
- One allele from each parent will be found at the locus on the pair of chromosomes.
- Mitosis is the doubling division of somatic or body cells, each of which produces two daughter cells with 23 pairs of chromosomes.
- A diploid cell has 46 chromosomes or 23 pairs.
- Gametes give rise to germ cells comprising the female ova and the male sperm.
- Meiosis is the process of reduction division that gametes undergo. Paired chromosomes separate, only half the quota (i.e. 23 chromosomes, one from each pair) going to each of four daughter cells. This means that each gamete makes four haploid germ cells. In males, all the haploid cells produced are viable. In females only one of the four germ cells from the gamete develops into an ovum.

Table 3 ABO blood groups, phenotypes and possible genotypes

Blood group phenotype	Possible genotypes
A	<i>AA, AO</i>
B	<i>BB, BO</i>
AB	<i>AB</i>
O	<i>OO</i>

Table 4 Punnett diagram: inheritance of ABO group

	Paternal	
	<i>A</i>	<i>O</i>
Maternal <i>B</i>	<i>AB</i>	<i>BO</i>
<i>O</i>	<i>AO</i>	<i>OO</i>

- A haploid cell has half the complement of chromosomes; instead of 23 pairs, it contains 23 single chromosomes, one from each of the 23 pairs.
- A zygote is formed when sperm and ovum unite. This is called fertilization. The zygote has the full quota of chromosomes, half inherited from each parent. It continues to develop into a new individual (offspring) with characteristics inherited equally from both parents.
- Genotype is the product of a pair of genes that together determine a particular characteristic. Genes and genotypes are expressed in italics when written, with superscripts rather than subscripts, when necessary to differentiate alleles. The genotype of an individual cannot necessarily be deduced by appearance or physical manifestation of a characteristic but may require family studies or molecular analysis in the laboratory for accurate verification.
- Phenotype is the observable characteristic, in which only dominant or co-dominant traits are manifested. Recessive (silent) characteristics will only become apparent if inherited in double dose, one from each parent. However, phenotype may be used to determine probable genotype.
- An individual is said to be homozygous for a particular characteristic when he/she has two identical alleles (double dose) for that characteristic.
- An individual is said to be heterozygous for a particular characteristic when he/she has two different alleles (and therefore a single dose) for that characteristic.
- The sex cells or gametes carry out meiosis, in which four daughter cells are produced that carry half the complement of genetic material. It is called reduction division.
- Crossing over occurs during gametogenesis when chromosomes are thickened and closely entwined. The chromosomes may break and then re-join with the other member of the pair, meaning that genetic material is swapped between the two.
- DNA is a nucleic acid composed of four nucleotide bases: purines (adenine [A] and guanine [G]) and pyrimidines: (thymine[T] and cytosine [C]).
- Adenine-Thymidine (A-T) and Cytosine-Guanine (C-G) are complementary and 5'-3' direction of binding is in opposite directions on the two strands of double-stranded DNA.
- At the junction of an intron, there is a sequence of two nucleotides called the donor splice site, and in the junction of an intron to an exon is another sequence of two nucleotides called the receptor splice site.
- The splice sites interact to excise the introns when transcribing genomic DNA to mRNA.
- A mutation is an unplanned/chance genetic change within the chromosome that may result in the production of a distinctly new characteristic, which, when associated with meiosis, results in this new characteristic being passed on to subsequent generations.
- Polymorphism refers to the occurrence in a population of allelic variations (two or more alleles at the same locus) producing different phenotypes.
- Dosage relates to whether one or two similar alleles are present for a particular blood group antigen. If the alleles are the same, there may be more antigen expressed on the red cells. This dosage effect is sometimes demonstrated by the difference in antibody titration endpoint between cells that are homozygous, as opposed to cells that are heterozygous. For example, red cells with a double dose of X antigen (homozygous – genetically XX) may give stronger reactions on titration with an anti-X antibody than cells with a single dose of X antigen (genetically Xx).
- A dominant allele is one that is always expressed, whether in single (heterozygous) or double (homozygous) dose.
- A recessive allele is only expressed in the double (homozygous) dose.
- A silent amorph is a gene that does not produce any detectable trait or characteristic, such as the O gene of the ABO blood group system.
- Co-dominant alleles are those which, when inherited, are expressed even if present in a single dose. Co-dominant alleles determining the same characteristic may or may not have a modifying effect on each other, which in turn may affect expression.
- X-linked characteristics are those that are linked to the genes found on the sex chromosomes. Haemophilia A, XG and KX blood group systems are examples of sex-linked characteristics.
- Genetic linkage has also been demonstrated with autosomal genes.
- The most common genetic variation responsible for differences in blood antigens are single nucleotide polymorphisms (SNPs) in the coding region of a gene.

Blood group systems

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Introduction

The red cell membrane carries a great variety of surface proteins, as well as proteins that cross the lipid layer of the cell membrane itself. It is these surface proteins and glycoproteins that carry the blood group antigens and their specificity is mostly determined by the sequence of oligosaccharides (e.g. ABO) or the sequence of amino acids (e.g. Kell, Duffy, Kidd, MNS). These antigens are assigned to blood group systems or collections based on their relationship to each other as determined by serological or genetic studies. As of June 2019, the Red Cell Immunogenetics and Blood Group Terminology working party of the International Society of Blood Transfusion (ISBT) recorded that 38 blood group system genes have been identified and all known polymorphisms (alleles) sequenced. This section will primarily cover the ten major blood groups systems (ABO, Rh, MNS, P1PK, Kell, Duffy, Kidd, Lewis, Lutheran, and I) and provide some information on some of the other blood group systems. Also included is basic information about Human Leucocyte Antigens (HLA) and platelet antigens (HPA). Some references will be made to basic molecular structures, but detailed molecular structures and recent advances in DNA technology are not within the scope of this publication.

Since blood group antigens are genetically determined, the frequency distribution of the antigens often varies in different populations. It is important to know what antigen frequencies apply in your local population. Testing should relate to the antigen frequency in the population, and this may present a challenge when using red cell reagents produced in other parts of the world.

Where feasible, two references have been used for the percentages of different groups in the major blood group systems; those appearing in the first edition of this publication, and those from the Blood Group Antigen Facts Book (full reference needed here). Where possible we have identified the geographical location from which the information is derived, and the local ethnic groups are identified as “Black”, “Caucasian” and “Asian”.

Learning objectives

- By the end of the section, the student should have a good understanding of the following:

- Blood group terminology
- Cluster of differentiation
- Functions of blood groups
- ABO and H blood group systems
 - ABO grouping
 - Inheritance of ABO bloods groups
 - ABO blood group frequencies
 - Production of ABO antigens
 - H-deficient phenotypes and O_h phenotype
- Subgroups within the ABO system
- ABO system antibodies
- Clinical significance of ABO system
 - Clinical significance in transfusion
 - Clinical significance in haemolytic disease of the fetus and newborn
- Lectins (plant agglutinins)
- ABH secretors
- Unique features of the ABO system
- Rh blood group system
- Rh genetics and inheritance
 - Molecular studies
- Rh terminology
- Rh frequencies
- Rh typing
- Rh antigens
- Clinical significance of Rh system
 - Clinical significance in transfusion
 - Clinical significance in haemolytic disease of the fetus and newborn
- Unique features of the Rh system
- Other major blood group systems
- Other blood group systems
 - MNS
 - P1PK
 - Kell
 - Duffy
 - Kidd
 - Lewis
 - Lutheran
- I
- Additional blood group systems/collections/antibodies reacting with high and low prevalence antigens
- Polyagglutination
- Human Leucocyte Antigen (HLA)
 - Disease association

- Transplantation
- Transfusion
- Pregnancy
- Parentage testing
- Human platelet antigens (HPA)
 - Fetomaternal alloimmune thrombocytopenia

Blood group terminology

As of June 2019, 38 different blood group systems are known, ten of which are considered in this publication to be major blood group systems.

In addition, there are various blood group antigens that have been allocated to collections (the 200 series), low incidence antigens (the 700 series) and high incidence antigens (the 900 series). The ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology develops and maintains guidelines for blood antigen and alleles nomenclature and assigns newly recognized antigens and alleles to the appropriate system. Currently known system and alleles are shown on the working party section of the ISBT website. Tables 1 and 2 list blood group systems and their main antigens.

There are strict criteria for:

- The allocation of a blood group antigen to a new or existing blood group system. The antigen must be shown to be an inherited characteristic, it must be defined by a human antibody, the gene encoding it must have been identified and sequenced, and the chromosomal location must be known.
- The establishment of a blood group collection, which requires two or more antigens that are related serologically, biochemically or genetically, but do not fulfil the requirements for a blood group system.
- Inclusion into the 700 series (an inherited antigen with an incidence of less than 1% in most populations tested and distinct from other systems and collections).

- Inclusion into the 900 series (an inherited antigen with an incidence of >90% in most populations tested and distinct from other high frequency antigens).

Various terminologies have been used to describe the different blood group systems and their antigens and respective antibodies ever since the ABO blood group system was first described in 1900 by Karl Landsteiner. In 1980 an ISBT committee was tasked to devise a genetically based numerical terminology for red cell antigens. This is an ongoing process and new information regarding the antigens and candidate new antigens are reviewed by the committee on a regular basis.

The numerical terminology was primarily designed to facilitate computer input. The alternative terminologies are commonly used, both in everyday communication, in laboratories and in publications. In this section the ISBT terminology for the blood group system will be shown in brackets, preceded by "ISBT" for clarity. This terminology consists of one or more letters, a space and three digits e.g. ABO 001. We will use the more 'user-friendly' alternative names.

Note: The term group or type can be used interchangeably when discussing blood groups or types. Further notes on Rh terminology will be found in the Rh section.

The number of antigens within a blood group system, collection, and series varies tremendously from 1 in the I (ISBT I 027) system to 55 in the Rh (ISBT RH 004) and 49 in the MNS (ISBT MNS 002) system.

Cluster of differentiation

The cluster of differentiation (abbreviated to CD) is a protocol for the identification of cell surface molecules that provide targets for the phenotyping of cells. CD molecules often act as receptors for various other molecules, and some play a role in cell signalling. Some examples are CD8 found on cytotoxic T-cells and NK cells, and CD4 found on T-helper cells. In order to distinguish these cells from one another, they may be referred to as CD8 cells and CD4 cells. Some CD molecules carry blood group antigens, such as CD235a which carries the MN antigens of the MNS blood group system (ISBT MNS 002) and CD235b which carries the Ss antigens of the MNS blood group system. At the time of this publication, more than 370 unique CD clusters and sub-clusters have been identified.

Drawn by Elisabet Sjöberg Webster and reproduced with permission.

Functions of blood groups

The structures of the different blood group carrier molecules and their antigens have been studied extensively, and a wealth of information has become available, particularly since the development of molecular genetic techniques and the data from the human genome project.

Table 1 Ten major blood group systems

ISBT no.	Name of blood group system	Major antigens	Chromosome location no.
001	ABO	A, B, A ₁	9
002	MNS	M, N, S, s, U	4
003	P1PK	P ₁ , p ^k	22
004	Rh	D, C, E, c, e	1
005	Lutheran	Lu ^a , Lu ^b	19
006	Kell	K, k, Kp ^a , Kp ^b , Js ^a , Js ^b	7
007	Lewis	Le ^a , Le ^b	19
008	Duffy	Fy ^a , Fy ^b , Fy ₃	1
009	Kidd	Jk ^a , Jk ^b , Jk ₃	18
027	I	I	6

Table 2 Other blood group systems

ISBT no.	Name of blood group system	Main antigens	Chromosome location no.
010	Diego	Di ^a , Di ^b , Wr ^a , Wr ^b	17
011	Yt	Yt ^a , Yt ^b	7
012	Xg	Xg ^a	X
013	Scianna	Sc1, Sc2	1
014	Dombrock	Do ^a , Do ^b , Gy ^a , Hy, Jo ^a	12
015	Colton	Co ^a , Co ^b , Co3	7
016	Landsteiner-Wiener	LW ^a , LW ^{ab}	19
017	Chido/Rodgers	Ch/Rg	6
018	H	H	19
019	Kx	Kx	X
020	Gerbich	Ge2, Ge3, Ge4	2
021	Cromer	Cr ^a	1
022	Knops	Kn ^a , Kn ^b	1
023	Indian	In ^a , In ^b	11
024	Ok	Ok ^a	19
025	Ralph	MER2	11
026	John Milton Hagan	JMH	15
028	Globoside	P	3
029	Gill	GIL	9
030	RH associated glycoprotein	RHAG	6
031	Forssman	FORS	9
032	Jr	Jr ^a	4
033	Lan	Lan	2
034	Vel	Vel	1
035	CD59	CD59.1	11
036	Aug	Aug1	6
037	Kanno	Kanno	20
038	Sid	Sid	17

However, only a little is known about the function of the blood groups.

The red cell is a complex structure, and the red cell membrane contains many surface proteins that are anchored to the membrane, cross the lipid bilayer one or more times or are adsorbed onto the surface of the red cells. Many of the proteins expressed on the surface of the red cells are polymorphic and carry the different blood groups. Figure 1 shows the red cell membrane with representative blood groups.

The functions of some of the red cell membrane proteins have been identified, such as the carrier molecule of the Jr^a antigen of the JR blood group system (ISBT JR 032) that was identified as ABCG2, a breast cancer resistance protein (BCRP) that makes cancer cells more resistant to anti-cancer drug therapy. It has been designated as CD338.

Studies on the null phenotypes that occur in most blood group systems have contributed to knowledge of their function. For example, the Rh protein, which assists in the transport of carbon dioxide across the cell

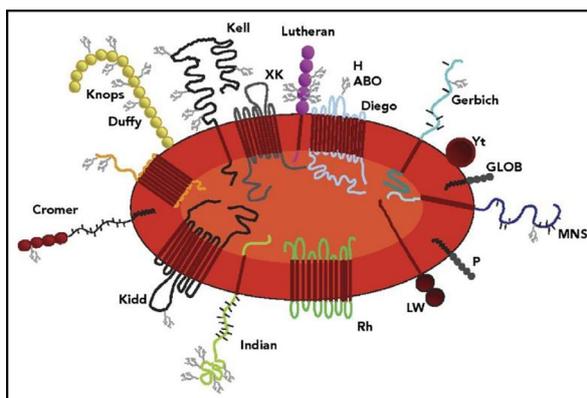


Fig. 1 RBC membrane with representative blood group antigens.

membrane, also has a structural role in maintaining the flexibility and flattened shape of the red cell. Absence of Rh antigens (i.e. the Rh null phenotype) is associated with structural changes to the red cell membrane that can produce haemolytic anaemia. The ABO, H, I, P1PK blood groups are carbohydrate structures on the red cell membrane glycolipids and glycoproteins and less is known about their function. Table 3 provides a list of the functions of the blood groups.

The format for describing the blood group systems in this publication is as follows:

- Antigen frequencies are given as approximate percentages, simply to make them easier to remember. This will sometimes result in the total being slightly more or less than 100%. The figures for Blacks apply to published data, or to surveys performed in southern Africa or published in the Blood Group Antigens Fact Book.
- When an antibody has an optimum reaction temperature of 37°C using the indirect antiglobulin technique (IAT), it is presumed to be an IgG antibody. These are generally clinically significant, being capable of causing *in vivo* destruction of antigen positive red cells.
- When an antibody is described as a saline agglutinin, reacting optimally at 4°C, it is presumed to be an IgM antibody. These are generally of no clinical significance (with the exception of ABO antibodies) unless they show reactivity at temperatures above 30°C or are capable of activating complement.

The ABO and the Rh blood group systems are the most clinically significant blood group systems.

Table 1 shows the major blood group systems. Note that H antigen is in a separate system, H (ISBT H 018), and is not part of the ABO system (ISBT ABO 001) Table 2 provides information on blood group systems other than the ten major systems.

Table 3 Functions of blood groups

Blood group system	Red cell function
Rh, Kidd, Kx, Diego, Colton, Gill, Jr, Lan, Aug, Kanno, RhAg	Membrane transporters
Kell, Yt, Dombrock	Membrane bound enzymes
MNS, Gerbich, Vel	Structural proteins
Duffy	Chemokine receptor
Lutheran, Landsteiner-Wiener, Xg, Indian, Scianna, Ralph, John Milton Hagan, Ok	Cell adhesion molecules
Cromer, Knops, CD59, Chido/Rodgers	Complement regulation
ABO, H, I, P1, P Sid, Forssman, Lewis	Carbohydrate structures which contribute to the glycocalyx

ABO and H blood group systems (ISBT ABO 001 and H 018)

Although the ABO and H are two different blood group systems genetically, they will be described together as they are closely related, both at the biochemical and phenotype level.

The ABO system is the most important blood group system in transfusion therapy and was the first blood group system to be described. This great contribution to medicine was made by Karl Landsteiner in Vienna, Austria, in 1900 when he observed that 'the serum of healthy humans not only has an agglutinating effect on animal blood corpuscles, but also on human blood corpuscles from different individuals'. The following year, in 1901, Landsteiner was able to recognise two antigens on the red cells by separating and mixing the cells and sera of several individuals. He called the antigens A and B. Those individuals with the A antigen on their red cells were called Group A; those with the B antigen, Group B. Many individuals lack the A and the B antigens and were termed Group C, which was later termed Group O (for the German "ohne" meaning "without" or "null"). The least common group, called AB, was found by two of Landsteiner's students in 1902. Group AB individuals express both the A and the B antigens on their red cells. Landsteiner found that the serum of an individual always contained antibodies to the antigen which was not expressed on that individual's red cells. Thus, Group A individuals will have anti-B antibodies in their serum and Group B individuals will have anti-A antibodies in their serum. These facts became known as Landsteiner's Rule which states, '(In the ABO system) the antibody to the antigen lacking on the red cells is always present in the serum or plasma.'

The regular presence of anti-A and/or anti-B antibodies means that it is critical for patient safety and good transfusion practice that ABO groups are performed, recorded and interpreted correctly prior to transfusion. ABO incompatibilities are responsible for the majority of

serious and/or fatal transfusion reactions and are usually caused by technical, clerical or administrative errors.

In 1930 Karl Landsteiner received the Nobel Prize in Physiology or Medicine for his work on blood types.

ABO grouping

As mentioned above, the ABO system is unique in that whenever the A or B antigens are not present on the red cells, the corresponding antibody is present in the plasma. Anti-A and anti-B isoagglutinins (also known as iso-haemagglutinins) are often referred to as being 'naturally occurring'.

ABO grouping can therefore be performed by:

- Typing the red cells for the presence or absence of the A and/or B antigens. This is known as forward grouping.
- Testing the serum/plasma for the presence or absence of anti-A and/or anti-B. This is known as reverse grouping.
- The forward and reverse grouping results should correlate; refer to Landsteiner's rule.
- The general population can then be divided into four ABO groups as shown in Table 4, based on the forward and reverse grouping.

It should be noted that the anti-A,B produced by a group O individual is different from anti-A + anti-B, which is a mixture of anti-A from one source and anti-B from another source. Anti-A,B detected in group O individuals is an antibody that will react with group A and group B cells. More information on ABO typing can be found in *Section 10: Donation testing*.

Inheritance of the ABO blood groups

The ABO genes are located on chromosome number 9 (9q34.1-q34.2). The inheritance in the ABO system is controlled by various alleles, four of which are common: A^1 , A^2 , B and O and a series of rare alleles, for example A^3 , A^x and A^m with a total of 286 alleles and 537 variants

Table 4 ABO groups

ABO Group	Antigens on red cells	Antibodies in serum/plasma
A	A	Anti-B
B	B	Anti-A
O	None	Anti-A, B
AB	A and B	None

being reported by March 2019. The *O* allele (which does not produce an antigenic product) is recessive to the *A* and *B* alleles, which are co-dominant. The ABO phenotype is shown by the grouping laboratory with ABO testing of a blood specimen, but the genotype of the individual is not obvious from these results. For example, the phenotype A_1 can result from one of several genotypes such as A^1A^1 , A^1A^2 , A^1A^3 , A^1A^x , and A^1A^m or A^1O .

Although each individual has two *ABO* genes, serological tests do not reveal the *O* allele in the *A* and *B* phenotypes, nor can an allele producing a weak form of *A* be recognised if an allele higher in the scale of *A* antigen production is simultaneously present. The genotype can, however, be determined by DNA analysis of the gene or may be determined by family studies. Table 5 shows ABO blood group phenotypes with possible genotypes (simplified), including some of the rare alleles.

ABO blood group frequencies

The frequency of the ABO blood group genes varies between different populations. Note the variation shown in Table 6 as an example of ABO blood group distribution.

Production of ABO antigens

The ABO red cell antigens expressed on the red cells are dependent on the presence of both the *H* (or *FUT1* gene) as described below, and the *ABO* genes. The loci for the *ABO* and *FUT1* genes are not linked (although they are functionally related) and they are therefore allocated to two separate blood group systems. The *FUT1*, *A* and *B*

Table 5 ABO group/phenotype and possible genotypes (simplified)

Blood group/phenotype	Possible genotypes
A_1	$A^1A^1, A^1A^2, A^1A^3, A^1A^x, A^1A^m, A^1O$
A_2	$A^2A^2, A^2A^3, A^2A^x, A^2A^m, A^2O$
B	BB, BO
AB	A^1B, A^2B, A^3B
O	OO

Table 6 Example of ABO blood group distribution in percentage (The Blood Group Antigen Facts Book, used with permission)

Group	Caucasian	Black	Asian
A	43	27	27
B	9	20	25
O	44	49	43
AB	4	4	5

genes do not code directly for red cell antigens, but for enzymes known as transferases. The H-transferase (fucosyltransferase 1, hence *FUT1* as the proper name for the gene) adds the sugar L-fucose to a precursor substrate, which is a carbohydrate chain already expressed on the red cell membrane. Once this has been performed, the 3- α -N-acetyl-galactosaminyltransferase (the enzyme produced by the *A* gene and for simplicity called A-transferase) and 3- α -galactosyltransferase (the enzyme produced by the *B* gene and for simplicity called B-transferase) can act. The A-transferase adds another sugar residue called N-acetyl-D-galactosamine, which results in the expression of A antigen on the red cells. Similarly, the B-transferase adds the sugar residue D-galactose and the cells then also express the B antigen. These red cells, as a result of the actions of the H-transferase, the A-transferase and the B-transferase, type as group AB.

Therefore, the group A antigen is expressed when the H- and A-transferases are the two enzymes present; the group B antigen is expressed when the H- and B-transferases are the enzymes present, and in the case of group O only the H-transferase is present. Figure 2 shows a simplified diagram to indicate the structural differences in the molecules that result in ABH antigen expression.

The expression of A, B or AB antigens results in a relative “masking” of the H antigen. Thus, A_1 , B or A_1B cells

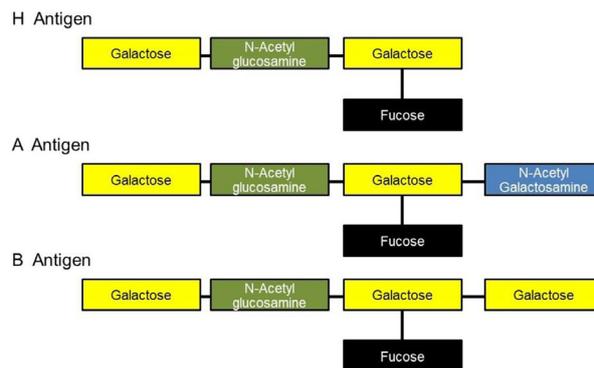


Fig. 2 Simplified diagram to indicate structural differences in ABH antigen composition.

express only small quantities of H antigen and Group O cells express the most.

The A^2 allele is less effective than the A^1 allele in masking the H determinant. A_2 cells therefore express considerably more H antigen and less A antigen than do A_1 cells. A_1 individuals express approximately 1 000 000 A antigens per red cell whereas A_2 individuals express only around 250 000 A antigens per red cell. The O allele in the homozygous state leads to the expression of H specificity alone, resulting in group O individuals having abundant H antigen.

The amount of H antigen that is detectable on red cells of different ABO groups, from left to right in decreasing order is as follows: most H antigen: O → Weak A → A_2 → A_2B → B → A_1 → A_1B → least H antigen.

The A, B and H antigens are detectable long before birth, although are expressed less strongly on the red cells of children than those of adults. The ABH antigen strength usually peaks at between two and four years of age and then remains relatively constant in most individuals. It may not be possible to distinguish serologically between group A_1 and A_2 groups at birth as the antigens may not yet be fully expressed.

H-deficient phenotypes

Although the ABO and H are two different blood group systems genetically (ABO Blood Group System: Number 001 and H Blood Group System: Number 018), they are closely related at the biochemical and phenotype level. The H-deficient phenotypes are very rare and include a total deficiency in H antigen (the O_h phenotype, often called the “Bombay” phenotype) or a partial deficiency (“para-Bombay” phenotype).

The O_h phenotype (Bombay phenotype)

The O_h phenotype, in which the cells lack the H antigen, arises when the individual has not inherited the very common *FUT1* gene. As there is no *FUT1* gene present, the H-transferase enzyme is absent. The precursor substance on the red cell remains unchanged and no molecules of L-fucose are present on the precursor substrate in the red cell membrane. The individual may have inherited the A and/or B genes, which code normally for the appropriate transferases. However, without the single terminal carbohydrate L-fucose at the α -1,2- position of the substrate protein, these transferases are non-reactive. The O_h phenotype, therefore, results when the individual has inherited homozygosity for the rare null allele *h*. The null *h* gene does not code for H-transferase. Individuals who have inherited one or two *FUT1* produce normal amounts of H-transferase.

O_h individuals are extremely rare. Those who were originally shown to carry the trait were individuals born

in India, whose ancestors originated in Bombay (now Mumbai), hence the “Bombay blood group”. There have been rare cases of O_h phenotype individual throughout the world, some with no apparent southeast Asian heritage, such as Italians living in Europe.

Their red cells are not agglutinated by anti-A, -B, -A,B or -H. O_h individuals usually have powerful anti-H and -A,B antibodies in their serum/plasma. To avoid serious transfusion reaction, recipients can therefore only be transfused with group O_h blood. Table 7 shows the difference between group O and group O_h blood.

Subgroups within the ABO system

Subgroups of A

About 10 years after the description of the ABO groups, the first subgroup of A was described. It was observed that not all group A bloods gave identical results when tested with anti-A from Group B individuals. It was realised, furthermore, that the common A antigen occurred in two forms: A_1 and A_2 . Later studies on transferase enzymes of A_1 and A_2 individuals showed that fewer antigenic sites are produced in group A_2 individuals as the enzyme is less effective in converting the precursor H substance into A antigen. However, with the use of monoclonal anti-A blood grouping reagents, little if any, difference between the reactions of A_1 and A_2 cells can be detected in the laboratory. In one survey in Southern Africa, about 99.9% of all group A bloods from Caucasians and about 96% of group A bloods from Blacks, were either A_1 or A_2 , with A_1 being more frequent than A_2 in both populations. A higher incidence of A_1 was detected in the Black population.

The anti-A found in the serum/plasma of group B individuals consists of two separate antibody specificities, anti-A and anti- A_1 , the latter being specific for the A_1 type. Group A or AB individuals who do not express the A_1 antigen may form an irregular, normally “cold-reacting”, anti- A_1 antibody in their serum/plasma. The lectin *Dolichos biflorus* (from which an anti- A_1 reagent can be prepared and standardised) or monoclonal anti- A_1 reagents, are usually used to type red cells for the A_1 antigen.

Table 7 Differences in blood groups O and O_h

Forward grouping	Reverse grouping						
	Anti-A	Anti-B	Anti-A,B	Anti-H	A cells	B cells	O cells
O	0	0	0	4	4	4	0
O_h	0	0	0	0	4	4	4

Further subgroups of group A and subgroups of group B

A number of other subgroups of group A and B have been described. The subgroups are caused by genetic variations that result in a variety of weakened expressions of the antigens. The subgroups cannot be detected when the gene for the weak antigen is inherited together with a normal *A* or *B* gene. The subgroups may be detected in the laboratory when weak or unexpected negative results are obtained with the forward grouping and/or anomalous results with the reverse grouping. For subgroups A_{e1} or B_{e1} , the presence of *A* or *B* antigens can only be demonstrated by an adsorption and elution technique with the corresponding antibodies.

Weak A

The term weak A covers a large range of reactivity, some bloods giving clear (although weak) results and other bloods giving such weak reactions that detection may prove difficult.

The weak A types include A_3 , A_m , A_x , A_{bantu} , A_{e1} , A_{finn} and A_{end} . Weak A type A_3 gives a characteristic mixed field agglutination pattern when tested with polyclonal anti-A and anti-A,B grouping agents. However, stronger agglutination is detected when using monoclonal blood grouping reagents. Table 8 compares reactions between groups and subtypes. Anti- A_1 may or may not be produced, although it is often produced by A_x individuals. Note that type A_3 shows mixed field agglutination with anti-A and anti-A,B and that type A_x reacts macroscopically with monoclonal anti-A,B

Weak B

Subgroups of group B are suspected when the expression of the B antigen is weak or cannot be easily detected. Subgroups of B are very rare and are found mainly in populations where the frequency of group B is high as in African and Far Eastern populations. The subgroup cannot be detected if inherited with a normal *B* allele. The

weak B subgroup may be inherited with an *A* allele giving rise to a normal A, weak B phenotype, AB_{weak} .

Acquired-B

Acquired-B is caused by the action of enzymes that deacetylate the group A_1 antigen N-acetyl-D-galactosamine to D-galactosamine which is similar to the structure of the group B antigen sugar residue (D-galactose). Some anti-B reagents, especially monoclonal reagents that contain clone ES4, react with the acquired-B phenotype and a group A individual could be incorrectly grouped as group AB. It is important to select anti-B grouping reagents carefully to ensure that they do not react with the acquired-B phenotype. The condition is rare but may be associated with gastrointestinal bacterial disease or caused by bacterial contamination of a blood sample. The individual's red cells often become polyagglutinable.

ABO system antibodies

Healthy adults who do not express a given ABO antigen on their red cells usually have the corresponding antibody in their serum/plasma as a result of stimulation from the environment, such as exposure to certain bacteria or food that may express A-, B- or H-like substances. Additional exposure to the antigen can result in more potent antibody formation.

This immune response may be induced by:

- Presence of ABO incompatible fetal red cells in the maternal circulation during pregnancy and/or at delivery.
- Injection of A or B substances that may be found in vaccines, either in the culture medium or on the micro-organisms themselves.
- The accidental transfusion or injection of ABO incompatible red cells.

Isoagglutinins that are weak or missing in adults may occur in weak subgroups of A or B, either hypogammaglobulinaemia or agammaglobulinaemia (patients with no, or low levels of serum globulins), twin chimerism, old

Table 8 Comparison of reactions: group A and subtypes

Monoclonal reagents Red cell phenotype	Reactions of serum/plasma with reagent red cells							
	Anti-A	Anti-B	Anti-A, B	Lectin anti- A_1	A_1	A_2	B	O
A_1	4	0	4	4	0	0	4	0
A_2	4	0	4	0	+ or 0	0	4	0
A_3	1 mf	0	1 mf	0	+ or 0	0	4	0
A_x	Micro+/1	0	1*	0	+ or 0	0	4	0

* A_x red cells can, and often do, give quite strong reactions with anti-A,B.

age or treatment with immunosuppressive drugs, or as the results of a bone marrow/stem cell transplantation.

Isoagglutinins in infants

Isoagglutinins are not normally detected in newborn infants but develop after three to six months of life due to exposure to A-like and B-like antigens in the environment. If ABO antibodies are detected in neonatal blood samples, they are usually agglutinating IgG antibodies of maternal origin. Table 9 shows the normal grouping results of a group B newborn and an infant of six months of age.

Anti-A₁

Individuals of phenotypes A₂, A₂B and weaker subgroups of A may have anti-A₁ in their plasma. This antibody will react with group A₁ cells. Anti-A₁ is usually a “cold-reacting antibody”, which is not of clinical significance. As it seldom reacts above 25°C, it is unlikely to cause transfusion reactions or haemolytic disease of the fetus and newborn (HDFN). It may, however, mask a clinically significant antibody.

Anti-A₁ occurs naturally in the plasma of about 2% of A₂ individuals and 26% of A₂B individuals. The antibody occurs more frequently as the strength of the A antigen decreases, therefore weak A (or weak AB) individuals are more likely to have anti-A₁ in their serum/plasma than A₂ (or A₂B) individuals.

Anti-H (other than O_h anti-H, -A, -B)

As individuals of group A₁, A₁B and B have very little H antigen expressed on their red cells, they sometimes develop anti-H in their plasma. This antibody can be recognised by its strong reaction with O red cells, a weaker reaction with A₂ cells and usually a failure to react with A₁ or B red cells. Anti-H of this nature, which is formed by individuals who are not H-deficient, is usually a benign autoantibody.

Group O serum

Group O serum is not a simple mixture of anti-A and anti-B. It cannot be separated by selective adsorption

using either group A or group B cells and is a cross-reacting antibody generally known as anti-A,B. Various theories have been suggested to explain this cross-reactivity (including Wiener's C theory) and it appears that the anti-A,B produced by group O individuals detects a structure common to both A and B antigens.

Clinical significance of the ABO system

Clinical significance in transfusion

Of all the blood group systems, the ABO is the most important in transfusion because the isoagglutinins are normally present in the absence of the corresponding antigen. Strong reactions take place when incompatible bloods are mixed with each other, not only *in vitro*, but also *in vivo*. Even an initial transfusion of group A blood into a group O or group B patient may be disastrous, because the naturally occurring anti-A in the blood of the recipient would react immediately with the incoming group A cells, activating complement and causing haemolysis of the donor cells. This would lead to an acute haemolytic transfusion reaction which may be fatal.

Universal blood donor

Group O individuals of are sometimes termed universal blood donors, as their blood can usually be safely infused into recipients of other ABO groups (heterologous group transfusion) because:

- They do not have A or B antigens on their red cells to react with antibodies within the circulation of the recipient.
- Their naturally occurring anti-A,B antibodies are not usually harmful to the red cells of the recipient if whole blood is transfused, provided the isoagglutinins are ‘low titre’ i.e. have a low level of ABO haemolysins.

However, whole blood from ‘high titre’ group O donors, which contains immune anti-A and/or -B, may only be transfused into group O recipients (homologous group transfusion). This is because these ‘dangerous’ universal donors have potent isoagglutinins with haemolysing characteristics in their plasma, which may cause severe haemolytic reactions when infused into recipients with A and/or B antigens on their red cells. The risk of transfusing harmful

Table 9 Newborn and infant ABO grouping results

Forward grouping Age of infant	Reverse grouping Anti-A	Interpretation					
		Anti-B	Anti-A,B	A cells	B cells	O cells	Group
Newborn	0	4	4	0	0	0	B
Infant of 6 months	0	4	4	3	0	0	B

anti-A and anti-B in blood group O whole blood can be reduced by the transfusion of group O red cell concentrates, from which most of the plasma has been removed.

In practice, however, it is better to transfuse a patient with blood of the same ABO group (ABO identical) and to conserve stocks of group O blood for group O patients and for emergency use.

Clinical significance in haemolytic disease of the fetus and newborn (HDFN)

Some individuals produce potent, high titre anti-A and/or anti-B, consisting of a mixture of IgM and IgG antibodies, with haemolysing characteristics in the presence of complement. This immune anti-A and/or -B in pregnant women can cause ABO HDFN with varying degrees of severity, although the fetus is rarely affected *in utero*. ABO HDFN typically develops within a few days of birth. See *Section 7: Haemolytic diseases*, for more information.

Lectins (plant agglutinins)

Certain plant extracts (usually seeds) agglutinate human and animal red cells. Two names have been suggested for these plant agglutinins: phytagglutinins and lectins, the latter term used for those which show red cell antigen specificity. Note that these substances are not antibodies. Lectins are sugar-binding proteins or glycoproteins of non-immunological origin. Some lectins are described in succeeding discussions:

Lectin anti-A₁

The most useful lectin, anti-A₁, is extracted from the seeds of *Dolichos biflorus*: the extract strongly agglutinates A₁ and A₁B cells; it reacts less strongly with A₂ cells and very weakly with A₂B cells. The extract can therefore be standardised by dilution as a specific anti-A₁ reagent. It also reacts with uncommon red cells that express the polyagglutinin antigens Tn or Cad.

Lectin anti-H

Lectin anti-H can be extracted from the seeds of *Ulex europaeus* or the common European gorse. *U. europaeus* is invaluable for the classification of group O secretor/non-secretor saliva (or group O secretor status) and confirming an O_h phenotype.

ABH secretion

In addition to being expressed on the red cells, A, B and H antigens are also expressed on most other tissues as

glycolipids and glycoproteins. Soluble blood group substances of the same ABO group as the red cells may also be found in the serum/plasma and are readily detectable in the saliva and other body fluids of most individuals.

The secretor status is controlled by the *SE* (or *FUT2*) gene on chromosome 19. *SE* is a dominant hemizygous gene and is responsible for the secretion of A, B and/or H. Approximately 80% of the general population secrete ABH substances (in the form of water-soluble antigens) in abundance in almost all their body fluids (not found in cerebrospinal fluid). There is no *se* allele and therefore “*se*” is used only to indicate the absence of *SE*. As there is no *SE* gene product in the absence of the *SE* gene i.e. in those individuals designated *se*, these individuals are “non-secretors” and produce no water-soluble ABH antigens. The ABO group of a secretor may be determined by testing the saliva to determine the presence or absence of A, B and H substance. The remaining 20% of the population are termed non-secretors. Table 10 shows the soluble antigens secreted according to ABO group.

Unique features of ABO and H systems

The critical unique feature of the ABO and H blood group systems is that unlike other blood group systems, the anti-A and/or anti-B isoagglutinins are invariably present in the serum/plasma of every healthy adult when the corresponding antigen is absent from their red cells.

As the ABO and H antigens are widely distributed throughout the body, the ABO group must be considered in organ transplantation. Some organs, e.g. the heart, must be ABO compatible with the recipient. In bone marrow transplantation, ABO incompatibility is acceptable because of the lack of expression of ABO on stem cells, but precautions need to be taken such as removal of the unwanted donor red cells or plasma. Note: Anomalous red cell typing may be seen post transplantation when an ABO incompatible graft was used.

Practical application

The cornerstone of safe blood transfusion practice is to transfuse safe blood of the compatible ABO group. It is

Table 10 Soluble ABH antigens according to ABO group

Group	Soluble antigens present
A secretor	A and H
B secretor	B and H
O secretor	Abundant H
AB secretor	A, B and a little H
Non-secretor	Not readily detectable

critical that the ABO group on all samples, whether from a patient or a donor, is correct, as ABO group mistyping can have fatal consequence

Rh blood group system (ISBT RH 004)

Number of antigens: 55 (2019)

CD numbers: CD240

The discovery of the Rh groups by Karl Landsteiner and Alexander Wiener in 1940, together with the work of Philip Levine and Rufus Stetson in 1939, heralded the greatest discovery in the blood grouping field since Landsteiner described the ABO system in 1900.

In 1939 Levine and Stetson described how the mother of a stillborn fetus suffered a severe haemolytic reaction when transfused with her husband's blood. The mother, who obviously lacked some 'new' antigen, must have been immunised by her fetus that expressed this antigen, having inherited the gene encoding it from the father. When the ABO compatible husband's blood was transfused, the maternal antibody reacted with this same antigen expressed on his red cells.

In 1940 Landsteiner and Wiener, having immunised rabbits with the blood of a rhesus monkey (*Macaques mulatta*), discovered that the resulting antibodies agglutinated not only the monkey red cells but also the red cells of about 85% of the Caucasians tested.

Later work, however, showed that the red cell antigens detected by the human-derived antibody and the animal antibody were not identical and in fact belonged to two different blood group systems. The blood group system detected by the human-derived antibodies is now known as Rh (not Rhesus or rhesus) and the antigen is called D. The antigen originally described by Landsteiner and Wiener is designated LW in the Landsteiner-Wiener blood group system (ISBT LW 016). The two systems are serologically, biochemically and genetically different from one another. The locus for the Rh genes (*RHD* and *RHCE*) is on chromosome 1 (1p36.11) and is linked to the gene for elliptocytosis. The locus for the *LW* gene (now called *ICAM-4*) is on chromosome 19 (19p13.2).

It was soon realised that the Rh antibodies produced in humans were not as simple as they had first appeared, and that many sera contained antibodies of more than one specificity. Many related antigens were found by workers in England and in the United States of America. This led to the discovery of the five major Rh antigens: D, C, E, c, and e. The Rh blood group system has now been shown to be one of the most complex multi-allelic blood group systems with 55 antigenic specificities having been described by 2019.

Rh genetics and inheritance

Most individuals are either D positive or D negative and the expression or absence of the D antigen on the red cells results from the presence or absence of an *RHD* gene. A D positive individual may inherit two *RHD* genes, one from each parent (in which case the individual is homozygous) or only one *RHD* gene from either parent (in which case the individual is hemizygous as no allele is present on the second chromosome). The two pairs of antithetical antigens C and c, and E and e are controlled by the various *RHCE* alleles. The *RHD* and *RHCE* alleles are inherited as a gene complex or haplotype. Note that genes are allelic, but antigens are antithetical (or allelomorphous). Genes cannot be antithetical (or allelomorphous), and antigens cannot be allelic.

Two different theories were initially proposed for the genetics and inheritance of the Rh blood group system, but these have subsequently been disproved by molecular genetic studies. They are described here because they are historically interesting, and because the terminology inherent in the theories is still widely used.

Fisher Race theory (UK – theory of three pairs of linked genes)

In 1943 the British statistician Ronald Fisher, studying the results of Robert Race and co-workers in England, noticed that some reactions were antithetical (opposite), and he theorised that there were three sets of alleles involved: C and c, D and d, E and e. Fisher assumed that the three genes, if separable, must be very closely linked, for no crossing over had been observed.

The CDE nomenclature was devised and although it did not accommodate subsequent complexities in the Rh system it was easy to use.

Although this theory suggested that antibodies to all the antigens described are able to be stimulated in individuals lacking the corresponding antigen, no anti-d has ever been found.

Wiener theory (USA – theory of multiple allelic genes)

This is a theory of multiple allelic genes occurring at a single chromosomal locus (rather than at three closely linked loci). One gene complex is inherited from the mother and one from the father. It was thought that each gene complex produced an agglutinin which had several serologic specificities (i.e. several distinct antigens). One agglutinin could react with various antibodies because it had as part of its structure more than one

antigen. The Rh-Hr nomenclature was developed to describe the gene complexes, agglutinogens and antigens.

Molecular studies

Following studies on the Rh blood group system at the molecular level, it has been shown that there are two Rh genes, *RHD* and *RHCE*, at the *RH* locus. The *RHD* gene primarily encodes for the D antigen. At the *RHCE* gene locus, depending on the allele present, one of four alternative antigenic combinations are primarily encoded for, namely *ce*, *Ce*, *cE* or *CE*. D positive individuals inherit two Rh genes: *RHD* coupled with one of the alleles of *RHCE* from each parent. In most D negative Caucasians, *RHD* is deleted, and individuals possess the *RHCE* genes only. As a result, most D negative individuals lack the total D protein (the D antigen) on their red cell membrane, but this does not appear to have an adverse effect on the cell function. Note that the genetic mechanism in many D negative Blacks is not deletion of the *RHD* gene, but the presence of the *RHD* pseudogene (*RHD*Ψ*) containing a 37 base pair duplication and a nonsense mutation. Figure 3 illustrates genomic organisation of RH genes.

The *RHD* and *RHCE* genes produce separate proteins, that are located in the red cell membrane next to each other, forming a complex of antigens. As the two gene loci are in such close proximity, many of the unusual Rh variants are the result of various genetic occurrences within the two loci, such as unequal crossing over, or mutations. Both the D protein and the RhCcEe proteins comprise 416 amino acid residues each. Whereas D- individuals generally lack the entire D protein, the difference between *ce* and *Ce* proteins are differences in four amino acid residues, three of which are transmembranous, and

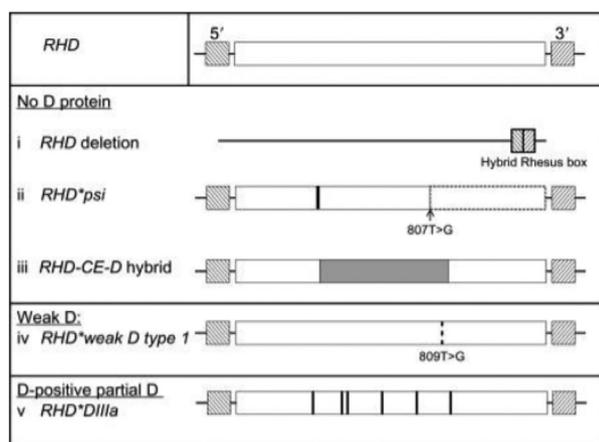


Fig. 3 Genomic organisation of RH genes in D positive and D negative haplotype and *RHD*Ψ* that contains 37 base pair duplication and a nonsense mutation from the Black ethnicities with the D negative phenotype.

only one extracellular, and *ce* and *cE* proteins differ by one amino acid residue, which is extracellular.

The presence or absence of the *RHD* gene, together with one of the four possible alleles of the *RHCE* gene, results in eight possible gene combinations or complexes. The Rh genotype is therefore a combination of any of the eight possible haplotypes, shown in Table 11. One *RH* gene complex or haplotype is inherited from each parent.

Rh Terminology

The eight possible gene complexes, or haplotypes are shown in Table 11. The symbol “d” denotes the absence of the D antigen. There is no *d* gene or *d* gene product and *d* therefore represents an *RHD* deletion or an inactive *RHD* gene. The eight Rh gene complexes, or haplotypes have each been allocated a shorthand notation, the symbol *R* indicating the presence of an *RHD* gene and D antigen and *r* the absence of the *RHD* gene and the D antigen. Table 12 shows phenotyping results using Rh antisera and the frequencies of some Rh genotypes in a UK population.

Numerical terminology

In 1962 Richard E Rosenfield introduced a new terminology for the Rh system based on a numerical system. Each antigen was numbered, as was the antibody detecting it. For example, the D antigen is Rh:1 and anti-D is anti-Rh1. Blood lacking the D antigen is noted as Rh:–1 (minus one). This numerical terminology lends itself to computerisation and is now the basis for the ISBT terminology for all the blood groups.

Rr and CDE Terminology hints

The Rh terminology can be very confusing and there are several different ways of documenting Rh. Below are

Table 11 RH genes together with gene complex (haplotype) and shorthand nomenclature

	Rh genes present			
	<i>RHD</i> gene	<i>RHCE</i> gene	Gene complex/haplotype	Shorthand nomenclature
D positive	<i>D</i>	<i>Ce</i>	<i>DCe</i>	<i>R</i> ₁
	<i>D</i>	<i>cE</i>	<i>DcE</i>	<i>R</i> ₂
	<i>D</i>	<i>ce</i>	<i>Dce</i>	<i>R</i> ₀
	<i>D</i>	<i>CE</i>	<i>DCE</i>	<i>R</i> _z
D negative	<i>d</i>	<i>Ce</i>	<i>dCe</i>	<i>r</i> '
	<i>d</i>	<i>cE</i>	<i>dcE</i>	<i>r</i> ''
	<i>d</i>	<i>ce</i>	<i>dce</i>	<i>r</i>
	<i>d</i>	<i>CE</i>	<i>dCE</i>	<i>r</i> ' <i>γ</i>

Table 12 Frequencies of some Rh genotypes in a UK population

Rh genotype	Phenotype results using Rh antisera					Percentage frequency
	Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	
R_1R_1	+	+	0	0	+	18
R_2R_2	+	0	+	+	0	3
R_1R_2	+	+	+	+	+	13
R_1r	+	+	0	+	+	35
R_2r	+	0	+	+	+	12
R_0R_0	+	0	0	0+	+	2
rr	0	0	0	+	+	15
$r'r$	0	+	0	+	+	0.4
$r''r$	0	0	+	+	+	0.8
$r'r'$	0	+	0	0	+	<0.1
$r''r''$	0	0	+	+	0	<0.1
$r'r''$	0	+	+	+	+	<0.1
R_2r	+	+	+	+	+	very rare
$r^y r$	0	+	+	+	+	very rare

several points that may be of assistance when using the shorthand notation:

- Whenever D is present, use the uppercase letter R together with the appropriate number or symbol (see second, third and fourth bullet points).
- The C antigen is associated with either $_1$ or $'$ (single prime). If C occurs with D use $_1$. When D is absent use $'$ (e.g. DcE = R_1 and dCe = r'). The $_1$ or $'$ indicates the C antigen and the e antigen are present.
- The E antigen is associated with either $_2$ or $''$ (double prime). If E occurs with D use $_2$. When D is absent use $''$ (e.g. DcE = R_2 and dcE = r''). The $_2$ or $''$ indicates the c antigen and the E antigen are present.
- When C and E are absent, but D is present, the notation R_0 is used. This indicates that the D antigen, c antigen and the e antigen are present.
- The phenotypes dCe and DCE are both very rare, so it seems logical to use y and z to describe them (r^y and R_z) the y and the z indicate that the c antigen and the e antigen are present.

Frequencies

The frequency of the eight possible Rh gene haplotypes varies between populations. For example, the gene combination R^0 is seen more frequently in various Black groups, particularly in sub-Saharan Africa than in Caucasian populations, whereas the haplotype r is more frequent in Caucasians than in Blacks. Table 12 gives the frequencies of most of the Rh genotypes in the UK population.

Rh typing

D typing

The D antigen is the most clinically significant antigen in the Rh system. Individuals are divided into D positive or D negative based on serological D typing results that detect the presence or absence of the D antigen. The frequency of the D antigen varies in different populations, e.g. in a mostly Caucasian population, 85% are D positive and 15% D negative but in Asians D negative individuals are extremely rare. On the other hand, in certain parts of the Iberian Peninsula, the percentage of D negative individuals approaches 25%.

Donors

Ideally a donor sample should be tested with two different monoclonal anti-D reagents and, if the test results concur, then the sample can be designated D positive or D negative. Anti-D reagents for donor typing should be selected to detect the majority of weak and partial D types, including D^{VI} . Donations giving weak or equivocal reactions are labelled D positive to avoid the risk of transfusion to D negative recipients.

Transfusion recipients

Patients who are to receive a blood transfusion should be tested with anti-D reagents that detect all commonly encountered D types. These reagents should NOT detect partial D^{VI} . It is not necessary to detect the very weak D variants because such patients should be typed D negative and should receive D negative blood.

Prenatal testing

Routine D typing is performed in pregnancy to determine the possibility of haemolytic disease of the fetus and newborn (HDFN) based on alloimmunization to D. Testing is performed using reagents that detect all commonly encountered D types, but not partial D^{VI} . Management may vary depending on weak D subtypes (see Clinical significance of weak D and partial D below).

Rh phenotyping

The Rh phenotype can be determined by typing the red cells with specific reagents; anti-D, anti-C, anti-E, anti-c and anti-e. Positive and negative test results using these reagents denote the presence or absence of the Rh antigens and this is known as the Rh phenotype.

Table 13 shows the variation in percentage frequency of various Rh phenotypes in one study of southern African populations. The symbol d is used to denote the absence of the D antigen.

Table 13 Variation in frequency of Rh phenotypes: a study of southern African populations

Rh reagent antisera					Phenotype	Ethnicity: percentage frequency			
Anti-D	Anti-C	Anti-E	Anti-c	Anti-e		Caucasian	Black	Asian	
+	+	0	+	+	DcE/dce	R ₁ r	33	13	30
+	+	0	0	+	DcE/DcE	R ₁ R ₁	18	< 1	48
+	0	+	+	+	DcE/dce	R ₂ r	13	15	4
+	0	+	+	0	DcE/DcE	R ₂ R ₂	2	< 1	< 1
0	0	0	+	+	dce/dce	Rr	14	2	3
+	0	0	+	+	Dce/dce	R ₀ r	3	66	2

It is not possible to determine the genotype of an individual from the red cell phenotype result, but the most probable genotype can be deduced using the haplotype frequency data. The phenotypes shown in Table 13 do not reflect one specific genotype. For example, cells which are phenotypically R₀ may be genotypically R₀R₀ or R₀r. Therefore, samples typed serologically as C-D+E- should ONLY be reported as R₀, NEVER as R₀r (or R₀R₀).

However, as the genotype incidence varies between different populations and ethnic groups, the 'probable genotype' result should be treated with reservation. It is an advantage to know the ethnicity of the individual being typed so that the appropriate frequencies can be used. The phenotype cannot determine if the individual is homozygous or hemizygous for the *RHD* gene as there is no *d* gene product. Accurate genotype determination can only be established by the use of molecular genetic techniques or by informative family studies.

Note that homozygous, heterozygous and hemizygous refer to the genes on the respective pair of chromosomes. Red cells cannot be homozygous or heterozygous/hemizygous for any blood group antigen. At best, they can have homozygous or heterozygous/hemizygous expression of an antigen.

Table 14 shows an example of a Rh phenotype and the possible genotype options, using figures derived from UK statistics. The probable genotype can then be determined.

Table 14 Rh phenotypes and possible genotype using UK-derived statistics

Rh reagent antisera					Phenotype	Possible option for Rh genotypes based on phenotype		Known frequency (%) of genotype (Caucasians)
Anti-D	Anti-C	Anti-E	Anti-c	Anti-e				
+	+	0	+	+	D+C+ c+e+	DcE/dce	R ₁ r	33*
						DcE/DcE	R ₁ R ₀	2
						Dce/dCe	R ₀ r'	< 0.1

*This is the probable or most likely phenotype for Caucasians.

It should also be noted that the presence of the very rare variant haplotypes, such as --- (*Rh_{null}*), Dc- and D- result in all or some of the Rh antigens not being expressed on the red cells and this will affect the possible genotype calculations.

Rh antigens

The Rh antigens are encoded by the *RHD* and *RHCE* genes, each of which produces a separate protein that is inserted into the red cell membrane. The D protein crosses the red cell membrane 12 times, giving rise to six extracellular domains. Despite many studies, the exact function of the Rh proteins within the red cell membrane is unknown, but their structure suggests a transmembrane transporter function. The structure and function of the D and CcEe proteins appear similar. In cases of the very rare type Rh_{null}, the absence of the Rh proteins has shown that the red cells are abnormal morphologically and individuals often suffer from some degree of haemolytic anaemia.

D antigen

The vast majority of all populations are D positive. The D antigen is extremely immunogenic and is likely to stimulate antibody production in D negative individuals exposed to the D antigen. It is the most clinically significant of the Rh antigens and plays a significant role in HDFN (refer to *Section 7: Haemolytic diseases*).

Weak D (previously termed D^u)

Weak D describes a weaker than normal expression of the D antigen, where fewer D antigen sites are expressed on the red cell, compared to normal D positive. Studies have shown that red cells of the presumed R₁r phenotype have about 10 000 D antigen sites per cell, whereas red cells of the presumed R₂R₂ phenotypes have about 33 000 D antigen sites per cell. Weak D cells have far fewer than this number, although the number is variable (between as few as 200 and 10 000). Weak D expression primarily results from single point mutations in *RHD* that encode amino acid changes predicted to be intracellular or in the transmembrane regions of D. These affect the efficiency of insertion, and, therefore, the quantity of D protein in the membrane, reflected in the reduced number of D antigen sites on the red blood cells. See Fig. 3 for an illustration of the *RHD* genes.

The term D^u for weak D is now obsolete. It was used to describe those forms of the D antigen that reacted weakly in laboratory tests when tested with different polyclonal anti-D reagents. With the use of monoclonal anti-D reagents many weak D types now react as D positive and cannot be distinguished from normal strength D in a routine laboratory. The identification of a weak D type will depend on the anti-D reagents selected for use and the technique used. If two potent, IgM agglutinating monoclonal anti-D reagents are used routinely for patient testing, most weak D samples will type D positive. Only the weakest form of weak D will be identified as weak or may be typed as D negative and such patients will receive D negative blood. The weak D type should, however, be detected in donor samples by either proceeding to the second (IAT) phase of a monoclonal blend reagent or by using agglutinating monoclonal anti-D reagents specifically selected for the ability to detect weak D.

Partial D

In 1953 there was a report of a D positive individual with anti-D in the plasma. Since then, many examples of D positive with anti-D have been reported, although overall it is a rare occurrence.

The term partial D is used to describe the phenotype of those rare individuals, whose red cells lack one or more of the D epitopes. The D antigen is considered to be a mosaic of epitopes. If some D epitopes are missing, or are mutated, then the individual can make an antibody specific for the missing epitope/s if they are exposed to normal D positive cells and, potentially, red cells expressing one of the weak D types, or even red cells from a different form of partial D. The anti-D produced in this way reacts with all normal D positive cells, which have all the

epitopes, but fails to react with their own cells and cells of the same or similar partial D types.

In their studies in the late 1950s, Alexander Wiener and Lester Unger used the Rh^A, Rh^B, Rh^C and Rh^D classification to describe the D mosaic antigen, but this terminology is now obsolete.

Patricia Tippett and Ruth Sanger used the Category Classification to describe partial D. Their original classification, using human sera, divided partial D bloods into six categories: I, II, III, IV, V and VI, with category VI expressing fewer D epitopes than the others. Note: category I is now redundant. The categories were shown to be inherited. The original framework of this study has allowed for the addition of further partial D type complexities, as more information became available. The use of epitope-specific monoclonal anti-D has enabled the partial D to be categorised further into categories II, IIIa, IIIb, IIIc, IVa, IVb, Va, VI, VII and many others (such as DAR and DOL). Panels of monoclonal anti-D reagents are now available to classify partial D types. The molecular structures of the many partial D types have been extensively studied and it has been shown that various *RHD* and *RHD-CE-D*, and *RHCE-D-CE* hybrid genes give rise to different partial D types.

Molecular *RHD* blood typing is very efficient for managing patients and donors carrying any of the various molecular types of weak D or partial D. With the advance of Next Generation Sequencing (a technique that has made genetic sequencing extremely rapid), the whole *RHD* and *RHCE* genes can be sequenced to identify the mutation.

Clinical significance of weak D and partial D

Weak D individuals do not usually produce anti-D, although isolated cases have been reported, whereas partial D type individuals may develop clinically significant anti-D.

Significance in patients:

- Weak D patients very rarely form anti-D. Antenatal patients identified to be 'weak D' will usually be regarded as D positive and therefore do not require antenatal or post-natal anti-D immunoglobulin. In particular, prenatal patients with weak D types 1,2, or 3, the most common forms of weak D in Caucasian populations, can be classified as D positive.
- Patients with partial D types other than D^{VI} are most commonly identified when an apparently D positive individual makes anti-D. Routine anti-D typing reagents will react with the majority of non-D^{VI} partial D types. Partial D individuals may be stimulated to produce anti-D if transfused with D positive blood. The anti-D can cause severe transfusion reactions.

- A partial D mother will not be identified as a candidate for anti-D immunoglobulin. Should she deliver an infant with normal D she may be stimulated to develop anti-D to the missing epitope(s). Future pregnancies may be complicated by HDFN.
- The most common partial D is D^{VI}. However, reagents selected for patient typing do not detect D^{VI} therefore D^{VI} patients will be managed as D negative.

Significance in donors:

- Blood donors should be tested for weak D, and some countries perform genotyping for *RHD* and, if weak D is present, their blood is labelled as D positive.
- Most partial D blood donors will be typed as D positive.

Trans effect of *RHCE**C

A weak D phenotype can occur as a result of the *trans* effect of *RHCE**C. (The * indicates that the letter or symbol following is the allele present in the gene complex. Thus *RHCE**C means that the *RHCE* gene complex contains the C allele.) If the haplotype encoding the D antigen is in *trans* (on the opposite chromosome) with a haplotype encoding the C antigen, but not the D antigen (e.g. *dCe*), the expression of the D antigen may be weak. This is known as the Ceppellini effect, after the principle author (the Italian geneticist Ruggero Ceppellini) on the first paper, published in 1955, to describe this effect. Family studies have shown that when the haplotype encoding the weak D is not in *trans* with C, in other words, it is in the *cis* position, then the D antigen is expressed normally. For example, *Dce/dCe* (D and C in *trans*) may type as weak D, whereas *DCe/dce* (C and D in *cis*) will type as normal D.

Cc and Ee antigens

The Cc and Ee antigens are less immunogenic than the D antigen and may demonstrate dosage effect depending on the reagent used. A number of variants, particularly variants of the e antigen, have been described, such as the rare hr^S and hr^B found mainly in Black populations.

C^w antigen and anti-C^w

Although the C^w antigen was originally thought to be antithetical to C, it has been shown to be antithetical to high frequency Rh antigen MAR. C^{w+} cells are almost always C+ although very rare examples of C^{w+}, C- individuals have been reported. Anti-C^w is not necessarily produced in response to a known red cell stimulus and may occur in combination with other antibodies to low prevalence antigens.

G antigen and anti-G

Red cells that are C+ or D positive are generally G+, although very rare exceptions have been reported. The Ce and CE proteins and the D proteins share an amino acid

sequence that is recognised by anti-G. Many if not most anti-C+D sera may therefore contain anti-G.

Anti-G will appear to be anti-C+D in routine laboratory testing. Transfusion management in all cases is therefore straightforward with C-D- blood.

A potential complication arises in the case of an antenatal patient with apparent anti-C+D because of the possibility this may be anti-G or anti-C+G without anti-D. In this case, the patient remains a candidate for anti-D immunoglobulin to prevent anti-D formation. Anti-G can be isolated from anti-G+C, anti-G+D and anti-G+C+D mixtures by a sequence of absorption and elution.

Clinical significance of the Rh system

Rh antibodies are most likely to be immune IgG antibodies that have been stimulated by exposure to foreign red blood cells either through pregnancy or transfusion. Examples of 'naturally occurring' IgM anti-E and anti-C^w are however, common.

The antibodies in the Rh blood group system can cause severe transfusion reactions and are second only to the ABO and H systems in this regard.

Practical transfusion measures to avoid Rh sensitisation

Because D antigen is so highly immunogenic, transfusion of D positive blood to D negative individuals should be avoided. In some circumstances, mainly as blood stock conservation measures when there is a shortage of D negative blood, D positive blood may be transfused into D negative patients. This can only be done when consequent development of anti-D is unlikely to be a major concern. For example, in emergency situations, D positive blood may be transfused to D negative males or to D negative women who do not have child-bearing potential.

Other Rh antigens are also immunogenic, though to a lesser degree than D. There is a risk that patients who lack the C, E, c or e antigens will be exposed to these antigens during transfusion. This often leads to the production of the corresponding antibodies.

While it is unnecessary for hospital laboratories to type all patients for Rh antigens other than D, there is a sound argument in favour of determining the Rh phenotype of patients likely to require repeated transfusions over multiple transfusion episodes, in order to prevent additional risks of sensitisation to Rh antigens other than D. For example, in the UK, it is standard practice to phenotype and/or genotype sickle cell disease patients for CeEe antigens as well as K and provide CcEe and K matched red cells to reduce the risk of alloimmunization.

All Rh antibodies reactive by IAT should be regarded as clinically significant and antigen-negative blood selected for transfusion into such patients. Weak, 'naturally occurring' Rh antibodies such as examples of anti-E and anti-C^w demonstrated by saline or enzyme techniques only, that fail to react by IAT, can be ignored. ABO group blood, which is D positive and untyped for these benign antigens maybe selected for crossmatch, and if compatible by IAT, may be safely given.

Clinical significance in haemolytic disease of the fetus and newborn

Rh antibodies, particularly anti-D and anti-c are capable of causing HDFN. (See *Section 7: Haemolytic diseases* for details.) Anti-D is the most common cause of severe HDFN and may be combined with other Rh antibodies, e.g. anti-D + anti-C.

Although 'enzyme only' reacting Rh antibodies may be detected (they often have anti-E specificity), the Rh antibodies that are always clinically significant and cause HDFN are IgG and are best detected using the IAT.

A fetomaternal haemorrhage (FMH), either during pregnancy or during delivery, acts in exactly the same way as a transfusion, although the volume of cells is usually much smaller. The mother may be exposed to antigens she lacks, but which the fetus expresses. The most common example of this is the D negative mother who has an FMH of D positive blood and develops anti-D, which may cause HDFN in subsequent pregnancies if the fetus is D positive.

HDFN usually increases in severity with each succeeding D positive pregnancy. The anti-D increases in titre and avidity with every FMH, followed by additional immunisation of fetal cells, so that eventually they may be of sufficient potency to cause intrauterine death of an affected fetus. Before the advent of prophylaxis (preventative treatment) with Rh immunoglobulin, anti-D was responsible for about 90% of severe HDFN cases.

Other Rh antibodies, particularly anti-c, may cause severe HDFN. For example, it is possible for a D positive (*DCe/DCe*) mother who has been stimulated to produce anti-c, to give birth to a D positive (*DCe/dce*) infant suffering from HDFN caused by anti-c.

Other major blood group systems

MNS blood group system (ISBT MNS 002)

Number of antigens: 49 (2019).

CD numbers: CD235a, CD235b

The M and N antigens were first described by Karl Landsteiner and Philip Levine in 1927. Although the MN

Table 15 MNS blood group frequencies in a Southern African population (excluding U which is a high prevalence antigen)

Phenotype	Caucasian	Black
M+N-	28	26
M+N+	50	44
M-N+	22	30
S+s-	11	3
S+s+	44	28
S-s+	45	69

types and Ss types are shown separately in Table 15, the two sets of antithetical antigens form a single blood group system. The MN antigens are situated on glycoporphin A (GPA, CD235a) and the Ss antigens on glycoporphin B (GPB, CD235b). GPA and GPB are sialoglycoproteins (sialic acid, sugar and protein) of the red cell membrane. The gene for the antigens within the MN blood group system is located on chromosome 4 (4q32.21). There are two amino acid residue differences between the M and N antigens on glycoporphin A and a single amino acid residue difference between the S and s antigens on glycoporphin B. Amino acid substitutions and hybrid GPA/GPB glycoporphins result in many of the large number of antigens identified within this system, e.g. M^g, Mi^a, St^a, and Dantu. The very rare null type M^k is the result of the absence of both red cell GPA and GPB.

The S and s antigens are associated with the high prevalence antigen, U. The rare phenotype S-s-U- occurs in 0.25% of Blacks and a rare variant form of U gives rise to an S-s-U+ phenotype, as a result of a mutant form of GPB.

Antibody characteristics

- Treatment of red cells with some proteolytic enzymes, such as bromelain, papain and ficin, denatures M and N antigens, variably denatures the S and s antigens, but not the U antigen, resulting in anti-M, anti-N, anti-S and anti-s not usually being demonstrable by enzyme techniques.
- Anti-M and anti-N are usually "cold agglutinins" reacting by saline agglutination techniques. Anti-N seldom reacts above 20°C.
- "Cold-reacting" anti-M often contains a large proportion of IgG immunoglobulins.
- Reactivity of some examples of anti-M and anti-S is enhanced by lowering the pH of the serum/plasma.
- Gel column technology is very sensitive for the detection of anti-M, because the gel has a low pH.
- Occasionally, anti-M and anti-N react by IAT.
- Anti-M frequently occurs in children where it may not be immune in origin and is often reactive by IAT at 37°C.

- Although anti-S may be naturally occurring, it is usually a complement-binding type IgG antibody. Anti-S is often produced together with antibodies to low prevalence antigens.
- Anti-U is usually demonstrable by IAT and enzyme methods.
- Monoclonal anti-M, anti-N and anti-S reagents are available for red cell typing and lectin anti-N (*Vicia graminea* or *Vicia unijuga*, *Bauhinia purpurea* and *B. variegata*) is available for N typing.
- Anti-s is quite rare.

Clinical significance

Clinical significance in transfusion.

- Anti-M and anti-N, unless reacting at 37°C, are generally considered not to be of clinical significance and have rarely been the cause of transfusion reactions. IAT crossmatch compatible blood, rather than antigen-negative blood, is usually given.
- Anti-S, anti-s and anti-U are considered clinically significant and may cause moderate to severe transfusion reactions. Crossmatch compatible and antigen-negative blood should be selected for transfusion.

Clinical significance in HDFN.

- Anti-M and anti-N have occasionally caused mild HDFN but are not considered to be of obstetric significance. However, there is gathering body of evidence that anti-M may be of greater clinical significance within Japanese and Chinese populations.
- Anti-S, anti-s and anti-U may cause moderate to severe, even fatal HDFN.

P1PK blood group system (ISBT P1PK 003)

Number of antigens: 3 (2019)

The P1PK blood group system was formerly called the P system and contained three antigens: P, P₁, P^k. In 1994 the P antigen was reallocated to the Globoside system (ISBT GLOB 028). In 2011 the NOR antigen was assigned to the P system, which was renamed P1PK in 2013.

The P1 blood group was described by Karl Landsteiner and Philip Levine in 1927, after injecting rabbits with human red blood cells and screening for antibodies by testing the rabbit sera against human red blood cells from different individuals.

The antigen was named P because this was the first letter after the already assigned M, N and O.

P1 is encoded for on chromosome 22 (22q13.2) The *A4GALT* gene encodes a 4- α -galactosyltransferase enzyme, which adds an α -galactose to paragloboside on the red cell membrane to create the P1 antigen.

The P1, p^k and NOR antigens are not primary gene products. They are located on glycolipids. The terminal linkage of each antigen is synthesised by the primary gene product (4- α -galactosyltransferase). All antigens in the P1PK systems are based on lactosylceramide, which is also the immediate precursor for the p^k antigens.

P and P_k are high frequency antigens. (P is the only antigen in the Globoside blood group system ISBT GLOB 028.)

The frequency of the P₁ antigen varies in different populations as shown in Table 16.

It is important to understand the differences in characteristics and clinical significance of anti-P and P1.

Antibody characteristics

- Anti-P₁ is naturally occurring, and usually an IgM antibody. It is commonly encountered as a cold agglutinin but occasionally reacts at 37°C. Some rare examples may bind complement and react by IAT.
- Anti-P₁ that binds complement and reacts by IAT may cause a transfusion reaction.
- Anti-P is found in the serum/plasma of all P^k negative individuals and will haemolyse P₁ and P₂ cells in the presence of complement. Anti-P is also found in cases of paroxysmal cold haemoglobinuria (PCH). PCH is a haemolytic disease that occurs mainly in children following a viral infection. The sera from such patients give a positive Donath-Landsteiner test (see *Glossary* for details).

Other antibodies that are not actually part of the P1PK blood group system are noted here:

- Anti-PP₁P^k (previously called anti-Tj^a) is a rare, potent antibody found in the very rare type pp individuals. The antibody reacts at all temperatures by all methods and is frequently present as a haemolysin. It causes transfusion reactions and is a potential cause of recurrent abortions. These abortions are induced because the placenta contains high numbers of P^k and P antigens which are targeted by the IgG antibodies. It rarely causes HDFN.

Clinical significance

Clinical significance in transfusion.

- Anti-P₁ is not normally considered to be clinically significant and it is not usually necessary to select

Table 16 Frequency of P1 antigen in percentage

Phenotype	Caucasian	Black
P ₁ + (or P ₁)	79	94
P ₁ - (or P ₂)	21	6

antigen-negative blood. Units found compatible by IAT at 37°C may be transfused.

- Anti-P and Anti-PP₁P^k are antibodies to high prevalence antigens and it is extremely difficult to find compatible blood for patients requiring blood transfusions.

Clinical significance in HDFN.

- Anti-P₁ has not been reported to cause HDFN.

Features of the P₁PK blood group system

- P₁ antigen is weakly expressed at birth.
- P₁ substance can be found in various flatworms, and hydatid (tapeworm) cysts in sheep livers. P₁ substance from avian sources, e.g. pigeon egg albumin can be used in inhibition tests.
- The frequency and avidity of anti-P₁ are increased in P₁- individuals suffering from helminth infestations (parasitic worm, e.g. hookworm).

Practical application

- The P₁ antigen varies considerably from individual to individual in antigenic strength and known strong P₁+ cells should be used for antibody detection.

Kell blood group system (ISBT KEL 006)

Number of antigens: 39 (2019)

CD number: CD238

The Kell blood group system was described in 1946, and was named after the proband, Mrs Kelleher. The antibody was named anti-K (Note: *NOT* anti-Kell). It caused HDFN in this case and has done so in numerous cases since. The Kell system alleles have been identified at the *KEL* locus on chromosome 7 (7q34). The Kell system is also associated with the Kx (ISBT XK 019) and the Gerbich (ISBT GE 020) blood group systems, which adds to its already quite considerable complexity.

The most commonly encountered antigens in the laboratory are the K and k antigens (Note: *NOT* Kell and Cellano). After the ABO and Rh antigens, the K antigen is the next most immunogenic. Table 17 shows the Kell blood group frequencies.

Many different Kell antigens are produced and the frequency of the antigens, other than the high and the low

Table 17 Kell blood group frequencies in percentage

Phenotype	Caucasian	Black
K+k-	0.2	0.1
K+k+	8.8	3.5
K-k+	91.0	96.5

prevalence antigens, varies greatly between populations. The well-known high prevalence antigens are k, Kp^b and Js^b.

The K₀ type is the rare null type in the Kell system and lack all the Kell antigens. K₀ individuals who have become immunised frequently produce anti-Ku, an antibody that reacts with all red cells except other K₀ cells. This makes the provision of compatible blood for such patients extremely difficult.

Red cells of the very rare McLeod phenotype show weakened expression of the Kell antigens. The degree of depression of the Kell antigens varies from individual to individual with the McLeod phenotype. Some express the antigens almost as strongly as normal individuals, while others express the antigens so weakly that by normal serological techniques they mimic K₀ red cells. The McLeod phenotype has an association with Chronic Granulomatous Disease (CGD), an inherited primary immunodeficiency disease, in which case the condition is known as the McLeod Syndrome.

Transient depression of Kell system antigens may also occur in warm auto-immune haemolytic anaemia, in microbial infections, and in cases of idiopathic thrombocytopenia purpura. Examples of auto-anti-Kp^b and anti-Ku have been reported.

Antibody characteristics

- Anti-K and anti-k are usually IgG immunoglobulins, reacting optimally by IAT and some examples also react by enzyme techniques.
- Some examples of anti-K react at temperatures below 37°C.
- Although anti-K is usually produced in response to stimulus by transfusion or pregnancy, 'naturally occurring' cases have been reported and are possibly due to bacterial infections such as *Escherichia coli*. This type of anti-K is usually transient.
- In the past, some examples of anti-K were reported to react weakly in tests using LISS or polybrene.
- If a patient develops anti-k it can be difficult to provide compatible blood because of the low frequency of k- blood. However, anti-k is a relatively rare antibody as few individuals are k-.
- The chemical dithiothreitol (DTT) denatures Kell antigens, so the detection of Kell antibodies is not possible if DTT is used to determine whether IgM or IgG antibodies are present in a sample or if a ZZAP solution that contains DTT and an enzyme such as papain or bromelain is used for auto-adsorptions.

Clinical significance

Clinical significance in transfusion. Anti-K and anti-k are clinically significant and can cause severe acute

haemolytic transfusion reactions as well as delayed transfusion reactions. K⁻ or k⁻ (i.e. antigen-negative) blood and crossmatch compatible blood should be provided to patients with anti-K or anti-k antibodies respectively.

The K antigen is highly immunogenic, and formation of anti-K has resulted from transfusion of K⁺ red cells units to K⁻ individual. Consequently, it is now common practice in the UK and many European countries to transfuse K⁻ females with child-bearing potential with K⁻ red cells to avoid the formation of anti-K. In patients requiring long term transfusion therapy, units matched for K (as well as the various Rh antigens) should be provided. In the UK appropriately 1/500 donors would be expected to be k⁻ and "wet" units are usually available from rare blood stocks.

Clinical significance in HDFN. Anti-K antibodies differ from the other blood group system antibodies that cause HDFN as they destroy early erythroid progenitor cells and suppress erythropoiesis, causing severe anaemia, and occasionally death of the fetus. High bilirubin levels are not a characteristic as the early erythroid progenitor cells are destroyed prior to haemoglobinisation. Bilirubin is therefore not released into the amniotic fluid as with Rh mediated haemolytic disease and consequently measurement of bilirubin concentration in amniotic fluid does not give an accurate indication of the severity of the disease. See *Section 7: Haemolytic diseases*, for more information.

Kp^a and Kp^b

The Kp^a and Kp^b antigens result from the presence of two allelic co-dominant genes in the Kell blood group system. The Kp^a antigen is a comparatively low prevalence antigen, being expressed on the red cells of about 2% of Caucasian individuals and is very rarely expressed on the red cells of Black individuals. The Kp^b antigen is a high prevalence antigen expressed on the red cells of more than 99.9% of all individuals. The antibodies are IgG, reacting optimally by IAT and are clinically significant both in transfusion and HDFN. The provision of Kp(b⁻) blood to individuals who have become immunised is difficult. Antigen-negative and IAT crossmatch compatible blood should be transfused when the intended recipient has the corresponding antibodies.

Js^a and Js^b

The Js^a antigen is expressed on the red cells of approximately 20% of Black individuals and less than 0.1% of Caucasian individuals. Anti-Js^a is commonly detected in a mixture of antibodies, rather than as a single specificity, especially in sickle cell disease patients, which increases the difficulty of finding suitable blood for transfusion.

The Js^b antigen is a high prevalence antigen found in more than 99.9% of all populations. The antibodies are IgG and react optimally by IAT. All examples of anti-Js^b have been detected in Black individuals.

Both antibodies have caused HDFN and transfusion reactions. Antigen-negative and IAT crossmatch compatible blood should be transfused. If the rare type Js(a+b⁻) blood is required it may be obtained through a rare donor registry.

General comment

The antibodies to the Kell system are usually of clinical significance and may cause severe or delayed transfusion reactions. The respective antigen-negative and IAT crossmatch compatible blood should be transfused. If the antibody is to a high frequency antigen in the Kell blood group system, it may be necessary to obtain the antigen-negative blood from a rare donor registry.

Duffy blood group system (ISBT FY 008)

Number of antigens: 5 (2019).

CD number: CD234

The Duffy blood group was described in 1950 by Marie Cutbush and Patrick Mollison. Anti-Fy^a was found in a haemophilia patient who had received multiple transfusions and the system was named after this patient. The Fy^a and Fy^b antigens are encoded by a pair of co-dominant allelic genes located on chromosome 1 (1q23.2). The difference between Fy^a and Fy^b is caused by a single amino acid residue variant within the protein.

The Duffy protein spans the red cell membrane seven times and the major (or α) protein contains 336 amino acid residues. There is also a minor (or β) protein containing 338 amino acid residues. The Duffy antigen, also known as the Duffy antigen receptor for chemokines (DARC), is a receptor of both the sub-families of chemokines (C-X-C and C-C).

The frequency of the Duffy blood group system antigens varies greatly between different populations. The silent recessive *FY* gene is very rare in Caucasians but occurs frequently in Blacks, particularly those living in malarial areas. Red cells lacking Duffy antigens are refractory to invasion by malaria parasites (such as *Plasmodium vivax* and *Plasmodium knowlesi*). This suggests an adaptive response to the disease or selective pressure due to the parasite; consequently, the proportion of individuals in certain African populations who do not express the DARC protein in their erythrocytes is high. Homozygous expression of the *FY* gene results in the Fy(a-b⁻) phenotype. Most examples of Fy(a-b⁻) in African Blacks are as a result of a homozygous mutation of the *GATA-1* gene in the promoter area of the Duffy gene, which

prevents the expression of the Duffy antigens *ONLY* on red cells (it is erythrocyte specific). The *FYB* gene is usually present (except in individuals from Papua New Guinea, where the *FYA* gene is often present, instead of the *FYB* gene). Such individuals rarely produce an anti-Fy3, however often they are stimulated to so do.

Table 18 shows the Duffy blood group frequencies.

Antigen characteristics

- Fy^a and Fy^b antigens are denatured by enzyme treatment (i.e. bromelin, ficin and papain, but not trypsin).
- Fy3 is expressed on all red cells, apart from those of the Fy(a-b-) phenotype and is resistant to enzyme treatment.
- Fy3 negative i.e. Fy(a-b-) is rare in Caucasians but not uncommon in Blacks where it may reach 68%.

Antibody characteristics

- Anti-Fy^a and anti-Fy^b are IgG and can be stimulated by transfusion and pregnancy, and both can cause HDFN.
- Anti-Fy^a and anti-Fy^b react by antiglobulin technique and are not detected by most enzyme techniques.
- Anti-Fy^a is often detected together with other red cell antibodies, particularly Rh antibodies.
- Anti-Fy^b is not frequently encountered but is not uncommon in Chinese populations.
- Anti-Fy3 was first found in an Fy(a-b-) Caucasian Australian multiparous woman who had also been previously transfused.
- Anti-Fy3 is a rare antibody found in Fy(a-b-) populations.
- Anti-Fy3 from individuals who not from one of the Black ethnicities reacts strongly with cord blood cells, while anti-Fy3 from Black ethnicities does not react or reacts only weakly with cord cells.
- Anti-Fy3 formation is usually preceded by formation of anti-Fy^a.
- Anti-Fy3 can cause haemolytic transfusion reactions and mild HDFN.

Kidd blood group system (ISBT JK 009)

Number of antigens: 3 (2019)

The Kidd blood group system was described in 1951 by Fred Allen, Louis Diamond and Beverly Niedziela and the first antigen found was named after the initials of the sixth child (John Kidd) of the first proband to make anti-Jk^a, which resulted in haemolytic disease of newborn. The blood group system was named after the proband.

Table 18 Duffy blood group frequencies in percentage

Phenotype	Caucasian	Black
Fy(a+b-)	20	9
Fy(a+b+)	47	1
Fy(a-b+)	33	22
Fy(a-b-)	0	68

The antigens Jk^a and Jk^b of the Kidd blood group system are encoded by a pair of co-dominant alleles located on chromosome 18 (18q11-12).

The frequency of the Kidd blood group system antigens varies between different populations. The Jk^a antigen is expressed more frequently in Black individuals, than in Caucasian.

The rare Jk(a-b-) or Jk:-3 phenotype (the null type in the Kidd blood group system), has been reported in some Polynesian and Finnish populations, and less frequently in other populations. It should be recognised that the 0.9% of Polynesians with the Jk(a-b-) phenotype could be an exaggeration as, according to one source (Issitt and Anstee), kinship was not taken into account. The urea lysis test is a useful test for screening for the rare Jk:-3 phenotype, as Jk:-3 cells, unlike Jk(a+) and/or Jk(b+) cells, are resistant to lysis by 2 M urea.

Antibody characteristics

- Anti-Jk^a and anti-Jk^b are IgG antibodies that can activate complement and are stimulated by transfusion or pregnancy.
- The detection of these antibodies can be difficult, because frequently they are weakly reactive and may show dosage, i.e. antibody reacts more strongly or reacts only with homozygous red cells, i.e. those carrying a double dose of the antigen.
- The antibodies react optimally by IAT, although some weak examples react only by enzyme IAT techniques and solid phase technique.
- Some examples may be complement-dependent and require the presence of activated complement before they can be detected.
- They are relatively rare antibodies and are most often present in serum/plasma containing other blood group antibodies.
- The avidity and the titre of the antibodies often diminish rapidly, and the antibodies may not be demonstrable in subsequent samples from the patient.
- Anti-Jk3 can be detected by IAT.

Clinical significance

- Both anti-Jk^a and anti-Jk^b (including weak examples) have caused severe acute haemolytic transfusion reactions due to an anamnestic response.

- Kidd antibodies can cause delayed haemolytic transfusion reactions.
- Antigen-negative and IAT crossmatch compatible blood should be transfused to patients with these antibodies. If further transfusions are required at a later date the antibodies may be difficult to detect at the time of the crossmatch or may no longer be demonstrable by routine serological techniques. Antigen-negative blood should be transfused to prevent a delayed transfusion reaction. Good records of patients with clinically significant antibodies should be kept so that the appropriate blood can be cross-matched. Anti-Jk3 should also be considered to be clinically significant. Compatible rare type Jk:–3 blood may need to be obtained from a rare donor registry.

The antibodies rarely cause HDFN. Table 19 shows the Kidd blood group frequencies.

Lewis blood group system (ISBT LE 007)

Number of antigens: 6 (2019).

The Lewis blood groups system was first described by Arthur Mourant in 1946 and named after one of the two donors in whom anti-Le^a was first identified. Lewis antigens are not actually intrinsic to the red cell membrane but are adsorbed onto the red cells from the plasma. The presence or absence of the Le^a and Le^b antigens is determined by genes at three different loci on chromosome 19:

- *H (or FUT1)*: responsible for production of H substance (the precursor substance for the A and B antigens).

Table 19 Kidd blood group frequencies (approximate) in percentage

Phenotype	Caucasian	Black	Asian
Jk(a+b–)	26	51	23
Jk(a+b+)	50	40	27
Jk(a–b+)	23	8	49
Jk(a–b–)/Jk:–3	Very rare	Very rare	<1*

*Polynesians.

Table 20 Summary of LE, A, B and H and SE gene interactions

Phenotype	Genes present	Comment (ABH)	Comment (Lewis)
Le(a+b–)	<i>LE sese</i>	ABH non-secretor	<i>LE</i> gene product acts on H precursor substance
Le(a–b+)	<i>LE Se</i>	ABH secretor	<i>LE</i> gene product acts on secreted H substance
Le(a–b–)	<i>lele Se</i>	ABH secretor	No <i>LE</i> genes are present
Le(a–b–)	<i>lele sese</i>	ABH non-secretor	No <i>LE</i> genes are present

- *SE (or FUT2)*: enables the A, B and H antigens to be secreted in most body fluids.
- *LE (or FUT3)*: which is the gene for the Lewis alleles. The symbol *le* is used to show the absence of the *LE* gene.

When the *Le* gene is present, the enzyme produced reacts with one of two possible H substrates. The preferred substrate is secreted H substance. This is produced when both *H* and *SE* genes are present. The secreted H substance is then converted into the Le^b antigen. If the secreted H substance is not present as a result of the absence of an *SE* gene, (or because of the inheritance of the very rare *hh* genes, i.e. O_h type), the enzyme product of the *LE* gene acts on the precursor H substance instead, giving rise to the Le^a antigen.

If no *LE* gene is present, neither the Le^a or Le^b antigens are produced, resulting in the Le(a–b–) phenotype.

Table 20 provides a summary of the Lewis blood groups, together with their interactions with the ABH and secretor genes.

The Le^a and Le^b antigens are adsorbed onto the red cells from the plasma, but the antigenic strength is variable and may change under various circumstances, e.g. pregnant women often type as Le(a–b–). Cord cells type as Le(a–b–) as the red cell Lewis antigens only develop during the first 12–15 months after birth.

The Le(a–b–) phenotype occurs more frequently in Black individuals than in Caucasians, as shown in Table 21.

Unusual phenotype of Le(a+b+)

The Le(a+b+) phenotype is caused by mutation(s) in secretor *FUT2* which reduces the efficiency of the *FUT2* enzyme, and as a result the Lewis *FUT3* enzyme become relatively more efficient and is thus able to compete more

Table 21 Lewis blood group frequencies in percentage

Phenotype	Caucasian	Black
Le(a+b–)	22	23
Le(a–b+)	72	55
Le(a–b–)	6	22

effectively for precursor. More Le^a means less H type 1 is made with less Le^b and ABH substance, hence the association of this phenotype with the partial/weaker secretor phenotype.

Antibody characteristics

- Anti-Le^a and anti-Le^b are often IgM, naturally occurring antibodies. They are usually developed by type Le(a–b–) individuals. The antibodies generally react best at temperatures below 37°C and react by saline and enzyme techniques. Some examples of anti-Le^a and anti-Le^b are IgG and react by IAT. Strong reacting IgG antibodies may cause *in vitro* haemolysis in the presence of complement.
- In the presence of multiple antibodies in a patient's plasma, the presence of Lewis antibodies can interfere with antibody identification; the use of Lewis substance for neutralisation can be helpful in resolving the problem.
- Some anti-Le^b reacts better with group O or group A₂ Le(b+) cells than with group A₁ or group B Le(b+) cells. These are termed anti-Le^{bH} as they react with cells with the most H antigen.

Clinical significance

- The majority of Lewis antibodies do not cause transfusion reactions.
- Generally, patients with anti-Le^a and anti-Le^b which reacts at 37°C can be transfused with crossmatch compatible blood. There are, however, some rare examples of Lewis antibodies, which react strongly at 37°C by IAT and activate complement. These antibodies should be considered to be of potential clinical significance, and it may be wiser to select antigen-negative units of blood for crossmatch.
- Lewis antibodies do not cause HDFN as fetal and newborn red cells do not normally express Le^a and Le^b antigens.

Lutheran blood group system (ISBT LU 005)

Number of antigens: 27 (2019)

CD number : CD239

Anti-Lu^a was described by Sheila T. Callender and Robert Race in 1945, and should have been named Lutheran, after the first Lu(a+) donor, but the writing on the label of the blood sample was misread as Lutheran.

The Lutheran glycoproteins (CD239) are immunoglobulins that bind the extracellular glycoprotein laminin. The protein is encoded by the *LU* gene which is located on chromosome 19 (19q13.2). Lu-glycoproteins may be involved in facilitating movement of maturing erythroid cells from the erythroblastic island of the bone marrow to

the peripheral circulation and may play a role in the migration of erythroid progenitors from fetal liver to the bone marrow. The *LU* locus is part of a linkage group situated on Chromosome 19, which includes the *SE*, *LE*, *H* and *LW* loci.

In addition to the two major antigens, Lu^a (a low prevalence antigen) and Lu^b (a high prevalence antigen), there are three further pairs of allelic co-dominant genes that control the antithetical Lu6 and Lu9 antigens, the antithetical Lu8 and Lu14 antigens, and the antithetical Au^a and Au^b antigens.

Nineteen other high prevalence antigens are part of the system. There is a very rare recessive type, which genuinely expresses no Lutheran antigens, and these individuals can be stimulated to produce anti-Lu3. Additionally, there is the In(Lu) dominant type, which is itself rare, but not as rare as the recessive type. This type does express Lutheran antigens very weakly (and normally types as Lu(a–b–) by routine serological techniques), but the antigens can be detected by adsorption elution. These individuals cannot produce anti-Lu3.

Table 22 shows the Lutheran blood group frequencies. The expression of Lutheran antigens is variable, and dosage effects may be seen.

Antibody characteristics

- The antibodies may be stimulated by pregnancy or transfusion.
- Anti-Lu^a is usually an IgM immunoglobulin reacting by saline techniques.
- Reactions with Lutheran antibodies often shows a typical 'mixed field/stringy' form of agglutination when viewed microscopically.
- Anti-Lu^b is usually an IgG immunoglobulin reacting optimally by IAT.
- Anti-Lu^a is seldom seen and rarely causes a problem in the crossmatching laboratory as compatible blood can be easily found.

Clinical significance

- Both anti-Lu^a and anti-Lu^b have been reported to cause mild or delayed transfusion reactions.
- IAT crossmatch compatible blood should be transfused. The provision of Lu(b–) blood may be

Table 22 Lutheran blood group frequencies in percentage

Phenotype	Caucasian	Black
Lu(a+b–)	<1	<1
Lu(a+b+)	7	7
Lu(a–b+)	93	93
Lu(a–b–)	Very rare	Very rare

difficult, but the antibody is seldom seen. In the UK, Lu(a-b-) or Lu(b-) red cell units are readily available from the rare blood stock upon special request.

Lutheran antibodies have not been reported to cause severe HDFN as the antigens are only weakly expressed on cord cells. In addition, even if the maternal antibodies are IgG, which is relatively rare, they are thought to be adsorbed onto fetal Lutheran glycoprotein occurring on the placental tissue.

I Blood group system (ISBT I 027)

Number of antigens: 1(2019)

The I blood group system was promoted to its own system in 2002 after the *I* (*GCNT2*) gene located on chromosome 6 (6p24.2) was identified as encoding the glycosyltransferase responsible for converting i-active straight oligosaccharide chains to I-active branched chains. Although the I blood group system is not one of the major blood group systems, it is included in this section as it is of practical importance. The I antigen is expressed on all normal adult red cells. The expression of I antigen varies with age and with disease, and the degree of expression varies considerably between individuals.

The I blood group system is associated with “cold-reacting” antibodies that are usually only detected at low temperatures and in some haematological diseases, where the thermal range may broaden.

All cord cells type as I–, because the I antigen is not well developed at birth. Cord cells may therefore be used as a source of I– cells for antibody identification tests. Within 18 months of birth, the I antigen develops and replaces the i antigen, because of the action of a-transferase enzyme using the i antigen as a substrate.

Rare variants of the i antigen exist; for example, the antigen i_1 is found as a rarity in Caucasian individuals, and the antigen i_2 is found as a rarity mostly among Black individuals. Natural antibodies to I are found in the serum/plasma of adults who express high levels of the i antigen; the presence of the i antigen in adults is caused by mutation of the I gene (*GCNT2*). Initially, no association between the adult i phenotype and disease was detected, but since 1972, it is known that there is a close link between the adult i phenotype and congenital cataracts in the Japanese population.

Antibody characteristics

- Anti-I is usually an IgM immunoglobulin that occurs frequently as a “cold-reacting” autoantibody or a “cold agglutinin”. It is seldom seen as an alloantibody.
- However, wide thermal amplitude auto-anti-I can cause cold auto-immune haemolytic anaemia, if the antibody can be detected at 30°C and above and may also be present in mixed type cold and warm reactive auto-immune haemolytic anaemia. Auto-anti-i is occasionally found in patients recovering from diseases such as infectious mononucleosis (glandular fever). Anti-I may be associated with anti-H, forming antibodies with anti-HI specificity.

Clinical significance

- Although most anti-I antibodies are clinically insignificant, some examples of auto-anti-I, have a wide thermal range (see above). Should these patients require blood transfusions, they may be given the “least incompatible” blood, warmed in a validated blood warmer before infusion. The patient’s samples may be difficult to type if the patient’s cells are auto-agglutinated. The red cells may need to be washed with warm saline before testing.
 - Crossmatching must be very carefully performed to ensure that the auto-anti-I is not masking clinically significant antibodies.
- I system antibodies have not been implicated in HDFN.

Additional blood group systems/collections/antibodies reacting with high and low frequency antigens

The blood group systems discussed so far in this section are either of clinical or practical importance. However, patients may produce antibodies to many other blood group antigens.

As the frequency of antigens may vary between population groups, it is often important to know the ethnicity of the patient, particularly when trying to find compatible blood for a patient with antibodies directed against an apparent high prevalence antigen or antigens. Such samples are usually referred to a reference laboratory that has rare high prevalence antigen-negative panel cells available.

Antibodies directed against high prevalence antigens are usually detected when the serum/plasma reacts with all samples tested, but the auto-control is negative. This indicates the presence of an alloantibody, not an autoantibody. Further tests are required to identify whether the antibodies are directed against a high prevalence antigen or whether they are a mixture of antibodies of different specificities. On rare occasions, such as anti-At^a in the Augustine blood group system (ISBT AUG 036), the underlying pathology can also give a clue to the probable specificity (in this case, pseudo-gout), as can the sex and

age, in many cases of anti-JMH in the John Milton Hagen blood group system (ISBT JMH 026).

Antibodies to the high prevalence antigen, Kn^a (Knops blood group system, ISBT KN 022) may be referred to as high titre, low avidity (HTLA) antibody. Chido/ Rodgers antibodies directed against the high prevalence antigens Ch and Rg (ISBT CH/RG 017) react with the complement C4 protein, which is adsorbed onto red cells. In the presence of these antibodies, exclusion of other antibodies, that may be clinically significant, is important. Antibodies directed against antigens within the Knops blood group system can be neutralised by CR-1 protein, while anti-Ch/Rg can be neutralised by ABO compatible pooled plasma. The identification of these antibodies may be time-consuming, and few are clinically significant.

The antibodies may be directed against the high prevalence antigens mentioned previously in the various blood group systems. Many of these antibodies are clinically significant and can cause severe transfusion reactions and HDFN. It may be extremely difficult to find compatible, antigen-negative blood and donations may have to be obtained from a local, national or international rare donor registry/frozen storage facility. For example, in the Ok blood group system (ISBT OK 024), initially a total of eight families with Ok(a-) individuals were identified in Japan. A further two cases, one from an Iranian and another of Hispanic origin, have been identified. Family members who would be suitable donors may also be tested to see if they lack the particular high prevalence antigen, as siblings are more likely to be compatible than donors in the random population.

Such samples are usually referred to a reference laboratory with a supply of rare panel cells of the null phenotypes such as Lan, Vel-, Jk(a-), and U-.

If the patient has an antibody directed against a low prevalence antigen, the provision of compatible blood should not be a problem. Antibodies to low prevalence antigens may be found by chance when crossmatching random units of blood or may be detected when using a panel that includes a panel cell sample positive for a low prevalence antigen.

Table 23 lists some of the rare phenotypes associated with different ethnic groups and the increased likelihood of patients producing antibodies of the corresponding specificity. Note that this should be used as a guideline only as there are always exceptions.

Polyagglutination

Polyagglutination is the term used to describe red cells that are agglutinated by almost all samples of adult human sera/plasma, but not by autologous serum/plasma or sera/plasma from newborn infants. The

Table 23 Rare phenotypes associated with different ethnic groups and increased likelihood of antibody specificity

Ethnic group	Increased likelihood of rare type		
	Blood group system	Red cell phenotype	Antibody specificity
Black	Rh	hr ^{S-}	Anti-hr ^S
	Rh	hr ^{B-}	Anti-hr ^B
	MNS	U-	Anti-U
	Kell	Js(a+b-)	Anti-Js ^b
Indian	H	O _h	Anti-H, -A, -B, -AB
	Indian	ln(a+b-)	Anti-ln ^b
	Kidd	Jk(a-b-)	Anti-Jk3
Caucasian	Kell	K+k-	Anti-k
	Kell	Kp(a+b-)	Anti-Kp ^b
	Vel	Vel-	Anti-Vel
	Yta	Yt(a-b+)	Anti-Yt ^a

polyagglutinable state may be transient or persistent. Transient polyagglutinable results from the exposure of normally crypt antigens by bacterial enzymatic activity (T, Tk, Th) during the course of an infectious process. For example, naturally occurring anti-T is present in the plasma of all individuals. It is formed after the exposure to T-antigens present on many gram-negative bacteria and vaccines. However, in paediatric patients with necrotising enterocolitis (NEC) and atypical Haemolytic Uremic Syndrome (aHUS), neuraminidase removes the sialic acid residues on red cells and exposes T-antigens. Transfusion of blood components with a high level of anti-T may result in severe haemolysis.

Persistent polyagglutination may be a consequence of somatic mutation leading to a cellular lineage characterised by an enzyme deficiency that results in exposure of a normally crypt antigen, Tn. Most human sera contain anti-Tn. Tn polyagglutination is regularly accompanied by leucopaenia and thrombocytopenia and has been associated with leukaemia. Other forms of persistent polyagglutination are due to the inheritance of rare blood groups or are associated with a hematologic dyscrasia.

Polyagglutination can be resolved with the help of a lectin panel. These are carbohydrate binding proteins, which are usually obtained from plant seeds or monoclonal antibodies, for example (anti-T).

Table 24 provides a summary of lectins that may be used to identify the specificity of polyagglutinable red cells.

Human Leucocyte Antigen (HLA) System

The human leucocyte antigen (HLA) system is the most polymorphic and complex region of the human genome.

Table 24 Lectin panel for polyagglutinable RBC

Lectin extracts	RBC					
	Normal	T-positive	Tk-positive	Th-positive	Tx-positive	Tn-positive
<i>Arachis hypoea</i>	0	+	+	+	+	0
<i>Salvia sclarea</i>	0	0	0	0	0	+
<i>Salvia horminum</i>	0	0	0	0	0	+
<i>Glycine soja</i>	0	+	0	0	0	+

The HLA system is part of the Major Histocompatibility Complex (MHC), a region of the human genome found on the short arm of chromosome 6 which contains 47 genes and pseudogenes with 13 023 alleles. The MHC genes and molecules are involved in regulating inflammation, the complement cascade, and the adaptive immune response.

The human MHC may be divided into 3 regions, class I, class II and class III. Figure 4 illustrates HLA locus, antigens and proteins products.

MHC class I genes:

Humans have three major MHC class I genes, known simply as HLA-A, HLA-B and HLA-C. These genes code for proteins found on the cell membranes of all nucleated cells and platelets, but not on red cells. On the cell membrane these proteins bind to viral and other fragments that have been transported from within the cell, and they are presented to the cells of the immune system.

MHC class II genes:

Humans have six major MHC class II genes, known as HLA-DPA1, HLA-DPB1, HLA-DOA1, HLA-DOB1, HLA-DRA, and HLA-DRB1. These genes code for proteins that are present on the surface of some of the cells of the immune system such as macrophages and dendritic cells. On the cell membrane these proteins present viral and

other fragments to the cells of the immune system, particularly to Helper T-cells.

MHC class III genes:

This region does not contain any HLA genes, but rather genes that encode different proteins with immune function, such as complement factors (C2, C4, Factor B) and tumour necrosis factor (TNF).

The HLA system is one of the most polymorphic human systems as it needs to recognise foreign antigens in the form of peptides from a wide variety of different pathogens. The differences between HLA proteins are localised primarily to the extracellular region of these molecules, which bind peptides and interact with T-cell receptors. The high degree of HLA polymorphism is likely the result of positive selection for human survival by enhancing the diversity in the repertoire of HLA-bound peptides. This is reflected in the many different HLA types found throughout the world. Some HLA types are more frequently found in certain populations.

Human leucocyte antigen Alloimmunization

The basis of HLA alloimmunization is the high degree of polymorphism since most individuals are likely to have different HLA molecules on the surface of their cells. Hundreds of HLA alleles have been identified through high-resolution typing, making the chances of two individuals having identical HLA phenotypes extremely low.

Exposure to foreign HLA molecules can occur due to pregnancy, transplantation and transfusion and can result in the production of HLA antibodies and activated lymphocytes in immunologically competent individuals. Female patients that have been primed by pregnancy are more likely to make HLA antibodies in response to transfusion or transplantation if the HLA mismatches are present in the transfusion or allograft.

Human leucocyte antigen alloimmunization is influenced by several patient and blood component factors and can be lessened (but not eliminated) by transfusion of leucocyte-reduced blood components.

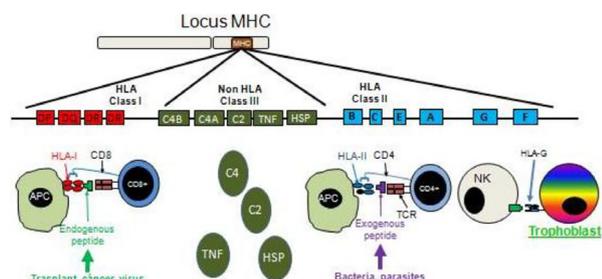


Fig. 4 Human leucocyte antigen (HLA) locus, antigens and proteins products.

Transplantation

The HLA system is one of the main barriers to transplantation between individuals. The immune response produced when a graft containing foreign HLA antigens is transplanted into an unrelated recipient can result in the production of donor-specific HLA antibodies or T-cells by the recipient to reject the graft. Graft survival can be improved when the donor and recipient are HLA matched. For example HLA matched sibling transplants have improved survival when compared to unrelated haemopoietic stem cell donor transplants.

The T-cell activation by the donor's HLA molecules after clinical transplant can be controlled by using appropriate immunosuppressive therapies. However, the major long-term problem is the development of donor-specific antibodies against mismatched HLA antigens. Controlling the antibody responses to mismatched HLA molecules is a challenge and needs continuous monitoring of their development.

Solid Organ Transplantation

Human leucocyte antigen matching has varying degrees of importance in solid organ transplantation depending on the type of organ being transplanted. Three major reasons why organs may be rejected, include:

- Hyperacute rejection where pre-formed antibodies directed against ABO blood group antigens and/or HLA class I antigens on the graft mediate irreversible damage to the transplanted organ within minutes or hours.
- Acute rejection predominately involves a cellular immune response, but donor-specific HLA antibodies can also be involved in binding to HLA molecules on the graft and mediate rejection through complement activation or antibody dependent cell-mediated cytotoxicity, usually weeks or months following transplantation.
- Chronic rejection is a long-term deterioration in graft function due to immune and non-immune causes. The number and severity of acute rejection episodes, infection and drug toxicity are just a few of the factors that can contribute to chronic graft rejection, months to years following transplantation.

Haemopoietic Stem Cell Transplantation

The only potential curative treatment for many patients with bone marrow and some metabolic disorders is haemopoietic stem cell transplantation (HSCT). The stem cells used for transplantation can be obtained from bone marrow, peripheral blood and umbilical cord blood.

The HLA system is the primary immunologic barrier to successful HSCT. Successful transplantation is dependent

on optimising the histocompatibility matching between the patient and the donor. This matching requirement is more stringent than for solid organ transplantation. International registries of millions of typed individuals exist to provide a possible matched donor for patients who do not have a matched family donor. Similarly, cord blood banks contain HLA typed cord blood units for use in transplantation of unrelated recipients.

Transfusion

Patients with an intact immune system, who receive multiple transfusions of platelets or leucocyte concentrates, or red cells containing platelets and/or leucocytes, can produce HLA antibodies directed against the mismatched HLA antigens present on leucocytes in the transfused blood product. Anti-HLA antibodies may lead to various types of transfusion reactions, discussed in detail in *Section 14: Risks of transfusion and haemovigilance*. The frequency of alloimmunization is reduced by the use of leucodepleted blood products.

Human Platelet Antigen (HPA) Systems

The molecular basis for the human platelet antigen (HPA) systems has been determined and a total of 39 platelet-specific alloantigens have been defined and their underlying molecular basis has been resolved. The systems are less polymorphic than HLA. Most antigens result from a single nucleotide polymorphism (SNP), which leads to a single amino acid substitution. HPA frequencies vary in different populations. Exposure to foreign platelet antigens following pregnancy, transfusion, or organ transplantation can result in alloimmunization.

The platelet antigens are located on the platelet membrane. The majority of the HPAs are located on the glycoprotein IIb/IIIa complex (integrin IIIb β_3). The detection of platelet antibodies is complex, and most methods are performed in specialized laboratories. With the development of molecular testing, it is possible to determine the HPA genotype.

In cases where patients develop platelet refractoriness during transfusion therapy, it may be necessary to provide both HLA and HPA matched blood products.

Fetomaternal alloimmune thrombocytopenia (FMAIT)

- The most common cause of severe thrombocytopenia in the fetus and newborn is FMAIT.
- Although FMAIT has been regarded as the platelet equivalent of HDFN, it occurs frequently during first pregnancies. In FMAIT, the antigen that stimulates

the maternal antibody response is found on fetal platelets where the fetus has inherited an HPA type from the father, lacking in the mother.

- In Caucasians, the most common antigens to stimulate the production of platelet antibodies are HPA-1a and HPA-5b which are the most immunogenic platelet antigens. FMAIT can also be caused by antibodies to low frequency HPA antigens.
- Paternally inherited platelet antigens, lacking in the mother, may stimulate the production of IgG maternal antibodies as a result of FMH. These IgG antibodies are able to cross the placenta and sensitize fetal platelets, causing thrombocytopenia.

- The diagnosis is usually made after birth when the neonate shows symptoms of thrombocytopenia, and platelet-specific antibodies are detected in the maternal serum/plasma.
- Because of severe thrombocytopenia, fetal/neonatal intracranial haemorrhage may occur.
- The antenatal administration of intravenous immunoglobulin to the mother may lead to an increase in circulating platelets in the fetus, reducing the likelihood of fetal intracranial haemorrhage. The reason for this is perhaps the modulation of maternal antibody production that may be induced by the administration of immunoglobulin.

Table 25 Some of the main characteristics of blood group systems and the corresponding antibodies

System	Antibody	Predominant type	Clinical significance	Comments
ABO	Anti-A			
	Anti-B	IgM/IgG	HTR and HDFN	Can cause fatal HTR and severe HDFN. Antibodies may be haemolytic.
	Anti-A,B			
H	Anti-A ₁	Mainly IgM	Rare	
	Anti-H	Mainly IgM	Low risk	Weakest reactions with group A ₁ and B cells.
	Anti-H ₁ -A ₁ -B	IgM/IgG	HTR and HDFN	Bombay O _h type
Rh	Anti-D	Mainly IgG	HTR and HDFN	Main cause of HDFN.
	Anti-C			Often occurs with anti-D.
	Anti-E	IgG/IgM		May be naturally occurring and react with enzymes only.
	Anti-c	IgG		Severe HDFN may be produced with anti-E.
	Anti-e			Not often seen. If individual is a variant e type, antibodies may appear to be anti-e but individual types e ⁺ .
MNS	Anti-M	IgM	Rare HTR	Antigen denatured by enzyme treatment. May show dosage.
	Anti-N		Low risk	
	Anti-S	IgG/IgM	HTR and HDFN rare	Antigen denatured by enzyme treatment.
	Anti-s		HTR and HDFN	Rare antibody
	Anti-U	IgG	HTR and HDFN	Antibody to high frequency antigen, compatible blood from rare blood registry.
P ₁ antigen	Anti-P ₁	IgM	Low risk	Common cold agglutinin produced by P ₁ - individuals. P ₁ substance used in inhabitation tests.
Lutheran	Anti-Lu ^a	IgM mainly	Low risk	Low frequency antigen
	Anti-Lu ^b	IgG/IgM	HTR and HDFN rare	Antibody reaction and clinical significance variable.
Kell	Anti-K	IgG	HTR and HDFN	Can cause death <i>in utero</i> .
	Anti-k			Rare antibody, difficult to find compatible blood.
	Anti-Kp ^a			Rare antibody
	Anti-Kp ^b			Rare antibody, compatible blood from rare donor registry.
	Anti-Js ^a			Very rare antibody
	Anti-Js ^b			Antibody to high frequency antigen, compatible blood from rare donor registry.
Lewis	Anti-Le ^a	IgM usually	Type IgG: rarely causes HTR	Antigen absorbed from plasma onto red cells.
	Anti-Le ^b			Antigen loss in pregnancy.
	Anti-Le ^a + Le ^b	IgM/IgG		Produced by Le(a-b-) individuals.
Duffy	Anti-Fy ^a	IgG	HTR and HDFN	Antigen denatured by enzyme treatment.
	Anti-Fy ^b		HTR	Antigen denatured by enzyme treatment, rare antibody.
Kidd	Anti-Jk ^a	IgG	HTR, delayed HTR, and HDFN	May require complement for IAT detection.
	Anti-Jk ^b			
I	Anti-I	IgM usually	Low risk	Often detected, seldom clinically significant. Soluble I antigen is secreted in human milk.

- Although the introduction of antenatal screening has been considered, there is generally a lack of optimal antenatal treatment. The safety of fetal blood sampling and platelet transfusion is also a concern. Most laboratories are unable to predict the severity of FMAIT based on antibody characteristics.
- As in the case of HDFN, premature induction of labour may also be recommended. It appears that caesarean section may be preferred to natural birth, in order to avoid cranial trauma at the time of delivery.

The practical problem is that most cases are diagnosed postnatally. Time is important as the condition should be treated promptly, preferably with HPA compatible platelets although random donor platelets, or (rarely) washed and irradiated maternal platelets, may be used. Most transfusion services do not have HPA matched platelets available as this would require the use of a panel of HPA typed donors donating regularly to provide an available unit in stock or available on call.

Summary of section: Blood group systems

Table 25 summarizes the characteristics of the blood group systems and their corresponding antibodies. Haemolytic transfusion reaction has been abbreviated as HTR.

Key Points

- The major histocompatibility complex (MHC) contains the genes of the highly polymorphic human leucocyte antigen (HLA) system and is located on the short arm of chromosome 6.
- The MHC also contains the genes for some cytokines and various complement components.
- The HLA gene encodes two types of molecule, HLA class I (HLA-A, B, C) and HLA class II (HLA-DR, DQ, DP).
- HLA class I molecules are found on nearly all nucleated cells and both HLA class I and II molecules are found on cells of the immune system which is a major immunological barrier to successful transplantation.
- The role of HLA is to present peptides to the immune system. These peptides may be derived from pathogens, altered self-cells (virus-infected, tumoral) or foreign tissue.
- The HLA system needs to be very polymorphic to be able to recognise the many different types of pathogens that humans may encounter throughout the world. This is why HLA matching between unrelated individuals is difficult.
- Exposure to foreign, mismatched HLA can occur as a result of pregnancy, transfusion or organ transplantation and can lead to the production of antibodies or the development of immune cells that are directed against the foreign HLA.
- The introduction of universal leucodepletion for blood and blood products has reduced the incidence of alloimmunization to HLA in some patient groups.
- The human platelet antigen (HPA) systems are less polymorphic and the frequency of the different genes varies in different populations.
- Exposure to foreign HPA following pregnancy, transfusion, or organ transplantation can result in alloimmunization.
- The HPA-1a and HPA-5b are considered to be the most immunogenic platelet antigens in populations of Caucasian origin and most cases of FMAIT are the result of anti-HPA-1a.
- In cases where patients develop platelet refractoriness during transfusion therapy it may be necessary to provide both HLA and HPA matched blood products.
- The most common cause of severe isolated thrombocytopenia in the fetus and newborn is FMAIT. Although it has been regarded as the platelet equivalent of HDFN, FMAIT occurs frequently during first pregnancies.
- The diagnosis is usually made after birth when the neonate shows symptoms of thrombocytopenia and platelet-specific antibodies are detected in the maternal serum/plasma.
- The practical problem is that most cases of FMAIT are diagnosed postnatally. Time is important as the condition should be treated promptly with HPA compatible platelets. Most transfusion services do not have HPA matched platelets available.

Haemolytic diseases

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Introduction

Anaemia has a variety of causes with iron deficiency and anaemia of inflammation being the most common worldwide and across all age groups. Haemolytic anaemias, while not as common, are an important subtype of anaemia which may have both inherited and acquired causes.

Haemolytic anaemia is characterised by enhanced destruction and clearance of red cells *in vivo*. Normal red cells survive for approximately 120 days in the circulation. In haemolytic anaemia, the premature destruction of red cells leads to a significantly shorter lifespan. The classification of haemolytic anaemia can be based on the mechanism of enhanced red cell destruction and whether the problem is intrinsic or extrinsic to the red cell. In practical terms, it is important to distinguish causes of haemolysis which have an immune-mediated cause, as these conditions rely on blood bank testing for diagnosis and management.

In this section an approach to the classification of haemolytic anaemia will be provided followed by a more detailed discussion of immune-mediated haemolytic anaemia with a focus on haemolytic disease of the fetus and newborn (HDFN) and autoimmune haemolytic anaemia (AIHA).

The discussion of HDFN will include an overview of how antibodies from the mother cause harm to fetal red cells. The laboratory testing and strategies needed to identify and prevent this disorder will be reviewed. A brief discussion of treatments for HDFN, both *in utero* and in the postnatal period will be provided, including the role of intrauterine and exchange transfusion.

Causes of AIHA will be explored (warm autoantibodies, cold autoantibodies and drug-induced immune haemolysis) along with the role of routine and more specialised blood bank tests in the diagnostic workup. The key clinical features and management of these conditions will be discussed briefly.

Learning objectives

By the end of this section, the student should be able to describe the following aspects of these conditions:

[Correction added on 17 December 2020, after first online publication: Melanie Bodnar has been added as 'Reviewer for Second Edition'.]

- Approach to haemolytic anaemia
 - Types and mechanisms of non-immune haemolytic anaemia
 - Intrinsic red cell disorders causing haemolysis
 - Paroxysmal Nocturnal Haemoglobinuria (PNH)
 - Pathophysiology
 - Diagnostic testing
 - Treatment
 - Extrinsic factors causing haemolysis
- Haemolytic Disease of the Fetus and Newborn (HDFN)
 - Pathophysiology
 - Clinical progression
 - Commonly implicated antibodies
 - Routine antenatal laboratory screening tests
 - Antenatal clinical management
 - Postnatal laboratory testing
 - Postnatal management of neonatal anaemia and hyperbilirubinaemia
 - Prevention of HDFN due to anti-D
- Autoimmune-mediated haemolytic anaemia (AIHA)
- Classification
- Warm autoantibodies
 - Pathophysiology and clinical features
 - Laboratory testing
 - Management and transfusion
- Cold autoantibodies
 - Pathophysiology and clinical features
 - Laboratory testing
 - Management
- Paroxysmal Cold Haemoglobinuria (PCH)
 - Pathophysiology and clinical features
 - Laboratory testing
 - Management
- Drug-induced immune haemolytic anaemia
 - Pathophysiology and clinical features
 - Laboratory testing
 - Management

Approach to haemolytic anaemia

There are many ways to classify haemolytic anaemias: inherited versus acquired, acute versus chronic, immune versus non-immune, and intravascular versus extravascular site of destruction. An approach based on whether the cause of haemolysis is intrinsic to the red cell (related to

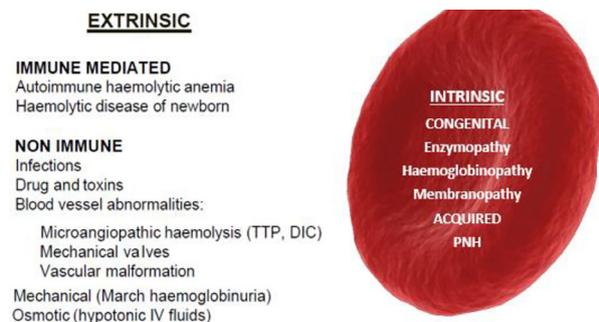


Fig. 1 Approach to haemolytic anaemia.

a defect within the red cell) or extrinsic to an otherwise normal red cell is highlighted in Fig. 1.

Haemolysis caused by factors intrinsic to the red cell

Most of the causes of haemolysis that are intrinsic to the red cell are inherited conditions with which the patient is born. PNH is a notable exception, see below. While some of these inherited conditions may present in infancy, in others, the diagnosis is not made until later in life when an infection, drugs or pregnancy unmask the problem.

Intrinsic red cell abnormalities that lead to premature red cell destruction include the following:

- Enzymopathies such as glucose-6-phosphate dehydrogenase (G6PD) deficiency: Red cell enzymes are important in maintaining the integrity of the red cell. Patients with inherited G6PD deficiency are susceptible to oxidative haemolysis triggered by certain drugs, foods or chemicals. The degree of severity can be quite variable.
- Haemoglobinopathies such as sickle cell disease: Inherited defects in the genes encoding the haemoglobin alpha or beta chains may lead to changes in red cell, shape, survival and function.
- Membranopathies such as hereditary spherocytosis: This group of red cell disorders is caused by mutations in genes that encode the proteins of the red cell membrane leading to red cell deformity and shortened red cell lifespan in the circulation.

Paroxysmal nocturnal haemoglobinuria (PNH)

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare acquired clonal bone marrow disorder that manifests as haemolytic anaemia, thrombosis and cytopenia. It can occur on its own or in association with other bone marrow failure syndromes such as aplastic anaemia and myelodysplastic syndromes. An acquired somatic

mutation in the *PIG-A* gene leads to the absence of several blood cell membrane proteins, notably CD55 and CD59 on the red cell, which results in uncontrolled complement activation and red cell haemolysis. Both intravascular and extravascular pathways of haemolysis occur. When intravascular haemolysis is exacerbated, the plasma and urine become red (paroxysms): this is most dramatically evident when the patient passes the first urine in the morning, however, haemolysis is an ongoing clinical problem for these patients and in some cases, can become life-threatening.

Traditionally, the Ham's acid haemolysis test was used to diagnose this condition. Many laboratories now use a flow cytometry-based test to look for a characteristic deficiency of proteins on the surface of red cells and leucocytes. PNH does not affect routine blood bank tests, but patients are at risk for red cell alloimmunisation given the need for chronic transfusion support in some cases. Additional management of these patients includes anticoagulation to prevent thrombosis, corticosteroids, the use of Eculizumab (a monoclonal antibody that inhibits complement activation) and in some cases, bone marrow transplantation.

Haemolysis caused by factors extrinsic to the red cell

Extrinsic causes of haemolysis are related to environmental factors external to an otherwise healthy, normally functioning red cell. These can be further classified as immune versus non-immune causes. Non-immune causes include mechanical shearing of red cells by vascular abnormalities such as microangiopathic haemolysis. This condition is caused by the presence of thrombotic deposits in small vessels and is seen in conditions such as disseminated intravascular coagulopathy (DIC) and thrombotic thrombocytopenic purpura (TTP).

Other causes of extrinsic, non-immune-mediated haemolysis include: red cell intracellular parasitic infections such as malaria; toxins released by infectious agents, spider bites or venoms; infusion of hypotonic IV fluids; and disruption of red cells by persistent vibration as described in "march haemoglobinuria".

Immune-mediated haemolysis is characterised by the presence of an antibody to an antigen on the surface of the red cell which tags the cell for destruction by the immune system (opsonisation). The source of the antibody varies depending on the type of immune haemolysis, but the final common pathway of enhanced red cell clearance leading to anaemia is the same. In HDFN, the antibody originates in the mother, crosses the placenta and leads to enhanced clearance of fetal red cells. In autoimmune haemolytic anaemia, the

patient makes an antibody which targets self-antigens present on the patient's own red cells. Immune haemolytic transfusion reactions (both acute and delayed) represent another sub-category of immune haemolysis where patient-derived antibody reacts against transfused donor red cells expressing foreign antigens. These alloimmune antibody mediated transfusion reactions will be discussed elsewhere (see *Section 14: Risks of transfusion and haemovigilance*).

Haemolytic disease of the fetus and newborn

Pathophysiology

Passive transfer of maternal IgG antibodies through the placenta to the fetus is part of normal fetal and neonatal physiology. Maternal IgG antibodies provide passive immunity to the fetus which persists in the neonatal period for as long as the maternal IgG antibodies remain. Sometimes this may be up to six months post-partum.

A fetus carries antigens encoded by genes inherited from both the mother and the father. This can result in fetal antigens which are paternally derived and foreign to the mother. The mother, if exposed to these antigens through the normal passage of fetal red cells through the placenta, may become immunised to these foreign antigens.

Haemolytic disease of the fetus and newborn occurs when a pregnant mother has or develops an alloantibody directed against a paternally derived antigen present on fetal red blood cells. When the antibody is an IgG antibody it can cross the placenta, bind to the antigen on the fetal red cells and cause rapid clearance of the affected red cells from the fetal circulation. This can result in mild to severe anaemia in the fetus, and hyperbilirubinaemia in the newborn, and in untreated cases can lead to fetal or neonatal death.

The antibody most commonly associated with HDFN is anti-D. Since approximately 10–15% of Caucasian individuals are D negative, it is common for a D negative female to have a D positive partner. The fetus can then inherit the D antigen from the father and in a first pregnancy the mother will be exposed to the paternally derived D antigen when fetal cells enter the maternal circulation, in a normal physiological process termed fetal maternal haemorrhage, (FMH). This can lead to maternal anti-D alloimmunisation.

Although fetal red blood cells may enter the maternal circulation throughout pregnancy, the largest number of cells and the greatest potential for alloimmunisation are in the perinatal period. For this reason, it is unusual for a first pregnancy to be impacted by HDFN. Alloimmunisation typically occurs at the end of the first pregnancy. See Fig. 2, Progression of HDFN.

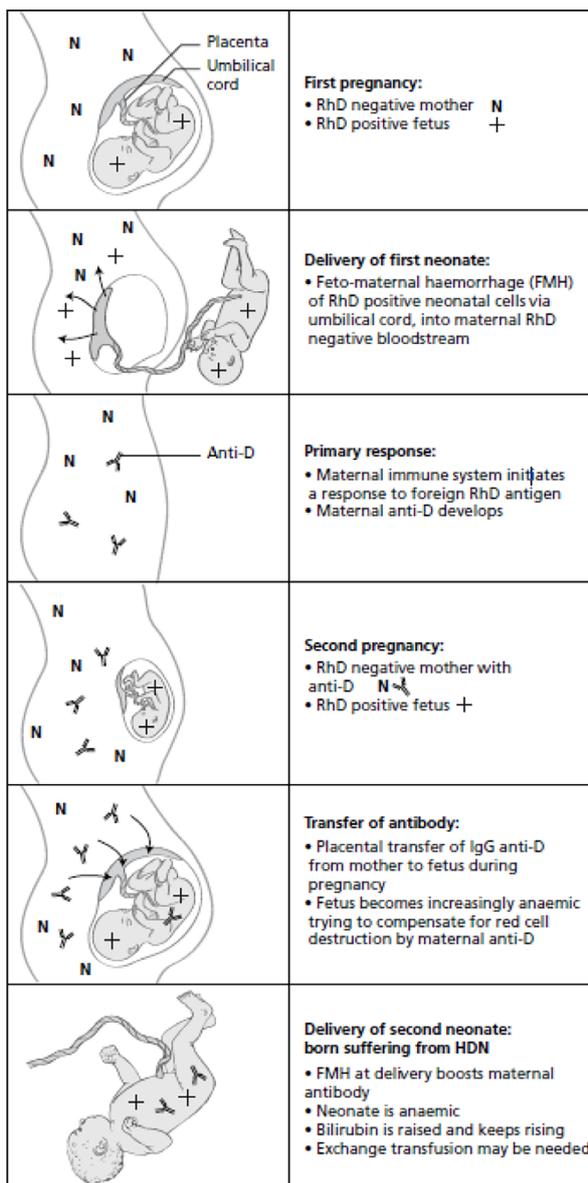


Fig. 2 Progression of HDFN.

Clinical Progression

In subsequent pregnancies where the fetus is D positive the pre-formed maternal anti-D crosses the placenta and binds to fetal D bearing red cells. The D positive cells of the second and any subsequent fetus may also boost the production of anti-D in the mother by providing for a secondary immune response. In this setting the amount of anti-D present in the mother is amplified by subsequent pregnancies with increasing antibody levels, and potentially increasing fetal haemolysis in later pregnancies.

The opsonized fetal cells are cleared by the spleen and liver resulting in anaemia in the fetus. Immature red cells are released into the fetal circulation to compensate for the anaemia in a process called erythroblastosis fetalis. When severe and untreated, fetal anaemia contributes to progressive cardiac failure with hepatosplenomegaly and significant swelling, or hydrops. Bilirubin rises as the opsonised red cells are haemolysed. In severe cases, sometimes called hydrops fetalis, there may be fetal intrauterine death. If the fetus survives and is delivered the ongoing haemolysis may result in hyperbilirubinaemia that cannot be adequately conjugated and excreted by the immature neonatal liver. Severe hyperbilirubinaemia causes brain injury because of deposition of bilirubin in the basal ganglia, called kernicterus. Phototherapy and/or exchange transfusion are used to reduce harmful levels of bilirubin as kernicterus may lead to long term neurological effects.

Haemolytic disease of the fetus and newborn due to other antibodies

Haemolytic disease of the fetus and newborn due to anti-D is the most common and severe form of HDFN. Other antibodies (typically IgG) for which the corresponding antigen is present on fetal red cells can also cause mild to severe HDFN. See Table 1, Summary of differences between ABO and Rh HDFN.

While HDFN has been reported with many antibodies, some of the important ones include:

ABO

Antibodies to ABO antigens are always present in Group O, A and B individuals, and may cross the placenta, however severe disease related to fetal maternal ABO incompatibility is uncommon. In Group O individuals anti-A and anti-B are often at least partly IgG in nature and can cross the placental barrier. It is common for Group A or B infants of a Group O mother to have a positive direct

antiglobulin test (DAT) at the time of delivery. Despite this, the likelihood of anaemia or severe hyperbilirubinaemia requiring treatment is very low. This may reflect the relatively poor development of ABO antigens on fetal red cells until late in gestation. In some populations anti-B antibodies from Group A mothers may also result in a positive DAT and neonatal anaemia and hyperbilirubinaemia.

While some have recommended routine assessment of the DAT in infants born to Group O mothers, this can be misleading as most infants, even with a positive DAT, will not require treatment for hyperbilirubinaemia. Early monitoring of bilirubin levels and assessment of the neonatal DAT in only those babies with a high or rising bilirubin level is a more prudent approach.

Kell

Anti-K can cause very severe and early onset HDFN. In addition to binding to the K antigen on fetal red cells with clearance and haemolysis, anti-K has been shown to suppress fetal erythropoiesis resulting in severe anaemia without concomitant hyperbilirubinaemia. This may occur even at low titres such that the role of titration for anti-K antibodies in the management of the condition is uncertain. Early doppler ultrasound monitoring is often recommended for affected pregnancies, even those with low titres of antibody.

Other Rh antibodies

Most antibodies in the Rh blood group system have been implicated in HDFN. Anti-E is commonly encountered in pregnancy however most anti-E antibodies do not reach a critical titre and do not cause significant anaemia nor hyperbilirubinaemia. Anti-c can cause severe disease.

Kidd and Duffy

Alloimmunisation to Kidd (particularly Jk^a) and Duffy (particularly Fy^a) antigens and subsequent HDFN are less frequently seen. However severe disease due these

Table 1 Summary of differences between ABO and Rh HDFN

Feature	ABO HDFN	Rh HDFN
Frequency and severity	Most common – usually mild	Most severe
Pregnancy initially affected	First and subsequent with ABO incompatibility	From second pregnancy onward when fetus inherits corresponding antigen
Neonatal DAT	Negative or weakly positive – poor diagnostic test	Strongly positive – good diagnostic test
Antenatal impact	None	May require antenatal intervention
Postnatal impact	Possible but often self-resolving	May cause hyperbilirubinaemia, brain damage or death
Treatment	Phototherapy and possible treatment with IVIG; rarely top up or exchange transfusion	Phototherapy with possible exchange and top up transfusions
Prophylaxis	None available	Rh immune globulin for non-immunised D negative mothers

antibodies can occur and monitoring of antibody levels and or doppler ultrasound if antibody levels are critical levels is important.

MNS

Anti-Mi^a is a common cause of HDFN in some populations with a higher prevalence of Mi^a positive individuals (such as China and South East Asia).

Anti-M is very commonly seen in pregnancy. As it is often predominantly IgM in nature, the likelihood of HDFN is low. In addition, it is uncommon for anti-M to reach critical antibody levels. If anti-M is identified in pregnancy it is helpful to assess whether the antibody is predominantly IgG or IgM in nature and to follow only those patients with IgG anti-M. Techniques for distinguishing the antibody type include dithiothreitol (DTT) treatment of the serum/plasma which denatures IgM but not IgG antibodies. This is followed by repeat titration with the DTT treated (IgG containing) serum/plasma.

Laboratory testing in pregnancy (antenatal testing)

To prevent the devastating outcomes of HDFN routine pregnancy monitoring must be available. An obstetrical history, including any prior pregnancies affected by fetal or neonatal anaemia is important. Several laboratory screening tests including some for infectious diseases or inherited disorders which could impact the fetus may be performed according to local policies. Laboratory tests relevant for diagnosis and management of HDFN are outlined here.

ABO and D grouping

Both ABO and D blood grouping are often part of routine antenatal assessment. The ABO blood group of the mother may impact the likelihood of jaundice in the neonate. The Rh blood group is important as it will inform the need for treatment to prevent D alloimmunisation.

Antibody screening and identification

Antibody screening early in pregnancy is also an important test. When an unexpected alloantibody is detected during antibody screening, the antibody is identified. The quantity or titre and nature of the antibody must then be assessed.

To be implicated as a cause for HDFN an antibody must be partially or completely IgG in nature, as only IgG antibodies cross through the placenta into the fetal circulation; and the antigen must be expressed on fetal red blood cells. If the antigen not expressed on fetal cells the antibody will not cause haemolysis or anaemia.

For antibodies where these conditions are met, the antibody is considered clinically significant as a risk factor for HDFN.

Determination of the quantity of antibody is an important next step. This can be done through comparison with known standards (for anti-D and anti-c), or through titration.

Titration/Quantification of antibody

Titration involves using patient serum/plasma, serially diluting it with saline (neat, 1:2, 1:4, 1:8, 1:16, 1:32 etc.) and incubating with red cells that are positive for the corresponding antigen (whether cells used should be homozygous or heterozygous for the antigen is controversial). The test is then read at the indirect antiglobulin test phase, following addition of anti-human globulin. The highest dilution of the serum/plasma where a macroscopic agglutination reaction occurs is called the titre of antibody (See *Section 4: Principles of Laboratory Techniques* for details).

For antibodies that meet a certain threshold, sometimes referred to as the critical or "cut off" titre, the likelihood of HDFN is increased and additional clinical monitoring of the fetus is needed. For anti-D a titre of approximately 16 (range of 8–32 depending on method) has been established as the level above which HDFN may occur. This is when clinical monitoring should commence. For other non-D antibodies, often the same titre is used as a cut off although the clinical impact at a specific antibody titre is less well established.

Often the measurement and identification of antibody quantity is done at regular intervals. For example, an initial antibody screen may be done at the end of the first trimester with repeat testing and titration at monthly intervals to determine the presence of additional antibodies and identify any increase in the antibody quantity.

Patients with clinically significant antibodies, and especially those with increasing quantity or critical titre of antibody should be referred to a specialist fetal maternal clinic.

Paternal red cell typing

When a clinically significant antibody is present in a pregnant mother, the blood type of the father provides important information. If the father is positive for the antigen corresponding to the maternal antibody, an assessment of paternal zygosity can determine the likelihood of the fetus inheriting the antigen and the risk of HDFN. For example, if the maternal antibody is anti-K, and the father is K negative, the fetus will not be at risk. If the father is heterozygous for the K gene, then the fetus has a 50% chance of being K positive and affected by the antibody. If the father is homozygous for K then the fetus

will be K positive and at risk for HDFN. See Table 2 for an example of maternal and paternal blood type and likelihood of HDFN.

This assessment of paternal zygosity is complicated in the case of D and other Rh antigens. The ethnicity of the individuals will impact the probable Rh genotype that would account for a particular combination of D, C, c, E, and e antigens. Generally, all five of these Rh antigens would be determined in both mother and father to help with the assessment of fetal impact of a maternal Rh antibody. See *Section 6: Blood Group Systems* for additional information.

Because of the complexity of the *RHD* and *RHCE* combinations along with the large number of variant genes which may impact the phenotype, non-invasive testing of maternal serum/plasma for the fetal genotype may provide for better prediction of the fetal phenotype.

Non-invasive perinatal testing, or cell free fetal DNA testing of maternal serum/plasma is routinely available in some countries and may be used to predict the fetal D status and therefore the requirement for RhIg prophylaxis. In addition, it can be used to predict fetal K or D antigen status to determine whether pregnancy monitoring is required in mothers with antibodies.

Antenatal clinical management of alloimmunised pregnancies

Ultrasound and doppler ultrasound

Obstetrical ultrasound is a non-invasive scan that is performed regularly in pregnancy and that can be used to determine gestational age and fetal size as well as detecting hydrops fetalis.

For patients with antibodies that meet the pre-established critical threshold, specialised clinical monitoring of the fetus using doppler ultrasound is recommended. This technique is a non-invasive way to monitor the fetus for anaemia, by measuring the velocity of blood flow in the fetal middle cerebral artery. An increased flow rate is correlated with increased cardiac output that can arise with anaemia. Using a gestational age-adjusted nomogram the

peak flow velocity can be converted to an estimate of the haemoglobin concentration.

Amniocentesis

Amniocentesis involves collection of a sample of amniotic fluid by passing a needle into the uterus. The amniotic fluid collected may be analysed to determine the bilirubin level of the fetus. The bilirubin level is correlated with the degree of anaemia and predicts the onset of hydrops, especially in Rh related disease. For many years the level of amniotic fluid bilirubin was considered the gold standard in determining the severity of HDFN, but this invasive technique has now been replaced by non-invasive doppler ultrasonography in most cases. In addition to the risk of pregnancy loss posed by an invasive procedure, amniocentesis may also result in FMH thus boosting the maternal antibody level or leading to alloimmunisation to additional red cell antigens.

Induction of labour

When anaemia is confirmed by ultrasound, next steps may involve delivery through early induction of labour, if the fetus is sufficiently developed. Following delivery, no further placental transfer of maternal antibody will occur and the passively acquired maternal antibody levels will gradually diminish, along with the degree of haemolysis. Generally, delivery is not contemplated for a fetus at less than 32 weeks gestation.

Cordocentesis

For a fetus impacted by HDFN before 32 weeks gestation, fetal blood sampling from a cord blood vessel (called cordocentesis or percutaneous umbilical cord blood sampling (PUBS)) to confirm the haemoglobin level may be required. This is generally done with ultrasound guidance, when the doppler ultrasound suggests severe anaemia. The sampled blood is tested for haemoglobin and bilirubin along with ABO and Rh typing and DAT testing. See Table 3 for a summary of antenatal testing for HDFN.

Table 2 Example of maternal and paternal blood type and likelihood of HDFN

Maternal type	Paternal type	Comment on possible blood type of fetus and related likelihood of HDFN
K- with anti-K	K+k+	Fetus has 50% likelihood of being K+ as father has a single dose of K. If K antigen was inherited, HDFN is likely
	K-k+	Fetus will be K- and therefore unaffected. Supposition: Maternal anti-K may have occurred as the result of a previous blood transfusion with K+ red cells, bacterial infection or a previous K+ pregnancy with a K+ partner

Intrauterine transfusion

If the haemoglobin assessment from the cordocentesis sample confirms anaemia, an intrauterine transfusion will typically follow immediately through the same needle into the umbilical vein. This technique is difficult and special expertise is required to successfully transfuse while avoiding premature labour or fetal demise.

This technique is typically performed in a fetus after 20 weeks gestation and repeated periodically until fetal maturity, when delivery can be safely accomplished.

Intrauterine transfusion may also involve injection of red blood cells into the fetal peritoneal cavity, or, in some cases both intravascular and intraperitoneal transfusion is provided. Red cells in the fetal peritoneal cavity are absorbed through the lymphatic system into the fetal blood vessels.

The volume of red cells introduced is typically small and it is often necessary to give sequential intrauterine transfusions at weekly or bi-weekly intervals until safe delivery can be induced.

The red cells transfused must be specially prepared for this very small and relatively immunosuppressed transfusion recipient. The amount of blood required is determined by using the measured haemoglobin and haematocrit, the haematocrit of the transfused red cells and the required increase in haemoglobin concentration to calculate the total amount of blood required.

Group O D negative red cells that are antigen negative for the maternal antibody, freshly donated, concentrated to a haematocrit of 0.80 and manipulated to remove red cell additive solution, are preferred. The red cells must also be modified to prevent cytomegalovirus (CMV) transmission (donors anti-CMV antibody negative and /or red

cells leucoreduced by filtration with a leucoreduction filter). The unit must be irradiated to prevent Transfusion Associated Graft Versus Host Disease (TA-GVHD). See Fig. 3 for an illustration of intrauterine transfusion into the fetal peritoneal cavity.

Plasmapheresis and intravenous immunoglobulin (IVIG)

Other maternal interventions that are used in exceptional circumstances include plasmapheresis and IVIG therapy. Plasmapheresis may be used in alloimmunised mothers with a high titre antibody and a history of fetal loss due to HDFN. The plasmapheresis removes antibody by exchanging maternal plasma with albumin. Antibodies typically rebound and may increase in quantity quickly following plasmapheresis, so this treatment is usually carried out in repeated cycles beginning early in pregnancy with or without the use of IVIG. The mechanism and efficacy of IVIG in this setting are uncertain.

Postnatal laboratory testing

Following delivery, a sample of the cord blood is often made available for blood grouping and additional testing, as required.

Cord blood typing is particularly valuable when the maternal blood group is unknown, when the maternal blood group is known to be D negative and when the mother is known to have a clinically significant alloantibody.

If a cord blood sample is unavailable, or in the days following delivery, blood group testing as well as haemoglobin and bilirubin assessment should be performed on capillary (heel prick) or venepuncture samples.

Table 3 Summary of antenatal testing for HDFN

Test descriptions	Comments
Rh typing	To identify D negative pregnant women
Antibody screening	To detect irregular red cell antibodies
Antibody identification	To identify irregular red cell antibodies detected on screening, to ascertain whether or not they are of obstetric significance
Antibody titration/qualification	To determine titre or strength of obstetrically significant red cell antibodies
Paternal red cell typing/phenotyping	To determine whether or not the father carries the corresponding antigen, and the chances of the fetus inheriting it
Amniocentesis	When HDFN is strongly suspected, amniocentesis may be performed to determine bilirubin level, to assess whether intrauterine transfusion is needed. This is an invasive measure and therefore carries risks.
Obstetric ultrasonography	Non-invasive technique to examine the fetus for possible hydrops fetalis
Doppler technique	Non-invasive technique to measure speed of blood flow in fetal vessels, as increased flow rate suggests severe anaemia
Cordocentesis (PUBS)	PUBS is an invasive procedure performed only when HDFN is suspected, to provide a blood sample for the laboratory determination of fetal Hb and blood type, and for DAT to detect <i>in vivo</i> red cell sensitisation.

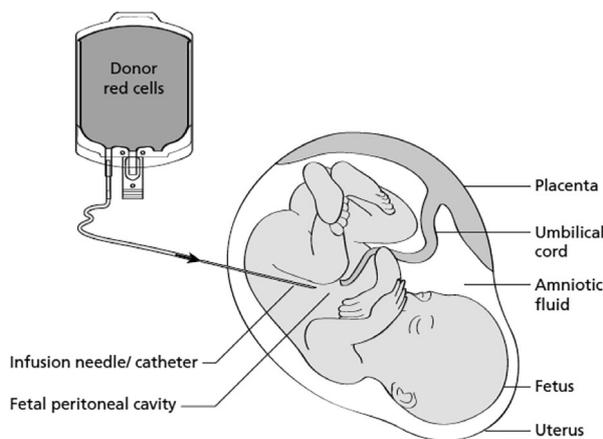


Fig. 3 Intrauterine Transfusion.

Maternal and neonatal ABO

If not available from prenatal testing, the maternal ABO should be performed on a postnatal sample. The neonatal ABO should also be evaluated. Maternal-neonatal ABO incompatibility may contribute to neonatal hyperbilirubinaemia, especially when the maternal blood group is O and the neonatal group is A or B. Group O individuals are more likely to have IgG anti-A and anti-B which could cross the placenta and bind to ABO incompatible fetal red cells. Since fetal cells have poorly developed ABO antigens these antibodies rarely cause significant haemolysis in the fetus but may contribute to an increase in postnatal bilirubin. For pregnancies in which the fetus is treated with intrauterine transfusion, the cord or neonatal ABO type will not be valid.

Maternal and neonatal D

If not available from prenatal testing, the maternal D type should also be determined. For D negative mothers, the cord or neonatal D type is also a necessary test. This cord typing will determine whether the mother is a candidate for Rh immune globulin prophylaxis to prevent development of anti-D antibodies that could impact the next pregnancy. When the mother is D negative, and the infant is D positive, Rh immune globulin should be provided to the mother.

Neonatal DAT and antigen typing

At the time of delivery, when a maternal antibody is known to be present, typing of the cord blood or a neonatal sample with a DAT is recommended. This test will determine whether the maternal antibody is bound to the neonatal red blood cells.

If the DAT is positive, antigen typing (phenotyping) of the neonatal cells is also helpful to confirm that the antibody present is the one seen in the maternal serum/plasma.

A neonatal red cell phenotype is helpful if the positive DAT could be explained by ABO incompatibility or when the mother has multiple antibodies. Determining the antibody responsible for the positive DAT may be helpful in predicting whether neonatal hyperbilirubinaemia and anaemia are likely. Different antibodies pose different risks for haemolysis. For example, a positive DAT due to ABO incompatibility is less likely to contribute to neonatal jaundice than an anti-D in a D positive neonate.

Red cell antigen typing of cells with a positive DAT requires appropriate monoclonal reagents. In some cases, an eluate of the neonatal red cells may be required to determine the separate antibody specificities responsible for the positive DAT. See *Section 4: Principles of laboratory techniques*.

If the DAT is negative in a neonate where the mother has a known alloantibody, it is likely that the neonatal red cells are not sensitised. This may reflect absence of the corresponding red cell antigen.

The DAT may also be negative or show a mixed field pattern when an intrauterine transfusion has been part of the treatment for the fetus in an alloimmunised pregnancy. This result is because the transfused cells are Group O and are antigen negative for the maternal antibody and are therefore not sensitised by the maternal antibody.

An unexpectedly positive DAT found through investigation of neonatal jaundice, when there is no history of a maternal alloantibody, should be investigated. In some cases, the mother may have an undetected antibody to a low frequency antigen not found on antigen screening cells but inherited by the baby from the father.

A DAT can also be positive for non-specific reasons including some congenital infections or maternal medications.

Neonatal haemoglobin

The neonatal haemoglobin should be determined to assess the severity of anaemia in HDFN when the mother has a clinically significant alloantibody. The haemoglobin concentration will be used to help determine whether neonatal transfusion is required.

Neonatal bilirubin

In a neonate, conjugation of bilirubin to its soluble form, for excretion, may be delayed due to immaturity of the fetal/neonatal liver. Bilirubin levels may rise rapidly in the first few days of life. An untreated and marked increase in bilirubin levels can lead to deposition in the basal ganglia of the brain and to a neurological syndrome called kernicterus.

Total bilirubin levels are assessed over time in the first hours and days following birth. The rate of rise and the

level of bilirubin, together with the neonatal size and gestational age are used to determine whether neonatal treatment is required. Bilirubin levels may be measured non-invasively using a transcutaneous measurement or may be confirmed by measuring serum/plasma bilirubin from a capillary or venous blood sample.

Fetal maternal haemorrhage (FMH) assessment

The volume of fetal maternal haemorrhage when a D negative mother has a D positive baby may be assessed through a rosette screening test, the Kleihauer-Betke test or by flow cytometry methods. This testing is important in guiding the dose of Rh immune globulin required by the mother.

These tests may also be used in assessment of FMH when antenatal procedures such as amniocentesis are done or following abortion, miscarriage or trauma in a pregnant mother.

The rosette test is a semiquantitative slide test which results in microscopically visible rosettes of D positive red cells on a D negative background following addition of anti-D antibody coated indicator cells.

The Kleihauer-Betke test is a differential acid elution test that allows fetal and adult cells on a blood film to be counted separately. An acid elution clears haemoglobin A from the adult cells while fetal haemoglobin remains. Following staining, the maternal ghost cells can be distinguished from the fetal haemoglobin containing cells. The cells in a predetermined area on the slide are then counted and a calculation performed to determine of the volume of FMH. See Fig. 4, Differential acid elution stain (Kleihauer-Betke).

Flow cytometric immunophenotyping also allows distinction of fetal from maternal cells and is an automated and accurate means of determining the volume of FMH. Table 4 summarises postnatal testing for HDFN.

Postnatal treatment of neonatal anaemia and hyperbilirubinaemia

Phototherapy

Mitigating hyperbilirubinaemia in a neonate affected by HDFN and prevention of kernicterus may be accomplished through the use of phototherapy to aid in conjugation of bilirubin. This therapy uses timed exposure to specific wavelengths of light in a specially designed incubator. See Fig. 5, Neonatal phototherapy.

Intravenous Immunoglobulin

Intravenous immunoglobulin therapy may be used in the treatment of HDFN after delivery. The mechanism and efficacy of this treatment are not clearly established.

Red cell transfusion

Treatment of anaemia may require a top up transfusion if the neonatal haemoglobin falls below a pre-established threshold. When intrauterine transfusion has been required, neonatal erythropoiesis is often suppressed by the Haemoglobin A containing donor red blood cells. Persistent passive maternal antibody may also contribute to neonatal anaemia. When the anaemia persists one or more top up red cell transfusions may be required.

Exchange transfusion

In cases where the bilirubin continues to rise despite phototherapy, an exchange transfusion may be required.

In this technique neonatal blood is withdrawn in small aliquots and following each withdrawal an equal aliquot of ABO compatible donor red cells is transfused. This is usually done through an intravenous catheter with a stopcock, introduced into the umbilical vein. The blood is infused through a blood warming device. This gradually reduces the level of bilirubin in the circulation. In severe cases exchange transfusion may be repeated several times or may be followed by top up transfusion over the ensuing days. See Fig. 6, Neonatal exchange transfusion.

Exchange transfusion replaces anaemic neonatal blood with a higher concentration of donor red cells. Both bilirubin and maternal antibody are removed from the neonatal circulation. Sensitised, DAT positive red cells are replaced with antigen negative, unsensitised donor red cells.

The transfusion volume is calculated based on the blood volume of the neonate. Like the donor blood selected for intrauterine exchange transfusion, the donor red cells should be freshly donated to maximise the post transfusion red cell survival and minimise the supernatant

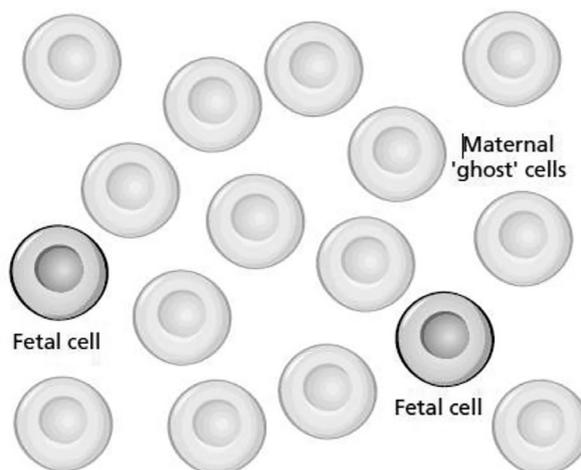
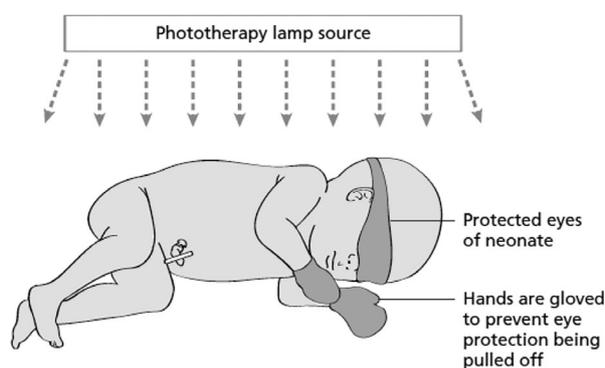


Fig. 4 Differential acid elution stain (Kleihauer-Betke); a microscope slide view showing fetal cells in maternal circulation following FMH.

Table 4 Summary of postnatal testing for HDFN

Test descriptions	Comments
ABO grouping	The ABO group of both mother and neonate are determined as ABO HDFN is the most common cause of non-physiological neonatal jaundice
Rh typing	Rh typing is performed to detect combinations of D negative mother/D positive neonate because this has the greatest potential of HDFN Rh typing also determines which unimmunised mothers require prophylaxis (Rh immunoglobulin)
DAT – neonate	Neonatal DAT is performed to detect the presence of <i>in vivo</i> sensitisation, indicating HDFN
Additional red cell antigen typing - neonate	If an obstetrically significant antibody other than anti-D is present in maternal serum/plasma, additional antigen typing may be performed on the neonate if the neonatal DAT is positive, an elution could be performed instead
Maternal red cell antibody detection, identification and titration	Irregular antibodies detected on screening should be identified and if obstetrically significant, titrated
Haemoglobin - neonate	Neonatal Hb level should be performed to indicate severity of anaemia and to guide the clinician in treatment required (i.e. need for transfusion)
Bilirubin – neonate	Bilirubin levels should be assessed serially to indicate rate of rise over the first few days of life to guide the clinician in determining the method of treatment (i.e. phototherapy or exchange)
Fetal maternal haemorrhage	The Kleihauer-Betke test may be performed to determine whether a significant FMH has occurred Alternatively the investigation of FMH can be performed by other methods such as flow cytometry Assessment of FMH provides a guide to dose of Rh immune globulin needed to prevent development of anti-D antibodies in a D negative mother with a D positive infant

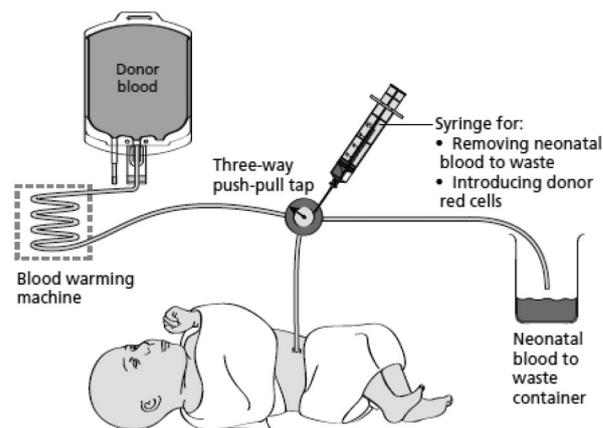
**Fig. 5** Neonatal phototherapy.

potassium in the donor unit. Donor blood should also be leucoreduced by filtration and irradiated.

Prevention of HDFN due to anti-D

Rh immune globulin, or RhIg, is a polyclonal human plasma derived concentrate of anti-D antibodies manufactured from the pooled plasma of many D negative alloimmunised donors. Administration of this passive anti-D antibody prevents sensitisation following exposure to the D antigen through FMH.

Post-partum RhIg prophylaxis is given when the mother has no anti-D on prenatal serological testing and after cord blood typing confirms that the neonate is D positive. If the neonate is D negative, no RhIg is required, as the mother is not at risk for alloimmunisation.

**Fig. 6** Neonatal exchange transfusion.

Addition of a dose of RhIg in mid pregnancy at 28–32 weeks gestation further reduces the risk of anti-D alloimmunisation. This antenatal plus postnatal prophylactic therapy has dramatically reduced the incidence of anti-D mediated HDFN and the associated fetal and neonatal complications.

A prenatal mother is considered eligible to receive antenatal RhIg when this mother:

- Is known to be D negative.
- Has no evidence of prior sensitisation to the D antigen.
- Has a fetus with unknown D type, or the fetus has been predicted to be D positive through non-invasive

perinatal testing of maternal serum/plasma for the fetal *RHD* gene.

Non-invasive prenatal testing for fetal D prediction is routinely available in some countries and allows targeted antenatal prophylaxis.

Assessment of postnatal alloimmunisation and the need for RhIg can be complicated by the antenatal RhIg dose. Many mothers who receive RhIg at 28 weeks gestation will have a positive antibody screen at the end of pregnancy. The anti-D seen in this setting should be considered passive, and additional doses of RhIg given with reassessment for the presence of immune anti-D six months post-partum.

In addition to a routine postnatal and antenatal RhIg at 28 weeks, additional RhIg is recommended for any prenatal event which may cause FMH including: obstetrical manipulation, amniocentesis, abdominal trauma and abortion or miscarriage.

Generally, RhIg should be administered within 72 h of delivery or of any suspected FMH. A standard dose (1500 IU) is enough to prevent immunisation by 30 ml of fetal blood. For suspected FMH that occurs after 20 weeks gestation, a test to quantify the amount of FMH must be performed and additional RhIg dose calculated and administered to ensure coverage for the volume of the bleed that exceeds that covered by the standard RhIg dose.

Autoimmune haemolytic anaemia (AIHA)

In AIHA, the immune system inappropriately makes antibodies to red cell antigens present on the patient's own red cells leading to their premature destruction and anaemia. Often these antigens are ubiquitous and are present on transfused red cells as well. AIHA may be triggered by infections (especially viral illnesses), drugs, or associated with underlying conditions such as lymphoma or connective tissue disorders such as lupus. In many instances, the AIHA is not clearly associated with any underlying condition and the trigger is unknown. It is important for the clinician to look for potential causes as this may influence the management of the patient.

Management of autoimmune haemolytic anaemia is complex and requires the involvement of a specialist: only a brief overview is provided here.

Classification of autoimmune haemolytic anaemia

Clinically and from a transfusion serology perspective, autoimmune haemolytic anaemia fits into three broad categories:

- Warm autoimmune haemolytic anaemia: autoantibody reacts best at 37°C and is typically IgG.

- Cold autoimmune haemolytic anaemia: autoantibody reacts best at low temperature and often has an IgM component that may lead to complement fixation.
- Drug-induced autoimmune haemolytic anaemia: presence of a drug triggers autoantibody formation and subsequent haemolysis.

Warm autoantibodies: Pathophysiology and clinical features

Warm autoantibodies are the most common cause of autoimmune haemolytic anaemia and involve an IgG autoantibody that reacts best at body temperature (37°C). Binding of the autoantibody to the red cell surface enhances red cell recognition and removal by the immune system. Typically, haemolysis is extravascular with red cells being destroyed by haemophagocytic cells in the spleen or in other tissues such as the liver. Intravascular haemolysis (red cells are lysed directly in the circulation) may rarely occur but is not the dominant mechanism of red cell destruction. The haemophagocytic cells (i.e. macrophages) remove portions of red cell membrane causing spherocytes to form. This change in red cell morphology on the peripheral blood film is an important diagnostic finding. The increased destruction of red cells leads to anaemia, stimulation of the bone marrow to make more red cells (increased reticulocyte count), and the release of red cell contents causing abnormalities in the biochemical markers of haemolysis (increased bilirubin and LDH (lactate dehydrogenase); low haptoglobin). Haemoglobinuria (the presence of free haemoglobin in the urine) is uncommon but may be seen if the haemolysis is very brisk.

Warm autoantibodies: Laboratory testing

The key serologic findings of warm autoimmune haemolytic anaemia are summarised in Table 5.

Classically, the poly-specific DAT is strongly positive and monospecific DAT testing may reveal IgG alone or both IgG and complement coating the red cells. A positive DAT alone does not confirm a diagnosis of immune haemolytic anaemia and is a non-specific finding in many patients.

In warm AIHA the antibody screen is usually positive as the optimal reactivity of these antibodies is 37°C. Follow up testing with extended panels of reagent cells typically reveals a pan-reacting pattern with all cells showing a similar strength of reaction, including the autocontrol.

Occasionally, the autoantibody may exhibit specificity, such as an apparent anti-e in an e positive individual. Other autoantibodies with specificity have also been described. An autoantibody with specificity should be considered when there is no prior sensitising event, a

Table 5 Comparison of autoimmune haemolytic anaemias

	Warm AIHA	Cold Haemagglutinin Disease	Paroxysmal Cold Hemoglobinuria	Drug-induced
Autoantibody	IgG	IgM	Biphasic IgG (Donath-Landsteiner)	IgG
Hemolysis	Usually extravascular	Often extravascular, but intravascular may occur	Acute onset intravascular with hemoglobinuria	Variable onset; typically extravascular
DAT	IgG +/- C3-	C3 alone	C3 alone	IgG (rarely C3 alone)
ABO typing	No interference	May interfere	No interference	No interference
Screen	Panreactive	Often nonreactive at 37°C	Nonreactive	Variable
Special studies	Phenotyping Adsorption studies	Titre at 4°C Thermal amplitude	Donath-Landsteiner Assay	Eluate may be negative
Possible autoantibody specificity	May see relative specificity for Rh antigens	Ii antigens	P antigen	Rare

positive DAT and the presence of the corresponding antigen in the patient.

The presence of an underlying alloantibody is difficult to recognise in the presence of an autoantibody. A pattern of stronger reactions on a subset of cells “shining through” the background reactivity of the autoantibody may be suggestive of an additional underlying red cell alloantibody. However, there is no guarantee that the alloantibody will react more strongly than the autoantibody.

Two additional serologic tools may be of use in this setting:

Autoadsorption. If the patient has not been transfused in the past three months, an autoadsorption may be performed. By adsorbing the autoantibodies on to the patient’s red cells and effectively clearing them from the serum/plasma, an underlying alloantibody may become detectable. If a patient has recently been transfused, allogeneic absorptions may be performed but these procedures are complex and time-consuming and often only available in specialised reference laboratories.

Elution. Antibodies eluted off the red cell tend to be concentrated and therefore show stronger reactivity. If the eluted antibodies react with all reagent red cells, then these antibodies are said to show no evidence of specificity. If the DAT was strongly positive and there is no evidence that antibodies were eluted, an alternate elution technique should be considered, or there is the possibility that the AIHA is drug-induced.

To summarise, the hallmark of a warm autoantibody includes (1) a positive DAT (2) pan-reactive antibody screen and panel, and (3) crossmatch incompatibility with all donor red cell units. If any of these three features are missing, the diagnosis of warm AIHA should be questioned and other serologic possibilities explored.

Management of patients with warm autoantibodies (including transfusion requirements)

The presence of an autoantibody makes it difficult to reliably exclude the presence of an underlying red cell alloantibody. The autoantibody in the serum/plasma of the patient will typically react with donor red cells and lead to serologic crossmatch incompatibility.

Obtaining an accurate transfusion and pregnancy history and reviewing blood bank results for previously identified alloantibodies is important.

While it is ideal to avoid transfusion unless clinically necessary, warm AIHA can cause severe and potentially life-threatening anaemia. Transfusion should not be withheld if the patient shows signs of haemodynamic compromise from severe anaemia with impaired organ function. Chest pain, decreased level of consciousness, increase in heart rate and a precipitous drop in haemoglobin are indications for urgent treatment, including transfusion.

Transfusion of crossmatch incompatible blood may be required in this circumstance. Although most recipients do not react, there is potential risk for a severe haemolytic transfusion reaction. The medical officer of the blood service should discuss this risk with the prescribing clinician and the transfusion should be monitored closely.

The following steps can be taken to minimise potential adverse events from transfusion:

- Provide donor red cells that are phenotype matched to patient red cells for Rh and Kell antigens (consider more extensive phenotype matching if feasible). It is important to perform red cell phenotyping before the first RBC transfusion. If the patient has been transfused, the sample may still be sent for genotyping. Transfusion may stimulate the development of new alloantibodies.
- Transfuse slowly and under close supervision. Discontinue the transfusion at the first sign of reaction.

If the patient reacts to the transfusion it may be difficult to determine the cause.

- D negative women should not be given D positive blood even if it appears to be the least incompatible. This may potentially occur where the autoantibody exhibits “e” specificity: in this situation, it is preferable to give blood that is antigen matched for the patient’s Rh phenotype.
- Give only the minimum amount of blood that is required to address the patient’s symptoms and bring the haemoglobin into a safe range. This is because transfusing red cells can cause them to be haemolysed and a large volume red cell transfusion can potentially make the haemolysis worse. Ensure that the patient is well hydrated as increased levels of free haemoglobin in the plasma can be harmful to the kidneys.
- Minimise the frequency and quantity of blood draws for ongoing testing (for example, use smaller paediatric sized tubes).
- Optimise red cell production by ensuring iron and vitamin B12 stores are adequate and providing routine supplementation with folate.

More definitive treatment to reduce autoantibody production may be prescribed and may include corticosteroids therapy, immunosuppressive therapy and therapeutic monoclonal antibodies that target B cells (for example, rituximab, an anti-CD20 monoclonal agent). In some refractory cases, splenectomy may be considered. IVIG is not routinely indicated in the management of autoimmune haemolytic anaemia.

Cold autoantibodies: Pathophysiology and clinical features

Cold autoantibodies are frequently encountered in the laboratory and the majority are clinically insignificant. They may interfere with routine blood bank testing. On rare occasions, a cold autoantibody may cause haemolytic anaemia. This is more likely when the antibody has a broad thermal amplitude or high titre.

Cold autoimmune haemolytic anaemia, referred to as cold haemagglutinin disease (CHAD), can occur as an isolated condition or may be secondary to an underlying lymphoma, autoimmune disease or infection. Infections with *Mycoplasma pneumoniae* or Epstein-Barr Virus (EBV) are frequently associated with cold autoantibodies although rarely associated with haemolysis. While acute self-limited CHAD can be seen in children, chronic CHAD is more often seen in the elderly and in some cases may be the first clinical manifestation of lymphoma.

The clinical and laboratory features of CHAD reflect the ability of the cold autoantibody to react best at lower

temperatures. The autoantibody is usually of the IgM class although IgG and IgA have been described.

While the core body temperature hovers around 37°C, the temperatures in the extremities such as ears, nose, fingers and toes can be much cooler facilitating antibody binding to red cells. These IgM autoantibodies fix complement and enhance red cell clearance primarily through extravascular haemolysis (complement on the surface of the red cell tags the red cell for clearance by the haemophagocytic cells of the immune system). Less commonly, complement cascade activation may proceed to completion with ensuing intravascular haemolysis. The cold autoantibody may dissociate in the warmer central circulation and become available to re-attach to unsensitised cells. An exacerbation of CHAD may occur when the patient is exposed to cold.

The pentameric structure of IgM allows it to effectively agglutinate red cells. On the peripheral blood film, this causes red cell agglutination. The presence of accompanying red cell spherocytes suggests that active immune-mediated haemolysis is occurring. In vivo, red cell agglutination in the small blood vessels of the extremities can cause symptoms of vaso-occlusion such as pain, bluish discoloration and ulceration in advanced cases.

Cold autoantibodies: Laboratory testing

The DAT is often positive with complement alone, but in some cases, it may be impossible to interpret the DAT because of room temperature spontaneous red cell agglutination. Washing the red cells using 37°C saline prior to performing the DAT may be helpful along with appropriate controls.

It may also be difficult to perform ABO grouping in patients with cold autoantibodies if the antibody reacts with reverse grouping cells incubated at room temperature. Additional measures for managing ABO discrepancies may be required.

Specimens collected from a patient suspected of having CHAD should be maintained at 37°C until the plasma can be separated from the red cells to prevent adsorption of antibody and complement in vitro. This can be done by warming all collection tubes in advance in the lab and using a pre-warmed thermos for specimen transport.

Causative antibodies typically have very high titres (>1:1000) with titration performed at 4°C using established methods. Thermal amplitude can be assessed by incubating cells at progressively warmer temperatures, but this is not required to establish a diagnosis.

Antibody specificity may sometimes be seen. Anti-I is the most common specificity and is commonly associated with *Mycoplasma* infection. Anti-i is more commonly associated with infectious mononucleosis due to EBV infection. Cord red blood cells express i antigen while

adult cells express I. Comparative testing of patient serum/plasma against adult Group O cells and cord Group O cells can help establish this specificity. Establishing an antibody specificity is not necessary for transfusion purposes or for the treatment of CHAD.

Compatibility testing performed at 37°C may yield compatible results, or a cold autoantibody with a high thermal amplitude may result in serologic crossmatch incompatibility. This may require release of least incompatible red cell units with the same transfusion concerns of masking an underlying alloantibody as described above.

Management of patients with cold haemagglutinin disease (CHAD)

An underlying cause for CHAD should be sought by the clinician for all patients. This includes appropriate microbiologic and serologic testing for infectious agents and investigation for lymphoma.

Establishing an associated diagnosis is important as the most effective therapy for CHAD is to treat the underlying cause. In the case of infection, this may include antibiotics or supportive care as the infection runs its course. Haemoglobin levels should be monitored and transfusion given only to those patients who are symptomatic or in danger of harm from severe anaemia. When performed, use of a blood warmer is recommended and the patient should be kept in a warm environment.

Steroids are not effective in cold agglutinin disease and there is no role for therapeutic splenectomy. Treatment of persistent disease or underlying lymphoma with rituximab or cytotoxic chemotherapy is considered first line therapy for cases that are not related to infection and are not self-limiting. (I have added rituximab to the Glossary)

Paroxysmal cold haemoglobinuria: Pathophysiology and clinical features

Paroxysmal cold haemoglobinuria (PCH) is an uncommon subtype of cold autoimmune haemolytic anaemia. PCH is caused by a biphasic autoantibody (also known as a biphasic haemagglutinin or the Donath-Landsteiner antibody) that typically shows P antigen specificity. The antibody is an IgG subtype that reacts optimally at cold temperatures and fixes complement on the red cell membrane. The antibody dissociates from the red cell upon warming to body temperature (hence the term “biphasic”) while complement activation proceeds to completion causing intravascular haemolysis.

In the early medical literature, this condition was associated with chronic, advanced syphilis and in these patients, cold exposure resulted in a paroxysm (or sudden onset) of haemolysis which manifested as haemoglobinuria (dark brown colour of the urine associated with the

excretion of increased heme pigments created as a by-product of intravascular red cell destruction).

Paroxysmal cold haemoglobinuria is most likely to be seen in young children following a viral or upper respiratory tract infection and has been associated with several different viruses and bacterial infections. The sudden onset of haemolysis with fever, malaise and jaundice, accompanied by haemoglobinuria is the classical clinical presentation. The disease is usually transient and self-limiting, but anaemia can be rapidly progressive and severe. In some instances, transfusion is required.

Paroxysmal cold haemoglobinuria: Laboratory testing

The poly-specific DAT is positive, and the monospecific DAT shows anti-C3 specificity only. Typically, there are no difficulties with ABO typing. The routine antibody screen at 37°C is negative and the serologic crossmatch is compatible.

In the literature, the classical finding on the peripheral blood film is erythrophagocytosis (neutrophils engulfing red cells) and occasional spherocytes may be seen; tests to confirm haemoglobinuria may be helpful.

If PCH is suspected, the Donath-Landsteiner test should be performed. This test highlights the biphasic nature of the autoantibody. Serum from the patient is added to test red cells at 4°C. The Donath-Landsteiner IgG autoantibody sensitises the red cell and fixes the early stages of complement. Haemolysis does not occur until the sample is warmed to 37°C. A source of complement (fresh serum) must be added to the test red cells for haemolysis to occur.

Management of patients with paroxysmal cold haemoglobinuria

Paroxysmal cold haemoglobinuria can present with mild to severe haemolytic anaemia and haemoglobinuria: the presence of an abrupt change in urine colour is an important clinical clue. Management consists of supportive care including keeping the patient warm. If transfusion is needed, serologically crossmatch compatible red cells should be provided, and a blood warmer should be used. There is no need to provide very rare P negative red cells. Most cases are self-limiting, and the use of steroids is rarely required.

Drug-induced haemolytic anaemia: Pathophysiology and clinical features

Drug-induced haemolytic anaemia can occur through a variety of immune and non-immune mechanisms. Non-immune-mediated causes include oxidative haemolysis and drug-induced microangiopathic haemolysis. Hereditary deficiency of the enzyme G6PD (glucose-6-phosphate

dehydrogenase) impairs the red cell's ability to cope with oxidative stress leading to premature red cell destruction. Certain medications with oxidative potential can induce haemolysis in susceptible individuals. Anti-malarial drugs and certain antibiotics are important causes of oxidative haemolysis. Drug-induced microangiopathic haemolysis is characterised by low platelets, anaemia and red cell schistocytes on the blood film and can be seen with some chemotherapy and immunosuppressant drugs.

Drug-induced haemolysis can occur through immune-mediated mechanisms whereby the drug triggers production of an autoantibody. Drug-induced autoimmune haemolytic anaemia is poorly understood and can occur with a variety of therapeutic agents as well as certain chemicals and insecticides. When evaluating any patient with new onset autoimmune haemolytic anaemia, a careful medication history should be obtained. Patients should be asked about newly prescribed medications, over the counter drugs, herbal remedies, potential chemical/environmental exposures and illicit drug use.

Mechanisms that may lead to drug-induced autoimmune haemolytic anaemia include the following:

- (1) Antibody to the red cell membrane: Binding of the drug to the red cell membrane triggers an antibody response that sensitises red cells for immune destruction (hapten effect). Penicillin is the classic example of this mechanism.
- (2) Immune complex formation: The drug does not bind directly to the red cells but induces antibodies which interact with the drug in solution. These complexes loosely adhere to the red cell with the ability to induce complement activation and intravascular haemolysis.
- (3) Antibody to the drug: Exposure to the drug triggers the formation of an autoantibody that exhibits cross reactivity to red cell antigens even in the absence of the drug through molecular mimicry.

Some drugs, such as the cephalosporins, may cause immune haemolysis by more than one mechanism. The onset of haemolysis can be highly variable, ranging from immediate onset within minutes of starting an IV administered drug, to more delayed onset occurring after several weeks or months of exposure. Both intravascular and extravascular haemolysis may occur.

Drug-induced autoimmune haemolytic anaemia: Laboratory testing

The polyspecific DAT is usually positive. A positive DAT does not always mean that haemolysis is occurring. Some drugs may cause a positive DAT without haemolysis. The monospecific DAT typically reveals IgG coating the surface of the red cell. Complement may or may not be detected with anti-C3d. Routine antibody screening may

be positive or negative. The results of eluate testing are highly dependent on the immune mechanism. In scenarios 1 and 2 above, the eluate will be negative, whereas in scenario 3, the eluate would be positive. The presence of a positive DAT with negative eluate should prompt consideration of a drug-induced AIHA. Some drug-induced antibodies may show specificity to red cell antigens (e.g. D and Duffy). Which scenarios are these? ones within this paragraph, or from the numbered list in the section above? (please clarify this reference)

Management of patients with drug-induced autoimmune haemolytic anaemia:

Discontinuing the administration of the offending drug is the key to management. The time required for the haemolysis to resolve and the serologic tests to normalise depends on the mechanism of the drug-induced AIHA, the half-life of the drug and its metabolites, and rate of antibody clearance. In most instances, routine pre-transfusion testing and the ability to provide crossmatch compatible units will not be an issue. In cases where the autoantibody is independent of the drug (scenario 3), the same principles for blood product selection in warm autoimmune haemolytic anaemia would apply.

Key points

- Haemolytic anaemias are conditions in which the destruction of red cells is enhanced leading to decreased *in vivo* red cell survival.
- Immune haemolytic anaemia may be caused by allo- or autoantibodies or may be drug induced.
- Haemolytic disease of the fetus and newborn (HDFN) may occur when the fetus inherits an antigen from the father that is lacking in the mother, and when the mother has an antibody corresponding to the paternally inherited antigen.
- HDFN may lead to fetal and or neonatal anaemia and hyperbilirubinaemia.
- Many antibodies may contribute to HDFN, but the most common severe form is related to maternal anti-D with a D positive fetus.
- ABO HDFN is common and is seen mainly when the mother is Group O and the neonate is Group A or B and is usually a mild disease with no need for treatment or antenatal intervention.
- Routine antenatal maternal laboratory testing for blood group and for irregular red cell antibodies can contribute to timely management of HDFN.
- Intrauterine transfusion can be provided to treat fetal anaemia in severe HDFN and requires special selection and preparation of red cells for transfusion to ensure the safety of the transfused fetus.

- Hyperbilirubinaemia in the postnatal period is treated by exchange transfusion when other non-invasive treatments are not successful.
- Phototherapy is a common non-invasive method for reduction of neonatal bilirubin and often prevents the need for exchange transfusion.
- Rh immune globulin is given prophylactically to D negative mothers with D positive fetuses or infants, to prevent the formation of maternal anti-D. Because of the success of Rh immune globulin prophylaxis, the incidence of Rh disease caused by anti-D has been significantly reduced.
- Haemolytic anaemias may be caused by either warm or cold autoantibodies or may be the result of drug-induced autoantibodies.
- In cases of warm AIHA the DAT is positive with anti-IgG AHG, antibody screen and panel cells are pan-reactive at the IAT phase and donor red cell units are crossmatch incompatible.
- In cases of cold AIHA, the DAT is positive with anti-C3 AHG when complement is cell bound.
- Transfusion may be needed to correct anaemia in AIHA and this is a serological challenge because the autoantibodies are often pan reactive and may mask underlying alloantibodies. Crossmatch incompatible blood may have to be used. Phenotypically similar red cells should be selected if possible.
- Transfusion should not be withheld from patients with warm AIHA who are haemodynamically compromised.

Blood donors

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Introduction

For blood transfusion to be of benefit to the recipient, it must do no harm and therefore the process of identifying safe blood donors begins at the time of recruitment. Selecting, assessing and retaining sufficient safe donors whose blood will do no harm to recipients must therefore be the aim of every blood service. It has been shown that non-remunerated blood donors, who give their blood voluntarily and regularly, have the safest blood. It should therefore be the goal of every blood service to attract and retain only those donors who give their blood for altruistic reasons, and whose lifestyle is such that they are unlikely to be carrying transfusion transmissible infections (TTIs).

A safe blood supply depends on:

- Recruitment and retention of an adequate number of donors who donate blood regularly.
- Growing the number of repeat donors, i.e. those who donate regularly, rather than new donors.
- Education of donors regarding transfusion risks.
- Selecting safe donors by asking donors about their health and lifestyle factors.
- Performing blood grouping and testing all donations for TTIs.
- Ensuring adequate donor, sample and donation identification and records to make sure donations are properly labelled and blood can be traced from donors to recipients (vein to vein traceability).

This section will focus on recruitment, retention, and selection of donors. Blood group testing and TTI testing are discussed in *Section 10: Donation testing*. Quality procedures are discussed in *Section 16: Quality*.

Learning objectives

After reading this section, the learner should be able to describe -

- Recruitment of voluntary non-remunerated donors.
- Types of donors.
 - Voluntary non-remunerated blood donors (VNRBD)
 - Family replacement donors
 - Paid individuals
 - Directed donors
 - Autologous donors
- Eligibility for blood donation.

- General eligibility requirements.
- Medical history interview
- General steps in donation

Recruitment of voluntary non-remunerated donors

Recruitment of voluntary non-remunerated blood donors (VNRBD) starts with donor education. Ideally this should begin at school, with the donors of the future, and in the community. The concept of donating blood for no reward other than restoring a patient to health is important. Education performed at schools and in the community should emphasise the importance of health and a healthy lifestyle, so that blood donated will be of benefit to patients. Donor education should stress that the blood service is not a testing centre for transmissible infection, and that patients have the right to safe blood transfusions. General population awareness of the importance of donation may also be increased using a website, advertising, and marketing initiatives.

Blood donor clubs have proved very successful in motivating young people to become blood donors. The blood service should empower such clubs by providing them with education about donating blood. It encourages teamwork and healthy individuals to become united in the cause of donating blood voluntarily; of helping to ensure that safe blood is available for all patients in need.

Part of ongoing donor recruitment may be performed by telephone. It is a good way to remind donors of their next donation. It is also a useful tool in thanking donors for giving a donation, and for answering donor queries. Ready access to contact telephone numbers is important for telerecruiting (contacting donors by phone), and mobile phones are ideal for sending text messages to large numbers of donors quickly, especially at times when emergency blood drives are being arranged.

Donor recruitment is an ongoing activity. Donor recruiters should attend blood drives, motivating and encouraging donors. They should have good interpersonal skills and keep giving educational messages so that enthusiasm for voluntary donation is not lost.

Blood services usually have fixed site blood collection areas, and equipped vehicles for mobile blood drives at temporary venues, or within the vehicle. Collection centres should open at times that suit donors. Mobile blood

drives should be held regularly in areas conveniently within the reach of safe donors and at times that suit them. Blood drives should always be advertised beforehand, such as in the local newspaper, and by using banners, posters and information leaflets, and through social media channels and radio.

To ensure attendance at mobile venues, a good relationship should be created and developed between donor recruiter and a contact individual from the mobile venue (e.g. the public relations officer of a shopping mall, or the receptionist within an office block). A well trained and experienced contact can encourage attendance at blood drives and help to provide a venue that meets the needs of the blood collection team. Table 1 summarises possible activities involved in donor recruitment and retention at the levels of general education and awareness, specific blood drives, and individual donors. Different strategies may be needed to attract first time donors, ensure that donors early in their donation career return, and retain long-term loyal donors.

Types of donors

Voluntary non-remunerated blood donors

Voluntary non-remunerated blood donors (VNRBD) who donate blood regularly do not expect anything in return except to be treated with respect by blood service personnel. Customer service is very important. The blood donor who feels good about the experience of giving blood and who feels respected and appreciated is likely to return and donate blood again.

Table 1 Examples of donor recruitment and retention activities

Education and general awareness
Promotional logo and advertising slogans
Printed materials (posters, pamphlets), donor pins
Website
Educational materials for students
Advertising (free or purchased) via radio, TV, local newspapers, health fairs
Well known media or sport personalities as spokespeople or Ambassadors
<u>Specific blood drives</u>
Donor recruiters find locations (schools, religious institutions, city hall, sports events)
Partner with local volunteers to plan and sponsor drives (hospitals, service clubs, Mayor, university)
<u>Individual donors</u>
Donor lists and contact information for future donations
Appointment booking using telephone, text messaging
Peer recruitment
Donor clubs
Recognition programmes for frequent donors

Blood services should aim to reach and maintain 100% VNRBD, to maximise the provision of safe blood to patients. A regular or active donor may be defined as one who donates blood at least once a year. A lapsed donor may be defined as one who gave blood in the past but has not done so within the preceding 12 months.

Many blood services are still faced with the challenge of achieving 100% VNRBD and donations continue to be taken from individuals who do not give blood solely for altruistic reasons. This is likely to reduce the level of safety and increase the chance of transmission of infection to recipients. Table 2 summarises characteristics of VNRBD as well as other types of donors.

Family replacement donors

Where there is lack of co-ordination or no regular supply of blood, family members of a patient who needs blood may be approached and asked to replace the number of units of blood likely to be needed. This may put the family in a difficult position; if they are not able to donate blood themselves, they may need to 'recruit' and maybe even 'pay' other individuals to do so. Studies have shown that individuals who are paid or coerced to give blood, or who do so because of payment – either in money or in kind – are more likely to carry TTIs. This is because they are unlikely to disclose health risks.

In some cases, however, family members are able to donate blood for a loved one and are healthy enough to do so. The experience teaches them the value of blood donation; in this way they become aware of the ongoing need for safe blood and may be encouraged to donate blood regularly as a result. Some countries, with predominantly family replacement donors, have been able to successfully convert them to VNRBD.

Paid individuals

If individuals are paid for their blood then it inevitably encourages those who find it difficult to earn money by other means, to sell their blood. The blood from these individuals may be the least suitable, as far as the safety of the blood is concerned. They too, are unlikely to disclose health risks. Infected or 'tainted' blood is known to spread hepatitis, human immune-deficiency virus (HIV) and other illnesses to recipients.

The practice of paying individuals for their blood has decreased in most countries in recent times. The establishment of a nationally coordinated blood service, supported by government, a national blood policy and with access to adequate funding, is the best strategy for the elimination of paid donation.

Table 2 Types of donors

Type and Definition	Advantages	Challenges
<p>Voluntary non-remunerated blood donors</p> <ul style="list-style-type: none"> ● Recruited by the blood service ● Unpaid VNRBD ● Donate for any patient in need ● Ideally, return to donate regularly 	<ul style="list-style-type: none"> ● Usually preferred donation type ● Lowest rate of TTIs, particularly if become repeat donors ● May become regular donors ensuring a stable blood supply 	<ul style="list-style-type: none"> ● Requires system of donor recruitment and retention
<p>Family replacement donors</p> <ul style="list-style-type: none"> ● Recruited by a patient, or a family member who needs blood ● Blood donation goes into general inventory to "replace" units used for the patient ● May be family members of the patient, or other individuals paid or recruited by the family 	<ul style="list-style-type: none"> ● May recruit healthy individuals to become blood donors ● May become VNRBD after successful donation 	<ul style="list-style-type: none"> ● Usually have higher rates of TTIs, when compared to repeat VNRBD ● May be less likely to disclose health risks due to motivation to donate and lack of confidentiality ● Very often donate only once
<p>Directed donors</p> <ul style="list-style-type: none"> ● Recruited by a patient or their family members who needs blood ● Donation is used specifically for that patient ● If donation is not needed for intended patient, it may be discarded or placed in general inventory 	<ul style="list-style-type: none"> ● May recruit healthy individuals to become blood donors ● May provide rare blood group unavailable in the general donor pool ● May become VNRBD after successful donation 	<ul style="list-style-type: none"> ● Cannot be done if blood is urgently needed ● Complicated procedures to ensure selected donors are correct blood type, blood is sent to correct hospital
<p>Autologous donors</p> <ul style="list-style-type: none"> ● Patients donate their own blood prior to scheduled surgery ● Donation is used only for that patient and discarded if unused 	<ul style="list-style-type: none"> ● May provide blood if inadequate VNRBD ● May provide rare blood group unavailable in general donor pool 	<ul style="list-style-type: none"> ● Only possible in scheduled, elective surgery ● Patient/donor assumes risk of donation, and often starts surgery at a lower haemoglobin level ● Complicated to ensure blood is sent to correct hospital pre-operatively ● High wastage

Sometimes 'voluntary' blood donation may encourage coercion by groups, such as a work team being jointly awarded a prize for giving blood. Every member of the team must therefore donate for the reward to be honoured.

There are also paid individuals who give their plasma regularly by plasmapheresis (their red blood cells are returned to them at the time of collection). Their plasma is used by commercial companies for the manufacture of pharmaceuticals. Before processing, the plasma is extensively tested, and only non-reactive units are used. Additionally, steps in the manufacturing process, such as solvent-detergent treatment, inactivate many pathogens.

Directed donors

Patients who doubt the safety of the blood supply and have sufficient time before a transfusion is required may wish to select individuals to donate blood for them. Prospective donors refusing to give blood for a friend or relative could lead to awkward questions regarding the reason for this refusal, and the exposure of a high-risk lifestyle. To avoid this, some individuals may hide risk factors, and donate. The result is that the blood provided for the patient, rather

than being safer than the supply in the blood bank, is more likely to carry infectious agents.

Studies have shown that the blood from directed donors is less safe than the blood from non-remunerated volunteers.

Conversely, some blood services operate very successful directed donation programmes by allowing a directed donation only if the selected donor is already a registered, active blood donor and disallowing the selection of directed donations from individuals who would be donating for the first time. This has the added advantage of allowing the blood unit(s) to enter the general stock if not used for the intended patient, as the donor has met all the requirements for VNRBD.

Autologous donors

Autologous donors are those who donate blood for themselves. This option is only possible for patients who are scheduled for elective surgery and whose clinician has advised them of the possible need for blood during the operation. Given enough lead time, and a system to support it, the clinician may arrange with the blood service

for the patient to donate his/her own blood and for it to be processed and stored until needed during surgery. Donations of this kind are only possible if they do not compromise the health of the donor/patient. Unused (surplus) autologous blood is usually discarded and not placed in available stock.

Although autologous donations may be seen as the safest donations, some risks apply as they would to regular donations. The donation may be bacterially contaminated during collection, switched with the donation from another individual on labelling, or incorrectly processed. However, provided the blood service operates within a well-managed quality system, such complications should not occur. With advances in patient blood management, such as ensuring patients are not iron deficient prior to surgery, surgical advances, and recognition that a lower haemoglobin level is well tolerated by most patients, the use of autologous donation has decreased substantially in recent years.

Patients receiving autologous (or directed) donations sometimes need more blood than is available for their use and have therefore to be supplemented with blood available in stock at the blood service. As with all other patients, they should have access to sufficient safe blood from VNRBD.

Eligibility for blood donation

Screening of blood donors should be carried out by a trained assessor of the blood collection service, using standard procedures and criteria. The goal of donor screening is to ensure that the donation will not compromise the health of the donor, the health of the recipient, or the quality of the product. Steps in donor assessment usually include a medical history interview, a mini-physical examination of the donor (general appearance, vital signs), and a haemoglobin screen. In some resource limited countries with relatively high TTI rates, a rapid screening test for infectious diseases such as HIV may also be done prior to donation.

Regulations on the suitability for blood donation and the reasons for temporary or permanent deferral differ from country to country. Potential donors should ideally be aware of the basic requirements to donate blood before attending a blood collection session. Basic requirements to donate include the acceptable age range, weight, general health, and lifestyle risks acceptable to donate. Many blood centres have information on their websites for potential donors, in sections entitled "Can I donate?" with criteria for common reasons for deferral listed in alphabetical order. Walk-in donors who have not had the opportunity of public education on blood donation may

still present to donate. All donors benefit from having education materials available on the clinic site describing health criteria, lifestyle requirements and what to expect during the donation process. The information and the environment should also provide the opportunity for unsuitable individuals to self-exclude and leave the venue without donating. The environment must also provide for confidentiality, so that sensitive conversations between donor and assessor are not overhead. Without making provision for confidentiality, sensitive information is likely to be withheld by the donor, who may proceed to donate an unsafe unit of blood.

Prospective donors should understand the health and lifestyle requirements applicable to safe donors, and consent to their blood being tested for blood group, antibodies, and TTIs. Most blood services permanently defer donors if abnormal TTI test results are obtained. If the donor is not prepared to participate in being assessed or does not agree to the testing of the donation, he or she should not be allowed to donate blood. Consent should be documented and kept on record.

The pre-donation signature of the donor provides confirmation of the following:

- The donor has read and understood all the education materials provided.
- The donor has answered all the questions honestly.
- The donor knows of no reason why his or her blood should not be transfused to a patient.
- The donor consents to having his/her blood tested for transfusion transmissible infections including HIV.
- The donor agrees to being notified of any unusual test results, by the blood service.
- The donor agrees that his/her donation be used for transfusion or for medical research purposes.

General eligibility requirements

Age

The age range for blood donation depends on local regulations. The lower limit for donation varies between 16 and 19 years old in various countries. Individuals who are eligible to give blood prior to the age of consent in the country may require the written consent of a parent or guardian to donate. Although many countries have an upper age limit of 65 years for donation, others do not have an upper age limit if the donor continues to be healthy and meet all other eligibility standards. The upper age limit may be lower for a new donor, compared to a regular donor with a history of many years of safe, successful donation.

Minimum interval between donations

The interval between whole blood donations should be at least eight weeks (56 days) to allow the donor time to replenish the loss of red blood cells. This means that theoretically, an individual in good health could donate six times a year. This frequency of donation may lead to depletion of iron stores and eventually to iron deficiency anaemia. Males are usually able to donate more frequently than females, because they typically have larger iron stores and lower iron needs compared to females of childbearing age. Females are at greater risk of iron depletion because of iron loss in menstruation and pregnancy. Therefore, in many countries, the minimum inter-donation interval is longer for females (84 or 120 days) than for males.

Estimated blood volume (EBV)

On average, an individual who weighs 50 kg may have a total blood volume of at least 3.25 l. Individuals cannot safely donate more blood at one donation, than 10.5 ml/kg of body mass, which is approximately 525 ml for an individual who weighs 50 kg. This volume includes the blood donated plus specimens for testing. Many blood services set the maximum volume of donation for a donor who weighs 50 kg at 500 ml. In some countries, such as Japan, where the average weight of healthy individuals is lower, the volume of donation would need to be less. Particularly for younger donors, a lower EBV has been associated with an increased risk of faint and pre-faint reactions (vasovagal reactions). Therefore, more stringent criteria, such as a minimum EBV of 3.5 l, may be required for donors under age 18 or under age 23. Tables of EBV are available based on donor sex, height and weight.

In some countries a formal estimate of blood volume is not carried out, and suitability for donation in this respect is based on a minimum haemoglobin (Hb) level (usually 125 g/l) together with a minimum body mass (usually 50 kg). Height, which is considered when calculating EBV, is ignored in this regard.

In some countries, such as the USA, individuals may donate double red blood cell (DRBC) donations using apheresis technology. DRBC donations require a higher EBV, higher minimum haemoglobin, and longer inter-donation interval compared to whole blood donation.

A donor should be deferred if there has been a recent unexplained weight loss exceeding 5 kg, as this may indicate undiagnosed illness.

Occupation

Donors with hazardous occupations or pastimes (e.g. crane operators, sky divers) or donors responsible for the

safety of others (e.g. airline pilots, bus drivers) are often advised not to return to work on the day of donation because the consequences of suffering from a faint reaction may compromise their own safety and the safety of others.

Donor questionnaire

Donors are asked to complete a health and lifestyle questionnaire. Table 3 provides sample questions that may be

Table 3 Sample questions on the donor questionnaire

Will donation be safe for the donor?
General Questions
Are you feeling well and healthy today?
Do you have flu, a sore throat, fever or infection?
Are you taking any medication?
In the last 6 months, have you consulted a doctor for a health problem, had surgery or medical treatment?
In the last 6 months, have you been pregnant?
Specific organ systems
Have you ever had epilepsy or fainting?
Have you ever had a coma or a stroke?
Have you ever had problems with your heart or lungs?
Have you ever had a bleeding condition or blood disease?
Will blood be safe for the recipient?
Specific infectious diseases
In the last 6 months, have you had hepatitis/ jaundice?
Have you ever had a positive test for HIV/AIDS virus?
Have you ever had Chagas' disease, Babesiosis, or Leishmaniasis?
Have you ever had malaria?
In the last 12 months, have you had or been treated for syphilis or gonorrhoea?
Risk factors for viral diseases (HIV, HCV, HBV)
In the last 12 months, have you had a tattoo, skin or ear piercing?
In the last 12 months, have you had an injury from a needle or come into contact with someone else's blood?
In the last 12 months, have you had sex with anyone who has HIV/AIDS?
In the last 12 months, have you paid or taken drugs for sex?
In the last 12 months, have you had a blood transfusion?
Have you ever taken illegal drugs with a needle - even one time?
Risk factors for prion diseases (CJD, vCJD)
Have any of your relatives had Creutzfeldt-Jacob disease?
Have you spent a total of 3 months or more in the United Kingdom from 1980 through 1996?
Risk factors for parasitic diseases (malaria, Chagas' disease)
In the past 3 years, have you been outside of the US and Canada?
Were you born in Mexico, Central America, or South America?
Have you spent a total of 6 months or more in a continuous period in Mexico, Central America, or South America?
Medication that might harm the recipient
In the last 3 months, have you had a vaccination?
Have you ever taken Tegison or Soriatane?
Theoretically, cancer could be transmitted to a recipient
Have you ever had cancer?

included in the questionnaire. It should be noted that YES answers to any of the questions posed, often require follow-up questions to ascertain important details. It is important that provision is made within the blood collection area for this activity to take place in private, so that answers cannot be observed by other donors. The completed questionnaire is then presented to the donor assessor, who should interview the donor in an area that provides privacy.

Medical history interview

Health history and lifestyle questionnaires used at blood collection centres are designed to establish that prospective donors are healthy enough to donate blood and that their blood will not harm a recipient. Examples of questions provided in this section should not be seen as all-inclusive. Questionnaires should be provided in all of the common languages used by the donors, and appropriate support must be provided for any donors who are illiterate or who are unable to completely understand the questions. Such support must be provided by the blood service staff, and relatives or friends of the donor should NOT translate or in any other way provide assistance to the donor as there will be no assurance that the correct information is being provided.

At the outset of the interview process, which should be undertaken in an area ensuring confidentiality, the following questions should be asked:

- Have you read and understood the educational materials? If not, then the individual should be given the opportunity to do so, because the signature of the donor on the health history and lifestyle questionnaire indicates that these have been read and understood.
- Have you ever been told that you should not give blood? A 'yes' answer needs investigation to determine the reason – it may be that the individual should be permanently deferred (excluded).
- How are you feeling today? This is an example of how important it is to word a question correctly. If the question was posed: 'You are feeling well today, aren't you?' then a 'yes' reply is implied. The donor should never be 'led' into the answer of a question by the way it is worded.
- Do you consider your blood safe for transfusion to a patient? If there is hesitation in providing the 'yes' answer to this question, maybe the donor is considering giving blood for the wrong reasons. A skilled interviewer will detect this and ask a follow-up question such as: are you comfortable with your answer, or would you like me to clarify it, or to discuss it further?

One-on-one interview and donor questionnaire

It is important that donor assessments are performed individually and allow for donor privacy. The assessor should be tactful and understanding, encouraging the prospective donor to feel at ease and answer queries openly during the one-on-one interview. This applies not only to medical issues, but also to questions about blood-borne diseases and lifestyle behaviours. It is helpful to use a separate room for assessment; otherwise screens may be used to partition off areas for privacy.

The donor questionnaire is usually completed by the donor and during the interview the questions posed by the assessor revolve around the written answers provided. Alternatively, the trained assessor may ask the donor each question and complete the questionnaire in the presence of the donor. It is important to make sure that the donor understands the words used in the questions. For example, 'Have you had any skin lesions?' The prospective donor may simply answer 'no' to such a question, as he/she does not know what it means, and it seems that 'no' is the best answer to give. It is important that questions are explained by the assessor, if there is any indication that the individual being interviewed has not clearly understood.

Specific questions aim to determine:

- Will donation be safe for the donor?
- Will the donated blood be safe for the recipient?
- Will the donated blood meet quality standards?

Examples of possible specific questions are shown in Table 3. Some conditions fall into more than one category. For example, a donor with a bacterial infection may be less able to tolerate donation, and their donation may contain bacteria and therefore be unsafe for the recipient.

Some answers may trigger further questioning. For example, if the donor says 'yes' to the question 'Are you taking any medication?' then further details would be required, and the assessor may need to ask follow-up questions to ascertain what medication was taken, why and when. Answers to questions are then used to determine if the donor is eligible to donate that day, or if they will be deferred from donation, either temporarily or permanently. Donor deferral criteria for common conditions are usually summarised for assessors in a criteria manual. The manual instructs the screener to either accept the donor, defer the donor for a certain period of time (e.g. until the donor has completely recovered from a surgery or for a certain number of months after the donor has had a tattoo), or permanently defer the donor (e.g. if the donor has had Hepatitis B or Hepatitis C).

Questions related to safety of donation for the donor

These questions aim to determine if a particular individual is at higher risk for a complication of blood donation (faint or pre-faint reaction, post-donation anaemia or fatigue) compared to an average donor. There are usually some general questions and some specific questions related to organ systems that are particularly important for withstanding phlebotomy. For example, donors may be asked a general question about taking medication, being under a doctor's care recently, or having an infection. Specific questions may relate to heart or lung problems, history of faints or stroke, and bleeding conditions or blood diseases. Certain conditions, such as acute asthma attack, or a bacterial infection may result in a temporary deferral until the donor has recovered. Others, such as congestive heart failure, symptomatic coronary artery disease, or a history of stroke may result in permanent deferral.

Questions related to safety of blood for the recipient

The safety of blood products will depend in part on identification and deferral of high-risk donors and in part on testing all or a subset of donors for transfusion transmissible infections (TTIs), discussed in *Section 10: Donation testing*.

Testing alone cannot eliminate all infectious risks for several reasons:

For some pathogens, such as prions (e.g. those causing Creutzfeldt-Jakob disease (CJD) and variant Creutzfeldt-Jakob disease (vCJD)), no test is available for donor screening.

A test may be available, but it may be more practical in a given country to identify at risk donors on the questionnaire and defer or test these donors. For example, Chagas' disease is common in certain areas of Mexico, Central and South America. In these high prevalence areas, it may be useful to test all blood donors for the antibodies to the infectious agent, *Trypanosoma cruzi*. However, in other countries where Chagas' infections do not occur locally, it may be more economical to question donors who were born in or lived in these endemic areas, and either defer or test this small subset of donors.

Even if testing is performed for a given TTI, there is a period of time soon after infection when an infected donor can transmit the infection to a transfusion recipient, but the pathogen is not yet detectable on testing. This is called the "window period". The length of the window period will vary depending on the test used. For example, the window period for HIV may be nine days

for nucleic acid testing (NAT) but close to three weeks for anti-HIV testing.

Relevant questions to be asked, deferral policies, and donor testing will depend on the epidemiology (incidence, prevalence, and risk factors) of various TTIs in the general population in a given country.

Donors may be asked if they have had a positive test for a given TTI, such as HIV, hepatitis C virus (HCV) or hepatitis B virus (HBV) in the past. Since these are chronic infections, donors who have had a positive test at any time are permanently deferred.

Other questions are designed to determine if the donor is in a high risk category for a given TTI. If infection is likely to be chronic and there is no test available, at risk donors will be permanently deferred. For example, donors who spent more than three months in the UK during the Bovine Spongiform Encephalopathy epidemic (BSE epidemic, or "mad cow" disease) from 1980 to 1996 may be at increased risk for vCJD and are permanently deferred from donation in many countries. If the risk is for a TTI where testing is performed (such as HIV, HCV, or HBV), the deferral may be temporary. In theory, these deferrals need only be a bit longer than the window period for infection. In practice, deferrals tend to be much longer than window periods, in part for historical reasons, and the length of deferrals may vary greatly between various countries. Examples of these type of risk factors include percutaneous blood exposure (tattoos, piercing, needle injury), receiving a blood transfusion, and use of illegal injection drugs or steroids. Many countries defer donors temporarily for a history of syphilis or gonorrhoea; in part this deferral is for lifestyle risk, since gonorrhoea is not transmissible by blood. Syphilis may be transmitted by very fresh blood components; however many countries also perform syphilis testing. For more information on testing, see *Section 10: Donation testing and transfusion transmissible infections*.

A prospective donor who has travelled to an area endemic for any blood-borne disease (for example, malaria) may have become infected and be carrying that disease in its incubation stage. A donation given during this asymptomatic window period could transmit infection to the recipient. It is therefore important to question the prospective donor on travels undertaken and consult a map if necessary to ascertain whether the travel destinations were within endemic areas. It is better to ask where the individual travelled than to ask, for example: 'Have you been to a malaria area ...?' when perhaps the individual is not aware of the boundaries of these and could answer 'no' when the answer should be 'yes'. The blood collection team should have access to current information on areas where blood-borne diseases are prevalent, particularly with respect to malaria, and/or maps to assist them in their determination of donor eligibility.

Donor screening for sexual risk factors is a difficult and contentious issue. Most blood centres defer donors who have a sexual partner who is HIV positive, who has utilised injection drugs, or who has paid money or drugs for sex. These may be temporary or permanent deferrals. Many also defer males who have sex with another male (MSM) for periods ranging from three to 12 months after the last sexual contact. Others attempt to use more gender neutral approaches and defer all donors who have had a new partner (same or opposite sex) or multiple or “casual” partners for a certain period of time. Policies in a given country will be determined by the epidemiology of HIV and applicable local standards and regulations. For example, in South Africa, MSM are not considered a high risk category for HIV and therefore are not deferred.

Vaccinations

Donors who have received a vaccine with a live infectious agent, such as mumps, measles, and rubella (MMR) will be deferred for several weeks after vaccination, since the attenuated viral agent used for vaccination may be transmitted to an immunocompromised recipient and cause a significant infection. Although the vaccine against Hepatitis B is not a live agent, it may cause false positive HBsAg results soon after vaccination. Therefore donors are usually deferred for several weeks post-vaccination.

Non-infectious risks associated with transfusion

Many blood centres ask donors about specific medication use. Donors who have taken medication that has been linked to fetal malformations when taken during pregnancy in humans (teratogenic effect) may be deferred. Some of these medications may persist in the body for a very long period of time, and therefore deferrals may be of long duration even after the donor has stopped the medication. For example, the AABB Uniform Donor Health Assessment Questionnaire has a medication deferral list which specifies which medications should be cause for deferral, and for what period of time. Since a pregnant recipient would be receiving a very small amount of the medication found in the blood component, only on one occasion, the actual risk to the fetus is likely extremely small. Other medication, such as anticoagulants, lead to deferral because of increased donor risk (bleeding at phlebotomy site) and decreased product quality (decreased coagulation factors in plasma).

Cancer has not been shown to be transmissible by blood transfusion. However, other than basal cell and squamous cell carcinoma of the skin and carcinoma of the cervix, most blood centres defer donors with a history of cancer. For many cancers, if the donor has been cured,

the donor may be accepted from 12 months to five years after cure, depending on the blood centre and applicable local regulations.

General steps in donation

General steps in donation are shown in Table 4. The registration of the donor includes the documentation of all demographic information, and the provision of a health history and lifestyle questionnaire for completion. For returning donors, the date of the last donation is checked to ensure that enough time has lapsed for the next donation to be given, and that there is no reason for deferral or exclusion.

Donor identification

Careful documentation of all identification details is done so that all blood donations and the components prepared from them can be traced back to the donors at a later stage if needed. Donors must also be correctly identified with the results of testing done on the donation. The information related to donors is kept confidential, with authorised access only, of blood service personnel. Donors do not get to know who receives their blood, and recipients do not get to know who donated the blood they receive. Further information about donor identification is found in *Section 9: Blood collection*.

General appearance and vital signs

The general appearance of the donor may be of concern. An individual who has not had breakfast and has eaten their last meal the night before, may be at higher risk of a faint. This is of particular concern at blood drives held in schools, where prospective donors may skip breakfast in order to reach school on time, and not have their first meal of the day until midday.

Temperature

Donors should have a normal body temperature. A febrile condition which causes a rise in body temperature, may

Table 4 Steps in donation prior to phlebotomy

Identify donor, obtain enrollment information
Verify records if repeat donor
Provide information about donation
Perform mini-physical exam (temperature, BP and pulse, general appearance)
Standard health assessment questionnaire completed by donor
Donor health assessment interview with staff
Provide fluids and snack to ensure hydration pre-donation

indicate that the donor is starting an infection. This donor should be deferred to protect the recipient from possible transmission of infection and also to protect the donor who is not fit to give blood while febrile. Many but not all blood collection centres measure donor temperature and defer donors who are febrile.

Pulse

Some centres routinely evaluate donor pulse, while others do not. If the pulse is measured it should be evaluated by a trained individual over a period of at least 30 s or may be measured with a digital blood pressure device. A high pulse rate (over 100 beats per minute) may be the result of nervousness, so the donor may be asked to sit quietly for a short while and then the pulse taken again. On the other hand, a low pulse rate (below 50 beats per minute) may be found in athletes and this should be noted for the record. Donors may be deferred for an irregular pulse (not just the occasional irregular beat), a consistently high pulse rate of more than 100 beats per minute, or a consistently low pulse rate of less than 50 beats per minute if the individual does not do regular exercise such as jogging. The rationale for taking the donor's pulse and deferring for high, low or irregular pulse is that these may indicate underlying heart disease, and donation may be less safe in these donors. However, in otherwise healthy individuals, the evidence of benefit for these deferrals is weak.

Blood pressure

Blood pressure is usually measured in donors using a sphygmomanometer or a digital blood pressure monitor. Systolic and diastolic measures are taken. Some blood centres routinely measure blood pressure while others do not. The rationale for measuring blood pressure is that individuals with high BP (usually considered as a systolic BP greater than 180 mm of Hg and/ or a diastolic BP above 100 mm of Hg) may have hypertension and be at higher risk of adverse reactions with donation. Individuals with low BP (usually considered a systolic BP below 90 mm of Hg or a diastolic BP below 60 mm of Hg) may be more likely to have a faint reaction with donation, particularly if they are not adequately hydrated. As with measurement and deferral for donor pulse abnormalities, the evidence level of the benefit of measuring blood pressure and deferring donors with high or low BP is weak.

Donor haemoglobin (Hb) screen

Donor Hb screen is an important part of donor assessment, both to protect donors who are anaemic and ensure

a certain minimum volume of red blood cells in the donation. The minimum acceptable Hb level in different countries varies from 115 to 125 g/l for females and 125 to 135 g/l in males. Hb is usually estimated using a finger prick capillary blood sample. Methods include a Hb colour scale, copper sulphate screening test, haematocrit, and portable haemoglobinometer. Portable haemoglobinometers are the most accurate of these methods, and provide an actual quantitative measurement of Hb, rather than a pass/fail result. No matter which method is used, some variability in results is due to the difficulty in obtaining a good capillary blood sample. Staff training is necessary to avoid milking of the finger to obtain a sample, diluting the blood with interstitial fluid and lowering the measured Hb. Some blood centres measure Hb on a sample taken from the blood donation using a laboratory analyser and use the result to qualify the donor for their next donation.

Haemoglobin colour scale

A small card is provided, with six shades of red to represent Hb levels ranging from four to 140 g/l.

- Place a drop of blood onto the test strip provided.
- Wait about 30 s.
- Match the colour of the blood on the test strip with the shades of red on the card.
- The matching shade of red provides the level of Hb in g/dl.

Copper sulphate screening test

The Copper sulphate (CuSO_4) method is based on specific gravity (SG). A drop of blood taken from a finger-prick is dropped into the CuSO_4 solution. It becomes encased in a sac of copper proteinate, which prevents any change in SG for about 15 s. If the drop of blood has a satisfactory SG it will sink within 10 s. If not, the drop will hesitate, remain suspended or rise to the top of the solution in 10 s. Movement of the drop after 10 s should be ignored as all drops will eventually sink to the bottom of the container. This is not a quantitative test, but a qualitative one, and will show only if the Hb is above or below the minimum limit. The SG of the CuSO_4 solution is adjusted to the lower levels of acceptance according to local regulations. For example, a SG of 1.053 is equivalent to a Hb of 125 g/l. Only those whose drops of blood sink within 10 s, should donate. Figure 1 illustrates the CuSO_4 test showing the finger-prick being carried out with a safety lancet (one that is designed to prevent accidental needlestick injury after use). In the example shown, the donor has passed the CuSO_4 test because the drop of blood has sunk within 10 s.

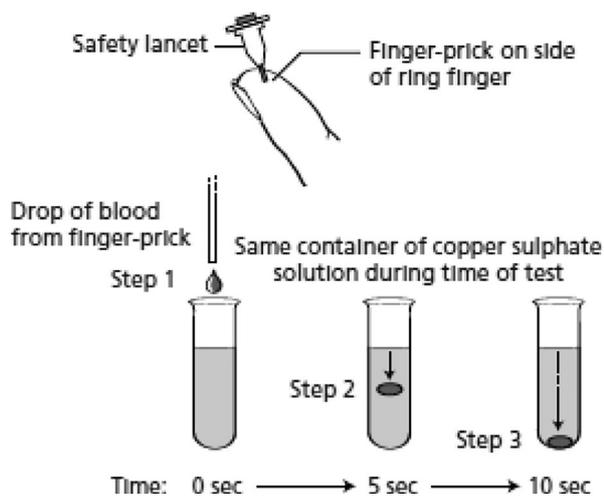


Fig. 1 Copper sulphate test showing finger-prick with safety lancet.

The Copper sulphate solutions should be stored away from light, in clean containers at room temperature and tightly capped to prevent evaporation. Individual containers should be checked to ensure they have not reached expiry date and should be used for a prerequisite number of tests only, depending on their volume and according to local quality protocols. Expired containers should be disposed of safely following the appropriate biohazardous waste disposal protocol, and a new container selected from the stock.

Although most variation in blood specific gravity is caused by Hb level, some variation in the result may be caused by the level of plasma protein. There will therefore be the rare occasion when an individual with a raised level of plasma protein but with a lower Hb than the cut-off level, passes the test. The CuSO_4 test is more likely, however, to fail donors who are above the Hb cut-off level and are therefore unnecessarily deferred from donation.

Portable haemoglobinometer

Although there are several different types of haemoglobinometer, the majority will consist of a microcuvette into which the sample to be tested is loaded and in which the actual test takes place, and a photometer which will determine the absorbance of the test solution and interpret the result. The result may be displayed on the instrument screen or may be printed onto a report. The haemoglobinometer must be calibrated before each batch of tests and quality control samples must be evaluated regularly to ensure that the instrument is performing as expected.

An example of a test method is as follows:

- Turn on the instrument and wait for "Ready" to appear on the screen.

- If testing a sample from a finger-prick, clean the finger with disinfectant and allow it to dry.
- Prick the finger using a lancet or similar device.
- Wipe away the first two drops of blood.
- Fill the microcuvette directly from the site of the finger-prick.
- Wipe away excess blood on the outside of the microcuvette tip using a tissue.
- Insert the filled microcuvette in the cuvette holder.
- Place the cuvette holder in its measuring drawer.
- After about 30 s the haemoglobin value of the sample will be displayed on the instrument screen.
- Record the result and discard the used microcuvette in a biohazard container.

Further steps in the donation process

Further steps in the donation process are explained in *Section 9: Blood collection*. *Section 9* also outlines counselling of donors who are either found to be ineligible to donate at the time of donation or have positive test results for a TTI.

Key points

- Prior to the blood drive, and even while still at school, prospective donors should be given general education on voluntary blood donation, what it means to be a safe donor, the meaning of a safe lifestyle, and what happens during the donation process.
- Donors who are voluntary, non-remunerated and who donate blood regularly are likely to have the safest blood.
- Other types of donors, whose blood may not be as safe, are family replacement donors, individuals paid for blood and directed donors.
- Prospective donors are assessed according to their general health, medical factors, and their risk for transmitting transfusion-associated infections to recipients, their lifestyle behaviours and travel history.
- Donors should be within the age range stipulated in the country, such as 16–18 years, to an upper limit of 65–70 years, meet or exceed the minimum weight requirement (40–50 kg, depending on country and volume of blood to be collected) and have a minimum Hb specified for the country, usually at least 120 g/l for females and 130 g/l for males.
- The minimum time lapse between two consecutive whole blood donations should not be less than 56 days.

- Whole blood donors should not donate more than 10.5 ml/kg body mass, which relates to approximately 500 ml of blood at a time, for a donor weighing 50 kg.
- The signature of a donor indicates agreement that health and lifestyle questions have been truthfully answered, that there is no known reason why their

blood should harm a recipient, that they understand their blood will be tested for ABO and Rh group, red cell antibodies, plus HIV and other TTIs, that their blood may be used for patients or for medical research purposes, and that the blood service may notify them of anomalous test results.

Blood collection

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Reviewer for Second Edition: Mindy Goldman

Introduction

The information in this section follows that in *Section 8: Blood donors* and starts with the assumption that the prospective donor has been assessed and found suitable to donate blood.

Aspects in this section that are of particular importance with regard to quality of the blood collection are:

- Cleaning the venepuncture site and taking all other measures to avoid contamination of the donation being collected.
- Mixing the blood and anticoagulant regularly during donation to ensure the best possible components, particularly platelets and plasma clotting factors.
- Making sure that the identity of donor, donation, specimen tubes and documentation correspond, so that test results will relate to the correct donor, the correct donation and the components to be processed from it.
- Giving the voluntary non-remunerated blood donor (VNRBD) a good experience so that they will want to return to donate again.

This section describes the principles of processes rather than providing information in the form of standard operating procedures.

Learning objectives

Describe all aspects of blood collection and give a clear account of the following:

- Blood collection area
 - phlebotomist's table
 - donor bed/chair
- Hand cleaning
- Blood collection bags and anticoagulant solutions – overview
- Donor identification
- The collection processes
- Venepuncture
- Taking the donation of whole blood
 - mixing the blood in the bag
 - checking the volume and flow
 - discontinuing the donation and collecting the specimens
- Apheresis blood donation – overview
 - Donor care and customer service

- donor education
- professionalism
- handling complaints
- refreshments for donors
- post-donation advice
 - post-donation request to discard a blood donation
- Adverse reactions to blood donation
- Haemovigilance
- Donor records
 - task tracking
- Handling of collected units – overview
- Transfer to processing centre

Blood collection area

The blood collection venue should be arranged in a way that promotes an efficient flow of donors from arrival, through registration, assessment, donation, post-donation care and refreshment to departure. Good workflow should be achievable at the blood centre, as fixed site workstations can be positioned where best suited and remain in place. Mobile blood drives require workstations to be set up for just one session, and then dismantled again. The mobile collections team may find that the area provided is not ideal. If such a venue cannot be changed, then the team should follow as efficient a layout as possible.

The collection venue should be clean, well lit, and well ventilated. The floor should be non-slip. It should be spacious enough to prevent donors from feeling claustrophobic. Figure 1 illustrates a venue layout that promotes workflow and efficient handling of donors from arrival to departure. It gives a suitable floor plan of a blood collection centre showing the likely positioning of the workstations. The entrance/exit leads to the reception desk for registration, preferably with a computerised system. This should provide an area where the donor health history and lifestyle questionnaire may be provided and completed, and the weight check performed. Seating, together with a supply of educational materials, should be provided for donors waiting to give their donation, or receiving refreshments after donation. Private cubicles are situated separately for health history assessment (review of the donor questionnaire), measurement of blood pressure (BP) and pulse, and finger-prick haemoglobin (Hb) check. Donors then proceed to the donor bed/chair and are attended to by the phlebotomist, who has a supply table at

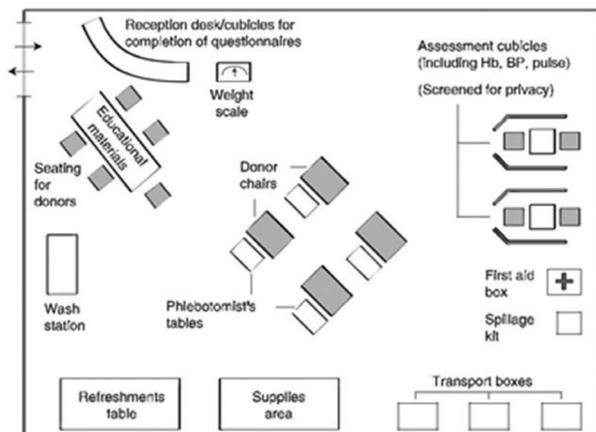


Fig. 1 Blood collection venue showing positioning of workstations.

hand. The wash station is conveniently situated for regular hand washing. Additional supplies are also nearby, as are the blood transport boxes containing coolants for temperature control of donated units and specimens awaiting transportation to the processing centre. Donors receive refreshments while donating or help themselves and take a seat in the reception area after completing their donation. It may also be helpful for donors to have some fluids before donating. A first aid box and spillage kit are also available if required.

One of the most important prerequisites of the collection area is cleanliness. Good hygiene is essential, with easy access to hand cleaning area, glove supply, spillage kit and waste containers as appropriate for disposal of biohazardous materials. Refreshment preparation should be carried out apart from other workstations to prevent cross-contamination with blood. There should be no electrical or other hazards that could jeopardise the safety of blood donors or collection team members.

Phlebotomist's table

The phlebotomist is the individual who carries out phlebotomy, i.e. venepuncture. There should be an area for materials and consumables used for taking blood donations, situated conveniently close to the donor bed/chair and the following items should be included:

- Supply of spare gloves.
- Blood collection bags.
- Needle guards (if not attached to blood bags).
- Sphygmomanometer and stethoscope or blood pressure monitor.
- Tourniquet or cuff.
- Cotton wool and sticking plasters or tape.
- Forceps (preferably plastic) for clamping tubing.
- Metal clips for sealing pilot tubing.
- Pair of scissors for cutting tubing after donation.



Fig. 2 Outline of donor chair for use at mobile venue.

- Antiseptic liquid for cleaning small spillages, forceps and scissors.
- Appropriate materials for disinfection of the venepuncture site prior to phlebotomy.
- Specimen tubes and test tube holder.
- Biohazard container for safe disposal of sharps such as venepuncture needles and lancets.
- Biohazardous waste container for used swabs and blood-stained materials.
- Disinfectant for cleaning donor bed/chair between donations if needed.
- Pilot tubing strippers (to ensure pilot tubing of bag contains anticoagulated blood, after donation).

The donor bed/chair should be plastic-coated (or coated with some non-absorbent material) for easy cleaning and should include the following attachments:

- Blood donation weight scale (if blood mixer is not in use).
- Easy adjustment for tilting into horizontal position.
- Armrest.
- Disposable towelling for head and footrest areas.

Donor bed/chair

Although a flat bed may be used, a chair that can recline is preferable, so that if a donor feels faint, the back of the chair may quickly be tilted back at the same time as raising the base. Figure 2 illustrates a typical donor chair that folds and is suitable for use at a mobile venue.

Hand cleaning

The decision as to whether gloves are required for certain procedures (such as when performing venepuncture, handing

blood specimens or blood donations), should be made by the management of the organisation. If the decision is that the wearing of gloves is compulsory, then all personnel should comply. The wearing of safety equipment should not be optional and should not be left to the discretion of the individual. Gloves should be changed as soon as they become soiled and in the case of blood collection personnel, new gloves should be worn for each donor.

Regular cleaning of the hands is mandatory for all members of the blood collection team. If gloves are worn, this does not mean that hand cleaning is no longer needed. All personnel who assist with the donation process should ensure that their hands and fingernails are clean. The following points with regard to hand cleaning are noted:

Washing hands in water

- Heavily soiled hands should be washed in water if possible.
- If there has been any contact with biohazardous substances, it is important that hands are washed immediately to avoid contamination of other items in the area.
- Hands should be washed in warm water, using a bactericidal/virucidal soap or solution and then rinsed with clean running water.
- Hands should be rubbed vigorously during cleaning, to create friction.
- The palms and back of hands should be rubbed, and between the fingers.
- After rinsing off all soap, hands should be rubbed dry using a disposable towel.

Cleaning hands with hand rub

- Hands can be effectively cleaned using an alcohol-based hand rub.
- The alcohol rub should be applied to the palms, back of the hands and fingers.
- The hands should then be rubbed together, between the fingers too.
- When the alcohol-based liquid has evaporated, hands are considered clean.
- Water is not used to wash off the alcohol rub and hands are not dried.
- Alcohol-based hand rubs may also be used on gloved hands.
- A bin should be conveniently located for the discard of used gloves and disposable towels.

Blood collection bags and anticoagulant solutions – overview

Historically, donated blood was collected into sterile glass bottles. These were discontinued many decades ago (1970s) and blood is now universally collected into plastic containers.

Donated blood is collected into specially designed single-use sterile polyvinyl chloride (PVC) plastic bags. Collection bags contain an anticoagulant/preservative solution, designed to prevent blood from clotting during storage and maintain viability of cells during storage. Anticoagulants for use in blood transfusion must be non-toxic so that they do not adversely affect the recipient. The first anticoagulant used was sodium citrate, which binds free calcium ions in the plasma and, because calcium is an essential component in the clotting cascade, it prevents clotting.

Since that time, many improvements have been made to anticoagulant/preservative solutions and additives such as those designed to maintain adenosine triphosphate (ATP) levels, which are necessary for red cell viability during storage have been introduced. Initially, at the time of collection, blood is drawn into citrate-phosphate-dextrose (CPD), acid-citrate-dextrose (ACD), citrate-phosphate-dextrose-dextrose (CP2D), or citrate-phosphate-dextrose-adenine (CPDA-1). The maximum storage time for whole blood in CPD, ACD, and CP2D is 21 days when stored at 1°C to 6°C, while whole blood stored in CPDA-1 has a maximum storage time of 35 days.

At first, the plastic collection bag was very simple, consisting of a single container with anticoagulant, and an integral pilot tube, at the end of which was the venepuncture needle. With the development of blood component therapy and the need to separate whole blood donations into their component parts, more complex collection systems evolved, allowing for the sterile separation of a whole blood donation into its various parts, including red cell concentrates, platelets and plasma. The sterility and integrity of the collection unit are maintained by means of a closed system of bags joined by integrally connected pilot tubing.

When whole blood is separated into components, additive solutions may be added to the red cells from an integrally connected satellite bag, after the plasma has been removed. Additive solutions introduced during processing of the blood into components further improve red cell viability during storage and extend the shelf life of stored red cells. Additive solutions may include adenine and mannitol. Additive solutions are described in more detail in *Section 12: Blood storage and transportation*.

Donated blood is collected into PVC bags that match the needs of the blood service and the components that are to be processed. Supplies of blood bags should be stored according to manufacturer's instructions, under cool conditions, and not left in the sun or in a transportation vehicle parked in the sun. It is important that quality checks are performed before use to ensure that blood bags meet quality standards. The integrity and sterility of blood bags are of critical importance to patient safety. Faulty bags that become contaminated, or sub-standard anticoagulant-preservative solutions, may compromise patient safety and cause fatalities. To maintain sterility, the blood container should not be entered except for blood collection or transfer of components to different containers for component production.

The venepuncture needle should be protected before use by means of a tamper-proof sheath, so that there is no possibility of it having been opened before the appropriate time. To prevent needlestick injury, a needle guard should ideally be fitted to the tubing, for use when the needle is withdrawn after donation.

A diversion pouch may be part of the blood bag unit. This allows for the collection of the first 30 ml of blood into a separate pouch for filling laboratory test tubes. The skin plug or any accidental contaminant from the venepuncture site enters the pouch and not the blood bag, making the process safer and reducing the risk of the donation becoming contaminated with skin bacteria. Figure 3 shows a diagram of the donation needle, needle guard, diversion pouch and specimen tube.

The correct blood collection bag for the components required should be selected by the phlebotomist.

Before use, the following should be checked:

- The bag has not reached batch expiry date as recorded on the bag.
- The sealed pouch of unused bags has not been unsealed for more than 14 days.

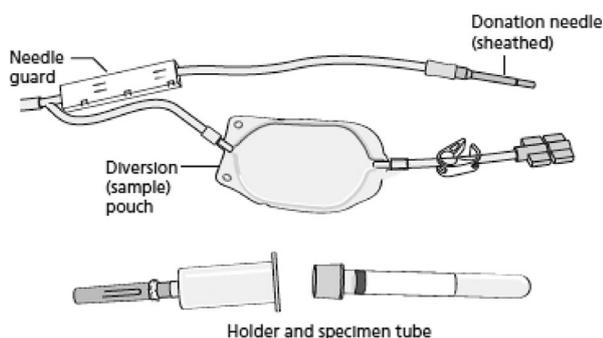


Fig. 3 Donation needle, needle guard, diversion (sample) pouch and specimen tube.

- The bag unit is not creased, and the pilot tubing is not badly bent or twisted.
- There is no evidence of holes or leaks in the bag or tubing.
- The anticoagulant-preservative is not discoloured or cloudy.
- The needle sheath is securely locked in place.

Donor identification

A suitable system for donor identification is a prerequisite for transfusion safety and a good quality system. Each donor should be assigned a unique donor number when donating for the first time. Every time this donor gives a donation, a unique donation identification number (DIN) is also assigned, preferably one that is both eye readable and bar-coded. By linking the donor number with the DIN of each of the donations made by that donor, it is possible to access the donor's entire donation history through the donor number. Thus, when the donor attends a blood drive, his/her record is accessed by the donor number, and the record of each previous donation can be referenced.

It is very important to be able to look back and trace all donations to the donors who donated each unit of blood. Likewise, all donations must be able to be traced to the recipients who received them. This is so that an investigation can be undertaken if a donor seroconverts and demonstrates the presence of a transfusion transmissible infection (TTI) marker, having donated blood previously as seronegative. For look-back purposes, transfusion recipients of window period donations may need to be traced to establish if they were infected as a result. Units from a previous donation may also need to be recalled if the donor calls the blood centre with more information affecting the safety of the donated product. Table 1 shows donor and donation numbers for a typical donor record of six donations. Although the record should contain additional information, such as date of each donation, for explanatory purposes, just the donor and donation numbers are shown.

An international coding standard (the ISBT 128 standard) is available for computerised donation and component identification. It is also available for manual systems

Table 1 Donor record of six donations

DONOR NO: 0823579	DONATION NO: 34722101
DONOR NO: 0823579	DONATION NO: 34777092
DONOR NO: 0823579	DONATION NO: 34783149
DONOR NO: 0823579	DONATION NO: 34788013
DONOR NO: 0823579	DONATION NO: 34790001
DONOR NO: 0823579	DONATION NO: 34792351

that can later be adapted to computerised systems. (Ref. www.iccbba.org).

A system should be in place to ensure that there is no possibility of duplication of donor or donation numbers. Donation numbers should be printed on high quality adhesive labels on a single sheet, and attached to the following items by a collection team member at the time the collection is made:

- Registration record card (may or may not be in use).
- Donation register (if records are not stored electronically).
- Health history and lifestyle questionnaire.
- Primary collection bag label.
- Specimen tubes.
- Remaining labels attached to reverse side of primary collection bag (for use on component bags at time of processing).

A DIN that has already been assigned to a particular donor should never be removed or replaced with another number. If, for any reason, the set of numbers assigned to a donation is not used, it should be discarded and documented as such.

Blood collection personnel must have a system in place to ensure that DINs cannot be switched between donors giving their donations at the same venue.

Implications of mistakenly assigning different numbers to donation and specimen tubes

If the specimen taken from 'Donor A' is inadvertently given the same number as the donation from 'Donor B', then the laboratory result for that specimen will not be linked to 'Donor A's' donation but will be linked to 'Donor B's' donation instead. If this specimen proves to be reactive for the human immunodeficiency virus (HIV), then the donation with the same number (i.e. 'Donor B's' donation) will be discarded, when in fact the donation from 'Donor A' was the HIV positive!

Because of the numbering error, the HIV positive donation is retained and placed in available stock. This is why it is so important *not* to make donor identification errors and to ensure that the blood bag and specimen tubes for a donation as well as all supporting documentation carry the same number and the blood from the same donor. Figure 4 illustrates the implications of a specimen numbering error. Similarly, label mix up errors can result in Donor A being labelled with the blood group of Donor B, with serious consequences.

The risk of errors of this type is increased if the numbered blood bag and specimen tubes are handed to the donor to convey to the donation chair, as two donors may swap specimen tubes, either intentionally or otherwise. Therefore, this practice should not be allowed.

The collection process

The venepuncture

Both arms of the donor should be examined for the best vein, in the antecubital fossa area (the inside of the elbow), from which to take the donation. This inspection also provides an opportunity to check for healthy skin, or evidence of rash, lesions or scarring that could indicate intravenous drug use. If the venepuncture site is blemished, or if there is evidence of drug use, the donation should not be taken.

The tourniquet or cuff is positioned around the upper arm and sufficient pressure applied to cause the veins to become engorged as the flow of blood out of the arm is restricted. This is assisted by the donor opening and closing their fist.

The venepuncture site may be cleaned using individually packaged swab sticks with an appropriate disinfectant. The most commonly used swab sticks contain a mixture of chlorhexidine and alcohol. Alternatively, cotton wool swabs and an appropriate disinfectant, or alcohol-based swabs individually sealed in sterile sachets may be used. If a liquid disinfectant is used, then dispenser containers should never be refilled from a stock bottle. When a container of disinfectant has been depleted, it should be discarded and a new one selected. Refilling and reusing the same container time after time introduces the risk of contamination by micro-organisms that proliferate in the container and disinfectant. When the arm is cleaned using contaminated disinfectants, this same contamination is then transferred to the venepuncture site, and in this way, the contaminant enters the donation. The micro-organisms thus introduced then multiply in the

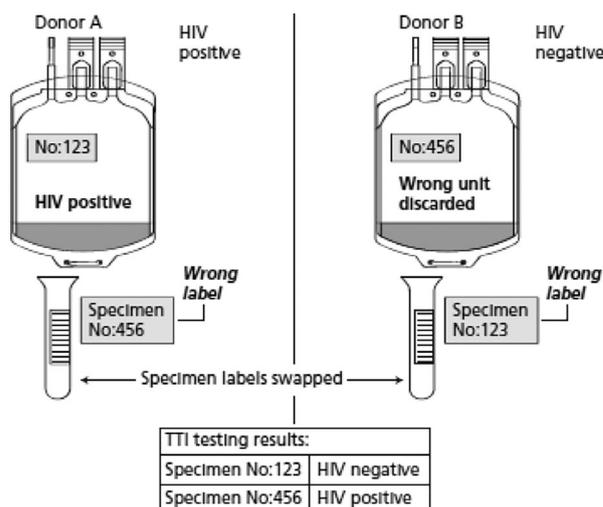


Fig. 4 Process of donation and specimen number error.

donated blood during storage and risk the development of septicaemia (infection in the bloodstream) in the recipient at the time of transfusion. Such disasters are likely to cause the death of the recipient.

Clean swabs, opened but unused at the end of the session, should not be replaced into a stock packet of clean swabs. They should be discarded as they could have become contaminated.

The phlebotomist should select the best vein in the antecubital fossa and clean the site according to protocol. The cleaning of the venepuncture area is the single most important step in the avoidance of bacterial contamination of the blood donation, so it is critical that it is performed properly. There should be a documented and properly validated standard operating procedure (SOP) for cleaning the venepuncture site, to which all phlebotomists are trained and comply. Ideally, alcohol alone should not be used for skin disinfection alone, since it does not inactivate all organisms. Alcohol combined with chlorhexidine or alcohol combined with an iodophor will cover a broader range of organisms. A "one step" procedure, using an individually packaged swab stick containing chlorhexidine and alcohol or an iodophor and alcohol may be used. Alternatively, an initial cleansing step may be followed by a second application with a new swab. The area is cleaned using swabs soaked in disinfectant, and applying firm pressure, from the vein chosen, radiating outwards, so that contamination from the surrounding area is not reintroduced to the selected venepuncture site each time a cleaning sweep is done.

The disinfected area must be allowed to dry completely, by waiting for at least 30 s after cleaning and before doing the venepuncture. Under no circumstances should the selected drying process be speeded up by blowing on it, or fanning it, as this may introduce micro-organisms to the

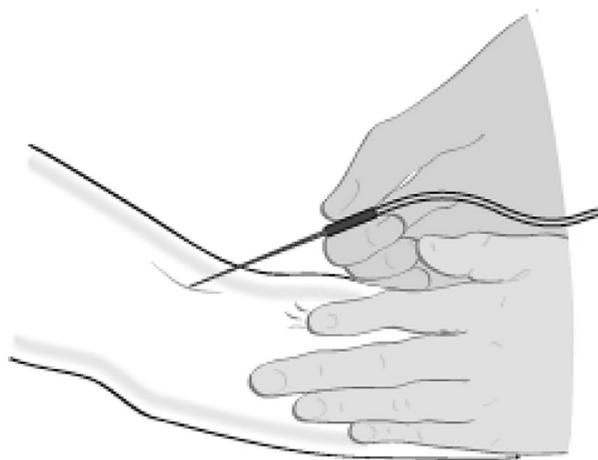


Fig. 5 Venepuncture.

skin, which then enter the donation bag at the time of venepuncture. For the same reason, the site must not be palpated (touched) again after cleaning, even if gloves are being worn. Figure 5 shows a venepuncture being carried out.

The donor should be asked to clench a fist while the needle is carefully removed from the tamper-proof sheath and the needle checked to ensure that it has no defects (such as a barbed tip). Using a 'no touch' technique, the phlebotomist should pull the skin of the lower arm tight (away from where the needle will enter) using a finger of the other hand, and then insert the needle quickly into the vein with a smooth motion, entering the skin and wall of the vein at an angle of about 30 degrees, and then straightening the angle of the needle to about 10 degrees as it enters the vein. This avoids exiting the vein on the other side, resulting in an unsuccessful venepuncture and the development of a haematoma (blood leakage from the vein into the tissues, with swelling and subsequent bruising).

Performing successful venepunctures is a practical skill that improves only with practice. It should be mastered under the supervision of an expert.

Taking the donation of whole blood

When the needle is in the vein, the tourniquet may be loosened slightly, and the donor asked to open and close their fist to promote blood flow. If fitted, the first 30 ml of blood is allowed to enter the diversion pouch. Once the pouch has been filled, that line is closed and the line into the blood bag opened so that the donation may be taken. If the collection unit does not have a diversion pouch, then the blood will start to flow directly into the primary bag.

Mixing the blood in the bag

It is important to ensure that the blood is adequately mixed with the anticoagulant from the time it starts entering the primary bag, by checking that the blood mixing apparatus is operating properly. If no mixing apparatus is available the bag should be hung upside down on the scale to allow the inflowing blood to mix with the anticoagulant immediately upon entering the bag, and the bag must be turned upside-down every 30 s during donation. Mixing is important to prevent the initiation of the coagulation process and the resultant consumption of coagulation factors. The initiation of clotting may not be visible at all, yet have major negative consequences including:

- Platelet concentrates prepared from such a donation will have a low platelet count.

- Fresh frozen plasma (FFP) prepared from such a donation will have low levels of coagulation factors, including factor VIII.
- Small clots that develop in the blood can later clog or block the filter of the administration set during transfusion.

Checking the volume and flow

The ratio between volume of blood collected and volume of anticoagulant in the bag should be correct. Too much blood in the bag (over-bleed) will mean that not enough anticoagulant is present, and the clotting process may be initiated; too little blood in the bag (low volume) will mean that there is an excess of anticoagulant that could damage blood cells during storage. In both scenarios, the blood would be unsuitable for transfusion.

The blood flow should be monitored to ensure that it is flowing freely into the bag. If it is not, the position of the needle must be checked and altered or rotated slightly to improve flow. The duration of the donation should be noted so that any donation that takes longer than 12 min is not processed into platelets and if more than 15 min is not used for FFP or cryoprecipitate production. No donation should take longer than 15 min; if it does, then the slow flow could have initiated coagulation. It may be convenient to note the start and stop time, or the duration of the collection on the blood bag so that this information is readily available to the component processing laboratory.

Blood mixers (blood shakers) mix blood and anticoagulant adequately and accurately measure the volume or weight of the donation, as well as monitoring flow rate and time taken for the donation. An audio-visual alarm should activate if there are problems with blood flow. When sufficient blood has entered the bag, an audio-visual alarm is activated, and the donation stopped. Figure 6 shows an example of a blood bag mixer.



Fig. 6 Blood bag mixer.

Discontinuing the donation and collecting the specimens

Specimens for laboratory testing may be taken into dry test tubes when post-donation testing will be performed manually but must be taken into the appropriate anticoagulant for automated testing. The test tubes must be labelled with the same number as the donation and should not contain the donor's name or donor number. If a diversion pouch is part of the blood bag unit, then the samples would be drawn from this pouch. Access to the pouch should not compromise the integrity of the hermetic seal and contents of the blood donation. (Note that the pouch does not contain anticoagulant.)

Once the required amount of blood has entered the donation bag, the blood mixer alarm should sound, or the counter-weight on the chair-side scale should tip. In both cases this should trigger an automatic clamping of the tubing, preventing further blood flow into the bag. If not, the tubing between needle and blood bag should be manually clamped to stop the flow.

The disconnection of the blood donation is performed by clamping the tubing, sealing it appropriately by means of a metal clip or knot, or using a dielectric sealer, and then cutting the tubing between the seal and the clamp. The needle is still in the vein when the donation is disconnected and with the cut and drip method, the samples would be collected at this point. Figure 7 shows a blood donation being disconnected.

Removal of the needle (after both donation and specimens have been collected)

- The donor should be asked to relax his/her fist and the pressure on the tourniquet or cuff should be released. Only after releasing the pressure should the needle be withdrawn. If this is not done, blood will spurt out, causing distress.
- The venepuncture site should be covered with cotton wool when the needle is withdrawn, and it should be withdrawn directly into the needle guard to prevent

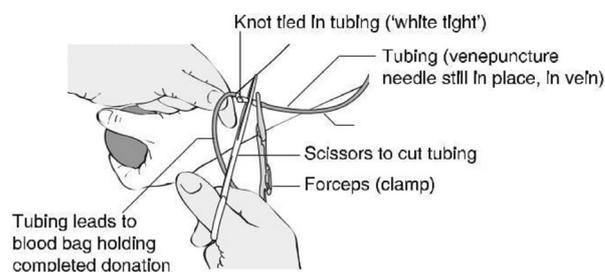


Fig. 7 Disconnecting the blood donation.

injury to donor or handler, and immediately discarded – together with remaining tubing – into a biohazardous sharps container. A needle must never be re-sheathed because of the danger of needlestick injury.

- Pressure should be kept on the cotton wool swab held over the venepuncture site for a few minutes to prevent oozing. The donor should be requested to keep his/her arm straight, elevated and not bend the elbow, which could cause a haematoma to develop. When the bleeding has stopped, the venepuncture site should be protected by applying clean cotton wool and a sticking plaster or bandage to keep it in place.
- To ensure that the blood in the tubing of the collection bag does not clot, it should be pushed (stripped) towards the bag. The pressure on the tubing is then released to allow it to refill with anticoagulated blood from the donation. This procedure should be repeated at least three times.
- The tubing attached to the collection bag should be sealed into segments. Knots, metal clips, or a dielectric sealer can be used to make the segments, which will be used for compatibility testing.

Apheresis blood donation – overview

Apheresis is the term used to describe the process of donation during which blood components are separated by a programmable machine called a cell separator (connected to the donor) into red cells, platelets and plasma. The component for donation is retained, and remaining components reinfused into the donor. For plasma and platelet collection, this allows for collection of a greater amount of the component of interest at each donation. Apheresis blood donation takes up to 90 min, whereas a whole blood donor would expect the process to take not more than 40 min from arrival to departure.

Plasmapheresis is the specific term used for the donation of plasma and the return of all blood cells to the donor.

Plasma may be collected for transfusion, or for pooling and further manufacture of plasma protein products such as albumin, factor VIII, and immunoglobulins. Plasma collected for further manufacture is known as source plasma. Cytapheresis is the general term used for the donation of cells and the return of the plasma to the donor. Plateletpheresis refers to the donation of platelets, while rarely performed granulocytpheresis refers to the donation of granulocytes. Red cells may also be collected by apheresis.

In general, the standards applicable to the selection and assessment of whole blood donors apply to apheresis

donors. They are usually regular donors with good veins. Most apheresis donors give either plasma or platelets. In both cases, they may donate more frequently than whole blood donors as their red cells are returned during the procedure. The volume and frequency of donation will depend on local regulations. As well as having their Hb checked prior to donation, regular plasma donors have total protein analysis (albumin and globulin levels) carried out. This is to ensure that they do not continue to donate if tests show that their levels have dropped. Plateletpheresis donors will have their platelet count measured to set the apheresis machine to collect the target number of platelets. Donors with higher platelet counts may be selectively enrolled on this programme.

Only automated collection should be performed, where the donor remains connected to the cell separator throughout the process. Apheresis should never be carried out using a manual process, in which the whole blood collected from the donor is taken away for processing and thereafter the red cells brought back and re-infused. This practice carries an unacceptable risk as the donor could accidentally be given blood from another donor, the consequences of which could be a fatal haemolytic transfusion reaction.

Cell separators use the differences in specific gravity or cell density of the formed elements (the red cells, and white cells and platelets of the buffy layer) to effect their separation, which in turn depends on the intensity and duration of the centrifugal force which the cell separator applies to the blood.

An anticoagulant is mixed with the donor's blood in a fixed ratio as the blood enters the chamber of the cell separator. When a standard volume of blood has entered the chamber, the blood is separated into its components by centrifugation. The components that are to be harvested are collected into one or more collection bags, whereas the remainder of the components is returned to the donor. This cycle may be repeated several times, depending on the component(s) to be collected. It is important that not more than 10.5 ml of blood per kg of the donor's body mass be collected into the cell separator at any one time, to prevent hypovolaemia. During plateletpheresis, white blood cells (leucocytes) are also removed from the donation, so that the platelet units are leucoreduced.

Donor care and customer service

All members of the blood collection team should receive regular training in customer service, so that they consistently keep the well-being of all donors as their focus. Donors who experience what they perceive to be poor customer service are unlikely to return to donate again.

They are also likely to relate a bad experience to other individuals, who in turn will not feel encouraged to donate their blood.

Donors should always be sincerely thanked for giving a donation at the time of leaving the collection centre. Thanking a donor again by telephone is an additional measure to apply to first time donors, or to those who experienced an adverse event, such as a faint or a haematoma.

Blood collection officers, like donor recruitment officers, are front-line representatives of the blood service and as such should receive adequate and ongoing training. The public is likely to make judgement on the whole organisation based on the attributes of these individuals, be they employees or volunteers. Customer service, with attention to detail to meet the needs of all donors at all times, is very important, as without blood donors there can be no blood transfusion service. Those who are responsible for attending to blood donors, from the time of their arrival at the blood drive or collection centre, to the time of their departure, are key contributors to the procurement of sufficient safe blood supplies. The receptionist, assessment officer, refreshment assistant, nurse and phlebotomist all play a vital role in the good or bad impression created, and the likelihood of VNRBDs returning to donate again.

Blood donors would like to feel assured that:

- They are sincerely appreciated.
- They will come to no harm through donating.
- The individuals attending to them are competent.
- Their donation will save a life.

Donors are sometimes confused into thinking that giving blood could expose them to infectious diseases. Collection team members should be able to confidently assure donors that all needles and other sharps used are brand new, sterile, used once only and then disposed of safely.

Donor education

The time that it takes for the donation to be given provides a good additional opportunity to educate the donor. It is inevitable that attendants will be asked questions by the donor at this time, so all collection team members should be trained to give the correct answers to technical questions in a simple way that is understandable to the public. Being able to answer questions competently creates confidence in the organisation.

Professionalism

The concept of professionalism and being professional should be promoted. Professional behaviour includes the following:

- Appearance: that the dress code and grooming meet documented standards.
- Behaviour: is in accordance with the professional guidelines of the organisation.
- Mission, vision and values: that all members of the blood collection team are able to describe the mission and vision of the organisation and are aware of and practice its values.
- Respect: that team members consistently show respect for each other, and for donors.
- Conversation: donors should not be witness to personal conversations between team members and should not be involved in or overhear inappropriate communication. Mobile phones should not be used in the collection area, other than in an emergency.
- Empathy: that team members listen to donors empathetically when they voice their concerns and rephrase issues to ensure understanding.
- Promises are kept: that team members do not 'over-promise and under-deliver'. For example, when a donor is told that an answer to a query will be given within a certain time period, then that promise must be kept.
- Body language: that team members are cheerful and enthusiastic at all times, with a genuine and ready smile to show donors that they are valued.
- Positive words: that team members never start a reply with a negative: ('No, we can't do that ...') or with a reply that shows indifference or incompetence ('I don't think we give badges to first time donors ...'), or with a reply that puts the donor's integrity or intelligence in question ('It's strange you should say that – you obviously don't understand our position on this ...') or by a response that puts the burden of action on the donor ('You will have to telephone our blood collection headquarters to find out when our next blood drive is scheduled ...').

Handling complaints

If the donor complains or is confrontational, this should always be handled with composure, and emotion should never be allowed to cloud the issue. The following behaviours may be helpful:

- **Listening:** it is important to listen with empathy to what the donor has to say, without argument or disagreement.
- **Expressing regret:** it is not necessary to defend an issue or argue with the donor. Simply saying sorry should defuse the situation and saying sorry does not mean taking the blame.
- **Clarifying information:** it is important that the problem is clarified, by respectfully asking follow-up questions to check for understanding.
- **Taking action:** the matter should be resolved as soon as possible, or a follow-up process should be in place if it is passed on to someone else, so that feedback is given.

Formal interactions with donors (be they positive or negative) should be documented according to quality requirements and include the following important steps:

- Identification of the issue or situation.
- Actions taken to resolve (or compliment a team member who is praised).
- Assignment of appropriate responsibilities for identified actions to be taken.
- Dates and outcomes of actions taken.
- Changes introduced to promote improvement.

Refreshments for donors

Before and after the donation, the donor should be offered refreshments (such as tea, coffee, or juice, and biscuits or sandwiches). Some studies have shown that having the donor drink 250–500 ml of water and ingest a salty snack immediately before donation can reduce faint and pre-faint reactions by approximately 25%. Having the donor move large muscles in their legs to return blood flow to the heart prior to getting up from the donation chair (applied muscle tension, or AMT) has also been shown reduce reactions after the donor stands up. It is especially important that first time donors remain seated for 5–10 min after donation, as getting up too quickly could precipitate a faint. No donor should leave the venue until collection personnel feel confident that he/she is well enough to do so, and that mishaps related to donation are unlikely to occur.

Post-donation advice

Donors should be given clear and simple post-donation advice including the following:

- Strenuous exercise should be avoided for the next 24 h.
- Plenty of fluids should be taken, particularly in the 4 h following donation.
- Heavy weights should not be lifted using the arm in which the venepuncture was performed for at least 4 h.
- Pressure should continue to be applied to the dressing on the venepuncture site for a short time after leaving the venue.
- The venepuncture dressing should be kept in place for the rest of the day.
- In the event of feeling faint, the donor should quickly sit down and lower his/her head.
- Any illness within 2 weeks of donation should be reported immediately to the blood service.
- Donors with hazardous occupations, such as bus drivers or heavy equipment operators, should not work for the rest of the day.

Post-donation request to discard a blood donation

There is always the possibility that someone who gives blood will fail to disclose a risk factor that could harm a recipient. Therefore, all donors should be given a telephone number to contact, if they later feel that their donation should not be transfused into a patient. It is not necessary for an individual to give their name, as long as they are able to quote their donation number, the donation can be located and discarded.

Note: The blood service should carry insurance that would cover eventual costs caused by adverse reactions to either blood donation or blood transfusion.

Adverse reactions to blood donation

Although blood donation is very safe for the vast majority of donors, adverse reactions can occur. The ISBT has developed a classification system for donor adverse reactions, that has been endorsed by many other international organisations, including the International Haemovigilance Network (IHN) and the AABB. If possible, the ISBT classification scheme should be used, so that reaction rates can be compared to other blood services. Use of clear definitions and reporting of reactions also allows blood services to follow rates over time and assess the impact of changes in eligibility criteria or procedures on adverse reactions (see Haemovigilance section below).

Broad reaction categories include the following:

- Systemic reactions (mainly faint and pre-faint reactions)
- Local arm complications:
 - (a) Bruising
 - (b) Haematoma
 - (c) Nerve irritation
 - (d) Accidental arterial puncture
 - (e) Allergic reactions

(f) Local thrombophlebitis.

Reactions, particularly faint reactions, may be complicated by donor injury.

Donors should be monitored throughout the donation process, as faint or pre-faint reactions (vasovagal reactions with or without loss of consciousness) are relatively common. Pre-faint reactions may occur in 1–5% of donors, while faint reactions with loss of consciousness may occur in 1 in 1000 to 1 in 5000 donations, depending on donor characteristics. Risk factors for faint and pre-faint reactions include first time donor status, estimated blood volume below 3.5 litres (can be calculated using donor sex, height, and body mass), younger age, and female gender. Therefore, first time, younger, female donors should be monitored particularly closely.

The causes of faint reactions are not totally understood. There are two main mechanisms. Fainting may be caused by nervousness, the sight of blood, blood donation by others and individual or group excitement. By engaging the donor in conversation, there is little time to worry about the blood going into the bag, or the large bore needle inserted into the arm. Fainting can also be caused by the reduction in intravascular volume (hypovolemia) caused by donation. It is important that the signs and symptoms of an untoward reaction are recognised quickly, so that steps can be taken to avoid a faint before it occurs. Taking appropriate action could also prevent a more serious reaction. Prompt and discrete action may also avert a mass fainting episode as the fainting of one donor has been known to result in observer donors fainting as well. The use of a screen to shield a donor having a reaction, from other donors at the venue, is advisable.

If the attendant is of the opinion that the donor is going to faint, then the donation should be stopped, and the needle with-drawn. Signs and symptoms of an impending faint include:

- Increasing pallor (paleness).
- Feeling of weakness.
- Cold and clammy to the touch.
- Dizziness.
- Trembling.
- Tingling of the fingers.
- Fall in blood pressure (hypotension).
- Fall in pulse rate (bradycardia).
- Sweating – particularly on the upper lip.
- Fidgeting and restlessness.
- Deep breathing.

Treatment options

A first aid kit to treat bad reactions should always be readily available for those trained and authorised to use

it. Ideally, a clinician should be in attendance or within easy call of the blood collection area, or an emergency medical service should be rapidly available if needed on the collection site. For serious reactions, it may be necessary to call a clinician, or an ambulance to take the donor to hospital. Although such incidents are extremely rare and may not occur for years, the collection team should have an action plan in place, and be trained in resuscitation, so that in such cases the appropriate care can quickly be given to the donor.

- Fainting: lowering the head and raising the legs should encourage the flow of blood to the brain and a return to consciousness.
- Haematoma: leakage of blood out of the vein and into the surrounding tissues with resultant swelling is often the result of a poor venepuncture. The donation should be discontinued, and the needle with-drawn. To contain swelling, it is helpful if ice can be applied. The donor should be told to expect bruising and be reassured that this will resolve over time.
- Allergy: donors could be allergic to latex in gloves, or iodine or chlorhexidine (in disinfectants) or adhesive plaster, for example. It is advisable to ask donors about allergies and if allergies exist, to notify the team leader and take action to avoid their use. Allergies should be noted on the donor record.
- Nerve irritation: When the venepuncture needle accidentally touches a nerve it causes severe shooting pain. The tourniquet pressure should be released immediately, and the needle safely withdrawn into the needle guard. Nerve irritation, although very painful, almost always resolves in a few weeks post-donation.
- In the event of arterial puncture, the blood will be bright red and will flow very rapidly into the collection bag. If noted early in the procedure, the collection should be stopped, and the needle removed immediately. The arm should be kept extended and raised above the head, and at the same time pressure applied to the venepuncture site for at least 5 min. A dressing should then be applied, and light pressure kept on the area. The donor should not leave the venue until bleeding has stopped.
- Citrate toxicity: this is sometimes a complication of apheresis donation, causing tingling and nausea when components are re-infused. It is caused by the presence of citrate (used for anticoagulation) in plasma returned to the donor. The return can be slowed down, and the donor given oral calcium supplements.
- Delayed complications such as infection at the venepuncture site, should be treated by a clinician if necessary. The donor should be asked to contact the

blood service to report any further complications, and collection personnel should contact the donor to follow-up the issue. The donor may need to be medically examined, and the cost of this should be covered by the blood service.

Haemovigilance

Haemovigilance is the term used to describe the noting of all adverse incidents related to blood transfusion, from the time of donation to the time of transfusion. It is important that a no-blame approach forms the culture of haemovigilance reporting, so that incidents are always reported and there is no motivation not to disclose them. Under-reporting defeats the quality objective of continuous improvement, which is that adverse events are used as the steppingstone to improvements in performance.

Donor reactions and incidents or errors involving blood at the time of donation should be analysed on a regular basis and form part of the annual haemovigilance report of a blood service.

The type of information given should include the numbers of the following events per 1000 donations:

- Faints, particularly those involving loss of consciousness.
- Unsuccessful venepunctures.
- Venepuncture with complications.
- Allergic reactions in donors.
- Over- or under-bleeds.
- Injury with sharps (including needlestick injury).
- Misidentification of blood donors.

The responsibility for gathering information and compiling reports should include representatives of the blood collection team and other appropriate staff members of the blood service, including the medical director and quality manager. A plan of action should be documented, suggesting actions to be taken to correct problems, such as extra training for a phlebotomist with poor venepuncture technique. Analysis enables year-on-year comparisons to be made, trends to become evident, and actions to be assessed according to their value.

Donor records

Donor records should be comprehensive and easily accessible, and should include the following information:

- Full name and residential address.
- Work address.
- Telephone numbers (Work and Home).
- Date of birth.
- Language preference (if applicable).
- Gender.

- Donor number.
- Blood group.
- Number and dates of previous donations.
- Blood collection area where donations were given.
- Active deferral (if applicable) – period and reason.
- Deferral history – dates and reasons.
- Deferral alerts for staff in code (such as 'do not take donation').
- Hb failures.
- Low or high blood pressure if performed.
- Pulse, if measured.
- Previous faint reaction.
- Poor veins and unsuccessful donations.
- Body mass history (to allow for weight loss trend to be noted).
- Code if positive TTI results.
- Comments.

Donor records, like any other medical records, are confidential and may not be disclosed to those who are not in authority to share them. For example, if a donor is reactive for hepatitis C and a team member who is a friend or family member of this donor finds out this information, it is never acceptable to approach this donor, no matter how good the intention, to discuss the result. This would be a breach of confidentiality and could have serious ethical and legal consequences.

Records of donors with positive TTI results should be coded in such a way that the code visible to the collection team members is 'Do not take donation' or similar – the actual reason for the code (e.g. anti-HCV reactive) should only be available to authorised personnel. If appropriate, such a donor may be referred for counselling, which should be performed before he/she returns to donate again.

A good policy is that collections staff and other staff who deal directly with the donors do not at any time have access to laboratory test results. Laboratory staff should not have access to donor information but work solely with Donor Numbers and DINs. They should not be able to link a test result, e.g. positive TTI, with a particular donor.

Task tracking

At each critical step in the process of blood donation, the individual responsible for that task should place his/her signature and identification code at the appropriate place on the documentation, such as the health history and lifestyle questionnaire. This is important for the subsequent tracking of those involved in taking the blood donation, should there be an enquiry after transfusion. There should also be a documented Standard Operating Procedure (SOP)

for each task, and team members should be fully trained. A system should be in place to assess ongoing competency.

Tasks, and the identity of personnel performing them, that should be trackable include:

- Registration of the donor (demographics, age, last donation and allocation of number).
- Provision of educational materials.
- Finger-prick haemoglobin assessment.
- Medical and lifestyle assessment (including body mass, BP and pulse if taken).
- Signed agreement (informed consent) by the donor that all aspects of the donation process are understood.
- Evidence of clarification of answers to health history and lifestyle questionnaire.
- Selection of collection bag (and attachment of donation number where required).
- Venepuncture and donor care (including handling of adverse reaction).
- Taking specimens as required for laboratory testing.
- Time taken for donation from venepuncture to discontinuation.
- Discontinuation of donation (including tube stripping).
- Preparation of donations for storage and transportation.
- Training of personnel and ongoing competency assessment in these tasks.

Handling of collected units – overview

Donated blood would be expected to cool after collection, from body temperature, which is 37°C. The ambient temperature at the venue could vary considerably, however, depending on the location and the time of the year. As soon as possible after collection, blood donations should be placed in a controlled temperature environment to prevent deterioration.

Time and temperature requirements for the storage and transportation of donated blood to the processing centre or main storage area depends on local regulations and on the nature of the components into which the blood is to be separated. The temperature control of donated units is detailed in *Section 11: Blood processing and components* and *Section 12: Blood storage and transportation*.

Transfer to processing centre

Blood transport boxes should be of validated quality and specification and able to maintain the required temperature range during transportation.

The blood collections team leader should check each consignment of blood before it is sealed for

transportation, to ensure that it contains all blood, specimens and documentation records.

On arrival at the processing centre, the temperature of the contents should be determined and documented. Only if contents are within the appropriate temperature range, should the units of blood be accepted for processing.

The processing team should be provided with the expected date and time of arrival of consignments of donated blood, as this influences their workload. On arrival, contents should be checked for the following:

- Blood and specimens should have been packed appropriately to prevent damage in transit.
- The transport box should be identifiable with the collection area of origin.
- Blood units enclosed should be identifiable as being from a particular venue.
- The name and contact details of the blood collections team leader should be noted in case of queries on arrival.
- The specimens for laboratory testing should be shipped with the donations to which they relate.
- The documentation related to the donations should accompany the consignment.
- The time of the first and last donation should be noted on the documentation.
- The temperature of the blood should be noted on arrival, at the processing centre.

Key points

- Anticoagulant-additives used for blood donation must be non-toxic and able to maintain ATP levels during storage.
- The design of the collection area should promote good workflow for the efficient handling of donors in a safe environment, avoiding delays.
- Hand cleaning is an essential preventative measure to avoid contamination of blood donated.
- If gloves are worn, it does not mean that hand washing may be neglected.
- Blood bag units are made of PVC with interconnected tubing in an hermetically sealed environment, allowing for the sterile collection and processing of blood into components.
- Blood bag units may be fitted with needle guards and may have diversion pouches.
- If needle guards are not part of the bag unit, they should be clipped onto the tubing adjacent to the needle at the time of venepuncture, as a safety measure to avoid needlestick injuries.
- Donor identification is critical so that the blood in the specimen tube correlates with the blood in the donation bag. Errors may result in an incorrect

- donation being deemed TTI-reactive and being discarded and the actual TTI-reactive donation being transfused.
- The cleaning of the venepuncture area is the single most important step in the avoidance of bacterial contamination of the blood donation, so it is critical that it is performed properly.
 - Mixing the blood in the bag during collection is essential to prevent the initiation of coagulation and the loss of functional platelets and clotting factors in the donation.
 - The volume of blood collected must be sufficient for the volume of anticoagulant.
 - Blood mixers determine rate of flow and volume being donated and mix the contents of the donation bag with the anticoagulant.
 - The venepuncture needle must be safely discarded immediately after removal, in a biohazard container designed for sharps. No needle should ever be re-sheathed because of the danger of needlestick injury.
 - Apheresis donations are those in which the donor gives only the component required, rather than whole blood, using automated procedures.
 - Plasmapheresis refers to the donation of plasma; plateletpheresis is the donation of platelets.
 - Donor care involves customer service and ensuring that all donors are treated with respect.
- Donors should be given refreshments and post-donation advice, including the facility to contact the blood service later if they feel that their blood is not safe for transfusion.
 - Adverse reactions may be experienced by donors, so blood collection personnel should be trained in first aid and resuscitation.
 - Haemovigilance is the recording of adverse events related to blood donation and transfusion. Monitoring and analysing incidents allows for the identification of actions that may be taken to reduce or eliminate problems and in so doing, improve safety.
 - Donor records are essential for the tracing of donors to donations and donations to recipients.
 - Donor records, like other medical records, are confidential.
 - Collected units should be kept under controlled temperatures from the time of donation, and while being transferred to the processing centre.
 - Four critical control points for quality in blood donation are:
 - (a) prevention of contamination of the donation being collected
 - (b) mixing the blood and anticoagulant during donation
 - (c) correct identification of donor with donation and specimens
 - (d) giving the donor a good experience.

Donation testing and transfusion transmissible infections

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Reviewer for Second Edition: So-Yong Kwon

Introduction

The accurate testing of blood donations plays a vital role in the provision of safe blood for transfusion. This section covers testing that should be carried out on every unit of blood donated.

All donations should be routinely tested for the ABO and Rh blood groups, screened for red cell antibodies and should be tested for transfusion transmissible infections (TTIs) including the human immunodeficiency virus (HIV), hepatitis B and C and syphilis. Anomalous test results should be investigated and resolved before blood is deposited in available stock.

A range of systems, equipment and techniques are available for both the red cell serological testing and the testing for TTIs. Fully automated computerised systems are used for all aspects of the testing in large-scale testing. In some organisations manual techniques may be used, e.g. for red cell serology tests, test tubes, microplates or microcolumns, (gel technologies) are used.

Anticoagulated samples are required when testing is automated. Clotted samples are suitable for blood grouping performed manually.

Each donation must have a unique identification number (see *Section 9: Blood collection*). When the donation samples taken from the blood donors at the time of collection arrive at the testing laboratory, they should be correlated with the respective donations to check that the batch of blood donations received relates exactly to the accompanying samples. Samples should be transported to the testing laboratory in accordance with procedures that ensure a constant approved temperature and secure confinement. Samples should be stored at $4 \pm 2^\circ\text{C}$ and tested as soon as possible, and within the time limits specified in test or kit manufacturer's instructions.

Learning objectives

By the end of this section, be able to describe:

- Red cell serology testing
 - ABO and Rh typing
 - red cell antibody screening
 - ABO antibody strength (haemolysing potential)
 - red cell antigen screening
 - automated testing for red cell serology
- Transfusion transmissible Infections (TTIs)

- Window period
- Testing algorithms: screening and confirmatory testing
- Specific TTIs
 - human immunodeficiency virus (HIV)
 - hepatitis B (HBV) and Hepatitis C (HCV)
 - syphilis
- Other transmissible infections
 - other transmissible viruses
 - transmissible parasites
 - prions
 - bacteria
- Emerging pathogens
- Lookback and traceback procedures

Red cell serology testing

The tests described in this section are based on manual testing. The principles, however, apply to the testing in general. An overview of automation will be provided at the end of this section.

ABO and D typing

The ABO and D typing should be carried out on every donation. Results should be compared with previous results if possible, and anomalies brought to the immediate attention of the individual member of staff responsible for investigation and resolution.

ABO typing

Saline methods are used to perform ABO grouping tests. Both forward and reverse grouping is performed. The results of forward and reverse grouping must correlate for the result to be acceptable. Reagent antisera should be standardised for both rapid spin and longer incubation techniques, as a rapid spin technique is not practical when dealing with large numbers of samples. Therefore, batches of tests may be incubated at room temperature for about an hour (depending on manufacturer's instructions) before reading.

The ABO grouping result for a repeat or lapsed donor should be compared with the result from the previous donation to check that it correlates, and action taken if it does not. In the case of an anomaly, the senior

technologist should be responsible for investigating and resolving the problem. If the anomaly cannot be resolved, the donation should not be used.

Correct grouping of subgroups of A

Group A or AB individuals, lacking the A₁ antigen, sometimes develop anti-A₁, usually as a cold IgM antibody. When the A antigen is very weak and is not readily detectable on initial testing, even when using avid blood grouping reagents, the presence of anti-A₁ in the donor serum/plasma may complicate the interpretation of the ABO blood group.

On repeat testing of anomalous groups, it is therefore preferable to use both group A₁ and A₂ reagent red cells, together with the reagent group B cells. Using both group A₁ and A₂ reagent red cells would enable clarification of subgroups of A as shown in Table 1.

A very weak subgroup of A, with anti-A₁ in the serum/plasma may be mistaken for a Group O if the weak agglutination is not detected in forward ABO grouping, and Group A₁ and B reagent red cells are used for the reverse grouping. However, an anomalous grouping result is noted when Group A₂ and B reagent red cells are used instead. The use of Group A₂ reagent red cells alerts the technologist to the fact that further testing is needed to resolve the ABO group, and prevents the incorrect interpretation of the blood group as Group O.

Similarly, if the very weak AB is not detectable in forward ABO grouping, and anti-A₁ is present in the serum/plasma, it appears as Group B when group A₁ and B reagent red cells are used and gives an atypical grouping result when Group A₂ and B reagent red cells are used instead.

Reverse or serum grouping is indispensable as a means of confirming the forward or cell grouping of blood donations. Isoagglutinin tests should be read blind with no preconceived idea of what results should be. This also applies to records of ABO groups – the technologist reading the test should not have knowledge of the previous ABO group at the time of reading. Every atypical result should be investigated, including weak agglutination results where strong agglutination should be apparent. Once the ABO group of a donor has been confirmed, his/

her record should be appropriately documented for future reference.

Unusual or anomalous ABO types on donations need to be resolved before blood may be labelled and placed in available stock. With problematical groups, a sample from the pilot tube of the donation should also be tested. On rare occasions when the blood type cannot be interpreted with confidence, the donation should not be used, and the anomaly may need to be resolved at the time of the next donation.

Table 2 shows an example on an anomalous ABO grouping result. Even though it appears from the forward grouping that the result is Group A, the group cannot be interpreted as the reverse grouping does not confirm the Group A type.

Blood grouping records for repeat donors should be consulted to check that the group on record is the same as the group for the current donation. If it is not, then the pilot tube of the blood unit should be grouped. If this differs from the group on the sample, then a switch is confirmed. However, if the group using the pilot tube is the same as the group using the sample, then the group on record may be incorrect, or the current donation misidentified. The implications of such errors could be far reaching, and results on other units of blood donated at the same session, put into doubt. The individual in charge is responsible for carrying out an investigation in such cases. If there is no conclusive resolution, the blood cannot be used.

D typing

Donations are separated into two D types: D positive and D negative based on the presence or absence of the D antigen, respectively. D typing results that indicate a D variant should also be classified D positive. This is to ensure that D negative recipients do not receive blood containing the D antigen. Some D variants, such as DEL, are not detected by routine serologic methods. Prevalence of the DEL phenotype differs significantly among populations, with 10–30% of D negative East Asians having the DEL phenotype. In the Caucasian population, only 0.1% of D negative people express a DEL phenotype and among African populations, there are no reports of DEL phenotypes.

Table 1 Agglutination results on testing weak subgroups of A

ABO group	Reagent antisera			Reagent red cells			Interpretation
	Anti-A	Anti-B	Anti-A, B	A ₁	A ₂	B	
Weak subgroup of A with anti-A ₁ in serum/plasma	0	0	0 (or very weak positive)	2 or 3	0	4	Mistaken for group O if only A ₁ and B reagent cells are used
Weak AB with anti-A ₁ in serum/plasma	0 (or very weak positive)	4	4	2 or 3	0	0	Mistaken for group B if only A ₁ and B reagent cells are used

Table 2 Example of an anomalous ABO grouping result

Reagent antisera			Reagent red cells				Comments
Anti-A	Anti-B	Anti-A, B	A ₁	A ₂	B	O	
4	0	4	0	1	4	2	On initial testing, interpretation appears to be group A with an unexpected antibody; the specificity of the antibody should be determined, and the reagent red cells can be tested to determine if the cells are positive or negative for the antigen corresponding to the antibody identified

If D negative patients are transfused with D positive red cells, this could stimulate the production of anti-D. This may subsequently have serious implications for D negative patients, such as:

- Anti-D present in a D negative woman, pregnant with a D positive fetus, could lead to haemolytic disease of the fetus and newborn. See *Section 7: Haemolytic diseases* for further details.
- Anti-D present in a D negative patient transfused with D positive blood in an emergency, or in error, could cause a haemolytic transfusion reaction.

D typing results on repeat donors should be compared with the results from previous donations to check that they are the same, and action taken if they are not. In the case of an anomaly, the individual in charge should be responsible for investigating and resolving it. If it cannot be resolved, the donation should not be used. Such donations should be flagged so that further testing may be carried out at the donor's next donation to investigate the anomaly.

D typing is usually carried out using IgM (saline reacting) commercial reagents. Donors should be typed with a minimum of one selected monoclonal anti-D reagent and negative results should be confirmed using a second appropriate reagent, which could be an anti-D blend reagent, using the indirect antiglobulin test (IAT) or a saline reactive monoclonal reagent known to detect weak D.

If the initial D typing is negative and additional testing using the IAT is positive, further testing should be performed to determine whether the red cells were sensitised prior to testing, in which case the result would be invalid. This may involve performing a direct antiglobulin test

(DAT) on the donor red cells or repeating the test with the appropriate typing control according to manufacturer's instructions.

Table 3 gives examples of additional testing carried out to determine the D type. Depending on the anti-D reagents in use, the original test may be taken to the IAT phase or the test may be repeated including the first saline phase. There are many variations in procedure depending on the source of the monoclonal anti-D selected for use in the laboratory. It is important to follow the manufacturer's instructions/laboratory protocol.

Controls for ABO and D typing

Positive and negative controls should be performed on all reagents used, in parallel with every batch of tests, or at least once a day at the same time of the day, for standard ABO and D typing reagents. If the reagents perform as expected, this will provide the assurance that the reagents meet sensitivity and specificity requirements and that test results produced using the reagents can be accepted. Any reagent, in which controls show anomalous results, should be removed from circulation to avoid its continued use until an investigation has been carried out to resolve the problem. When faulty reagents are used for testing, all results for that batch are invalid.

Red cell antibody screening

Irregular red cell antibodies in donor plasma may have an adverse effect on a recipient with the corresponding antigen especially when whole blood or plasma

Table 3 Examples of additional Rh typing when an IAT reacting reagent is used

Anti-D reagents			Donor red cells		Comments
Original IgM (saline)	Repeat IgM (saline)	IAT method	DAT	Typing Control	
0	0	1	0	0	Confirmed weak D positive
0	0	1	1	1	Further investigation needed
1	1	1	1	1	Further investigation needed
0	1	1	0	0	Confirmed weak D positive
0	0	0	0	0	Confirmed D negative

components are transfused. Plasma containing strong irregular antibodies may not be suitable for the preparation of fresh frozen plasma or for transfer to a fractionation facility for the preparation of plasma derivatives.

A set of reagent Group O screening cells is used to test for irregular red cell antibodies, as described in *Section 4: Principles of laboratory techniques*. When large numbers of samples are tested manually, it may not be feasible to use the IAT method, which may be the most sensitive but is also the most laborious. There is a variety of different techniques for automated systems depending on the instrument in use. These include the use of bromelin-treated cells, gel cards or solid phase technology.

When the red cell antibody screen is positive, antibody identification should be performed to determine antibody specificity. Suitable records should be kept for donors with antibodies so that they are recognised at each donation and antibodies of known specificity are not re-identified at every donation.

Donations showing the presence of cold autoantibodies, which are not of clinical significance, should be tested to ensure that the cold autoantibodies do not mask a clinically significant antibody. Whole blood donations with strong autoantibodies should not be transfused to patients undergoing hypothermia.

ABO antibody titre (haemolysing potential)

To detect potentially harmful anti-A and anti-B, samples may be tested for haemolysins or for high titre anti-A and/or anti-B antibodies. High titre antibodies can be detected by using a single dilution (e.g. 1 in 128, in saline). At this dilution, strong isoagglutinins should still be able to agglutinate group A₁ and B reagent red cells and should be classified as 'High Titre'.

In the haemolysin test, complement is triggered by the reaction of donor immune anti-A and/or immune anti-B with reagent red cells, causing the red cells to haemolyse.

It is important that donors with haemolytic or high titre anti-A and/or anti-B are detected so that their blood/plasma is not used for heterologous group transfusions.

Group A₁B (or A₁ and B) reagent red cells are used for the selected tests as they contain the strongest A (A₁) and B antigens to react with either anti-A or anti-B donor isoagglutinins. When used for haemolysin tests, reagent red cells may have to be washed to remove preservative fluid if it is anti-complementary.

Plasma samples are unsuitable for haemolysin tests, as anticoagulants are anti-complementary. Time also negatively affects complement. Provided that serum is used, and the sample is less than 24 h old, it is not necessary to add complement from an external source. When testing

samples older than this, the addition of extraneous complement is required. Without active complement, haemolysins are indistinguishable from non-haemolysing isoagglutinins.

Donations identified as containing potentially harmful anti-A and anti-B, either by titration or haemolysin test, are labelled 'high titre' for homologous group transfusion only. Other donations, that do not demonstrate high titre/haemolysing isoantibodies, are labelled 'low titre'.

Red cell antigen screening

As an ongoing proactive measure, selected donations (e.g. Group O donations) may be screened for additional red cell antigens, such as those within the Rh and Kell systems. This may be carried out by using reagents with specificities such as anti-C, anti-c, anti-E, anti-e and anti-K. Results of extended antigen screening should be added to the records, and subsequent donations from the same donor flagged so that the donations can be identified from available stock, and selected if they fit the type required to resolve a compatibility problem. This avoids time-consuming screening that may be needed at the time of crossmatch, to find compatible blood. Alternately the donors may later be traced to donate blood for patients with antibodies but there is a time delay between calling the donor to give blood, and the fully tested donation being available for crossmatch and issue.

Ongoing screening of donations may be performed to find donors who lack high incidence antigens so that rare donations identified in this way may be stored frozen in glycerol in a low temperature freezer for future use. When a patient with antibodies to a high frequency antigen requires blood, suitable blood can be requested from the rare donor registry. The blood will need to be thawed and deglycerolised prior to crossmatch and issue.

Automated testing for red cell serology

All the tests described previously may be fully automated or partially automated according to the systems and modules selected. For information on automation, see *Section 4: Principles of laboratory techniques*.

Automated testing of all donations in batches to perform the red cell serology tests is much quicker than manual testing and suits laboratories that handle many samples. Most automated instruments require special reagents with optimum reaction temperatures and techniques. It is important to note that some reagents require dilution before loading into the instrument, and manufacturer's instructions should be closely followed.

Automation saves time as the instrument is programmed to rapidly interpret all standard reaction patterns for the tests. This process categorises the majority of donations into one of the four ABO groups (A, B, O or AB), one of the two D types (D positive or D negative) and identifies antibody screen positive and high titre donations. The computer program linked to the automation set-up should be designed to detect and flag anomalous results so that they can be investigated further.

Quality controls for ABO and Rh typing

Automated instruments require the inclusion of specific quality control samples of known ABO and Rh type, in the batch being tested, to ensure that all reagents are working correctly. Controls should be included in every batch and when large numbers of samples are tested, after a certain number of tests (such as every 200 tests) and should always be repeated when reagents are replaced, or new dilutions made.

Quality problems

When automated control results fail, it is important to establish the root cause of the failure. Each laboratory should have a troubleshooting procedure in place that is applicable to the specific instrument in use. The training and operating manuals should provide such information.

Problems with automated instruments may need to be resolved by technical personnel from the supplier or manufacturer of the instrument. Common problems may, however, be identified by suitably trained technologists working with the instrument in the laboratory, but it is critical that qualified and experienced support from the supplier or manufacturer is readily available.

When blood grouping is automated, technologists should regularly perform manual testing, so if the instrument fails and is not operational for an extended period, they retain competency in manual techniques and are able to continue testing blood donations.

Transfusion transmissible infections (TTIs)

TTIs fall into two main categories:

- Those that result in chronic infection in the donor: The donor may have been asymptomatic or experienced an acute illness, but may feel well for years post-infection, and therefore present to donate. Most TTIs fall into this category. Examples include Hepatitis B (HBV), Hepatitis C (HCV), HIV, and Cytomegalovirus (CMV).
- Those that result in a short period of viraemia, which then resolves: The donor may remain asymptomatic

or have symptoms of infection at the end of the period of viraemia. There is no chronic phase of infection. Examples include West Nile Virus (WNV), Zika virus, and Hepatitis A (HAV).

Blood safety depends on both donor health screening (deferring donors who are aware they are infected by a TTI or are in a high-risk group for a TTI), and donation testing. The strategy employed for a given infectious agent will depend on the epidemiology of the particular agent in a given donor population, blood processing steps that might reduce transmission (such as pre-storage universal leucoreduction), and the availability of testing equipment and kits adapted for donor screening. Sometimes a combination of approaches may be used. For example, in countries where there is no local transmission of malaria, donors may be asked about their travel history, and malaria testing may be performed only on at risk donors (selective testing). See *Section 8: Donor selection*, for details about donor health screening, and *Section 4: Principles of laboratory techniques*, for more details about donation testing. Testing for TTIs is subject to ongoing change and improvement, as additional and more sensitive tests and automated test systems become available or new risks of possible infections are identified. Tests may detect viral nucleic acid (NAT testing), a viral component (Hepatitis B surface antigen, or HBsAg; the p24 antigen of HIV), or the host's immune response to the infection (antibody testing performed using Enzyme linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA)/chemiluminescence assay (CLIA) for antibodies such as anti-HIV, and anti-HBV). A donation with a confirmed reactive result for a TTI may not be used for transfusion.

Window periods

The window period refers to the period of time during which the donor may transmit infection, but testing performed will be negative. It is a latent period of immunosilence shortly after infection when laboratory tests for markers of the infection are non-reactive. Figure 1 shows the window period of immunosilence. A blood donation given during the window period will test negative, be classified safe for use and be placed in available stock yet may be infectious. The term seroconversion refers to the time when the blood of an infected individual shows the presence of antibodies to the infection.

The biological attributes of host–virus interaction, replication times, infectivity dose, natural history of infection, serum volume used for *in vitro* testing, and the relative sensitivity of each assay employed to detect an infection present in a blood donor are highly variable. As illustrated in Table 4, the lag time (cumulative window period) for ability of each serological test to detect an infection

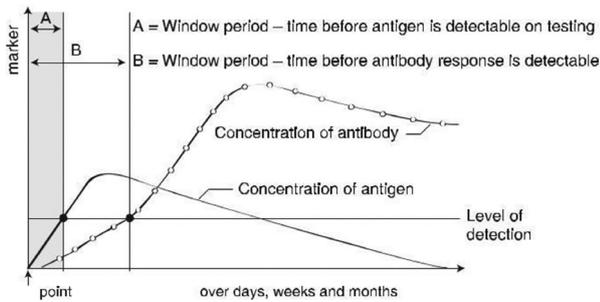


Fig. 1 Window period of immunosilence.

present in a donor’s blood is based on the window period data for HIV, HBV and HCV published in 2016 by WHO (see reference related to Table 4). In general, window periods are shortest for NAT testing, longer for antigen testing, and longest for antibody detection. Thus, the lag time for anti-HIV to detect HIV infection can be as long as 21 days, for HBsAg test to detect HBV infection as long as 42 days, and for anti-HCV to detect HCV infection as long as 60 days. This lag period gets truncated (shortened) by direct tests for viral gene amplification with NAT. Single unit NAT testing, where each donation is tested separately, is slightly more sensitive than minipool testing, when a small number of donor samples are pooled for testing. The window period is of great concern to blood services, as it is not possible to detect an infected donation of blood during this phase. Although laboratory tests today are very sensitive, and the window period may be narrowed to a few days, no matter how sensitive the test may be, there could still be a window period.

Testing Algorithms: Screening and confirmatory testing

The interpretation of results and the strategy for donor deferral or exclusion as well as the way in which donors are notified of anomalous or reactive results, is dynamic. Each laboratory/service should have protocols in place for both initial and confirmatory testing.

In the context of TTIs, an algorithm is the term used for a sequence of steps that is documented and followed in order when a donation is initially found to be reactive. Depending on the TTI concerned, the algorithm is unique. For example, the steps taken when a sample is HIV reactive may not be the same as steps taken when the sample is syphilis or malaria reactive. In general, initially reactive tests are usually repeated in duplicate on the same sample, using the same testing platform. If one or both of the repeat tests is positive, the sample is considered ‘repeat reactive’, the donation should be discarded, and the donor should be deferred. Repeat reactive donations are discarded using the appropriate disposal protocol/methods and according to local regulations for the disposal of bio-hazardous material.

Algorithms are usually complex and individualised for each TTI. In this publication, however, a single algorithm is shown that generalises and summarises the suggested flow of actions when a donation is reactive for a TTI. Figure 2 is a guideline algorithm summarising the sequence of actions that may be taken when a laboratory test is initially reactive for HIV, HBV or HCV. The algorithm does not describe the procedure to follow for a specific TTI.

Confirmatory testing

Partly because of the need for sensitivity in testing systems, TTI screening may lead to false reactive results, and biological false positives do occur. Many TTI test systems rely on cut-off values to determine whether a result is reactive or not, so some test results may fall close to a range of uncertainty (i.e. ‘indeterminate’ result). Extreme care needs to be taken with follow-up action, including confirmatory testing on the donation. The confirmed reactivity to certain TTIs such as HIV, HBV and HCV leads to permanent exclusion of the donor, whereas the risk of other TTIs such as malaria, may have specified time deferrals. For purposes of donor notification and counselling and possible actions to be taken regarding previous donations from the donor, confirmatory tests are done. Whenever possible,

Table 4 Length (in days) of the viraemic phase of diagnostic window periods (vDWP) for assay categories

	ID (individual donations) NAT	MP (small pools donations) NAT	Antigen EIA/CLIA	Combo EIA/CLIA	Antibody EIA/CLIA	Antigen RDT	Combo RDT	Antibody RDT
HIV	8	11	14	16	21	–	20	28
HBV	27	37	42	–	–	55	–	–
HCV	5	7	9	38	60	–	–	80

RDT, Rapid Diagnostic Tests.

Adapted from reference:

Guidelines on estimation of residual risk of HIV, HBV, or HCV infections via cellular blood components and plasma. Who Expert Committee on Biological Standardisation 67th report; WHO Technical Report Services #1004, 2017, Annex 4.

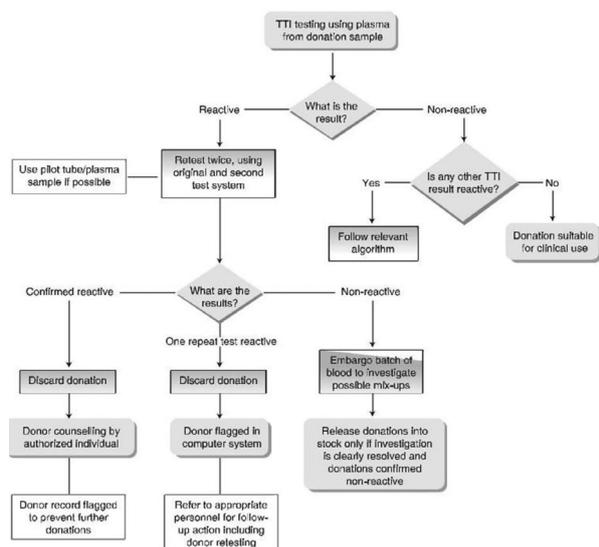


Fig. 2 Guideline algorithm for TTI testing: minimum steps only – actual process may be complex.

it is preferable to confirm initially reactive results by using a different confirmatory test system. The pilot tube from the donation is ideally used for follow-up testing, to check that blood bags and samples were not switched at the time of donation. However, this step is more difficult if testing is performed at a central laboratory and blood donations retained in the peripheral blood bank. Discrepancies between initial test result and confirmatory test result must be fully investigated and resolved before the final decision is made regarding the donation.

If there is any doubt about the result on repeat testing, the donation should also be discarded. Confirmatory testing may also be performed using a second sample from the same donor, if this is available, in case the sample used originally became contaminated in the laboratory or in transit. The information on all reactive units must be referred to the appropriate department for authorised donor follow-up (for more information, see *Section 9: Blood collection*).

Specific TTIs: Human Immunodeficiency Virus (HIV), Hepatitis B (HBV), Hepatitis C (HCV), and syphilis

Of all the transfusion transmissible infections, HIV, HBV, HCV and to a lesser extent syphilis, are the most important with regard to universal screening of blood donations. All blood services should test every donation for HIV, HBV, HCV and syphilis, all of which may result in chronic, asymptomatic infections in donors.

HIV

- HIV is spread by infected body fluids. The main routes for the spread of this infection are:
 - sexual contact
 - vertically from mother to child.
 - infected needles and syringes.
- HIV is also spread by the transfusion of HIV infected blood. Minimising the chance of this happening is of paramount importance to blood services.
- HIV positive individuals have a lifelong infection that eventually leads to the Acquired Immunodeficiency Syndrome (AIDS). This acquired immunodeficiency attracts opportunistic infections, which use the 'opportunity' of an immune system not capable of effective response, to thrive. Immunodeficient HIV positive individuals are therefore far more likely to become seriously ill with diseases such as malaria, tuberculosis and hepatitis B, as well as CMV and many others. Although similar infections may be found in immunocompetent individuals, they are far less likely to suffer the morbidity and mortality seen in AIDS patients.

Testing for HIV

Every donation should be tested for antibodies to HIV-1 and HIV-2 and common subtypes, using a test system such as ELISA or EIA/CLIA. Laboratory markers for HIV include anti-HIV, p24 antigen, and viral RNA. In order to reduce the window period for HIV, many blood centres use combination tests for HIV antibody and p24 antigen, and/or perform NAT testing in addition to antibody testing. Western blot is not used routinely for screening blood donations for viral markers. It is a gel electrophoresis technique and requires specialised equipment. It may be useful for confirmatory testing for HIV.

HBV and HCV

Hepatitis viruses cause inflammation of the liver.

- Viruses that cause acute disease and do not have a chronic phase of viraemia, such as HAV, cause epidemic jaundice. HAV is spread by contaminated food and water. It is rarely implicated in transfusion but is still a serious risk.
- Hepatitis viruses that are parenterally spread (by infected blood and body fluids gaining entry into the bloodstream of a non-infected individual) include HBV and HCV.
- HBV is primarily transmitted by sexual contact, from mother to child (vertical transmission), or using

contaminated needles and syringes (drug users). Body piercing or needlestick injuries can also result in transmission.

- HCV is similarly spread but is most likely to occur in drug users who share infected needles.
- Hepatitis B and C sometimes cause those infected to become chronic, asymptomatic carriers of the virus and as they appear well, they may enrol as blood donors.
- Chronic carriers of hepatitis B or C have lifelong infection that if untreated, may lead to liver cirrhosis or carcinoma.

Testing for HBV and HCV

Every donation should be tested for hepatitis B, using a test system such as ELISA, EIA/CLIA or NAT. Laboratory markers for HBV include HBsAg and viral DNA. Screening for anti-HBc is used in some countries and reactive donations excluded. However, in hyper-endemic countries, introduction of this marker to the screening algorithm might adversely affect the adequacy of the blood supply.

Every donation should be tested for hepatitis C, using a test system such as ELISA, EIA/CLIA or NAT. Laboratory markers for HCV include anti-HCV and viral RNA.

Syphilis

- All donations should be tested for syphilis. As it is a sexually transmitted disease, possibly indicating a high-risk lifestyle, those who are infected are considered to be more likely to be also carrying other sexually transmitted diseases, such as HIV and HBV. For this reason, syphilis is termed a surrogate test for HIV and HBV.

The syphilis spirochete (*Treponema pallidum*) is susceptible to time and temperature and will lose its ability to infect a recipient after several days of storage after donation, particularly if the storage is at $4 \pm 2^\circ\text{C}$. Serological tests for syphilis include:

- Rapid plasma reagent test (RPR).
- *Treponema pallidum* haemagglutination test (TPHA).
- Fluorescent Treponemal antibody absorption test (FTA).

Blood services may decide to use either RPR or TPHA as a first-line screening test and retest reactive specimens using FTA, which is less likely to give false positive results. However, if a confirmatory test is not performed, donors should not be notified of the infection solely on the result of a screening test, although reactive donations are not transfused. If feasible, the donor could be referred to their own doctor for further testing and possible treatment. The donor record should be flagged (in code) to

draw attention to the testing result when a subsequent donation is given.

Rapid plasma reagent test (RPR)

Serum/plasma from the donor is tested for antibodies to reagent and not for antibodies to *T. pallidum*. Reagent levels are raised in certain infectious conditions such as syphilis, and this causes an antibody response in the host. Reactive results are demonstrable as flocculation; a form of precipitation between antibody to reagent in the donor sample and the reagent reagent.

T. pallidum haemagglutination test (TPHA)

The TPHA test is used to detect antibodies to *T. pallidum*. The test uses avian (i.e. related to birds) erythrocytes coated with antigenic components of the *T. pallidum* organism. This test can be automated, and results produced are read either by the technologist or by instrument.

Fluorescent treponemal antibody absorption test (FTA)

Some blood services may use an FTA test, which measures specific antibody for *T. pallidum*, to confirm screen reactive. However, once reactive, an individual remains reactive for life, so the test does not differentiate between infectious and non-infectious individuals. Serum/plasma from the donation sample is mixed with *T. pallidum* and after processing, an anti-human globulin reagent labelled with a fluorescent indicator is added. If antibodies are present in the sample, the labelled AHG will act as an indicator, and fluorescence will be detected when using a specially designed microscope or appropriate reader. The FTA is costly and requires a degree of technical skill to perform.

Other transmissible infections

Screening donations for markers to infections that are endemic to certain geographical regions should be performed in addition to HIV, HBV, HCV and to a lesser extent, syphilis. For example, screening for Chagas' disease should be carried out in endemic regions of South America and is performed as a one-time donor screening in the USA. Screening for West Nile Virus (WNV) and Zika virus may also be required in some countries. Human T-lymphotropic virus (HTLV) -I and -II screening should be performed in areas, where it is regularly encountered in the population, such as in the Far East, and other countries where the prevalence warrants it. These and other TTIs are not described in detail in this publication, but some are mentioned briefly in succeeding discussions. Table 5 provides a list of microorganisms and other agents that may cause TTI.

Table 5 Micro-organisms and other agents that may cause TTI

Micro-organism	Transmissible agent
Viruses	1. Hepatitis A, B, or C (HAV, HBV, HCV)
	2. Human immunodeficiency virus type 1 or 2 (HIV-1, HIV-2)
	3. Human T-cell lymphotropic virus type I or II (HTLV-I, HTLV-II) – Far East, Caribbean, parts of Africa and South America
	4. Cytomegalovirus (CMV)
	5. Human parvovirus (B19)
	6. West Nile virus (WNV) – outbreaks in the Middle East, Europe, North America
Additional known viruses with potential to be transmitted	7. Zika Virus – outbreaks in Polynesia, central and South America
	8. Epstein-Barr virus (EBV)
Bacteria	1. Syphilis: <i>Treponema pallidum</i> (may also be classified as a spirochaete)
	2. <i>Yersinia enterocolitica</i> , <i>Streptococcus bovis</i> : contamination originates with a bacteraemic donor
	3. <i>Staphylococcus</i> , <i>Streptococcus</i> : contamination of donation from unclean venepuncture site
	4. <i>Pseudomonas</i> : widespread microbe that flourishes at cold temperatures
Parasites	1. Malaria: <i>Plasmodium falciparum</i> , <i>P. vivax</i> , <i>P. malariae</i> , <i>P. ovale</i> – <i>P. Falciparum</i> most likely to be implicated
	2. Chagas' disease: <i>Trypanosoma cruzi</i> – Central and South America
	3. Babesiosis: <i>Babesia microti</i> – Asia, Europe, some parts of USA
Prions (are not micro-organisms)	1. Creutzfeldt-Jacob Disease (CJD)
	2. Variant Creutzfeldt-Jacob Disease (vCJD)

Because of the increase in international travel, other transmissible infections too, are more likely to be spread amongst communities in which they were previously not detected. Individuals who are particularly vulnerable due to a lack of immunity to an infection rarely encountered in their home environment become increasingly exposed to these infections as a result of travel.

As the following extract from a World Health Organization (WHO) report shows, infection with multiple pathogens is becoming of increasing concern, particularly in developing countries.

Extract from a WHO World Health report 2007: the deadly interaction: HIV/AIDS and other diseases. *The interaction of HIV/AIDS with other infectious diseases is an increasing public health concern. In sub-Saharan Africa, for example, malaria, bacterial infections and tuberculosis have been identified as the leading causes of*

HIV-related morbidity. HIV infection increases both the incidence and severity of clinical malaria in adults. In some parts of Africa, falciparum malaria and HIV infection represent the two most important health problems.

Other transmissible viruses

Human T-cell lymphotropic virus (HTLV)

- HTLV-I is a retrovirus that may cause T-cell leukaemia and T-cell lymphoma and perhaps also a nervous system disease called tropical spastic paraparesis, usually after many years of incubation in the host.
- It is spread by vertical transmission, by sexual contact, or by contaminated blood (as a result of transfusion or by the sharing of needles). HTLV is mainly confined to leucocytes and is not transmitted by frozen plasma. Leucoreduction reduces the risk of transmission.
- HTLV-II is a virus that is closely related to HTLV-I and may also be a causative agent for paralysis. It predominates in some areas of the world and is rarely seen in others.
- If HTLV-I or HTLV-II is transmitted via blood transfusion, it may take years to become manifest in the recipient.
- Not all countries test for HTLV-I or HTLV-II. This may be because the prevalence of infection in the general population is extremely low. Additionally, universal pre-storage leucoreduction results in a significant reduction in the rate of transmission.
- Blood services in areas where HTLV has been shown to be present may include ELISA screening tests for antibodies to HTLV-I and -II, usually as a combined test, in their testing regime, with Western Blot as a confirmatory test. Testing may be done on first time donors or all donors.
- If a donation tests positive for HTLV-I, -II it must be discarded, the donor notified and counselled, and asked never to donate again.

Cytomegalovirus (CMV)

- CMV is commonly found in healthy individuals. Exposure is widespread and most individuals have developed antibodies as a result.
- CMV belongs to the herpes group of viruses and if transmitted by blood transfusion, usually presents as a mild disease in CMV-negative recipients who recover in a relatively short time period.
- However, in CMV-negative patients with an immune system that is not functioning properly (those who

are immunocompromised or immunosuppressed) the transmission of CMV infection can be very serious, causing morbidity and/or mortality. Examples of patients who are typically immunodeficient are untreated AIDS patients and newborn infants.

- Clear indications for the transfusion of CMV 'safe' blood or blood that has undergone pre-storage leucoreduction are neonatal exchange transfusion, intrauterine transfusion, and transfusion to immunocompromised patients.
- CMV 'safe' blood refers to either selected blood units that have been tested using antibody screening and shown to be from anti-CMV-negative donors, or donations that have been processed using pre-storage leucoreduction. As with HTLV, CMV is usually found within the leucocytes, and removing them before they disintegrate reduces the risk of transmission.

West Nile virus (WNV)

- As with malaria, mosquitoes are the vectors for WNV. A vector may be defined as the vehicle whereby a living microorganism – such as WNV – is transported from an infected host to another within the same or a different species, spreading and increasing the incidence of the disease. When a mosquito (that becomes infected with WNV from a bird or animal) bites a human, that human may also become infected.
- The appearance of WNV is seasonal, coinciding largely with the rainy season, or in North America, the summer and early autumn. WNV is widespread and currently causes periodic outbreaks in parts of Africa and the Middle East, Western Asia, Europe, and North America.
- The disease ranges from a mild to a serious form of encephalitis in a ratio of about 140:1. Many infected individuals are asymptomatic. Whereas mild forms of the infection can be asymptomatic or resolve in a few weeks, serious illness can lead to death.
- When infected individuals who do not have signs or symptoms donate their blood, the infection may spread to recipients, who in turn may not be seriously afflicted or on the other hand, may become seriously ill and die. However, there is not a chronic viraemic period, therefore donors who have recovered from infection may be able to donate again, after a defined temporary deferral period.
- WNV may also be vertically transmitted (from mother to fetus) and may infect recipients of organ transplants.
- Blood donations in areas where WNV is endemic or where WNV has been reported, should be screened

for the virus and donations that are reactive must be discarded.

Transmissible parasites

Malaria (caused by protozoa of the genus *Plasmodium*)

Transfusion transmitted *Plasmodium falciparum* may cause severe malaria and lead to the death of the recipient. Malaria should always be considered in a blood recipient who develops a febrile condition some weeks after transfusion.

- Malaria is spread to humans by infected female *Anopheles* mosquitoes and occurs mainly in tropical and subtropical areas. It is, however, a global problem and is responsible for the deaths of millions of individuals worldwide each year.
- Depending on the endemic area, the strain of *Plasmodium* could be *P. falciparum*, *P. vivax*, *P. ovale* or *P. malariae*. The parasite enters the body, proliferates in the liver and then infects the host red blood cells and destroys them.
- Red cells, whole blood, and platelets prepared from infected donations can infect recipients, but cell-free plasma from such donors should be safe for use. Red cells and whole blood are most likely to transmit infection.
- Individuals who have recovered from malaria may remain carriers for many years. They may experience occasional relapses or be asymptomatic. Those who are asymptomatic for some time may feel quite well and donate their blood, only to infect a recipient. Individuals who have recently become infected may also appear well and donate their blood. This is why it is so important to question prospective donors thoroughly about recent travel, or residence in a malaria area, and defer donation according to local guidelines.
- In endemic areas, most individuals, including donors, and patients who might be transfusion recipients, carry some immunity to the disease.
- The effort to maintain a malaria-free blood supply is an ongoing challenge for many blood services for two reasons: malaria in asymptomatic carriers is difficult to detect due to the very low level of parasitaemia, although even a single parasite transfused into a recipient can cause infection; and donors from endemic areas often have circulating antibody as a result of partial immunity to malaria. There does not seem to be one ideal laboratory test system for the screening of blood donations, although evaluation of new test systems is ongoing. Pathogen reduction

methods that can be performed on whole blood or red cells are under development and show promise in reducing transmission.

The options available to a blood service include the following:

- Deferral of donors at risk of carrying malaria. Some points related to deferrals are given here:
 - Having suffered from malaria: this often leads to deferral for a long period of time since the last attack, or permanent deferral.
 - Time of residence in malaria area: the longer an individual has lived in a malaria area, the greater the likelihood that he/she may be harbouring parasites due to semi-immunity and be in a state of asymptomatic parasitaemia.
 - Locality within malaria area: it is important to ascertain where the potential donor has been, rather than to ask during questioning, if the donor has visited a malaria area. Travellers may not necessarily be aware that the area they visited carried a risk of malaria. The whole country or regions within a country may be endemic. At certain times of the year (the rainy season) more individuals are likely to contract malaria, so deferrals may relate not only to area but to seasonality too.
 - Period since leaving malaria area: In non-endemic areas, deferral periods may be shorter for short term travellers, compared to individuals who lived in a malaria risk area for extended periods of time, who are more likely to be infected but asymptomatic. Deferral periods vary from six months to three years, depending on the blood centre and length of time the donor resided in the malaria risk area.
 - In endemic areas where it is likely that all available blood donors could have had exposure to malaria, it may be unavoidable to use them as blood donors. In this case, donations should be confined for use within the malaria area, for local patients, and the use of concomitant anti-malaria therapy advised for susceptible transfusion recipients.
- Screening of all blood donations, or at risk donations (at risk donations are those taken from donors who could have been exposed to malaria).
- Concomitant administration of malaria prophylaxis to recipients of at risk blood.
- A combination of the above, such as a three or four month deferral period, followed by antibody testing.

Testing for malaria

Although it is possible to test donations for malaria, some blood services do not have the facilities to do so or are operating in countries where the test is not licensed for use in blood donor screening.

The various screening tests available include the following:

- Screening for parasites found within the red cells.
- Screening for antibodies to the malaria parasite.
- Screening for the presence of malaria antigens.
- Screening for the presence of plasmodial DNA.

Comments on screening assays for malaria markers:

- Examining blood smears directly for intracellular parasites, with or without the aid of immunofluorescence, is suitable for the diagnosis of malaria in patients with clinical signs and symptoms, but the number of parasites in an asymptomatic blood donor may be too low for detection. The process of examining slides is also time consuming.
- The immunofluorescent antibody (IFA) and EIA tests may be more sensitive but are still not ideal. Although the IFA test is relatively simple it cannot be automated.
- EIA is suitable for the detection of antibodies to malaria, although a non-reactive result may be a window period donation and does not necessarily mean that the donor is free of parasites.
- Donors from endemic areas are likely to have antibodies to malaria, so testing blood donations taken from individuals who live and donate in malaria endemic areas will not differentiate between semi-immune and infected individuals, unless tests include a differentiation between IgM and IgG antibodies. The presence of IgM antibodies indicates a recent infection.
- Malaria antigen detection assays using commercially available monoclonal antibodies are available but are not necessarily more sensitive or reliable. A combined strategy of testing for both antigen and antibody may be considered a safe approach, together with accurate donor questioning prior to donation.
- Although NAT for the detection of plasmodial DNA has been developed, its value is not fully established, and this technology may not be available to some blood services.

Chagas' disease

Chagas' disease is seen mainly in poor rural areas of Central and South America, but due to immigration but is now becoming a global concern.

- The reduviid bug lives in the thatched roofs of rural dwellings, and carries the parasite, *Trypanosoma cruzi*. The actual bite does not transfer the parasite; when the bite itches and the host scratches it, the infected faeces that the bug deposited on nearby skin

are able to enter the host, either via the bite site or by hand contact transfer to the eyes or mouth.

- The disease may also be spread from accidentally ingesting infected bugs, or by placental transfer of parasites from mother to fetus.
- The acute phase of the disease may be mild and go unnoticed, but unless treated early, lifelong infection is the result. Chronic Chagas' disease can lead eventually to heart problems and/or digestive complications.
- Anyone who has had the disease may never donate blood.
- All blood donations collected in endemic areas should be tested for antibody to the disease, and any donation confirmed positive must be discarded, the donor contacted and counselled and asked never to donate again.
- In non-endemic countries, different testing strategies may be used, such as testing all donors once (USA) or selective testing of high-risk donors (Canada). Donors are asked if they, their mother, or their maternal grandmother were born in Central or South America, or if they resided in Central or South America.

Babesiosis

- This parasite (*Babesia microti*) is spread by bites from infected ticks, so is more likely to be a problem close to herds of animals, such as deer.
- It is found in red cells, and therefore, similar to malaria, may be transmitted by whole blood, red cells, and more rarely by platelet concentrates, but not by plasma.
- It occurs in Asia, Europe and some parts of the USA.
- It causes a disease similar to malaria but seldom causes fatalities except in individuals who have had a splenectomy.
- Anyone who has had the disease is permanently deferred from blood donation.
- Selective testing is done in the USA, in states where the parasite is endemic.

Prions

- Prion diseases, also called transmissible spongiform encephalopathies (TSEs), are believed to be caused by an abnormal form of a cellular glycoprotein known as the prion protein that alters brain and neural (nerve) tissue and are always fatal.
- Prion diseases occur in animals, such as bovine spongiform encephalopathy (BSE) in cattle,

commonly known as mad cow disease, and scrapie, in sheep.

- The two main prion diseases in humans are Creutzfeldt Jacob Disease (CJD), sometimes called 'classical' CJD, and variant CJD (vCJD).
- Classical CJD may be sporadic (unknown cause), due to genetic syndromes, or iatrogenic (caused by medication or medical treatment). Sporadic CJD is rare, occurring in approximately 1 in a million individuals. Iatrogenic CJD was primarily related to the use of human pituitary growth hormone and human dura mater (brain covering) grafts that had been inadequately processed to remove infectivity. Since 1985, recombinant growth factor has replaced use of growth factor of human origin, and dura mater grafts are synthetic or derived from screened donors and processed using inactivation steps. Incubation periods between iatrogenic exposure and development of disease may be very long. Therefore, although there have been no cases of transfusion transmission of CJD, many blood services defer donors who have received growth hormone or a dura mater graft. Donors with a family history of CJD are also deferred.
- vCJD is a novel prion disease that was found in humans, coincident with the epidemic of BSE in cattle in the 1980s and early 1990s in the UK. It is thought that eating infected beef products can cause variant vCJD infection. Most cases occurred in the UK, with a smaller number in countries that imported a substantial amount of beef from the UK (France, and other countries in Western Europe) and also had some BSE in their cattle herds. After the link between animal feed practices, BSE, and vCJD was established, measures were taken in the UK and the rest of Europe to stop BSE transmission in cattle. Although the incubation period between ingestion of beef and development of vCJD can be very long, the number of recent cases has decreased substantially.
- There have been a small number of cases of vCJD in patients who received blood from donors who subsequently developed vCJD. There is presently no laboratory test for those in the incubation phase of vCJD and as a result, those who are symptom-free may present themselves as blood donors. Therefore, many blood services question donors about residency or cumulative travel to at risk areas and defer donors with potential exposure to BSE. For example, many countries, including the USA, Canada, and Australia, defer donors who were born in or spent more than three months cumulatively in the UK between January 1980 and December 1996.

- Some countries have introduced universal leucodepletion of blood components partly to reduce transmission of vCJD, based on data from animal models of infection.

Transmissible bacteria

Blood components provide a hospitable, conducive environment for bacterial growth. This is especially true for platelets, since they are stored at room temperature. Bacteria present in the blood component may multiply during storage and cause severe transfusion reactions and septic shock when transfused; see *Section 14: Risks of transfusion and haemovigilance*. Blood collection kits are sterile, and steps are taken in donor screening, venepuncture, and processing and storage to ensure sterility; see *Section 9: Blood collection*. Despite precautions, occasionally bacterial contamination and proliferation does occur.

The main mechanism of contamination is inadequate skin disinfection, with primarily Gram positive organisms that are part of normal skin bacterial flora, such as *Staphylococcus epidermidis* and *Staphylococcus aureus* introduced into the blood collection bag at the time of phlebotomy.

Other mechanisms include intermittent donor bacteraemia with Gram-negative organisms, sometimes related to gastroenteritis (*Yersinia enterocolitica*) or chronic intestinal pathology, such as colon cancer (*Streptococcus bovis*). *Yersinia enterocolitica* in particular is cold tolerant and may survive and proliferate in refrigerated whole blood and red cells.

More rarely, contamination may occur with environmental contaminants (*Serratia liquifaciens*, *Pseudomonas*) during storage or processing of components, particularly if there is a defect in the storage bags, such as a micro-puncture or leaky seal.

Some blood services test all platelet components for bacteria. Most commonly, after a 24–36 h hold period, an aliquot of the main platelet component is expressed into an integral sampling pouch, which is then removed from the component in a sterile fashion. The sample is injected into one or several blood culture bottles that are incubated in an automated culture system. The platelets are put into inventory but removed from inventory at the blood centre or the hospital blood bank if the blood culture becomes positive. Co-components from the same donation are also recalled. Further confirmatory testing may be done to confirm that bacteria are present and identify the precise microorganism.

Emerging pathogens

An emerging pathogen is either a microorganism that has expanded in geographic range or pathogenicity, or an

entirely novel agent. A blood service should be constantly on the alert for the reported detection of emerging pathogens that may impact on the safety of the blood supply. When there is a risk that an infectious agent may be transmitted by blood transfusion, and this microbe is known to have entered the donor population, this may involve additional screening of donors and testing of blood donations. The best example in this regard was the introduction of HIV testing in the mid-1980s. Other examples of potential risks to the safety of the blood supply occurred with the advent of vCJD, a novel agent. Pathogens with expanded geographic range include WNV, Zika virus, and babesiosis. To mitigate the impact of these emerging pathogens on the safety of the blood supply in a timely and effective manner, a close relationship between blood services, regulatory authorities, public health, industry and other stakeholders is important. Pathogen reduction is a proactive strategy to deal with these threats (for more information, see *Section 11: Blood processing and components*).

Pandemics

A blood service should develop an action plan in case of pandemics that may occur, such as the outbreak of coronavirus disease in 2019/2020 (COVID-19) which was later declared a pandemic by the WHO. Although it was not immediately apparent whether or not the causative virus (designated Severe Acute Respiratory Syndrome Coronavirus 2 or SARS-CoV-2) was transmissible via blood transfusion, blood services acted quickly to implement strategies to identify at risk donors and defer them from donating.

Pandemics not only threaten the safety of the blood supply, but affect personnel within the blood service, and the availability of safe donors to provide blood. In the case of COVID-19, for example, it was necessary for blood services to introduce additional precautions to prevent possible transmission of the virus from occurring within their premises, between visiting donors and also amongst staff.

Lookback and traceback procedures

Due to the need for confidentiality of medical information, it is important that there is no visible link within the testing laboratory, between a donation that is reactive for a TTI, and the identity of the donor. As few individuals as possible should be able to link the donor with the sample that is reactive for a TTI; either in the laboratory or in the blood collection centre. Only authorised personnel should have access to this information. A designated individual should gather all the relevant results and refer them to a trained counsellor (with the consent of the

medical director) who should be the only one authorised to make contact with the donor or with a clinician identified by the donor.

Lookback procedures

Look-back investigations may be done when a donation from a donor who had given blood before, and which was then non-reactive for TTIs and therefore considered safe for transfusion into a patient, now tests positive for a TTI. Recipients of previous transfusions from the donor may be contacted and tested for the specific TTI.

Traceback procedures

When a recipient becomes positive for a TTI following a transfusion, donors who contributed components to the transfusion may be contacted and tested, if they have not already returned to donate.

National regulatory authorities should establish guidelines for blood services, to define the scope and algorithm for managing lookback and traceback investigations, and how earlier donations and recipients of blood products made from these donations are handled, when a donor has seroconverted and may have been in the window period of infection when the earlier donation was given. Both lookbacks and tracebacks require good records in both the blood service and hospital to ensure vein to vein traceability of blood, from donor to recipient. The yield of lookbacks and tracebacks will be highest when new tests are introduced, or there has been a major advance in test sensitivity. Yields are low when donations have been tested using sensitive methods, such as NAT, and donors were very unlikely to have been in the window period leading to infection of a recipient.

Key points

- ABO and D typing should be carried out on every donation. Results should be compared with previous results if possible, and anomalies brought to the attention of the individual in charge for investigation and resolution. If an anomaly cannot be resolved, the donation should not be used.
- Reverse or serum/plasma grouping is used to confirm the forward or cell grouping in ABO typing. Isoagglutinin tests should be read blind (i.e. without reference to forward, or cell grouping) so that there is no preconceived idea of what results should be. This also applies to records of ABO groups – the technologist reading the test should not have knowledge of the previous ABO group at the time of reading.
- Blood grouping records for repeat donors should be consulted to check that the group on record is the same as the group for the current donation.
- D typing results that indicate a weak D or D variant should also be classified as Rh-positive. This is to ensure that Rh-negative recipients do not receive blood containing the D antigen.
- Controls should be performed on all reagents used, in parallel with tests. If reagents perform as expected, this provides the assurance that they meet sensitivity and specificity requirements and that test results produced using the reagents can be accepted. When faulty reagents are used for testing, all test results for that batch are invalid.
- Irregular red cell antibodies in donor plasma may have an adverse effect if transfused to a patient with the corresponding antigen. Plasma containing strong irregular antibodies may not be suitable for the preparation of fresh frozen plasma or for transfer to a fractionation facility for the preparation of plasma derivatives.
- Whole blood donations with strong autoantibodies should not be transfused to patients undergoing hypothermia.
- It is important that donors with haemolytic or high titre anti-A and/or anti-B are detected so that their blood/plasma is not used for heterologous group transfusions.
- Selected donations may be screened for additional red cell antigens. Results of extended antigen screening should be added to the records, and a subsequent donation from the same donor flagged so that it can be identified from available stock and selected if it fits the type required to resolve a compatibility problem. This avoids time-consuming screening that may be needed at the time of crossmatch to find compatible blood.
- Ongoing screening of donations may be performed to find donors who lack high incidence antigens so that rare donations identified in this way may be stored frozen in glycerol in a low temperature freezer for long term future use.
- Automation saves time as the instrument is programmed to rapidly interpret all standard reaction patterns for the tests. The computer program linked to the automation set-up should be designed to detect and flag anomalous results so that they can be investigated further.
- Automated instruments require the inclusion of specific quality control samples of known ABO and

- Rh type, in the batch being tested, to ensure that all reagents are working correctly.
- Testing for TTIs is subject to ongoing change, as additional or more sensitive tests become available or new infections are identified.
 - The length of the window period varies by infectious agent and the test method used in screening, with Nucleic acid testing (NAT testing) having shorter window periods than antigen or antibody testing.
 - An algorithm is used for a sequence of steps to be taken when a donation is initially found to be reactive. Depending on the TTI concerned, the algorithm is unique.
 - Many TTI test systems rely on cut-off values to determine whether a result is reactive or not. Initially reactive results are repeated twice, and if are again reactive on one or both repeats, should be confirmed using a different testing system.
 - Every unit of blood should be screened for HIV, hepatitis B, hepatitis C and syphilis, as well as any other TTIs that relate to the geographical area of the blood service.
 - Screening for HTLV may be included in areas of high prevalence. Universal pre-storage leucoreduction is also an effective method of reducing transmission.
 - CMV is widespread and therefore presumed to be present in most donations. There are clear indications for the transfusion of CMV 'safe' blood (neonatal exchange transfusion, intrauterine transfusion, transfusion into immunocompromised patients). Universal pre-storage leucoreduction or blood tested and found negative for anti-CMV may be considered CMV safe.
 - Red cells, white cells, platelets and fresh (not frozen) plasma (which may contain a few red cells after processing) are all capable of transmitting malaria with red cells the most likely to do so.
 - With regard to malaria, options available are the deferral of donors at risk, screening of all blood donations, or at risk donations and/or concomitant administration of malaria prophylaxis to recipients of at risk blood.
 - Tests available for screening blood donations for malaria include screening for parasites within red cells, or antibodies in serum, or malaria antigens in the blood, or plasmodial DNA.
 - A blood service should be constantly on the alert for the emergence of new agents that may impact on the safety of the blood supply and take action to begin screening donations if possible. National requirements should be considered prior to introducing a new test.
 - Look-back investigations to identify and test previous recipients, may be done when a donation from a donor who had given blood before, and which was then non-reactive for TTIs and therefore considered safe for transfusion into a patient, now tests positive for a TTI.
 - Traceback investigations may be done when a recipient develops a TTI, which was potentially due to an undetected infection in a donor.
 - National authorities should establish guidelines to outline the scope and algorithm for managing the lookback and traceback processes.

Blood processing and components

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Introduction

A unit of whole blood collected from a donor is a precious asset. A donation of blood should be seen as a bag containing all the different constituents of whole blood – red cells, white cells, platelets, and plasma with plasma proteins such as clotting factors and protective antibodies. In modern transfusion medicine the aim is to transfuse the patient with only the component required as far as is practical. Component therapy also maximises the use of one donation. The products from a single donation can benefit multiple patients. See *Section 18: Indications for transfusion*, for more information about the use of whole blood and different blood components.

Whole blood may be processed into various components and each component can then be stored under ideal storage conditions (i.e. temperature and movement) to ensure that the product is most effective when it is used. Special preservative solutions and blood bags are used to lengthen the expiry time and improve product quality.

This section will cover very broadly, the processing of blood into components.

Learning objectives

By the end of this section, reader will be able to understand the processing of blood into components and to discuss the requirements of a component programme under the following headings:

- Processing blood into components

Sterile systems

Bag systems for blood collection

- Single bag
- Double bag
- Triple bag
- Quadruple (quad) bag
- Top and bottom bag

Principles of centrifugation

Blood processing equipment

- Blood bag centrifuge
- Scale
- Balances
- Plasma extractor
- Automated blood processing machines
- Pilot tube sealer
- Sterile connecting device
- Platelet agitator

- Plasma snap freezer
- Low-temperature freezer
- Laminar flow cabinet

Preparation of specific blood components

- Whole blood
- Red cell concentrate (RCC)
 - RCC in plasma
 - RCC, buffy coat removed, in additive solution (RCC, leucocyte-reduced)
 - RCC, filtered (RCC, leucocyte-depleted Prestorage)
 - On demand
 - In line
 - Add on
 - RCC, paediatric
 - RCC, cryopreserved (frozen)
 - RCC, washed
- Plasma
 - general information
 - fresh frozen plasma
 - fresh frozen plasma – for fractionation
 - freeze-dried plasma
 - cryoprecipitate
- Platelet concentrate (PC)
 - general information
 - preparation of PC by platelet-rich plasma method
 - preparation of PC by buffy coat method
 - conventional quad bag method
 - top and bottom bag method
 - single unit PC from buffy coat
 - pooled PC from buffy coat
 - pooling kits
 - chain method
 - apheresis platelets
 - quality control for PC

Irradiated products

Measures to prevent transmission of pathogens

Labelling and records

Overview of plasma fractionation

Processing blood into components

Figure 1 shows the three main component types (red cells, plasma, platelets) that can be processed from whole blood and lists some of the blood products that may be prepared by processing further.

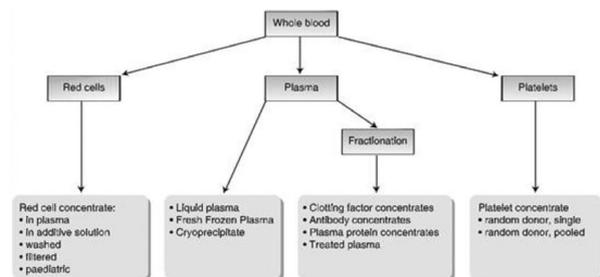


Fig. 1 Components from whole blood

To process whole blood into components requires a basic understanding of:

- Sterile systems.
- Blood bag systems.
- Principles of centrifugation.
- Blood processing equipment.

Sterile systems

Blood bag manufacturers must ensure that all blood bags plus anticoagulants and additive solutions are sterile (free of contamination by bacteria or viruses) and pyrogen free (do not contain endotoxins or micro-organism debris). Collection of whole blood is described in *Section 9: Blood collection*.

The routine processing of blood into components relies on the availability of closed blood bag systems. Individual bags in multiple bag systems are connected with one another via tubing and thus constitute a closed system. The advantage is that the components can be separated into the attached bags (after centrifugation) without affecting sterility, as the primary bag is never opened, and the entire process occurs in a closed system.

Bag systems for blood collection

A wide variety of polyvinyl chloride (PVC) plastic blood bag systems are available from many suppliers. The choice of bag type depends on the requirements of the particular blood service, based on the following criteria:

- Affordability.
- Clinical demand for components (such as RCC, plasma, PC).
- Whether units will be processed manually or automatically.
- Level of product storage to be achieved, such as the use of additive solutions and special plastic bags for platelets.
- Whether filtration to remove white cells (leucodepletion) is required.

Single bag

This is the simplest of the bags available. The donation is taken into the bag and the pilot tubing is then sealed. No further processing into components is performed and the unit is transfused as whole blood. The bag contains an anticoagulant solution (usually CPDA). CPDA contains sodium citrate that prevents clotting, and citric acid (C), monobasic sodium phosphate (P), dextrose (D), and adenine (A) that provide buffers and nutrients for enhanced red cell survival.

Double bag (two bag system)

In a multiple bag system, the bag with anticoagulant into which the donation is taken is referred to as the primary bag; in a double bag system, an additional empty bag is attached to the primary bag (called a transfer or satellite bag). Contents of the primary bag are prevented from entering the transfer bag by the presence of a breakable seal at the point where the transfer tubing joins the primary bag. After centrifugation of the whole blood, the seal between the two bags can be broken (without compromising the hermetic seal and the sterility of the system) and the plasma transferred through the tubing to the attached transfer bag creating two components, a red cell concentrate (RCC) suspended in a small amount of plasma in the primary bag and plasma in the transfer bag.

Triple bag and quadruple (quad) bag systems

A triple bag differs from a double bag only by having an additional transfer bag. Once plasma is separated into the first transfer bag, there is still another empty transfer bag attached to it. This configuration is used to manufacture PC from platelet-rich plasma, or to harvest cryoprecipitate from fresh frozen plasma. A quad bag system is similar to the triple bag system but has an additional bag containing red cell additive solution and is usually used in automated systems to prepare RCC, plasma, and platelets produced by the buffy coat production method. Quad bag systems may be designed to have transfer bags attached to both the top and the bottom of the primary bag (so called bottom and top method) or just to the top of the bag (so called top and top method). Details of how triple and quadruple bags are used to make components are described later in this section, under the heading *Preparation of specific blood components*.

Principles of centrifugation

Blood constituents can be separated by centrifugation because they differ in size and density and will sediment at different rates when centrifugal force is applied.

When whole blood is centrifuged, the red cells settle at the bottom of the blood bag because they have the highest density (have a greater mass/weight than the other components).

Being less dense, the white cells and platelets do not settle as quickly and remain in suspension for longer. As centrifugation continues, the white cells sediment above the red cells, and finally the platelets form a layer above the white cells and leave the original suspending fluid (now clear plasma plus anticoagulant) at the top. Figure 2 illustrates the separation of components as a result of moderate or hard centrifugation of a unit of whole blood.

It is important to select the correct speed and time of centrifugation to be used in order to separate the desired component. For example, if platelet-rich plasma is required then centrifugation should be such that the platelets are not sedimented. A lower centrifugation speed for a longer period is required. If, on the other hand, completely cell-free plasma is required then a faster centrifuge speed for an adequate time period would yield clear plasma and densely packed red cells with the white cells and platelets layered above and cell-free plasma on top.

It is important that the optimal conditions for a good separation be carefully evaluated for each centrifuge to obtain the desired components. To establish optimal centrifugation:

- Collect parameters that indicate the desired outcome of the procedure (e.g. whole blood centrifugation should yield RCCs with a haematocrit (Hct, also called the packed cell volume) between 0.65 and 0.75 and a certain volume of plasma (minimum 200 ml) as well as a buffy coat (BC) with a particular platelet yield. Hct is a measurement of the proportion of the blood that is made up of the red cells, expressed as a fraction. For example, an Hct of 0.65 means that there are 65 ml of red cells in 100 ml of blood.
- From the centrifuge manual or other procedure manuals establish a base line centrifuge setting with regard to speed (measured in revolutions per minute, i.e. rpm, or gravitational force equivalents, i.e. g) and time.
- Prepare a number of products (at least 10) using this setting and measure all parameters.
- Repeat the procedure with reduced or increased speed and time combinations and compare parameters until the optimum setting for the centrifuge is established.

After centrifugation, the bag system is gently removed from the centrifuge, taking care to prevent mixing or swirling, and the primary bag is placed in a plasma extractor, or an automated component extractor, for

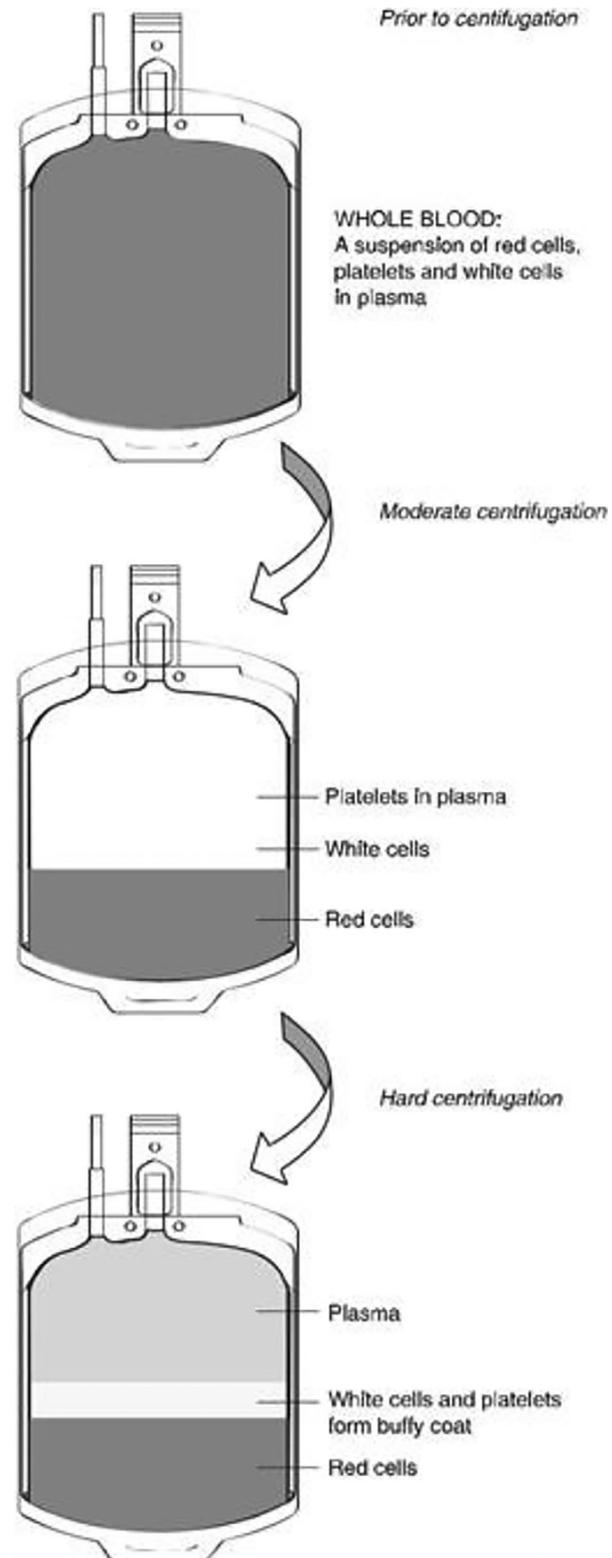


Fig. 2 Centrifugation of whole blood

separation. Pressure is applied to the bag and the component layers are then transferred, in order, into one or more of the transfer bags connected in the closed system.

Blood processing equipment

Blood bag centrifuge

Blood bag centrifuges are essential pieces of processing equipment. Several manufacturers market centrifuges capable of spinning between four and 12 blood bags at a centrifugal force of up to 5000 *g*. These are large machines that are usually floor-standing and require dedicated floor space and electrical supply. Professional installation and a good maintenance programme are essential to ensure staff safety and consistently reproducible centrifugation of product.

A typical centrifuge has an electric motor that turns a rotor housed in a very thick metal chamber. As the rotor is spinning the door on this chamber locks automatically to prevent access by the operator during operation as this would be extremely dangerous. The rotor is designed to hold a certain number of metal buckets. These vary in size and shape depending on the type of blood bag system being centrifuged (e.g. a quad bag system with additive solution will require a bigger bucket than a simple double bag). Each bucket has a plastic insert that can easily be loaded into, or unloaded from, the metal bucket. The inserts make the centrifugation process easier to perform and are easy to clean. Figure 3 shows a centrifuge bucket insert, metal bucket and rotor that fits into a centrifuge housing.

When loading a rotor, the bucket/insert/blood pack combinations that are placed opposite one another must be of equal weight. The metal buckets are rarely removed and should be placed in the rotor according to matching weights.

Two plastic insert and blood bag combinations are placed on a balance (or scale). Weight (mass) in the form of plastic or rubber strips, is added to the lighter combination until the two bag/insert combinations are of equal mass. The loaded and balanced inserts are then placed into the metal buckets opposite one another in the rotor. Failure to balance the buckets before operation, can cause the centrifuge to vibrate when spinning starts, and could cause serious damage to the centrifuge or injury to personnel. In addition, the product will not be adequately centrifuged. The use of water or other liquids is not recommended for balancing the opposing buckets as the liquid may become bacterially contaminated, and moisture may smudge or dislodge the blood bag label. The plastic or rubber strips used for mass correction should be disinfected regularly.

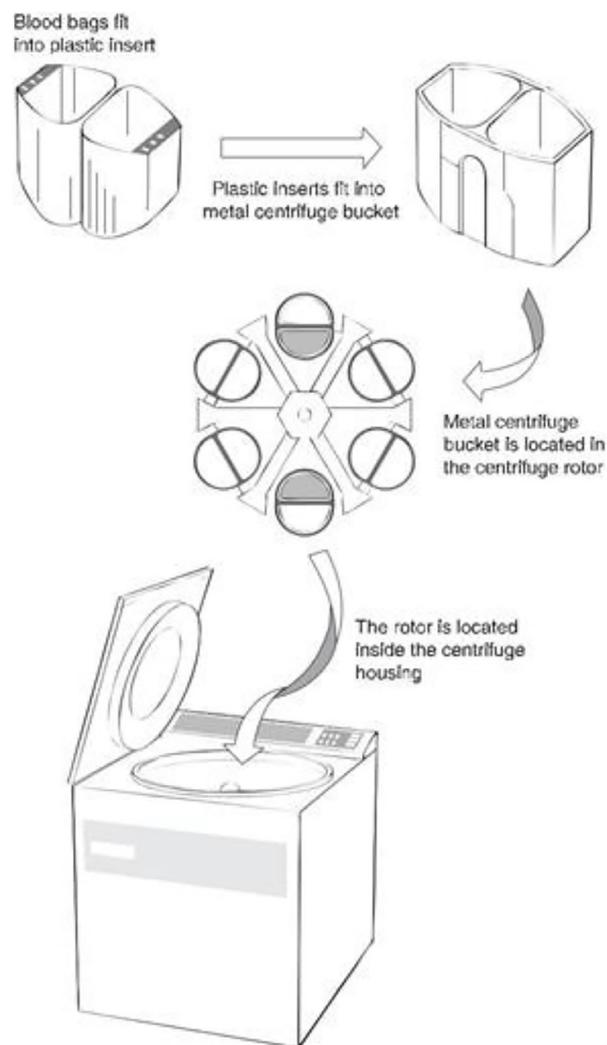


Fig. 3 Centrifuge insert, bucket and rotor

Blood pack centrifuges must also have a refrigeration capacity that enables the temperature in the chamber to be controlled during processing.

In order to make quality blood components, a centrifuge must be able to perform within tight parameters. The amount of centrifugation (i.e. spin) that a product requires can be measured in terms of speed and time (e.g. 2000 rpm for 10 minutes). This, however, is not the most accurate way, as the amount of gravitational force exerted is much greater in centrifuge heads with a longer radius. For example, 2000 rpm in a centrifuge with a radius of 30 cm produces less force than 2000 rpm in a centrifuge with a 50 cm radius. It is therefore better to calculate the *g*-force for a particular spin and specify the requirements in terms of gravitational force and time (e.g. 1900 *g* for 10 min). This figure takes the centrifuge radius into account and can be used to set

similar centrifuge settings on different makes and models. The handbook related to the blood pack centrifuge provides the formula to convert rpm to gravitational force for each specific centrifuge rotor. Some centrifuges have built-in software to do the conversion automatically. Centrifugation under a low g -force is referred to as a soft or light spin.

Some centrifuges also take into account the time taken to reach the desired speed (acceleration) and the time taken to stop (deceleration or braking), as these will vary according to centrifuge load. It is also possible to link computer software to a blood pack centrifuge and record all operational data for total process control. This means that the software is able to capture information like operator's name, date and time processed, and details of the centrifuge time and speed parameters against the donation identification number. In the event of quality problems, full traceability of the centrifugation data is thus available.

Scale

A laboratory scale capable of weighing components to at least the nearest gram is essential when making components. The mass forms a critical part of quality control, for example:

- Whole blood must meet mass requirements in order to be suitable for component production.
- Each component produced must fall within a specified mass range.
- The mass may be used to calculate the volume of a component (gross weight of component minus empty bag weight, multiplied by specific gravity of component).

Systems need to be in place to ensure the accuracy of the scale on an ongoing basis and a calibration/service should be performed at least once per year. Figure 4 shows a laboratory scale for weighing blood components.

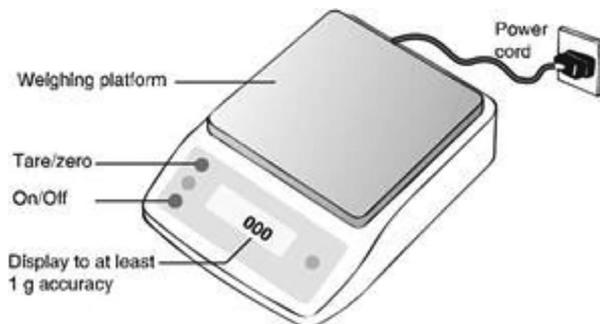


Fig. 4 Laboratory scale for weighing components

Balance

A balance has two weighing platforms and is used to prepare combinations of blood bags/centrifuge inserts of equal mass to be placed opposite one another in a centrifuge. The balance should ensure that combinations do not vary by more than 1 g. Clean plastic or rubber pieces are added to the side with the lighter combination until it is equal in mass to the heavier side. The display will indicate the mass difference in grams and give the operator a guideline as to how much balance material to add. A calibration/service should be performed at least once per year. Figure 5 shows an example of a balance with two weighing platforms for preparing blood bags of equal mass for centrifugation.

Plasma extractor

A plasma extractor (or blood press) is a commercially available device that is used to apply pressure to a centrifuged unit of blood in order to transfer part of it (e.g. plasma or BC) to an attached transfer bag. The design of the device is such that a controlled amount of pressure is applied to the bag that should allow reasonable flow of liquid from one bag to the next without danger of bursting the bag or causing excessive frothing of the component being transferred.

Regular cleaning and checking of the device are essential and if it is not performing correctly it should be repaired before use. Additional pressure should not be applied by squeezing the plates together by hand to 'speed things up' or compensate for a lack of pressure as a result of a defect. Figure 6 shows a typical plasma extractor.

To operate the plasma extractor, the following steps are taken:

- (1) Use the handle to open the front pressure plate.

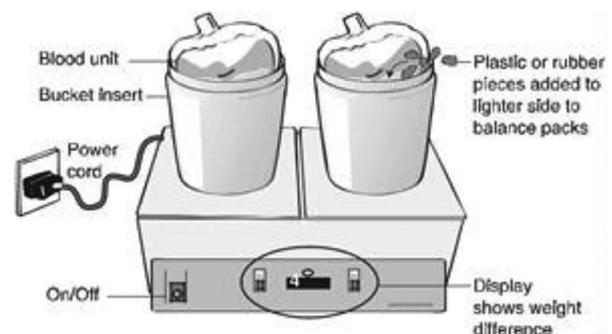


Fig. 5 Balance with two weighing platforms for preparing blood bags for centrifugation

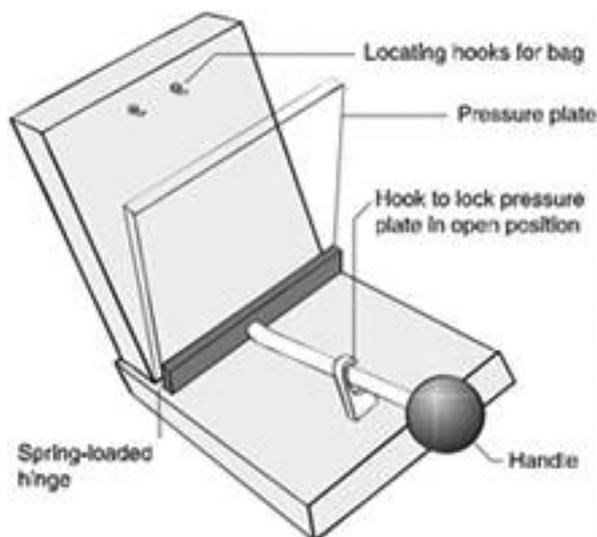


Fig. 6 Plasma extractor

- (2) Hold it in the open position using the hook provided.
- (3) Carefully remove the centrifuged blood bag from the centrifuge bucket.
- (4) Hang the primary bag on the hooks located on the backing plate. Great care must be taken not to disturb the interface between red cells and plasma.
- (5) Carefully reposition the transfer bags still attached to the primary bag and place them on the workbench next to the plasma extractor.
- (6) Release the handle and slowly allow the front pressure plate to apply pressure to the primary bag in the press (without disturbing the interface).
- (7) Once the pressure is applied, break the seal on top of the blood bag to allow plasma, and later BC if desired, to flow via the connecting tubing into the transfer bag. (Do not release pressure in mid-flow as this will cause mixing in the bag.)
- (8) Stop the flow at the desired point by using forceps or plastic tubing clamps to create a temporary seal in the tubing.
- (9) Now remove the separated bags of components from the extractor.
- (10) Permanently seal the blood pack tubing for the separation of the bags into individual components.

Automated blood component extractor

Automated blood component extractors are commercially available devices that can be configured to process a centrifuged unit of blood into the required components with little or no manipulation by the operator other than to load and unload the machine.

There are several different types available that vary from machines that perform basic separations to more sophisticated machines that perform advanced separations and record the details of each separation for total quality management purposes.

The machines use light sensors to detect blood cells in the primary bag and tubing to activate the selected programming that controls the opening and closing of tubing clamps and regulates flow between bags. Some machines also have built-in scales that weigh the product transferred to the bags (e.g. BC) and use this information to activate the clamps. Fully automated blood component extractors will also perform the sealing of tubing between bags as part of their process.

Automation is justified when:

- There is an adequate number of units requiring processing each day.
- The blood centre has made a decision to produce leucocyte-reduced RCC (i.e. BC removed) that are suspended in red cell additive solution.
- The blood centre wants to produce PC from BCs.
- Personnel are motivated by the idea of automation and the quality improvement that can be realised by moving to it.
- Adequate technical support is available in the area for the repair, maintenance and calibration of the machines.

The manual processing of units of whole blood into RCC suspended in additive solution, involving the removal of BC and plasma into separate transfer bags, is an extremely labour-intensive operation to perform routinely without the aid of automation.

Pilot tube sealer

During manual or semi-automated processing, the tubing between separated components is temporarily sealed using forceps or plastic clamps. These temporary seals are replaced with permanent seals in the tubing as soon as possible. Seals are also used in the tubing of prepared red

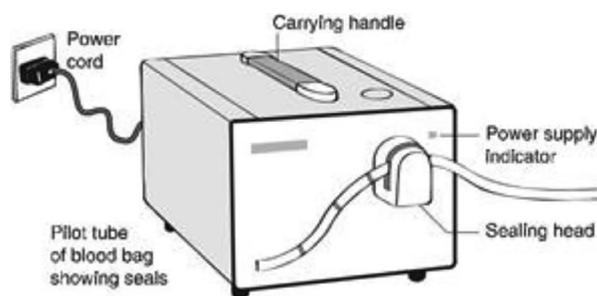


Fig. 7 Pilot tube sealer

cell products to make segments of approximately five cm in length, that contain red cells from the product for use in testing without compromising the sterility of the bag contents.

A tubing sealer is a fairly simple device that, by means of heat, creates permanent seals in the PVC tubing of blood packs. The ideal seal is made quickly without generation of excess heat and will be about two mm wide with a 'split line' down the middle to enable easy parting of the tubing when firmly pulled apart. Figure 7 shows an example of a pilot tube sealer.

There are several types available:

- Hand-held models that are either electric or battery powered and are used at the bedside and in operations where the sealer is brought to the bag.
- Bench models that are positioned in specific areas where the bags are brought for sealing and are used when a large number of seals need to be made. They are generally quicker and less prone to overheating than the hand-held machines.

Great care needs to be taken to ensure that the machines are functioning correctly. Without a proper cleaning, maintenance and quality checking system, blood products with faulty or leaking seals might get into the blood supply.

Sterile connecting device

A sterile connecting device (SCD) is used to attach an additional transfer bag (or bags) to a primary blood bag without breaking the sterile integrity of the system. The shelf life of components thus prepared is the same as if the product had been prepared in a closed system.

The pilot tubing of the primary bag is placed into a slot on the SCD. The tubing of the transfer bag to be joined is placed in an adjacent slot running parallel to the first. On starting the operation, a disposable wafer is superheated by the SCD and then drawn through the tubing in the slots. Simultaneously, the tubing is moved to align the ends to be sealed, and the wafer is withdrawn. The ends to be sealed are welded together instantly and the closed system is extended by another bag (or set of bags).

There is another technology available which uses a permanent heating element for the docking, avoiding the need for wafer. However, the manufacturer has introduced a number counter that obliges to equally pay for every seal made.

Figure 8 shows a sterile connecting device and illustrates how the tubing of two separate bags is joined to extend the closed system.

The ability to extend a closed system has many advantages as it allows the technologist to weld in bags with

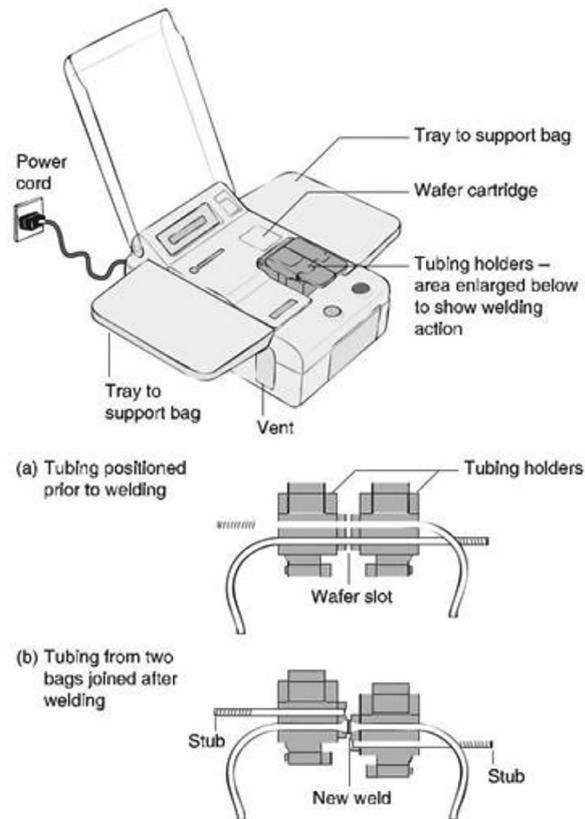


Fig. 8 Sterile connecting device

filters, smaller bags to create paediatric units, and to create platelet pools. All SCD welds must be inspected for quality, integrity, leaks, air bubbles and alignment. The consequences of passing a faulty weld can be very serious indeed, so procedures must be in place to ensure that the correct course of action is taken when faults are detected. Record keeping must include full documentation of products welded and weld quality control results.

Regular (once per year minimum) servicing and calibration of weld strength is vital to safe use of the SCD.

Platelet agitator

A platelet agitator is a device designed to fulfil the need for PC to be agitated during storage and is an essential part of the equipment in a modern component's laboratory. The best mixing action is provided by a machine that moves a flat tray/shelf in a gentle horizontally oscillating motion (side to side) at approximately 60 cycles per minute.

Other types, which rotate end over end, or in an elliptical action, are considered to have too robust an action and are not ideal. Figure 9 is a sketch of a small platelet agitator.

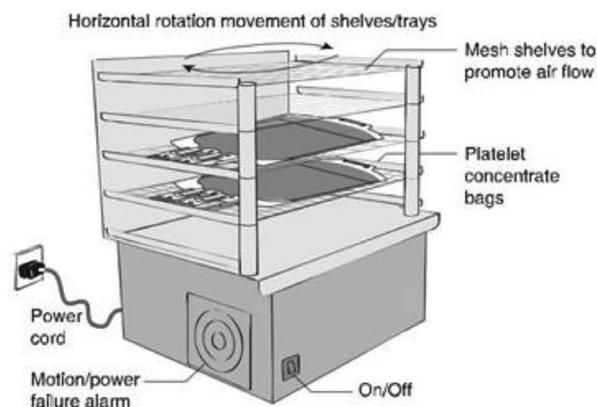


Fig. 9 Platelet agitator

The oscillating tray/shelf is made of mesh or stainless steel sheet with multiple holes punched through it so that when a PC bag is placed on it, air can circulate all around the bag. This helps to fulfil the need for platelets to exchange gas through the walls of the special bags used for platelet storage.

Another requirement for PC storage is that they be held at a controlled temperature of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Platelet agitators that are built into a temperature-controlled cabinet are ideal, but if the agitator is operated in a room where a controlled temperature is maintained (and recorded) they are not essential. See *Platelet Concentrate, General Information* later in this section for more on platelet storage requirements.

A strict cleaning routine according to the manufacturer's specifications should be in place to ensure that the platelet agitator (in particular the oscillating shelves) is clean and free of bacterial growth. Records indicating the frequency of cleaning and personnel responsible should be kept.

Checks need to be in place to ensure that temperature and oscillation requirements are being continually met, and these should be recorded and reviewed regularly by a senior technologist.

Plasma snap freezer

The main aim of freezing fresh plasma is to preserve the clotting factors (most notably factor VIII). During freezing, pure ice is formed and the solutes (salts) in the plasma are concentrated in the remaining water. If the plasma freezing is carried out in a conventional deep freeze, it could take up to 24 h (or longer) before the plasma is solidly frozen. Under these conditions, the factor VIII molecules are exposed to a high concentration of solutes for a prolonged period and become inactivated. This damage to clotting factors is avoided when the

plasma is frozen rapidly to a core temperature below -30°C in less than one hour from the time the freezing process commences (e.g. -32°C core temperature in 45 min would exceed the requirement).

A variety of machines that can achieve these specifications are commercially available:

- Blast freezers blow super-cooled air over the product that is placed in special cassettes to give the plasma units a uniform flat shape.
- Plate freezers hold plasma units between two super-cooled plates to achieve freezing and uniform flat shape.
- Some devices use liquid that is super-cooled mechanically or by the addition of dry ice (solid carbon dioxide). Care must be taken to ensure that the liquid used cannot penetrate the container or that the container is not in direct contact with the coolant liquid.

In all instances, the snap-freezing system must be validated to ensure that it can achieve the desired freezing rate. Every run should be recorded, and temperature and time data collected to ensure that the machine is operating within specifications

Low-temperature freezer

A freezer capable of cooling to temperatures as low as -80°C is required for cryopreserved (frozen) blood storage. See *Red cell concentrate, cryopreserved* later in this section. These freezers are usually set at approximately -70°C in order to ensure that the frozen blood units are constantly maintained at a temperature lower than -65°C , and that there is reserve cooling capacity. *Section 12: Blood storage and transportation*, provides more information about storage freezers.

Laminar flow cabinet

On rare occasions it may be necessary to perform a blood processing operation that cannot be made in a closed system or by the use of a SCD (e.g. addition of wash solutions from a bottle or preparing bottles of plasma for freeze-drying). In this event, every precaution should be taken to ensure that bacteria present in the air do not enter the bag.

A laminar flow cabinet provides an enclosed clean area that is kept clean when not being used by ultraviolet (UV) radiation. When the cabinet is being used, the UV light is switched off and air, sterilised by being passed through a series of filters capable of removing bacteria, is pumped into the cabinet creating a steady flow towards the operator. This positive pressure prevents micro-organisms from the environment surrounding the laminar flow cabinet from entering. The operator wears protective clothing and

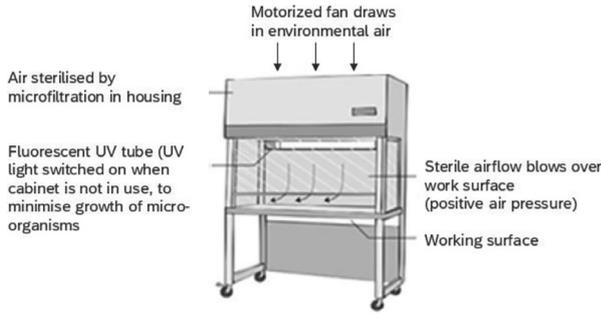


Fig. 10 Laminar flow cabinet

sterile gloves and works through the restricted space in front of the working surface of the cabinet. This reduces the likelihood of contamination of the product being processed, by the operator. Figure 10 is a diagram to show a laminar flow cabinet.

At the end of an operation the area under the hood and the working surface is cleaned thoroughly with a bactericidal agent and closed. The UV light is then switched on and remains on to inhibit bacterial growth until the hood is required again. The use of laminar flow may be needed in some processing laboratories but generally it is not an essential piece of equipment because multiple blood bags and sterile connecting devices, to maintain the closed system, are readily available.

Preparation of specific blood components

Blood components can be made in many different ways, which depend largely on the requirements of the blood service and availability of resources (donors, personnel, disposables, funding and space). The following explanations are not designed as standard operating procedures, but to provide students with an overview of each component and outline the processing methods (Fig. 11).

Whole blood

Whole blood is the source material for blood component preparation and if not processed any further maintains various properties for different periods of storage time at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. For example, red blood cells retain their oxygen carrying capacity for the entire duration of the storage period, labile plasma factors (particularly factor VIII) will decrease in concentration after the first 24 h of storage, and platelet counts will decrease but may retain haemostatic function for up to 2 weeks.

Red cell concentrate (RCC) in plasma

Red cell concentrate in plasma is prepared by removing part of the plasma from centrifuged whole blood. Enough

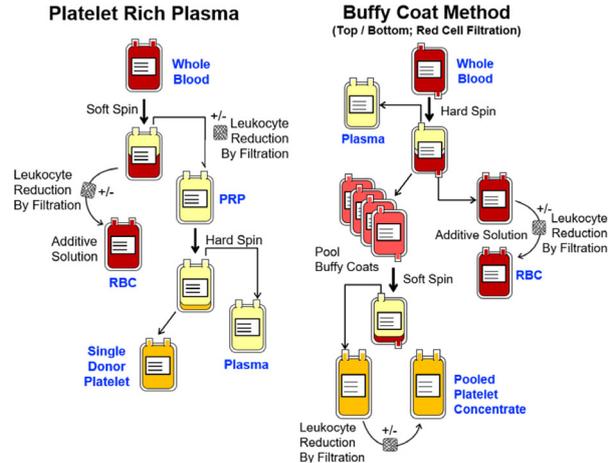


Fig. 11 The platelet-rich plasma and buffy coat methods of platelets preparation. (used with permission, Dr. Jason Acker)

plasma is removed to increase the Hct to between 0.65 and 0.75, and white cells and platelets remain with the red cells. This is a simple separation of whole blood that is usually collected into a double bag.

Red cells in plasma are used for replacement of blood or red cell loss. As white cells are not reduced and the storage medium for the red cells is not improved using additives, they do not offer much advantage over whole blood other than reduced volume in the transfusion. However, the separated plasma may be used as another component or forwarded to a fractionation facility.

Red cell concentrate, buffy coat (BC) removed, in additive solution (RCC, leucocyte-reduced)

For more processing options in the provision of components, blood collected into triple or quad bags is preferred.

These systems typically include an attached bag containing approximately 100 ml additive solution. Additive solutions from different suppliers may include sodium chloride, adenine, glucose, mannitol, citrate, phosphate or guanosine dissolved in water in differing combinations and amounts.

Some additive solutions have brand names, and, in some areas, personnel may refer to these solutions using these names, or they can simply be named according to their composition (e.g. SAGM contains saline, adenine, glucose and mannitol).

The component is derived from whole blood by centrifugation and removal of plasma and BC and the subsequent resuspension of the red cells in nutrient additive solution. The actual volume of additive in the concentrated red cells varies depending on the volume of whole blood collected from the donor (e.g. 500 ml collection = 111 ml SAGM; 450 ml collection = 100 ml). The

amount of the solution added is designed to give a final product haematocrit of 0.5–0.7 to facilitate good infusion flow rates and easy administration.

Removal of the BC creates a product with less than 1.2×10^9 leucocytes per bag, and this is considered to be leucocyte-reduced (not to be confused with leucocyte-depleted, which is a filtered product and has even fewer leucocytes, typically less than 1×10^6).

The main advantages of BC removal include:

- Microaggregate formation during storage is greatly reduced (compared to whole blood or RCCs stored with BC).
- The incidence of recipient febrile reactions is reduced.

Storage in additive solution can support red cell viability and function if stored at $4^\circ\text{C} \pm 2^\circ\text{C}$ for up to 42 days from the date of donation.

Red cell concentrate, filtered (RCC, leucocyte-depleted)

Red cell concentrate with extremely low white cell counts of less than 1×10^6 per unit are essential in the treatment of patients known to have leucocyte antibodies and to prevent alloimmunisation to leucocyte antigens in patients where transfusions are likely to be ongoing. The use of leucocyte-depleted red cells is considered an acceptable method to prevent the transmission of CMV.

In recent years many blood services (and indeed countries) have adopted a policy of universal leucodepletion of all cellular products in order to reduce febrile transfusion reactions, HLA alloimmunisation, CMV transmission, and possibly the risk of transmission of variant Creutzfeldt-Jakob disease (vCJD).

Leucocyte-depleted red cells can be prepared in a number of ways depending on the local requirements, policy adopted and available finance. Table 1 shows several ways in which blood services meet demand for filtered (leucocyte-depleted) RCCs.

Prestorage filtration means that the unit of blood is filtered as soon as possible, preferably within 48 hours of donation. Blood services applying this policy have usually decided to carry all, or part, of their stock as filtered RCCs and are able to supply the product routinely when ordered.

On-demand filtration: A blood service may apply an on-demand policy, filtering units only when requested. This has obvious cost-saving advantages as filters are expensive and there is little wastage because of expiry.

In-line filter systems are supplied with a filter built into the closed system to ensure sterility and ease of operation. The system may be designed to filter the whole blood donation prior to further processing or to filter the RCC after removal of the plasma and BC.

Add on filter systems involve a separate bag with integral filter that is attached to the unit to be filtered by pushing the spikes (cannulas) into its port or by connecting it using an SCD.

If the connection is made using an SCD then the shelf life of the component is unaffected. Other methods require that the product is used within 24 h of the 'add on' when stored at $4^\circ\text{C} \pm 2^\circ\text{C}$.

Prestorage filtration has several major advantages:

- Filtration is carried out when the unit is still fresh and white cells still intact.
- White cells are removed before they fragment and release micro-organisms into the plasma.
- White cells are removed before they release cytokines that can cause immune modulation in a recipient.
- Personnel, who are trained and certified competent, perform the procedure.
- Good laboratory practice (GLP) can be applied.
- Quality control of product is better. It is easier to collect quality testing samples from a representative number of filtered units when the process is performed in a processing laboratory. Samples are also taken into the correct test tubes after thorough mixing of the product.
- There is little storage lesion and red cell expiry time is unaffected.

Prestorage in-line products are used in two ways to filter blood:

- (1) A filter is located in the tubing between the whole blood donation bag and a second transfer bag. On receipt in the component's laboratory, the whole blood is filtered into the transfer bag, which then becomes the new primary container of filtered whole blood. Red cell and plasma components made from this filtered whole blood are leucocyte-depleted. Most

Table 1 Options for filtration of red cells prior to transfusion

Prestorage filtration	On-demand filtration				
	<i>In line</i>	<i>Add on</i>	<i>Add on</i>	<i>Add on</i>	<i>Add on</i>
Whole blood prior to processing	RCC during processing	Sterile weld	Processing laboratory	Blood issue laboratory	Bedside filtration

whole blood filters also remove platelets, so the method is unsuitable for making BC platelets.

- (2) A filter is located in the tubing between the primary bag and an additional attached transfer bag. On receipt at the components laboratory the whole blood is processed as usual into components (RCC, plasma, BC). The BC reduced RCC is then filtered through to the additional transfer bag that is then appropriately labelled leucocyte-depleted RCC.

Prestorage add on systems require a sterile transfer bag with a leucocyte filter in line. This is sterile welded to a BC reduced RCC prepared from top and top or top and bottom bags in a routine separation procedure. The red cells, generally after adding an additive solution, are then allowed to flow through the filter and into the transfer bag, which must be correctly numbered and labelled as leucocyte-depleted RCC.

A blood service may benefit from the flexibility of this system. With proper management, selected numbers and blood groups can be processed with little wastage resulting from expiry.

On-demand systems generally meet the need for filtration in situations where prestorage products are not available. On-demand filtration has several disadvantages:

- The unit can be at any stage of its shelf life at the time of filtration.
- A larger number of personnel in various laboratories and hospital wards need to be trained to perform the procedure.
- It is likely that personnel performing the task do so only infrequently, and competency may be reduced as a result of lack of practise.
- GLP is difficult to apply to a procedure performed in multiple centres.
- Taking the required number of quality testing samples in the correct way to obtain accurate counts is difficult if not impossible in some areas.
- White cell fragmentation occurs as a result of extended storage prior to filtration.

On-demand processing of filtered red cells in the Components Laboratory is the most practical way to supply the component when usage is low and only occasionally requested. In this instance the technologists who are most familiar with component production perform the filtration in a laboratory setting where GLP may be applied. Quality testing is possible because personnel are familiar with quality testing requirements and how to take the samples for testing. Connection of the filter/transfer bag can be achieved using an SCD (expiry date of component therefore unaffected) or by using the cannula (open system – expiry reduced to 24 h when stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$).

On-demand processing by blood bank technologists in a crossmatching laboratory may be practical in some

situations. Connection of the filter/transfer bag is likely to be made using the cannula just before issue (open system – expiry reduced to 24 h, when stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$). As the number of individuals performing the procedure is limited to blood bank trained personnel, there is some quality control and measure of GLP. It is likely that personnel performing the task do so only infrequently and competency may be reduced because of lack of practise.

On-demand bedside filtration is achieved by using a transfusion set with an in-line white cell filter, and the red cell product is filtered as it is being transfused. The blood service is not involved in the filtration process, training or quality control as this responsibility has shifted to the hospital personnel responsible for setting up the transfusion.

Red cell concentrate, paediatric

Neonates and very young children have a smaller circulating blood volume, generally higher haematocrit values, reduced metabolic capacity and immature immune systems. These factors need to be taken into consideration when preparing components for their use.

A paediatric dose of RCC is prepared either from RCC with BC removed, or filtered RCC by dividing the adult unit into quantities suited to the smaller patient's needs (usually from 25 to 100 ml).

Additional bags may be built into the blood bag system used for the collection to ensure that sterility is maintained during transfer of red cells (closed system). To use this method the need for the product must be identified at the outset and the special bag must be committed to the process at the time of blood collection. For this reason, it is more common to make paediatric units from stock blood by welding on additional bags using an SCD.

Premature infants are particularly susceptible to CMV infection. The use of filtered RCC, when preparing paediatric units minimises the risk of CMV transmission.

Care must be taken to ensure that separated paediatric doses of RCC are identified with unique numbers that can be clearly traced to the original donation.

When a paediatric patient requires blood over several days, all the split units should be reserved for that patient to limit the number of donor exposures received. The greater the number of donations used to provide components, the greater the risk of exposure to TTIs and also to donor-derived antigens.

Red cell concentrate, cryopreserved (frozen)

When frozen, the shelf life of red cells can be extended up to 30 years if the units are correctly protected during freezing, and ultra-low freezing temperatures (colder than -65°C) are maintained throughout the entire storage period.

Damage to red cells if frozen without protective solutions:

- Red cell dehydration: This occurs when extracellular water freezes before intracellular water, creating a difference in osmotic pressure between red cells and their surroundings, and resulting in intracellular water diffusing out of the red cells, collapsing them.
- Intracellular ice: The red cells rupture from mechanical trauma caused by ice crystals that form inside the cells.

The most commonly used cryoprotective solution is a high concentration of glycerol (40%). Other protective solutions such as low concentrate glycerol and hydroxyethyl starch are less frequently used and are not described. Glycerol consists of a small molecule that can cross the red cell membrane, and this is known as a penetrating cryoprotective agent. After entering the red cell, it provides an osmotic force that will stop the migration of water from the cell as ice is formed outside it. The high concentration of glycerol also prevents the formation of intracellular ice crystals and thus prevents cell membrane damage.

Frozen storage of red cells is used mainly to preserve units of blood with rare blood types. It can be used to stockpile blood for emergency use in disasters, but the high cost and short shelf life after recovery from the frozen state make it impractical as a routine tool to manage inventory.

Procedures for glycerolisation of cells vary. Usually, all plasma and/or additive solution is removed from whole blood or RCC within six days of collection. The glycerol solution must be added slowly with adequate, constant mixing to allow equilibration of solution and cells. Failure to equilibrate during addition results in a high degree of cell damage that is seen as excessive haemolysis and poor red cell recovery when the unit is thawed.

The original blood collection bag can be used for frozen storage, but the volume capacity is rather small to facilitate mixing of cells and glycerol so it may be preferable to use a larger volume bag (800 ml). Some polyvinyl chloride (PVC) bags can cause cellular damage during the freeze/thaw process so these larger bags are usually made of polyolefin plastic that minimises cell damage. Polyolefin bags are also less brittle when frozen to the very low temperatures required and are less likely to break during storage and transportation.

Donations destined for freezing can be collected into special primary packs with these specifications, but it is also possible to connect a suitable freezing bag to any stock donation.

Automated equipment and disposables that perform the entire freezing and thawing operation in a closed system are available.

Notes on freezing and thawing technique

- Red cells prepared with a final glycerol concentration of 40% (weight to volume) are placed in metal or cardboard protective canisters to minimise breakage during the long period of frozen storage during which they might be handled or transported several times.
- The canisters and units are placed in a mechanical freezer capable of operating at -80°C . Although the freezer can run at this temperature, it is more likely to run at about -70°C to maintain the temperature of the cells below the required -65°C .
- If temperatures are maintained continuously below -65°C the units have an expiry date set at up to 30 years from date of donation.
- Most donations that are stored frozen are rare blood types, so when this type of donation reaches expiry it is not discarded. Scientific evidence indicates that cell recovery and viability from units stored for up to 21 years is acceptable, so if a frozen rare donation is required after its official expiry, it may be used as long as the reasons are clearly documented (e.g. no frozen units less than 10 years in storage and no other compatible donations or donors available).
- When frozen cells are to be recovered (deglycerolised) the unit is thawed with gentle agitation at 37°C for about 20 min.
- The thawed cells contain a high concentration of glycerol that should be removed gradually by washing with sterile saline solutions of decreasing osmolarity to avoid red cell haemolysis.
- Using a typical three wash procedure, the cells are diluted with 12% saline for the first wash, with 1.6% saline for the second wash and finally with 0.9% (normal) saline for the third wash. After the addition of each wash solution and gentle mixing, the bag is centrifuged, and then the supernatant removed from the red cells and discarded. The solutions are always added slowly with mixing and plenty of time for osmotic equilibration.
- Whatever technique is used it should be properly validated for local conditions, and the final product should be free of cryoprotective agent, show minimal signs of haemolysis and yield at least 80% of the cells originally frozen.
- The freeze/thaw process involves adding solutions and extra bags and as this is usually performed using an open system it is carried out using a laminar flow cabinet. The red cells have a post thaw shelf life of 24 h at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- Automated equipment, disposables and solutions that perform the thaw operation in a closed system are

commercially available and provide a post thaw shelf life of up to 14 days at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Red cell concentrate, washed

Washed red cells are a specialised component prepared for patients with antibodies to plasma protein (e.g. anti-IgA) and those who have severe allergic reactions when transfused with blood products. This condition is uncommon, and components laboratories are not often asked to provide the product.

Washed red cells are usually prepared by further processing of RCC, BC removed, in additive solution. Some blood services use filtered RCC as the starting material.

- Approximately 250 ml of cold ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$), sterile, isotonic saline is added to the RCC and the contents gently and thoroughly mixed. The saline bag can be attached to the RCC bag using an SCD and in this way it is possible to maintain a closed system throughout the process. If saline is added by making connections using the spikes (cannulas) on transfer bags and transfusion sets, then the connections should be made under laminar flow conditions, and the expiry time of the product reduced to 24 h provided that temperature is kept between $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

After centrifugation in a refrigerated centrifuge set at approximately 4°C , the supernatant saline is removed and discarded. This is referred to as the first wash. The wash process (of adding saline, mixing and centrifuging) is usually repeated three or four times, with each wash resulting in the removal of more plasma protein from the product and finally producing washed red cells suspended in saline with less than 0.5 g protein per unit.

The technology is available to perform this wash procedure automatically but would require quite a large demand for the product to justify the cost of machine and disposables.

Plasma

General information

The first step in component processing is to remove the plasma from a centrifuged unit of whole blood. Fresh plasma contains proteins such as albumin, coagulation factors (most notably FVIII), and immunoglobulins. It is not practical to store plasma in liquid form as some fractions deteriorate rapidly, even if stored at refrigerator temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$). Liquid plasma stored for more than a few days at this temperature is suitable only as a blood volume expander. To preserve labile fractions, plasma must be stored frozen and then becomes known as fresh frozen plasma (FFP). If blood being donated is to be processed into FFP or cryoprecipitate, the time taken

to make the donation should not be longer than 15 minutes as poor flow during donation leads to consumption of clotting factors.

Fresh frozen plasma is used therapeutically or is a starting material for the preparation of plasma derivatives in a fractionation facility. See *Overview of plasma fractionation* later in this section for more detail.

- Plasma must be frozen within a maximum of 24 h from time of collection.
- From the time of donation to the time of freezing, the donation must be kept at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$. For details on storage, refer to *Section 12: Blood storage and transportation*.
- The time taken to freeze the plasma to a core temperature colder than -30°C must not exceed 1 h from the time freezing is commenced. Core temperature refers to the temperature in the centre of the unit – the warmest part of the plasma pack during the freezing process. See *Plasma snap freezers* earlier in this section for more detail.

Fresh frozen plasma

Fresh frozen plasma for therapeutic use is prepared by snap-freezing the plasma as soon as practical after collection. Specifications require that this time should not be longer than 24 h from time of collection.

Once frozen, units are best stored at temperatures consistently colder than -18°C , and under these conditions have an expiry date of 12 months after date collected in the US and Canada. In Council of Europe regulations, plasma stored at -18°C has an expiry date of 3 months, while plasma stored at -25°C has an expiry date of 36 months.

Fresh frozen plasma is often stored in cardboard or polystyrene protective containers that minimise the risk of breakage of the brittle frozen product during storage, handling and transportation.

Labelling of frozen bags of plasma is difficult because stick-on labels will not adhere securely. Units are either labelled before freezing, and a rigorous checking system put into place to verify safe donations once TTI testing results become available, or donations are labelled using tie-on tags.

In all instances the use of computer records to identify safe donations is essential. Manual tracking of donations in a quarantine system is not recommended.

Plasma for fractionation

Plasma not used therapeutically may be supplied to a fractionation facility for the extraction of clotting factors, albumin and immunoglobulins. See *Overview of plasma*

fractionation later in this section for more detail. Source plasma refers to plasma collected for further fractionation.

Fractionation facilities require bulk lots consisting of several thousand units of plasma frozen within 24 h of collection. Fresh plasma is preferred, as albumin, immunoglobulin and clotting factor concentrates can be made from the same pool.

Plasma that is separated and frozen after the deadline of 24 h from collection is considered to be 'outdated' plasma. Separation of this type of plasma may continue for up to 1 week after the expiry date of the whole blood provided that it was stored within the prescribed temperature range ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$). The outdated plasma is not used for fractionation.

Any supplier of plasma as a raw material to a fractionation facility must comply with legislation and the fractionator's guidelines. See *Overview of plasma fractionation* later in this section for more detail. The records of plasma forwarded to a fractionation facility must be supported by a comprehensive data capture system to ensure quality and traceability of every unit in the batch.

Freeze-dried plasma

A suitable product for use in outlying rural hospitals and emergency rooms can be manufactured using a process of freeze-drying (lyophilisation). By this method the clotting factors present in fresh plasma can be preserved in powder form and stored at ambient temperature (usually at 25°C or less). A measured amount of distilled water (the same amount as was lost during drying) is added to reconstitute the powder just before use.

In the process of lyophilisation fresh plasma is transferred to a glass bottle under laminar flow conditions and then frozen by rolling the bottles in a bath of alcohol and dry ice, which when mixed, results in a temperature of colder than -60°C . This process is called 'shell freezing' as the plasma freezes in a thin layer over the inner surfaces of the bottle so that a large surface area is made available for evaporation when the product is in the process of being dried.

After freezing, each shell frozen plasma bottle has its rubber bung replaced with a sterile vapour-permeable membrane, also under laminar flow conditions. The bottles are then loaded onto the shelves of a refrigerated freeze-drying machine, the door is sealed, and a vacuum is created in the chamber. In the presence of a vacuum, the plasma releases its water in the form of vapour, without passing through a liquid phase – the product does not melt. This process of sublimation (changing from a solid directly to a vapour) is called lyophilisation. The drying

process is accelerated by mildly heating the shelves on which the bottles are placed. The vapour migrates from the plasma shell, through the permeable membrane positioned over the neck of the bottle, to the coldest area in the freeze-drier (the condenser) where it forms ice again.

Freeze-dried plasma in single donation units is not as commonly used as in the past. It has been superseded by pooled fresh plasma that is solvent/detergent treated and dried in a fractionation facility.

Cryoprecipitate

When the plasma of freshly donated blood is frozen shortly after collection, and later slowly thawed at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, a white precipitate (called cryoprecipitate) may be seen in the plasma. This cryoprecipitate contains most of the factor VIII, von Willebrand factor and fibrinogen that was present in the original fresh plasma. After hard centrifugation at a temperature of $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ (the precipitate will go back into solution if the plasma is warmed) the precipitate is concentrated in the bottom of the plasma bag. The cryoprecipitate-poor ('cryo-poor') supernatant plasma is then removed into an attached bag leaving only about 40 ml for resuspension of the cryoprecipitate. This product is also called wet cryoprecipitate or 'wet cryo'.

Cryoprecipitate isolated from fresh plasma may be stored frozen for up to three years at temperatures colder than -25°C or for three months at temperatures of between -18°C and -25°C . Alternatively, it may be freeze-dried and stored for at least 1 year.

Platelet concentrates

General information

Platelets may be made by two main methods: random donor (or recovered) platelets refer to individual or pooled platelet products made from whole blood donations, while single donor platelets are made from one donor using apheresis technology.

If whole blood is being donated for the preparation of PC, the time taken to give the donation should not take longer than 12 min as poor flow during donation leads to consumption of platelets. Blood should be collected only from individuals who have not taken aspirin in the previous 72 h, since platelet function is adversely affected.

Whole blood used for the preparation of platelets should not be refrigerated, which would initiate clumping of platelets and reduce their functionality. Suitable donations should be stored in conditions validated to cool donated blood to $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ as rapidly as possible after donation and then maintain this temperature range for up

to 24 h. This is usually achieved using an insulated transport box and coolant packs (or plates) filled with a specially selected coolant that has an appropriate melting point (e.g. butane diol with a melting point of 20.1°C).

A standard adult therapeutic platelet dose is derived from four to six whole blood donations. The yield of platelets from a single donation of whole blood (approximately $50\text{--}70 \times 10^9$) can be recovered and concentrated in a small volume (50–60 ml) by centrifugation using various techniques, and then administered to the patient in a total volume of 250–300 ml.

Platelet yield is the total number of platelets present in the final storage bag and is calculated using the platelet count per litre and adjusting it according to the total volume in which the platelets are suspended.

Platelet concentrate may be issued for therapeutic use as individual units but are more likely to be pooled into a single bag to provide the therapeutic dose in a convenient package for easier administration. When pooled in the components laboratory the connections are performed using an SCD and product sterility and expiry time are not compromised. It is also possible to use a leucocyte filter during pooling and provide filtered (leucocyte-depleted) PC pools.

Once the PC has been prepared, the storage conditions required to maintain viability and haemostatic activity are very different from other components:

- The PC must be continually stored at a controlled temperature of $22^\circ\text{C} \pm 2^\circ\text{C}$. This temperature is best maintained in a temperature-controlled room or cabinet. Though this is the best temperature for platelet storage, it is also a temperature that will encourage rapid growth of bacterial organisms that may contaminate the donation from the phlebotomy site or the donor's circulation. For this reason, the maximum storage period for PCs is 5–7 days, depending on the licensure of the specific bag and storage solution, and the use of bacterial detection methods.
- The PVC bags used for platelet storage differ from collection and transfer bags in that they are made from special plastics that are permeable to gases and guarantee availability of oxygen to the platelets, thus preventing a drop in pH. The bags are large; a bag of approximately 1000 ml is used for the storage of 300 ml of pooled platelets, to enhance oxygen transfer over a bigger surface area. The amount of oxygen required depends on the number of platelets in the bag – more platelets need more oxygen. Bag manufacturers usually give guidelines on the capability of their products.
- During storage, gentle and continuous agitation on a flatbed agitator rotating at approximately 60 cycles

per minute is essential to prevent clumping of the platelets and to enhance oxygen transfer.

- the trays/shelves of the agitator should allow airflow to the underside of the bag (i.e. perforated or meshed).
- PCs must be placed individually on the rotator and not on top of one another as this inhibits airflow.
- the size and number of labels stuck on the platelet bag should also be kept to a minimum so as not to restrict airflow.

Methods of preparation: platelets

Figure 10 illustrates the production of whole blood-derived platelets by the platelet-rich plasma (PRP) method used in the USA, and the buffy coat method used in most other countries

Whole blood-derived platelets, Platelet-rich plasma method

A unit of whole blood is centrifuged at a speed and time validated to maximise the number of platelets and minimise the number of red cells and leucocytes in the plasma. This is the critical step in the production of PC by this method.

The centrifuged bag is then placed in a plasma extractor, or automated blood component extractor, and the platelet-rich plasma (PRP) is slowly expressed to an attached transfer bag. To minimise red and white cell contamination of the final product, great care must be taken not to disturb the interface between the red cells and plasma when loading, and the separation should be slow and stopped when about eight millimetres of plasma remains above the red cells.

The platelets in the PRP are sedimented to the bottom of the transfer bag by quite hard centrifugation (higher rpm or *g* for longer time). However, if the second centrifugation is too hard, then the platelet button formed in the bottom of the bag will not disaggregate. Care needs to be taken in selection of the correct centrifuge setting (see *Principles of centrifugation* earlier in this section). The supernatant platelet poor plasma is removed into an attached transfer bag leaving 50–70 ml of plasma with the platelet button.

Resuspension of the aggregated platelets can be difficult if the proper procedure is not followed. The bag must be allowed to rest undisturbed for between 1 and 2 h before gentle mixing by agitation on a flatbed agitator is commenced. Attempting to speed up the resuspension by manual manipulation of the platelet button is not recommended as this may cause irreversible aggregation.

After resuspension, four to six units of ABO-identical PCs may be pooled to make an adult therapeutic dose. In this process the PC bags are joined to a large volume platelet storage bag using SCD connections or by spiking the ports using laminar flow. SCD connections are preferable because the pool has the same expiry time as the individual units (5 days), whereas spiked pools are an open system and should therefore be used within 6 h of pooling.

As the first centrifugation is quite moderate, this method does not maximise the volume of plasma harvested from a donation. Removal of BC from an RCC and addition of red cell additive solutions is also more difficult when platelets are derived by this method, because the interface is not sharply defined.

Whole blood-derived platelets, Buffy coat method

Whole blood units stored for less than 24 h at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$, are centrifuged at a speed and time validated to sediment the platelets into the BC layer with the white cells. This centrifuged whole blood is usually separated using automated equipment but may be performed manually.

Buffy coats can be prepared by one of two methods:

(1) Top and top quad bag method

During separation of the centrifuged whole blood, all but a small amount of plasma is transferred to an attached bag and the BC, together with a small amount of plasma, is transferred to another attached bag, leaving the red cells in the original primary bag.

(2) Top and bottom bag method, illustrated in Figure 12

During separation of the centrifuged whole blood, all but a small amount of plasma is transferred to a bag attached to the top and the red cells transferred to another bag attached to the bottom leaving the BC (with the small amount of plasma) in the original primary bag.

Prepared BCs are stored at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ without agitation until they are further processed into PCs, preferably within 48 h of donation. The BCs can be processed as single or pooled units:

Single unit PC from buffy coat

The volume of plasma left in the BC should be 50–60 ml to ensure that the final product has sufficient volume to suspend the platelets. The BC bag should have an additional bag attached that is suitable for platelet storage. This bag may be an integral part of the bag system or added using a sterile connection.

After thorough but gentle mixing the BC is centrifuged at a speed and time validated to sediment the residual red cells and white cells whereas the platelets remain

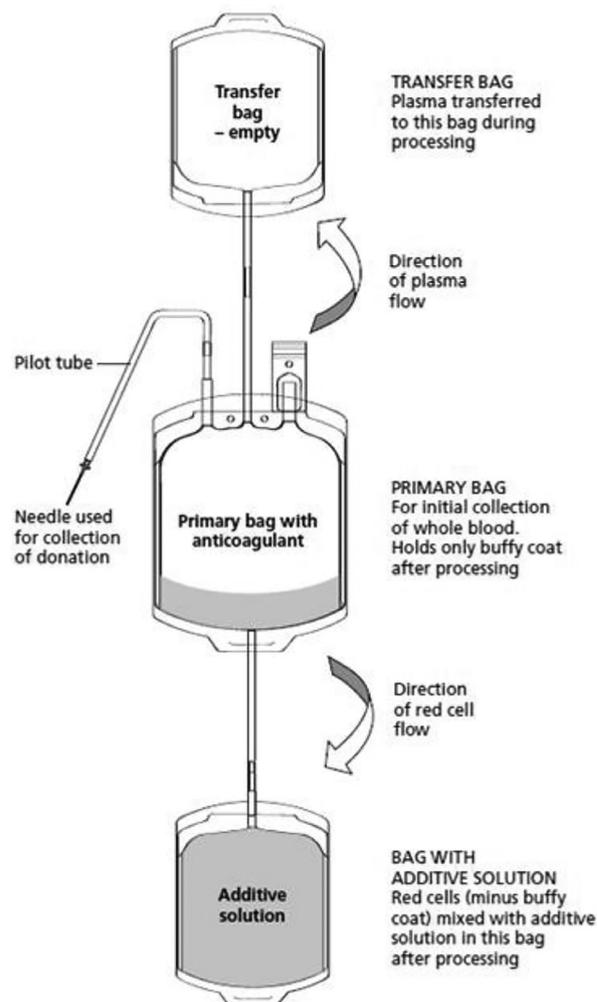


Fig. 12 Top and bottom bag system

suspended in clear plasma supernatant. This centrifuge setting is critical to the successful production of PCs containing a large number of platelets with a low white and red cell count.

Because the volume of BC is very small, the bag should be supported vertically in a centrifuge bucket insert to ensure that there are no folds to trap pockets of cells. There should be a distinct interface between residual red cells and platelet-rich plasma after centrifugation. A special plate, with hooks to hang the bag, or packing the bucket insert with dummy bags, are the usual means of supporting the PC at the time of centrifugation.

After centrifugation the clear supernatant plasma containing the platelets is transferred to the PC storage bag. The BC bag containing the sedimented red and white cells is discarded. The PC is stored on a flatbed agitator. After suitable labelling the unit is ready for transfusion.

Pooled PC from buffy coat

Four to six BCs of the same ABO blood group may be pooled together before centrifugation. BCs used to prepare these pools are approximately 50 ml each, with a haematocrit of 0.4–0.5.

The pool is diluted either with plasma (from one of the BC donations), or with platelet additive solution (PAS). Although many different formulations of PAS are available, they are all designed to maintain pH and platelet viability better than plasma, and more plasma is therefore available for therapeutic use.

BCs may be pooled using a pooling kit or the chain method.

(1) Pooling kits

- Pooling of BCs may be achieved using commercially available pooling kits, as illustrated in Fig. 13. Using an SCD, BC bags are welded individually to one of the multiple tubing ends of the pooling kit. The plasma or PAS is attached to a further tubing end.
- The BCs are drained into a 600 ml transfer bag. Ultimately the contents of each BC bag plus approximately 300 ml PAS or plasma are pooled into an attached transfer bag with a total liquid volume of approximately 600 ml.
- The pooling bag has an integrally attached platelet storage bag, and, if required, an in-line platelet filter may be included to provide a filtered pool.

The bag of pooled BCs (prepared using either the pooling kit or the chain method), with platelet storage bag attached, is centrifuged at a speed and time validated to sediment the red cells and white cells, leaving the platelets suspended in clear supernatant (plasma or PAS). The centrifuge setting is critical to the successful production of PCs with high numbers of platelets and low white cell and red cell counts. The centrifuged pool is carefully moved so as not to disturb the interface and is placed in a plasma extractor or automated blood processing device. Pressure is applied and the platelet-rich supernatant is transferred to the platelet storage bag. The transfer can be through a platelet filter or not depending on requirements. The BC bag containing the sedimented red and white cells is discarded.

During separation and pooling procedures critical labelling errors can occur. It is vital that strict checking procedures, that are preferably computer assisted, are in place to ensure that the identification link to the original donation is maintained for each unit in the pool.

Apheresis platelets (single donor platelets)

PC may be obtained by platelet apheresis of a single donor using automated cell separation equipment. In this procedure whole blood is removed from a donor and the

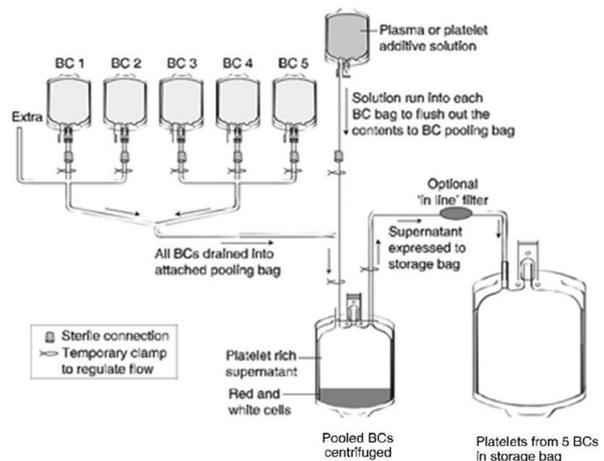


Fig. 13 Pooling of buffy coats using a pooling kit method

(1) Chain method

- BCs separated from the top and bottom bag method can be effectively and economically pooled using the chain method, illustrated in Fig. 14. Using this technique, the tubing at the top of the BC bag is joined to the tubing at the bottom of another BC bag using an SCD. In this way the desired numbers of BCs are joined together in a 'chain', and the plasma or PAS to dilute the BCs is attached to the tubing at the top of the chain. The chain is hung with the diluent at the top. All the BCs are allowed to drain into the bottom BC bag and then the diluent is run through the drained BC bags, one at a time, to rinse out remaining BC from them. The final bag has a total volume of approximately 600 ml of BC diluted in plasma or PAS.
- A platelet storage bag, which may have an in-line platelet filter if required, is welded (using an SCD) to the bag of pooled BC.

apheresis machine harvests the platelets. All other components are returned to the donor, so he/she can return to donate again more frequently than a whole blood donor. The platelets collected may be leucocyte-depleted by additional centrifugation or filtration as part of the collection procedure.

Apheresis platelets can be stored in plasma or in a mixture of plasma and PAS. The bags used for storage and the temperature and movement conditions during storage are the same as for pooled platelets. Generally, the apheresis procedure is a closed system, and the product has a 5-day shelf life at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

The yield of platelets obtained from a single donor can vary depending on the donor, type of machine, and procedure used but is equivalent to between three and 13 random donor PCs.

Dividing apheresis platelet donations

The yield of platelets collected from a single apheresis procedure may be too large for storage in one storage bag, and can be divided to provide two (doubles) or three

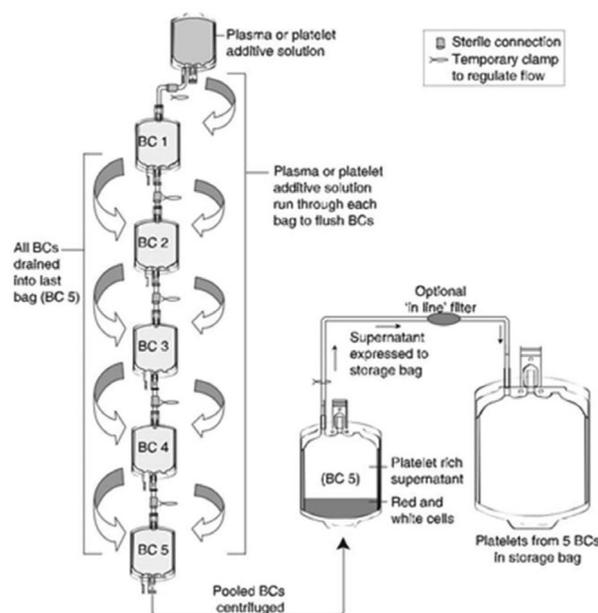


Fig. 14 Pooling of buffy coats using the chain method

(triples) adult platelet doses. Most platelet apheresis collection kits have two storage bags that allow for the product to be split and stored with adequate oxygen exchange. Often the yield of platelets in each bag is sufficient to provide a standard adult therapeutic dose (approximately 240×10^9 per pack, not less than 200×10^9) and each bag may be used for a different patient.

In other instances, the second bag may not be sufficient for an adult therapeutic dose and may be further split into smaller units suitable for paediatric use (more than 60×10^9 platelets in 40–60 ml plasma or PAS per bag).

Quality control for platelet concentrates

- Quality control (QC) testing should conform to local or international standards that set guidelines for the number of units that need to be tested, and the specification range for volume, platelet content, white cell content and pH.
- Samples for QC can be taken from fresh product an hour after completion of processing using an attached sampling pouch (welded or integral). This testing does not waste product and provides early assurance that production is within specification.
- Some QC testing of expired units is essential to monitor pH and platelet yield per pack after storage. Testing of expired units should not be carried out after day six, and the correct storage temperature

range and agitation must be maintained until samples are taken.

- It may not be possible to test every PC produced, so it is essential to keep daily records of QC testing and compare results over time to identify trends.
- A very simple non-invasive visual test can be performed on every PC at issue. By holding the PC up to a light source behind it and moving the contents in a circular motion with the thumb, a swirling phenomenon can be observed. This is based on light being scattered by platelets with normal morphology (discoid), and would be poorly, or not at all visible in units with only a few platelets, or with damaged platelets (spheres).
- Various strategies are used to test for bacterial contamination, including culturing a sample of all platelet concentrates, and/or performing a rapid test closer to the time of transfusion.

Irradiated products

Blood components that may contain viable lymphocytes could initiate graft vs. host disease (GvHD) in a recipient given the following circumstances:

- Recipient on immunosuppressive drugs.
- Neonate (poorly developed immune system).
- Recipient with immunodeficiency.
- Intrauterine transfusion.
- Component for transfusion is from close family member of recipient.

GvHD is an immune condition that occurs after transfusion when immune cells in the transfused blood (i.e. the graft) attack the tissues of the patient (the host).

In these conditions listed above the patients are immunocompromised and may not recognise foreign viable lymphocytes in the transfused blood that then proliferate in the host (patient) usually with fatal results.

Lymphocytes exposed to 25–50 Gy of ionising radiation (Gy, i.e. gray, is a unit of measurement for ionising radiation) will be rendered non-viable, whereas other components (red cells, platelets) are not significantly affected.

Irradiation is accomplished in blood irradiators designed specifically for this purpose or in a hospital radiotherapy unit. Protocols describe the procedure and standardise exposure time. Periodic validation of the exposure source is essential.

Radiation sensitive labels are commercially available and if attached to a unit being irradiated will indicate, by change of colour, that the process has been successfully completed.

Red cell products to be irradiated should be less than 14 days old and have a maximum of 28 days shelf life.

Measures to prevent transmission of pathogens

Pathogens are defined in this publication as micro-organisms with the potential to infect recipients of blood products or plasma derivatives, and include bacteria, viruses and protozoa. Section 8: *Blood donors*, and Section 10: *Donation testing*, explain steps in donor screening and testing to prevent pathogen transmission. There are also several steps in blood processing that contribute to reduced pathogen transmission:

- Avoiding the immediate refrigeration of donated blood so that white cells in the blood bag ingest pathogens, such as bacteria, present in the donation.
- Prestorage leucocyte filtration of blood donations prior to processing, to remove white cell-associated micro-organisms, such as CMV
- Storage of donated blood in a temperature range of $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for four days will prevent the transmission of *Treponema pallidum* (syphilis) because of its susceptibility to time and temperature.
- Storing and transporting blood components within the correct temperature range to minimise deterioration and the proliferation of bacterial contaminants.

Pathogen inactivation

Despite the measures listed above, some risks remain, such as pathogens not detected by current screening tests or pathogens that are not yet identified. Pathogens may also have been accidentally introduced into the donation at the time of phlebotomy. Bacteria present in a unit of blood may replicate during storage and this may not be detectable even with bacterial culture performed at the time of preparation.

Other technologies have relatively recently been developed for inactivating pathogens in labile blood components. Pathogen inactivation is aimed at preventing infectivity by damaging the nucleic acid of the pathogens. Unfortunately, it has been shown that some pathogens, such as small nonenveloped viruses including hepatitis A and hepatitis E, are not inactivated by currently available technologies.

Inactivation technologies include the following:

- Photochemicals, such as psoralen (amotosalen) with a high affinity for nucleic acid strands that under illumination with ultraviolet A (UVA) light, induce lesions to RNA or DNA, making the pathogen unable to replicate *in vitro* or after transfusion. Amotosalen and UVA light-based technology are able to inactivate bacteria, viruses and protozoa in plasma and platelet products. In this way both known and

unknown micro-organisms may be rendered harmless.

- Methods using photosensitisers such as methylene blue and riboflavin are also available for treating plasma (methylene blue) or platelets and plasma (riboflavin). In the presence of these molecules, the illumination with visible or UV light provokes the generation of reactive oxygen species, i.e. chemically reactive chemicals containing oxygen, that will induce lesions to the nucleic acid of the pathogen, thus preventing its replication.
- Methods, based on the addition of amotosalen and riboflavin, damage DNA, preventing replication of lymphocytes in treated blood components. Treated components should not be able to cause TA-GvHD, and there is a growing body of evidence that these products will not need to be irradiated.

Procedures for pathogen inactivation are relatively complicated and expensive and have started to be implemented in developed countries for plasma and PC. More recently, methods are being developed for pathogen inactivation of whole blood and RCC. Studies are being performed in Africa to assess the ability of these methods to reduce the risk of transfusion transmissible malaria.

For information on pathogen inactivation and removal in plasma derivatives, see *Overview of plasma fractionation* later in this section.

Labelling and records

The labelling of blood components should comply with the relevant legislation (local and national), as well as conforming to international agreements (e.g. for barcode generation).

A unique donation identification number that links the donation to records and test results should identify every blood bag. This kind of information can best be accurately kept and updated if stored in a computer database. All functions of the computer and the software should be validated to check that they do exactly what they are intended to do without corrupting or deleting stored data. Accuracy of computer records is dependent on the quality of the software and the quality of data input. Input errors can best be minimised (or eliminated) by the use of barcodes, which can be electronically scanned directly into the database.

The label on a blood component ready for use should contain information (preferably in eye and machine-readable codes) necessary for safe transfusion, including the following:

- Name of producer (collection organisation/blood service).

- The donation identification number should incorporate a code for the responsible collection organisation, the year of donation as well as a serial number. The number should be unique and never used again to label another blood product.
- There should be full traceability by number, from the recipient back to the donor and the collection, testing, processing, storage, release, distribution and transfusion of every single donation that was processed into components or transferred to a fractionation facility.
- The name of the component (e.g. red cells, leucocyte-depleted).
- The ABO and Rh(D) blood group.
- Titre (if applicable).
- Name and volume of anticoagulant and/or additive solution.
- Additional information such as that indicating irradiation, leucodepletion, washing.
- Volume (or weight) of component.
- Date of donation and date of expiry.
- Temperature for storage and temperature for transportation.

The blood service (collection organisation) should supply product information in a booklet and/or a product information leaflet format. This should include information about the various components that are made available to clinicians with regard to composition, indications, and storage and transfusion practices.

All blood components and plasma derivatives issued for transfusion should carry a warning that the product must not be transfused if there is any abnormality noted, such as haemolysis in red cell components, or some other evidence of deterioration, such as cloudiness in plasma or fractionated products. The warning should also state that all blood components should be administered through a 170–200 micron filter.

Quality control

Making safe and efficacious blood components requires the continual application of GLP and good manufacturing practice (GMP). For more details, refer to *Section 16: Quality*.

Overview of plasma fractionation

Blood services as they develop from the collection and distribution of whole blood towards a comprehensive component therapy programme inevitably will generate plasma surplus to the needs for therapeutic FFP. The more successful the component programme (higher percentage of RCC) the more plasma is potentially available for fractionation.

This can provide a means of cost recovery for the blood service, as costs incurred through recruitment, collection, processing and testing of blood are recouped through the sale of excess plasma to the fractionation facility. Large blood services can generate significant quantities of plasma for fractionation, and the opportunity to embark on 'contract fractionation' becomes an option to be considered. This is the process whereby the plasma is supplied to a fractionation facility, a manufacturing fee is charged to produce the fractionated products, and the products are returned to the blood service.

Plasma for fractionation is obtained either as 'recovered' plasma from whole blood donations, or as 'source' plasma from apheresis donations. If such plasma is frozen to a core temperature below -30°C within 24 h of donation, labile clotting factors may also be harvested; if plasma is frozen after 24 h of donation, labile clotting factors can no longer be extracted.

The pool of plasma used as the starting material consists of plasma from several thousand blood donations, all of which must have tested non-reactive for TTIs. The safety of plasma can be improved by employing a process of quarantining the plasma in a 'donor retest programme' as previously described.

As the fractionation of plasma is not the function of a blood service and is generally governed by legislation applicable to medicinal products rather than blood components, this overview does not address the complexities of this pharmaceutical process.

Requirements for supply of plasma for fractionation

Maximising the safety of starting plasma from the blood service includes the points listed in 'Measures to prevent transmission of pathogens' in this section. Although plasma that is transferred to the fractionation facility is considered safe and has been found non-reactive for TTIs, micro-organisms are still presumed to be present (window phase donations or organisms not tested such as hepatitis A), and every effort is therefore made to either remove or inactivate known and unknown micro-organisms.

Although inactivation or removal processes may make plasma derivatives less likely to transmit pathogens, it is critical that the starting material is tested for HIV, HBV and HCV plus other TTIs in the region, so that only plasma that is non-reactive is forwarded to the fractionation facility and pooled for fractionating. Starting pools of plasma are retested by the fractionation facility for the presence of HIV, hepatitis B and C prior to proceeding, so that only non-reactive pools are fractionated.

The complete history from the demographics of the donor base, the recruitment, selection, testing, look-back

procedures, traceability, separation, freezing, storage and transportation of plasma supplied, is generally contained in a Plasma Master File (PMF). This is prepared by the supplying blood service and is subject to audit by the fractionator to verify that the information contained in the PMF is correct and that the processes and testing procedures are GMP/GLP compliant.

Fractionation process

The most widely used method of separation is cold ethyl alcohol fractionation. This was developed by Cohn-Oncley in the USA (1940s), and Kistler-Nitschmann in Switzerland (1964). Both methods involve the addition of ethanol at varying concentrations, to a large pool of plasma while simultaneously cooling it and controlling the temperature, pH and ionic strength. The methods rely on the different solubility of the protein fractions in plasma, and their behaviour when subjected to varying concentrations of alcohol, degree of acidity or alkalinity (pH), temperature and salt concentration (ionic strength). Fig. 15 provides a flowchart of the Kistler-Nitschmann fractionation process for large pool plasma.

Cold ethanol fractionation results in the desired protein fraction being isolated, either by precipitation as a paste, or by retention in solution, while other protein fractions are precipitated. This is achieved without destroying their biological function by processing at cold temperatures so that in the final form they remain efficacious, suitable for infusion (non-toxic, non-pyrogenic) and remain stable when stored.

The yield for the different clotting factors and protein fractions is not the same for each; some are more abundant in unprocessed plasma than others, and this affects the yield, as does the actual process. Albumin is the most abundant and FVIII gives the lowest yield. The process of fractionation is time-consuming; it takes days to isolate a fraction and weeks or months before the final product is bottled and available for therapeutic use. Each derivative has a unique storage temperature and shelf life (see Table 2 for further information).

The first stage of fractionation is concerned with the isolation of coagulation factors, notably factor VIII and fibrinogen in cryoprecipitate and factor IX or prothrombin complex from the cryo-poor plasma. FFP from the blood service is control-thawed to isolate cryoprecipitate in bulk, using a process of continuous flow centrifugation. This is normally carried out prior to commencing fractionation with ethanol, because of the labile nature of the coagulation factors.

After these clotting factors have been separated, the residual plasma pool is cold ethanol fractionated to extract the predominantly protein groups; gamma

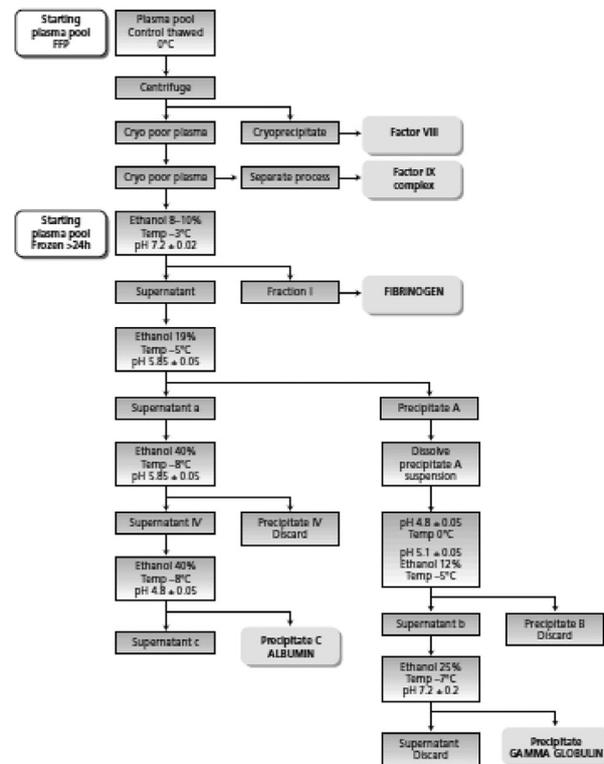


Fig. 15 Flowchart of Kistler-Nitschmann fractionation process for large pool plasma.

globulin (immunoglobulins) and albumin. This is a sequential process using conditions that will precipitate groups of proteins that are required and may then be isolated, or that are contaminants and must be removed, or that remain in solution for subsequent precipitation into a more concentrated form (paste). Once isolated into these groups other techniques are used to ultimately produce the final products.

Other processing techniques used in the fractionation of plasma

Although cold ethanol fractionation is the foundation of fractionation, refinements in protein separation techniques have added new benefits. These include new products from the plasma or plasma fractions, improved yields, increased safety, higher purity and enhanced stability. Of these, the developments in chromatography (such as size exclusion, anion exchange and immunoaffinity) and filtration (such as membrane filtration, ultrafiltration and nanofiltration) have had the biggest impact. This has enabled a better utilisation of the plasma and plasma fractions obtained from ethanol fractionation.

The development of these techniques also facilitated the introduction of viral inactivation/removal processes

Table 2 Guideline summary for storage of whole blood components and plasma derivatives

Blood component or plasma derivative		Shelf life	Storage temperature
Whole blood in CPDA-1		35 days	
Red cell concentrate	Separated from whole blood	Closed system: 35 days	4°C ± 2°C
	Buffy coat removed (leucocyte-reduction)	Closed system: 35 days	
	In additive solution, also leucocyte-depleted (filtered)	Closed system: 35–42 days	
	Washed	Open system: up to 24 hours	
		Closed system: according to validated methods used	
		Up to 28 days from donation	
	Irradiated: within 14 days of donation	Use as soon as possible after irradiation (up to 48 h)	
	Irradiated: within 5 days of donation for intrauterine transfusion and neonatal exchange transfusion		
	Cryopreserved in high glycerol	At least 10 years	–70°C ± 10°C
	Cryopreserved/thawed	Open system: up to 24 h	4°C ± 2°C
		Closed system: according to validated method used	
Fresh frozen plasma and cryoprecipitate	Frozen	3–12 months depending on national standards	–18°C to –25°C
	Thawed	1–3 years depending on national standards	Below –25°C
Platelet concentrate (in constant agitation during storage)	Isolated from platelet-rich plasma in a closed system	If not used immediately, 24 h	4°C ± 2°C
	Irradiated	5–7 days	22°C ± 2°C
	Recovered from buffy coat in a closed system		
	Pooled from buffy coats	Open system: up to 6 h	
		Closed system: 5–7 days	
Dried factor VIII (S/D treated)		Up to 3 years depending on manufacture	Below 25°C
Dried factor XI (S/D treated)			
Fibrinogen (dried)			
Albumin (liquid)			
Immunoglobulin, intravenous (dried)			
Immunoglobulin, intramuscular (liquid)			4°C ± 2°C

that significantly further enhance the safety of fractionated products.

Infection control: inactivation or removal of pathogens

Viral inactivation techniques are only considered effective if it can be demonstrated that they are able to destroy at least one element needed for viral replication (validated inactivation). Although not all viruses may be completely inactivated or removed, what is important is that the residual viral load within the pool is reduced to a level below which it could be infective when administered to a patient.

During the manufacturing process, chemical or heat treatment steps are included to inactivate both known

and unknown micro-organisms that may have been in the starting pool (from window period donations or contamination at the time of phlebotomy). Unlike blood components derived from single donations, plasma derivatives are manufactured in large batches, and every bottle or vial in the batch is the same.

Some inactivation steps are in-process during cold ethanol fractionation; others are additional processes. Depending on their physical nature, both known and unknown microbes are similarly affected by these processes. There are numerous techniques that may be used to inactivate or remove microbial agents in fractionated products, and descriptions of these are not included in this publication. However, a few notes on microbial control are included for information.

In-process

- The ethanol used in cold alcohol fractionation is in itself an effective virucide $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and antimicrobial agent.
- Pooled plasma is likely to contain donor antibodies (such as anti-HBs) and this assists in the immune neutralisation of viruses of corresponding specificity (such as HBV) that may also be present in the pool.
- The pooling of large quantities of plasma, the use of large volumes of water and ethanol has a dilution effect.
- The process of partitioning viruses during the precipitation of fractions and the isolation of precipitates has been demonstrated to result in significant viral reduction.
- Filtration to remove precipitates and retain the fraction in solution, such as immunoglobulin, also removes microbes.

Additional processes for plasma derivatives

Heat.

- Pasteurisation (60°C for 10 h) in the final container inactivates viruses and is used in the final stage of albumin production.
- Dry heat in the final container.
- Steam treatment of dry product in the presence of steam under pressure.

Chemical.

- Solvent/detergent (S/D) treatment disrupts the viral membranes of lipid-enveloped viruses (such as hepatitis B and C and HIV) by means of an organic S/D solution. The solvent breaks the protective lipid envelopes of the viral particles and the detergent retains the lipids in solution. S/D is then removed and the product (such as FVIII) is filtered to remove any viral debris that may be present.
- Low pH treatment ($\pm\text{pH } 4.0$) with or without pepsin, at a temperature between 30°C and 37°C , is used in the viral inactivation of immunoglobulin solutions.
- Beta propiolactone treatment followed by UV irradiation is performed using special equipment.

Filtration.

- Filtration using filters of appropriate pore size (nanofiltration) removes viruses with a protein membrane and not a lipid envelope.
- Aseptic membrane filtration (0.22 nanometres) is used to remove micro-organisms and sterilise bulk products prior to filling ampoules/final product containers.

Pharmacovigilance and product recall

Because large numbers of containers of product belong to a single batch of product and could potentially be infused into a large number of patients, it is essential to monitor the use and effect of the products on recipients. This is a formalised process called pharmacovigilance.

Pharmacovigilance is the structured approach to monitoring and assessing (measuring the effect) of medicinal drugs, including plasma derivatives, on patients. Reporting of untoward effects is encouraged by pharmacovigilance personnel and consideration is then given on how products may be improved, and reactions avoided. The process should be both proactive and reactive.

- Proactive measures include ongoing assessment of products before issue. Regular inspection of batches allows for the detection of deterioration, and batch recall.
- Reactive measures address reactions reported by clinical personnel. This may lead to a product recall to prevent further reactions of the same nature in other patients.

Abstract of blood storage conditions

Once blood has been processed to prepare components or has been fractionated to manufacture plasma derivatives, products are stored within the correct temperature range, and under the correct conditions. Information provided in this connection, within the preceding text, is consolidated and summarised in a table to review these unique conditions together.

Table 2 summarises the storage conditions required for blood components and plasma derivatives. Notes that relate to the summarised information in the table, are as follows:

- Local standards apply – these are general guidelines only.
- Shelf life of RCC in additive solution depends on the nature of the additive.
- When using an open system to prepare red cells, shelf life is reduced to a maximum of 24 h at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- When using an open system to pool platelets, shelf life is reduced to a maximum of 6 h.
- Closed systems must be validated to ensure that they are safe before being put into use.
- RCC divided into paediatric packs using an approved closed system may be used for 4 weeks if stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- Apheresis red cells and platelets have a similar shelf life and storage requirements as listed.

- Storage temperature of plasma derivatives depends on manufacturer's recommendation.

Key Points

- Whole blood may be divided into three main component types: red cell concentrates, platelet concentrates and plasma.
- Blood bags are available in different configurations where different numbers and types of bags are integrally attached with tubing in a sterile closed system. Each bag configuration is designed for use in a specific way according to processing requirements.
- Blood components can be separated as they differ in size and density and will sediment differently when centrifugal force is applied. A blood bag centrifuge is an essential item of blood processing equipment.
- A scale capable of weighing components to at least the nearest gram weight is essential when making components.
- A plasma extractor (or blood press) is a device that is used to apply pressure to a centrifuged unit of blood in order to transfer part of it to an attached transfer bag.
- Automated blood processing machines are devices that can be configured to process a centrifuged unit of blood into components.
- A tube sealer is a device that will place permanent seals in the PVC tubing of blood packs.
- Sterile connection devices are used to weld additional transfer bags to a primary blood bag without breaking the sterile integrity of the system.
- A platelet agitator is a device designed to fulfil the need for platelet concentrates to be kept in motion during storage.
- Plasma snap freezers are designed to rapidly freeze fresh plasma in order to preserve the labile clotting factors.
- RCC, buffy coat removed, in additive solution, may be considered the red cell product of choice. The component is derived from whole blood by centrifugation and removal of plasma and BC and the subsequent resuspension of the red cells in nutrient additive solution.
- RCC filtered (RCC, leucocyte-depleted) are depleted of white cells by filtration. They are used for patients with leucocyte antibodies and to prevent alloimmunisation in patients expected to receive repeated transfusions.
- Some organisations (and countries) have adopted a policy of universal leucodepletion of all blood donations in an effort to minimise the risk of exposure to vCJD.
- RCC, paediatric, is usually made from either BC depleted RCCs or filtered RCCs by dividing the adult unit into smaller quantities in several bags (usually from 25–100 ml).
- RCCs cryopreserved (frozen) have a shelf life up to 10 years or longer if units are correctly protected during freezing and ultra-low freezing temperatures of colder than -65°C are maintained during the entire storage period.
- Frozen storage of red cells is used mainly to preserve units of blood with rare blood types. It can be used to stockpile blood for emergency use in disasters, but the high cost and short shelf life after recovery from the frozen state make it impractical as a routine tool to manage inventory.
- RCCs, washed, are a specialised component prepared for patients with plasma protein antibodies (e.g. anti-IgA).
- The first step in component processing is to remove the plasma from a centrifuged unit of whole blood.
- Fresh plasma contains coagulation factors (most notably FVIII), protein (e.g. albumin) and immunoglobulins (antibodies). To preserve labile components such as FVIII the plasma is stored frozen and then becomes known as fresh frozen plasma.
- FFP for therapeutic use is prepared by freezing the plasma component of whole blood within 18 h of collection.
- Because plasma has a long expiry time when frozen and stored below -18°C , it is possible to minimise the risks of TTIs by instituting a quarantine and release 'donor retest' programme.
- Freeze-dried plasma is a suitable product for use in outlying rural hospitals and emergency rooms. Freeze-drying preserves the clotting factors present in fresh plasma in a powder form and may be stored at ambient temperature (25°C or below) before use.
- Platelet concentrate from a single donation of fresh whole blood (approximately $50\text{--}70 \times 10^9$) can be recovered and concentrated in a small volume (50–60 ml) by various techniques.
- PCs may be issued and transfused individually by hospital personnel or may be pooled in the components laboratory into a single bag to provide the therapeutic dose (4–6 units) in a convenient package.
- Whole blood used for the preparation of platelets should not be refrigerated, which would initiate clumping and
- reduce their functionality and should be stored in conditions validated to cool donated blood to $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ rapidly and then maintain this temperature range for up to 24 h.

- PCs should be continually stored at a controlled room temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$).
- BC platelets may be prepared as single or pooled units.
- BC platelets may be diluted in either plasma or platelet additive solution.
- Pooling of BCs may be achieved using commercial pooling kits or the 'chain' method.
- Platelet concentrates can also be obtained by platelet apheresis of a single donor using automated cell separation equipment.
- Quality control testing of PCs should conform to local or international standards that set guidelines for the number of units that need to be tested, and the specification range for volume, platelet content, white cell content and pH.
- The swirling test is a simple non-invasive visual check that may be performed on every platelet concentrate. The test is based on light scattering by platelets with normal morphology, and would be poorly, or not at all visible in units with damaged or few platelets.
- Irradiation of blood components is accomplished in irradiators designed specifically for this purpose or in a hospital radiotherapy unit.
- Systems that inactivate viral and bacterial pathogens in blood are available for some blood components (platelets) and are under development for others (red cells).
- The labelling of blood components should comply with the relevant legislation (local and national), as well as conforming to international agreements.
- Making safe and effective blood components requires the continual application of good laboratory practice (GLP) and good manufacturing practice (GMP).
- Ongoing product control should be carried out to ensure that the processing laboratory maintains a high and consistent quality of product.
- FFP (for fractionation) is supplied to a fractionation facility for the extraction of clotting factors, albumin and immunoglobulins. Fractionation requires bulk lots made up of several thousand units of 'safe' plasma.
- Component processing laboratories may be involved in the preparation of plasma for transfer in bulk to a fractionation facility, which has strict requirements related to quality and standards, and will audit potential suppliers to ensure that they comply.
- The most widely used method of separation of plasma fractions is cold ethanol fractionation, using ethanol at specific concentrations, together with pH and temperature control, to either precipitate a fraction or isolate it in the supernatant.
- Plasma derivatives manufactured in a fractionation facility are subjected to several steps, either in-process (such as filtration) or as additional processes, to remove or inactivate micro-organisms. Additional processes include pasteurisation and solvent/detergent treatment.

Blood storage and transportation

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Introduction

In terms of blood processing, quality may be defined as a series of processes that will ensure that preparation of blood components is controlled to achieve the required standard needed to improve the haematological status of the recipient. An important aspect of this process is the transport and storage of the product from the time of collection to the time of transfusion. Blood components may be collected and prepared correctly, but if storage and transportation conditions are not well managed, then the components transfused will not achieve this quality goal.

Anticoagulants and preservatives prevent clotting and maintain cell viability and function during component storage. Storage conditions relate largely to the maintenance of temperature from the time of collection, through processing, testing and labelling and transportation, up to the point of issue for transfusion into a patient. This is known as 'cold chain management'. Blood is of a fragile nature and components should be handled with due care. Mechanical and thermal trauma are detrimental to their viability and functionality. Rough handling may also damage the collection bags resulting in leakage and contamination.

As components are usually administered in hospital wards or operating theatres it is important to store and transport blood and components under stringently hygienic conditions to avoid their contamination and ensure that when they reach the patient, they are visually presentable and free of any sign of soiling. Moreover, satellite blood refrigerators are required in clinical areas, where blood may not be used immediately (such as in operating theatres), to maintain the components at the correct storage temperatures until use.

Learning objectives

By the end of this section, the student should be able to describe the concepts of blood anticoagulation and preservation, as well as storage and transportation requirements. From the viewpoint of the technologist, the student should be able to discuss the following:

- Anticoagulant and additive solutions
- Composition of anticoagulant preservative solutions
 - Volume of anticoagulant preservative solution
- Composition of red cell additive solutions
 - Volume of red cell additive solution
- Storage lesion
- Shelf life and storage temperature
 - Pre-processing transport and storage temperature
 - Processing temperature specifications
 - Storage temperature specifications
 - Shelf life and storage temperature specifications for blood components
- Storage equipment and environment
 - Blood refrigerators and freezers
 - Blood storage refrigerators
 - Plasma storage freezers
 - Room temperature storage facilities
 - Platelet agitator
 - Temperature-controlled room
 - Temperature-controlled working environment
- Design features of storage equipment and environment
 - Design features of blood storage refrigerators and storage freezers
 - Design features of cold rooms and freezer rooms
- Transportation of blood and blood components
 - Blood/blood component transport containers
 - Design features of a blood/blood component transport container
 - Selection of suitable coolants
- Transportation of fresh donated blood from collection area to processing centre
- Transportation of stored red cells or whole blood units from one storage venue to another
- Transportation of stored platelet concentrates from one storage venue to another
- Transportation of frozen plasma and cryoprecipitate from one storage venue to another
- Transportation of thawed plasma and cryoprecipitate from one storage venue to another
- Issuing blood components to clinical areas
- Container and coolant validation
- Temperature monitoring
 - Responsibility
 - Temperature measuring points
 - Temperature monitoring devices
 - Thermometers
 - Chart recorders
 - Electronic data loggers
 - Computer-linked software

- Alarms
- Installation and maintenance
 - Installation
 - Preventive maintenance
 - Repairs
 - Contingency measures
 - Prevention of product loss from equipment failure
 - Power failure
- Links in the blood cold chain
 - Collection
 - Processing
 - Storage
 - Transport
 - Transfusion
- Inventory management and control
 - Baseline usage figures
 - Daily stock count.

Anticoagulant and additive solutions

Whole blood is collected into a bag containing an anticoagulant solution. The anticoagulant solutions used in blood collection have been developed to prevent coagulation and to permit storage of red cells for a certain period of time. Anticoagulant solutions used for blood collection vary in composition; their main function is to prevent clotting and to provide nutrients and energy to maintain red cell viability and functionality throughout the storage period.

Energy-rich compounds in the preservative solutions (such as dextrose or glucose) are absorbed into the cells and are metabolised by enzymes to release their potential energy by a process called glycolysis. Adenosine triphosphate (ATP) is an energy-rich molecule used to support red cell function during storage.

2,3-diphosphoglycerate (2,3-DPG) affects the ability of haemoglobin to release bound oxygen. During storage, red cells rapidly lose 2,3-DPG leading to a proportionate increase in the affinity of haemoglobin for oxygen. Therefore, when transfused, these red cells cannot readily release oxygen to the tissues where it is required. However, once in circulation, stored red cells regenerate 2,3-DPG within 72 h.

Separating the plasma from the red cells in the preparation of components will remove more than half of the nutrients from the residual red cells. Thus, it may be more logical to provide the proper nutrients for the cells using a re-suspension medium instead of a small portion of the initial anticoagulant solution.

Composition of anticoagulant preservative solution

Anticoagulant preservative solutions vary depending on their composition. All of the anticoagulant preservative

solutions contain sodium citrate, dextrose and citric acid, and some of them may, in addition, contain phosphate and adenine.

- **Sodium citrate:** Citrate binds calcium and prevents clotting of the blood during storage. This is achieved by interfering with the calcium-dependent steps in the clotting cascade.
- **Dextrose:** is used by red cells during storage. Dextrose supports the generation of ATP by glycolysis and in this way provides nutrients that are required by the red cells.
- **Citric acid:** is added to obtain a suitable pH at the beginning of storage at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Without the addition of citric acid, blood is alkaline at storage temperature. In conjunction with sodium citrate and dextrose, it comprises the anticoagulant acid citrate dextrose (ACD).
- **Sodium phosphate:** in conjunction with citric acid, dextrose and sodium citrate, it comprises the anticoagulant citrate phosphate dextrose (CPD).
- **Adenine:** improves the synthesis of ATP to compensate for the dropping ATP levels during storage. (See *Composition of red cell additive solutions* below)

ACD was one of the earliest anticoagulants used for blood collection and storage. However, it is no longer commonly used, as better solutions are now available. This is because the resultant acidic pH of blood in ACD does not maintain adequate 2,3-DPG levels. CPD anticoagulant is now more commonly used, often in conjunction with red cell additive solution, as it has a more alkaline pH and maintains 2,3-DPG levels better than ACD. When adenine (A) is added to CPD, the anticoagulant is called CPDA-1 (the '1' signifies the version of the formula used).

Volume of anticoagulant preservative solution

The volume of anticoagulant required to prevent clotting and preserve red cells is dependent on the volume of blood collected from the donor. Some collection bags are designed for the collection of 500 ml blood and contain 70 ml anticoagulant; others are designed for 450 ml collections and contain 63 ml anticoagulant. If smaller quantities of blood are to be drawn, then the volume of anticoagulant would have to be reduced proportionately by the manufacturer of the collection bags.

Composition of red cell additive solutions

Red cell concentrates (RCCs) that are prepared from whole blood donations collected into CPD are suspended in additive solution to improve storage and shelf life. During the processing of donated whole blood, and when plasma

is removed after the centrifugation, a considerable amount of adenine and glucose is removed with the plasma. At this stage, blood has been effectively anticoagulated so the presence of CPD is no longer required by the red cells remaining in the primary collection bag. However, the red cells need nutrients to survive and to maintain their viability post-transfusion and should also be suspended in sufficient fluid to allow for normal flow characteristics during transfusion. This is achieved by the addition of an additive solution containing glucose and adenine to the red cells, within 24 h of collection. Other substances (e.g. mannitol, citrate) may be used to prevent *in vitro* haemolysis. Phosphate may be added to enhance glycolysis, and sodium chloride or disodium phosphate may be added to give the additive solution a suitable osmotic strength.

Red cell additive solutions vary in composition depending on the supplier. They are sometimes referred to by their brand names or simply as SAGM (saline, adenine, glucose and mannitol) or AS-1, AS-3, AS-5 and so on. The typical composition of an additive solution is as follows:

- **Saline:** the fluid in which the red cells are suspended to provide the desired flow rate conditions.
- **Glucose (or dextrose):** provides the basic nutrients for glycolysis.
- **Adenine and mannitol:** assist in the process of ATP generation.

Volume of red cell additive solution

The volume of additive solution required to preserve red cells during storage varies according to the volume of the whole blood donation. Red cells from a donation of 500 ml require about 111 ml of additive solution, whereas 450 ml donations need 100 ml.

Storage lesion

Changes alter the physiological properties occur in stored blood over time. This is known as storage lesion and includes the following:

- Oxidative damage to red cells leading to increased free haemoglobin and reactive oxygen species (these are unstable molecules containing oxygen that may cause damage to DNA, RNA and other proteins, and may even cause cell death).
- Rapid fall in 2,3-DPG. The red cells increase their affinity for oxygen and lose some viability.
- Reduction in intracellular ATP in the stored red cells.
- Fall in nitric oxide levels which plays a critical role in vascular reactivity due to the potent vasodilatory

effect of nitric oxide. Dilation of the blood vessels results in an increased blood supply, and thus better oxygenation of the tissues, and a reduction in blood pressure.

- Lactate accumulation and release of cytokines.
- Impaired enzyme activity and reduced antioxidant capacity. Antioxidants help to protect the cells from the damage that is caused by free radicals.
- Damage to red cell membranes and cytoskeleton and reduced deformability of the red cells.
- Haemolysis and release of potassium from the red cells.
- Rapid deterioration of coagulation factor activity (including factor VIII) in whole blood, particularly after the first 24 h of storage.

Shelf life and storage temperatures

Storage conditions for blood components are designed to preserve optimal viability and functionality during the entire storage period. Collection of blood into anticoagulant preservative and additive solutions maintains component function and viability only if storage is within the correct temperature range. The risk of bacterial contamination decreases substantially if appropriate storage conditions and temperatures are used. The shelf life of a blood component is the maximum allowable storage time that it may be stored, provided that the requirements of temperature, preservative solutions and physical environment are met. Storage areas must provide appropriate and secure storage for different components with access restricted to authorised personnel. Storage facilities should be clean and free from dust, litter and pests. Lighting, temperature, humidity and ventilation should be appropriate and such that they do not adversely affect components.

Control of temperature is vital to the successful shelf life of blood and components. Shelf life and storage temperature specifications must comply with local standards, provided that they are equal to or more stringent than internationally accepted norms. Temperatures should be maintained during transportation of blood and blood components. There should be a system in place to monitor, maintain and control the storage of blood components throughout their shelf life. For information and benchmarking, international standards should be consulted.

Pre-processing transport and storage temperature

Time and temperature limits should be defined for the processing of blood components. Due to the potential deterioration of viability and functionality of labile blood

components, the conditions of storage and time before processing are vital. Delays in preparation or unsuitable storage conditions may affect the quality of the final components adversely.

- Whole blood may be stored immediately after collection at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Alternately, it may be placed in a controlled environment of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$, after rapid cooling to this temperature using a method validated to maintain this temperature range. The option of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ storage for up to the first 24 h after collection is a prerequisite for the production of platelet concentrates.
- Rapid cooling of whole blood immediately after collection should result in its temperature reduction to $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in <4 h. Continued storage of whole blood at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for up to 24 h from donation allows more time for better scheduling of blood processing workload.
- Whole blood donation collected for processing of components other than platelets shall be cooled toward a temperature range of $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ within 24 h of collection until arrival to the processing laboratory.
- Whatever option is used, after the first 24 h all whole blood donations must be maintained at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Processing temperature specifications

- For preparing platelet units from whole blood using the platelet-rich-plasma (PRP) method, whole blood donations placed in an environment with a temperature range of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ should be processed within 8 h of collection.
- For preparing platelet units from whole blood donations using the buffy coat method, whole blood donations placed in an environment with a temperature range of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ should be processed within 24 h of collection.
- RCCs prepared from whole blood refrigerated immediately at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ after collection should be replaced at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ as quickly as possible after processing, and in total no longer than 1 h from the time the whole blood was removed from the refrigerator for processing. The centrifuge used to spin the whole blood for separation of the RCC should be set at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- RCCs prepared from whole blood stored at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for up to 24 h after collection, should be stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ immediately after processing. The centrifuge used to spin the whole blood for separation of the RCC should be set at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ when processing blood stored at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Shelf life and storage temperature specifications of blood components

- Whole blood and red blood cell concentrates must always be stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The shelf life varies according to anticoagulant preservative and additive solution used. The requirement that determines shelf life is that at least 75% of red cells transfused at the end of the proposed storage period must still be in detectable in the circulation of the recipient 24 h after transfusion. This interprets as a shelf life of 21 days for ACD/CPD/CP2D and 35 days for CPDA-1. When an additive solution is added to red cell concentrates (such as SAGM or AS-3), the shelf life of the red cell concentrate is 42 days.
- If red cell concentrates were processed in an open system (such as manual wash), the shelf life is reduced to 24 h when stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- The shelf life of irradiated whole blood or RCC is the original expiration date or 28 days from date of irradiation, whichever is sooner.
- Platelet concentrates derived from whole blood donations (single or pooled), or by apheresis are stored at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with continuous gentle agitation. The shelf life is determined by its efficacy when transfused, and concern over bacterial growth during storage. This may be related to platelet viability and function during storage in the correct conditions of temperature and motion. Most blood services allocate a 5 day shelf life to minimise the risk of bacterial growth resulting from the room temperature storage requirement, some have a 7 day shelf life with additional measures to detect or inactivate bacteria. The shelf life is not affected by irradiating the platelets.
- For plasma, the levels of stable clotting factors (FII, FVII, FIX, FX and fibrinogen) are quite well maintained at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. However, labile clotting factors (FV, FVIII) deteriorate to levels that are not useful if not frozen within 24 h of collection. Plasma units (whether separated within 8 h or 24 h of collection) and cryoprecipitate must be stored frozen in the blood bank at a temperature of -18°C or colder for up to one year from collection. Frozen plasma can be stored at -65°C or colder for up to seven years from collection.
- Thawed plasma may be stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for a maximum of 5 days provided that it was produced using a closed system.
- For reconstituted components (such as reconstituted blood for neonatal exchange transfusion), shelf life is 24 h.
- Cryoprecipitate prepared from fresh frozen plasma may be stored at -18°C or colder for up to 1 year.

Storage equipment and environment

Blood refrigerators and freezers

Blood components need a clean storage environment and careful handling to avoid physical damage and contamination. Refrigerators and freezers used for blood storage shall contain only donor blood, blood specimens, reagents or blood components. Access must be restricted to authorised personnel. Equipment should have temperature recording and alarm devices that are set to activate under conditions that will allow timely action to be taken before blood or blood components reach an unacceptable temperature. The alarm system in liquid nitrogen freezers shall be activated before the contained liquid nitrogen reaches a level that could result in thawing of the contained blood component. Provisions must be in place in the event of equipment or power failure to maintain blood components within the acceptable temperature range. Equipment should ideally be connected to a reserve power unit in addition to the main power supply, and that when the main power supply fails, there is automatic and immediate switchover to the reserve power unit. It is very important that there is prompt service for repair when problems are experienced, and that reserve power units are routinely tested for faults.

Blood storage refrigerators

The purpose of a blood storage refrigerator is to maintain whole blood, RCCs and thawed plasma at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. As correct storage is critical to the quality and functionality of blood components, a blood storage refrigerator is a basic requirement for the blood bank and its performance must be controlled very carefully.

There are different types of blood storage refrigerators:

- **Upright refrigerators:** are most widely used. These combine all the ideal design features, as noted in the *Design Features* subheading below. They vary in size according to workload and application. The design should minimise the need for door opening by having a glass door or solid (opaque) door covering an internal see-through glass or epoxy door. Large, double-door units are required for managing large numbers of components, such as in processing areas. An electric fan forces air circulation to ensure a uniform temperature throughout the cabinet.
- **Satellite blood refrigerators** are small and are based in the clinical areas in the hospital such as the emergency rooms and operating theatres. These should meet the same specifications of a blood bank refrigerator.

- **Chest refrigerators** are not commonly used. The feature of a top-opening lid rather than a door placed on the front allows for better retention of cold air when the lid is opened, and models with better insulation can be useful in areas where power supply is intermittent, as they are able to hold the temperature for longer periods during outages.
- **Walk-in cold rooms:** these are fixtures built into major blood centres. They vary in size and design and are usually constructed at the same time as the facility is built so that their features may be carefully planned. Cold rooms are set to the required temperature range for bulk storage of blood and components.
- **Solar and gas-powered refrigerators:** although in most applications blood storage refrigerators are electrically powered, it must be acknowledged that in some countries, gas or solar powered units may be the only way to store blood under controlled conditions. Solar powered refrigerators convert solar energy to direct current, as an alternative source of electricity to the main supply. Such units will meet basic requirements for blood storage. The insulation of the cabinet is better so that the hold-over time is at least 24 h. Generally, they are expected to hold fewer units and have very thick insulation to limit heat loss. They do not have blower fans to circulate air in the storage chamber. The energy requirements are low. The equipment shall have the same temperature monitoring devices as for the standard electric refrigerator.

Plasma storage freezers

The purpose of a plasma storage freezer is to store plasma for therapeutic use and bulk plasma for transfer to a fractionation facility at temperatures consistently at -18°C or colder. Freezers require a 'defrost cycle' to clear ice from the fan (blower) unit. However, it must be guaranteed that the low temperature is maintained during defrosting. During the defrost cycle the temperature in the freezer may rise approximately 5°C , so in order to maintain a temperature consistently at -18°C or colder, the operating temperature of the freezer should be set at -23°C or colder. The same requirements apply to cryoprecipitate storage freezers.

There are different types of plasma storage freezers:

- **Upright freezers:** these are convenient to use because the front-opening door allows easy access to stored plasma. This feature makes them popular in issue areas, and for holding quarantine units for sorting and checking before long-term storage. These

have more insulation compared to a blood refrigerator, in order to maintain the lower temperatures. The freezer has an internal fan to ensure even distribution of air in the cabinet.

- **Chest freezers (top-opening door):** these are commonly used for both small quantity and bulk plasma storage applications. These are efficient in maintaining the desired temperature. As cold air is heavier than warm air, it remains at the bottom of a chest freezer when the top-opening lid is raised. However, it is sometimes difficult to gain access to frozen components near the bottom of chest freezers.
- **Freezer rooms:** these are fixtures built into a blood centre. They vary in size and design and are usually constructed at the same time as the facility is built so that their features may be carefully planned. Freezer rooms are best suited to bulk storage of plasma that is destined for fractionation or large-scale quarantine of plasma in a 'donor retest' programme. They have thicker insulation for the colder temperatures required, than for walk-in cold rooms.

Room temperature storage facilities

The purpose of creating a room temperature storage facility is to provide a controlled environment, validated to maintain temperature at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$, under all ambient conditions (extreme heat or cold). Such an environment is required for:

- Storage of platelet concentrates on a platelet agitator housed in an incubator.
- Storage of fresh donated whole blood between receipt and processing in a temperature-controlled room if a 24 h hold at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ is used.
- Providing a temperature-controlled working environment that complies with good manufacturing practice (GMP) where units of blood are processed or handled in bulk (i.e. sorting of incoming fresh blood, labelling, preparing deliveries and so on). Access must be restricted to authorised personnel.

Platelet agitators

A platelet agitator can be housed in a closed incubator capable of maintaining a temperature of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Suppliers are able to provide this agitator/incubator combination, in various sizes depending on the number and type of platelet concentrates requiring storage. These should be opened for as short a time as possible when loading or unloading platelet concentrates. They should be fitted with a temperature monitoring device for a permanent record of the temperature reached and contain alarm systems for motion failure. They contain roll-out

trays and a glass door to allow inspection of the products. All types of platelets (random donor platelets, buffy coat platelets or apheresis platelets) should be stored in these units with continuous gentle agitation.

Features specific to these units are:

- Contents of the incubator are visible through a glass door.
- The agitator has a set speed of agitation to enable satisfactory mixing in the platelet bag and to avoid foaming.
- Pre-set alarm points at 20°C (low) and 24°C (high) with audio-visual alarms when temperature is out of range.
- Continuous recording of the temperature by computer or thermograph chart. Alternatively, the temperature of the incubators should be manually monitored and recorded every 4 h.
- A battery-powered alarm to notify personnel if the power supply to the unit is cut.

Temperature-controlled storage room

A dedicated room can be used in some blood banks for storage of platelets and freshly donated blood. The room should be large enough for several platelet agitators and/or, the storage of crates/trays of fresh blood (<24 h from donation), that are to be processed. The room should have a controlled temperature environment of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Features specific to a room of this type are:

- Moderately insulated walls
- Automatically closing door
- Sealed viewing window (no opening windows)
- Good lighting
- A top-quality air-conditioning unit that is able to maintain the room temperature at the desired and controlled range
- Pre-set alarm points at 20°C (low) and 24°C (high) with audio-visual alarms when temperature is out of range
- A battery-powered alarm to notify personnel if the power supply to the air-conditioning unit is disconnected
- Temperature display in 0.1°C graduations. Continuous recording of this temperature by computer or thermograph chart is desirable. The device should be manually monitored, and all parameters recorded at least twice per day (even with continuous monitoring).

Temperature-controlled working environment

To be GMP compliant, a well-controlled room temperature of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ is desirable in areas where units of

blood are processed or handled in bulk. This is to avoid the need to unload blood from an insulated transport container and handle it in a room that has no temperature control. Likewise, taking cooled blood from a refrigerator for labelling, or for despatch preparation, changes its temperature rapidly to that of the environment, which could be unfavourable (either too hot or too cold). Handling of stock blood is best performed in a room with a controlled temperature, and even then, steps should be taken to ensure that as few units as possible are removed from their controlled storage facility at one time. Features specific to the provision of a controlled working environment of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ include:

- An efficient and well-maintained air-conditioning system.
- Work areas with sealed windows and self-closing doors and air curtains to assist efficient air conditioning.
- Minimum of two temperature displays in 1°C graduations placed in separate locations to detect variation in temperature within the work area. These temperatures should be manually monitored, and all parameters recorded at least twice per day.
- Audible alarms and continuous monitoring of the temperature in the work area are not essential but could be useful in achieving high-level quality ratings. Personnel should have clear instructions on action required if the temperature moves out of range.

Design features of storage equipment and environment

Design features of blood storage refrigerators and storage freezers

- Good insulation is required to minimise heat transfer from the environment to the contents of the refrigerator/freezer. Good insulation also reduces the workload placed on the equipment's compressor, making its operation much more efficient and also improving its 'hold-over' time.
- Hold-over time may be defined as the time period that a fully loaded piece of equipment is able to hold its temperature— with the door closed — when there is a loss of power. A longer hold-over time is particularly useful in areas where electricity supply is unreliable, and ambient temperatures are usually high.
- Insulation used for freezers may consist of denser and thicker insulating material than that used for blood refrigerators.

- To protect the environment from harmful compounds, the compressor should use chlorofluorocarbon (CFC)-free gas.
- The motor should have sufficient reserve capacity to cool the refrigeration/freezing compartment efficiently.
- The motor/compressor should be controlled by a sensitive thermostat capable of holding the temperature within the required range for blood or maintaining a sufficiently low temperature for frozen plasma.
- A fan is used to efficiently circulate cold air within the refrigerator/freezer to ensure that uniform temperature is maintained in all parts of the interior.
- Shelving within the unit should be designed to fit blood trays/crates and may be of a roll-out design for ease of accessibility. The shelving should also be perforated and not made of solid sheeting and should be positioned to allow the free flow of circulating air.
- A visual temperature display and an audible alarm should be fitted to notify personnel when temperature is out of range, or when the electricity supply is cut.
- There should be an automatic temperature recording and an interface for attachment to an electronic recording device linked to a computer database.
- The interior and exterior of the refrigerator/freezer should be made of material that is easy to clean, and that will withstand strong detergents and regular cleaning. Stainless steel and aluminium are commonly used but other corrosion resistant metals are also acceptable.
- The storage area inside the refrigerator should be well lit to enable easy identification of contents, numbers and labels.
- Some models have double-glazed glass doors to enable viewing of the contents without opening the door. The product temperature is better maintained, and the compressor works more efficiently when the door is opened less frequently.

Design features of cold rooms and freezer rooms

- The room must have well-insulated walls, door, floor and ceiling. The insulation can be built into the brickwork, but it is more common to use interlinking pre-built panels to construct the room.
- The door could be either a conventional hinged type or sliding. The entrance should be wide enough to allow easy access by personnel (and wheeled blood trolleys if required). It is advisable to have either a

strip plastic curtain, or a blower unit mounted above the door which turns on when the door is opened and creates an air curtain across the doorway to prevent massive loss of cold air when the door is opened.

- For safety reasons,
 - The door locking mechanism must be designed so that an individual inside the room is able to open it.
 - Many installations have a 'emergency' alarm button located inside the room that can be activated by an individual unable to get out.
 - Some units have alarms that are automatically activated when the door is opened, and sound after a pre-set period of time if the operator has not manually reset the alarm on exiting again. If the alarm sounds, other personnel are alerted to check the cold room for trapped or injured personnel.
- Freezer rooms should ideally not open directly into a warm environment (i.e. open air or room temperature) as it is difficult for the cooling plant to compensate for the rush of warm air entering the room each time the door is opened (even with a strip plastic or air curtain fitted). To avoid temperature fluctuation and frosting up of the blower fan motor, a freezer room is often built off a 4°C cold room. Personnel entering the freezer room therefore enter the 4°C cold room first, and this forms an 'airlock' between the freezer and the external environment.
- Cold room and freezer room floors should be non-slip and have no step or ramp from the outside adjoining room. With no steps or ramps it is easy to wheel bulk components, placed on a trolley, in and out of storage.
- One of the biggest advantages of a cold/freezer room over freestanding refrigerators or freezers is that the operating plant (motor, compressor, control gear and so on) is located outside the work area. Therefore, noise, heat and dust accumulation are absent from the blood handling area, and refrigeration maintenance personnel are able to work on the operating plant without disruption to the work area.
- Cold/freezer rooms are frequently built with two cooling units that work in tandem. If one is out of operation, the other unit is able to provide back-up and maintain cold room and/ or freezer room temperatures on its own for an extended time period, while the other is undergoing repair.
- Installation of cold/freezer rooms should only be considered if the motors can be linked to a reliable emergency power supply (i.e. generator) capable of providing sufficient power during power outages. It

may be very difficult to find alternate storage for large amounts of blood and plasma in the event of an extended power failure. An assured power supply capable of maintaining the correct temperature is therefore critical, as these rooms usually hold the bulk supply of valuable blood and/ or plasma that would be extremely difficult and costly to replace.

- Continuous temperature monitoring should be carried out in at least two separate locations in the rooms to confirm that the correct temperature is evenly distributed throughout.
- The rooms should be well lit to enable easy identification of product labels and numbers, but the light source should not generate too much heat within the area.
- Shelving should be sturdy enough to support the weight of fully loaded blood trays. They should also cater for separate storage and restricted access of different categories of blood in clearly demarcated areas (e.g. units for issue, units awaiting completion of testing, and quarantine plasma).
- Personnel who work in cold/freezer room environments for extended periods must be provided with appropriate protective clothing.

Figure 1 is a diagram to show the ideal design for a freezer room, with a cold room airlock.

Transportation of blood and blood components

Introduction

If blood components are to be transported other than by the blood service personnel, care must be taken to ensure that the carrier is aware of the nature of the material being entrusted to them, and of the temperature and time constraints for handling the material. It is good policy,

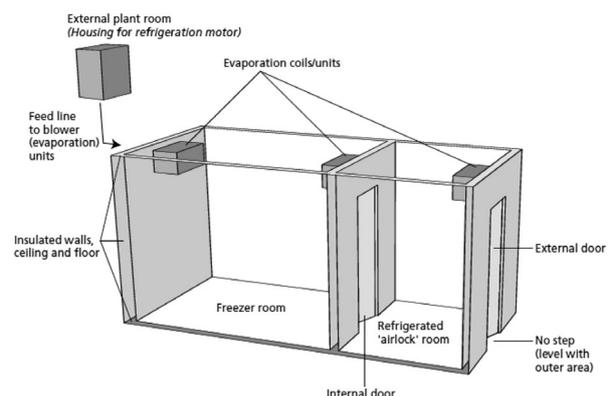


Figure 1 Ideal design of freezer room

where possible, to meet with the transport personnel to discuss and explain these restrictions and to ensure that the blood service meets all the carrier's requirements in terms of packaging and the associated paperwork. This is particularly important if the components are to be transported by air, as the carrier may otherwise refuse to convey the components at the last moment. Many countries have legal requirements for the transport of biological substances, including blood, and also on materials that may be used in the packaging of some components, such as dry ice (solid carbon dioxide, CO₂).

The transport of blood components should not be extended beyond 24 h. If a longer period is required, it may be necessary to replace the coolant packed with the components with fresh coolant that is at the correct temperature. When the components reach their destination, the temperature and condition of the components should be checked and the components placed in the requisite storage environment as soon as possible.

Following collection, blood shall be placed in a qualified container for a maximum of 24 h. The transportation container shall have sufficient refrigeration capacity to cool the blood continuously toward the required temperature range.

The purpose of a transport container is to provide a secure controlled temperature environment for blood and components in transit from one location to another. Transport containers should be used to transport blood and blood components between institutions. Transportation time should be kept to a minimum.

Containers used for the transportation of blood and blood components must be validated to ensure they are suitable for maintaining required temperatures. Validation data must be available to demonstrate that the method of transport used maintains the blood within the specified temperature range throughout the period of transportation.

The receiving facility shall maintain a system for checking that containers of blood and blood components arrive at their destination within the stipulated temperature range, and corrective actions are required if the container did not arrive at the required temperature.

Blood/blood component transport containers

A transport container consists of an insulated box that, when sealed, provides a space that is isolated from the external (ambient) environment. Blood transport containers must be specially designed with efficient insulating material to maintain the temperature inside the box between 2°C and 10°C for at least 24 h regardless of ambient temperature.

Transport containers should be stored in a clean, dry area, away from heat. This area should ideally be air-conditioned to maintain a temperature of 22°C ± 2°C so that

when blood components are loaded into it, the container is cool, giving the contents the best chance of being maintained at the correct range of temperature for the time period required.

Each blood transport box requires frozen coolants or ice packs in order to ensure maintenance of the temperature of blood components within the acceptable range. No matter how good the insulation, isolation from external temperature is never absolute, and gradually, over time, the temperature within the container will equilibrate to the external temperature. Placing frozen coolant inside the container extends the time taken for this process, as it absorbs heat already in the container, or that from the environment that is able to penetrate the insulation. The internal environment of the container attempts to cool to the melting point of the frozen coolant placed in it.

The quantity and type of coolants to be used will depend on the blood components to be transported and the time taken for the transportation and must comply with the qualification/ validation tests done on the container prior to putting it into use.

Figure 2 is a cross-section of a transport container with sealed lid and insulation, plus coolant, and illustrates how a temperature-controlled environment is created inside the container.

Design features of blood/blood component transport containers

- Sufficient insulation is needed to ensure the maintenance of desired temperature. The supplier should provide documented evidence that the container achieves the required storage temperature and time parameters.
- The supplier of the containers should be able to sustain ongoing supply of the transport boxes, as once

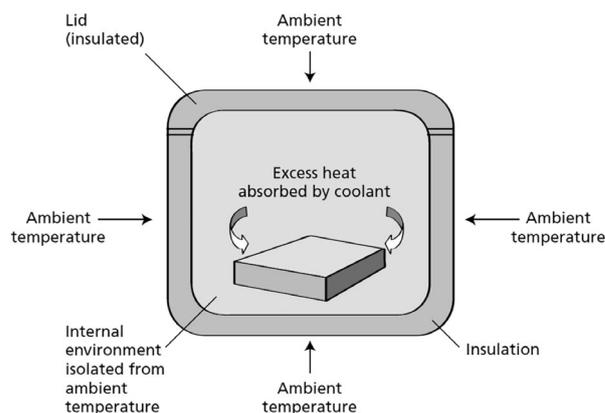


Figure 2 Temperature-controlled blood and component transport container

validated, the transport box becomes a critical piece of equipment for a blood service.

- The container should be well constructed and indicate its quality by its good appearance.
 - reusable containers are usually made of formed plastic or fibreglass filled with high-density polyurethane foam.
 - disposable containers are most frequently made of moisture-resistant cardboard boxes with polystyrene or polyurethane foam insulation.
- The container should be robust and able to withstand repeated handling during transportation.
- In the event of component breakage and leakage during transportation, the container should be able to contain the spill.
- The container's design should be practical, so that loading and unloading of product and coolant packs is facilitated. The container should not be too large so that when fully loaded it is difficult to lift. It should have sturdy carry handles for easier lifting.
- Reusable containers should be of a design that makes them easy to clean (no ridges) and store (optimum width, length and height for stacking).
- A tamper-proof sealing system should be included to prevent the contents from being accessed during transit. On receipt, when the seal is broken, the receiver is then assured that the container was not opened since despatch.
- The container should have a unique ID number, preferably eye readable and bar-coded, which can be recorded for tracking purposes.
- The quality image of the box may be enhanced by embossing the name and logo of the organisation on the container, together with instructions on handling.
- Provision for attachment of waybills, delivery notes and destination labels should be included.

Selection of suitable coolants

The melting point of frozen water is 0°C. At this temperature, ice stops getting warmer and begins to melt, losing its rigid shape and transforming into water (known as phase change). The energy (heat) used to transform a certain mass of frozen ice into a liquid, without changing its temperature, is called the latent heat of melting. Water requires a lot of heat energy (latent heat) when changing from solid phase (ice) to liquid phase (water) and will absorb as much heat energy in converting from a solid to a liquid, as it will take to warm the resulting water to about 80°C. Figure 3 is a graph that illustrates the principle of latent heat of melting.

This property of absorbing heat at a particular melting point is used in selecting liquids for use as coolants in

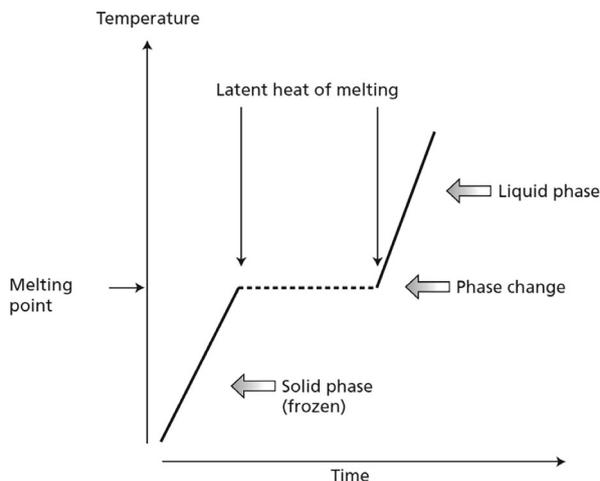


Figure 3 Graphic illustration of the principle of latent heat of melting

blood/ component transport containers. The selected coolant reaches its melting point and then stays at that temperature for long periods as it is converting from solid to liquid phase.

Plain water is in itself a good coolant to use, as it has the potential to reduce temperatures only to its melting point of 0°C. The melting point can be adjusted to a degree or two above 0°C by the addition of certain chemicals to the water, and this results in a more suitable coolant for red cell transportation, as it does not have the potential to cool the blood below minimum transportation temperature (1°C). In practice, the product temperatures in insulated containers are generally a few degrees warmer than the melting point of the coolant. For example, if packed in the right ratio of product to coolant, a plain water coolant would provide a product environment of approximately 4°C.

Some chemicals have melting points at temperatures well above 0°C and will convert to solid phase (freeze) at refrigerator temperatures. Butane-1,4-diol with a melting point of around 18°C is an example of a coolant solution that is useful in providing a 22°C ± 2°C environment in hot or cold climates. It is therefore useful for rapidly cooling fresh whole blood from body temperature to 22°C ± 2°C and to assist to maintain the temperature of fresh whole blood at this temperature during transportation from collection areas to processing centres. It also can be used for transportation of platelet units between the laboratory and patient recipient. Other chemicals can be used to provide a coolant with a melting point colder than -20°C, and these are useful for the transportation of frozen plasma.

It is, however, important to point out that the efficiency of the coolant depends on the insulating capacity of the blood transport container. Transport containers

should have sufficient refrigeration capacity to cool the blood continuously toward the required temperature range. Blood banks need to carry out onsite evaluations of the shipping conditions of the containers and coolants to ensure the cold life is maintained. Coolants are commercially available, reusable and therefore cost-effective.

Transportation of fresh donated blood from collection area to processing centre

- Containers and coolant packs used for this purpose are required to reduce the temperature of freshly donated blood from body temperature (37°C) to 22°C ± 2°C within 4 h of collection.
- The coolant used should have a melting point of approximately 18°C. They must be 'frozen' in an environment between 1°C and 10°C immediately prior to use.
- After this initial cooling, requiring considerable heat to be transferred to the coolant pack, the container is expected to maintain the temperature within that range for up to 24 h.
- Sufficient coolant units for initial cooling and subsequent storage should be provided. These quantities are best decided by validating potential systems under local conditions.
- Transport containers used should not hold more than 10–20 units of whole blood. Keeping the quantity low ensures that loaded containers are of manageable weight for blood collection personnel to handle.
- The containers are often used at venues where they are open to public scrutiny. Particular attention should be given to hygiene and image conveyed.
- Sufficient stocks of coolant units and coolants, for initial cooling and subsequent storage, should be provided.

Transportation of stored red cells or whole blood units from one storage venue to another

- Containers and coolant packs used for this purpose are required to maintain the temperature of previously cooled red cells or whole blood units between 1°C and 10°C during transportation.
- The coolant packs used should have a melting point of approximately 0°C–2°C. They must be frozen in a freezer set between –10°C and –20°C immediately before use. Care should be taken to ensure that these coolant packs are not too cold; ideally, they should be frozen solid, but close to melting point at time of use. This can be achieved by placing them in an environment between 1°C and 10°C immediately prior to use.

- To prevent accidental freezing of red cells, especially those located near the outer plastic of the blood bags, an appropriate insulating material such as foam sheeting or corrugated plastic must be placed between the coolant packs and the blood bags. The coolant can also be in a sealed pouch housed inside another bag in order to provide better protection.
- The ratio of blood to coolant needs to be calculated to maintain the correct temperature environment, for that number of units, for at least 24 h and preferably longer. These ratios are best decided by validating potential systems under local conditions.
- Transport containers can be used to transport a minimum of one RCC (or one whole blood), and a maximum of approximately 30 RCCs (or 20 whole blood) per container.
- It is advisable to use a 'space filler' such as shredded paper in a plastic bag, sponge sheets or bubble plastic to fill air space if the container is not full. This will prevent product movement during transit and will also result in improved temperature maintenance.
- Variable quantities of blood components will require the use of different size insulated containers and varying quantities of coolant.

Transportation of stored platelet concentrates from one storage venue to another

- Containers and coolant packs used for this purpose are required to maintain the temperature of platelet concentrates stored at 22°C ± 2°C (controlled room temperature) within the same range during transportation.
- This applies to the transportation of random donor platelet units, buffy coat platelets, pooled platelet units and apheresis platelets.
- Insulated containers may be used without coolant packs if having sufficient insulation to isolate the contents from cold or high ambient temperatures, while still maintaining the required temperature of 22°C ± 2°C. Depending on the time taken for the transport and the ambient temperature, coolant packs might be needed. Transport containers should then be packed in a ratio of coolant packs to platelet concentrates validated to maintain the correct temperature for the number of units, for at least 24 h. These ratios are best decided by validating potential systems under local conditions.
- It should be noted that the transportation requirement for platelet concentrates differs from the

transportation requirement for fresh whole blood from collection areas even though the temperature requirement ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$) is the same for both. Freshly collected whole blood is warm and requires more coolant to reduce its temperature and hold it at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ if intended for room temperature components. Processed platelet concentrates are of a much smaller volume and are already at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ prior to transportation, so require less coolant.

- Air circulation is a requirement for platelet concentrates stored under ideal conditions on an agitator in a temperature-controlled room or cabinet at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$. When placed in a transport container, air circulation around the platelet concentrates is greatly reduced. This is unavoidable using currently/routinely available transport containers.
- Variable numbers of platelet concentrates will require the use of different size insulated containers and varying quantities of coolant. It is advisable to use a 'space filler' such as shredded paper in a plastic bag, sponge sheets or bubble plastic to fill air space if the container is not full. This will prevent product movement during transit and will also result in improved maintenance of desired temperature.
- Platelet concentrates should not be in transit without agitation for longer than 24 h.

Transportation of frozen plasma and cryoprecipitate from one storage venue to another

- Containers and coolant packs used for this purpose are required to maintain frozen plasma and cryoprecipitate at temperatures similar to the required storage temperature or lower. For example:
 - Plasma stored in a freezer that runs at a temperature between -18°C and -25°C should be transported as close to this temperature range as possible, but never warmer than -10°C .
 - Plasma stored in a freezer that is colder than -25°C should be transported at temperatures as cold as practicably possible, but never warmer than -20°C .
- Transportation periods should be as short as possible, preferably less than 24 h. However, if a system using additional coolant packs and insulation is validated to maintain temperatures colder than -25°C for longer than 24 h, then this is acceptable. Coolant packs with low melting points (colder than -20°C) can be used to maintain plasma temperature for periods longer than 24 h but are not as effective as dry ice.
- Special care should be taken to ensure that the bags are secure and protected to prevent damage during

transportation, from expected jarring or as a result of rough handling:

- Therapeutic plasma is often packed in protective cardboard or polystyrene packaging.
- Plasma units for fractionation are usually packed tightly together to minimise movement while in transit. Layers of sponge sheeting, bubble plastic or other impact-reducing materials may be used between layers or as individual wrapping.
- Dry ice (frozen carbon dioxide) can be used to maintain temperatures of shipped frozen plasma. It has the potential to reduce temperatures to lower than -60°C if excess amount is used. When packing plasma bags with dry ice, just sufficient to maintain the minimum temperature for the maximum transportation period should be used. As a guideline, if 10 kg of frozen material (such as plasma) is packed in a well-insulated container, 15 kg of dry ice placed in the top of the container will keep the material frozen for at least 48 h.
- Although it maintains low temperature levels, the use of dry ice has several disadvantages:
 - It is expensive.
 - It is not always readily available.
 - PVC blood bags become brittle at low temperatures.
 - CO_2 emissions as dry ice vaporises can pose an asphyxiation risk in confined areas such as cold rooms or aircraft holds.
 - Operators must take special precautions to prevent freezer burn injury to skin and eyes.

Transportation of thawed plasma and cryoprecipitate from one storage venue to another

- During transportation, the containers and coolant packs shall maintain the temperature of thawed plasma and cryoprecipitate at temperatures between 1°C and 10°C .

Issuing blood components to clinical areas

- Whole blood or red cells should be issued in a cold box or insulated carrier which will keep the temperature between 1°C and 10°C .
- Platelet concentrates should be issued from the blood bank in a carrier that keeps the temperature at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- Thawed plasma and cryoprecipitate should be issued and transported to the ward in a cold box or insulated carrier which will keep the temperature between 1°C and 10°C .

Container and coolant validation

Any blood/component transport container shall be validated to ensure that it performs at the level expected. Validation indicates that the blood bank establishes recorded evidence that a specific process will consistently produce the desired outcome that meets its pre-specified requirements. In other words, an organisation should have proof that any transport container being used will perform according to a list of pre-set temperature and time requirements, for a certain number of transported units, with a certain coolant volume, at extremes of ambient temperature.

For example, the requirements for a container for red cell transportation may include the following:

- Maintain temperatures between 1°C and 10°C in all parts of the storage compartment when tested at cold ambient temperature (winter) and at hot ambient temperature (summer).
- Maintain these temperatures for a minimum time period of 24 h.
- Hold 10–30 units of RCC.

The principles for validation of transport containers is described below using the requirements for a container for red cell transportation as set out in the example above. This is not intended to be used as a guideline, but to illustrate parameters that are checked during a container validation process. Requirements are as follows;

- At least three tests per shipping container are required for both minimum and maximum product load. At minimum, each series of tests should be conducted during the warmest and coolest time of the year.
- If the shipping container will be used in multiple routes, determine and choose the worst-case shipping route and transport method for your validation. This will test your container at a maximum stress conditions in terms of temperature and time.
- A certified temperature monitoring device with the ability to monitor at least three points: two to monitor temperature in different locations within the container, and one to record ambient temperature. Multiple loggers may be needed to achieve this. The exterior logger should be positioned so that the logger's sensor has unobstructed access to the ambient air while taking into account the need to protect the device from damage during shipment.
- A device that is capable of reading data at each point, at least every half hour is adequate, although more frequent recording (e.g. every 5–10 min) is better. The resolution of the logger should be 0.1°C at minimum, over a temperature range approximately between –20°C and 50°C. The device should be able to download the data to a computer for easy analysis.

- A freezer to freeze the ice packs, set to operate at –10°C to –20°C with a recording device to confirm this operating temperature.
- Thirty expired or discarded red cell units (clearly marked as such) that are stored in a blood bank refrigerator (at 4°C ± 2°C). A recording device must provide proof of this temperature requirement.
- The insulated container under test is then loaded in different ways to monitor test parameters required for the validation:
 - 30 units, hot ambient;
 - 30 units, cold ambient;
 - 10 units, hot ambient;
 - 10 units, cold ambient.
- Pack each shipping container in accordance with the manufacturer's instructions and perform the validation along the predetermined route in the different test conditions. In addition to thermal monitoring, a visual inspection of the physical condition of the container at destination is required. The container should show no sign of damage or deterioration.
- A worksheet should be completed for each individual container system with documentation of the pre-conditioned refrigerant and product loads, the time at which the container system was fully packed and sealed, the serial number of the thermometers used, the package weight and the shipment tracking number. Clear instructions to the individual(s) responsible for receiving the container with instructions on downloading and recording temperature data obtained from the thermometers are required. When the validation studies are completed, the results are then compared with the pre-set requirements. If they comply, then two additional identical sets should be performed to confirm the original findings. Once confirmed, documentation should be prepared to collate all the findings, and this should be signed off by a senior technologist and the individual responsible for quality.

Once a validation has been successfully completed and approved, a standard operating procedure (SOP) outlining the exact packing sequence should be prepared. Thereafter, personnel are trained to pack exactly according to the SOP and are tested for the competencies. Once these steps have been completed, there is confidence that transportation will be correctly carried out.

If the validation fails, then changes need to be considered. The ratio of coolant volume to number of units may need to be altered, or the coolant used may need to be changed. With every change, the full range of validation data should be collected and compared against the pre-set requirements, until a compliant combination is found, or until the particular container is deemed unsuitable.

It is recommended that periodic monitoring is performed to determine the need of additional performance qualification of the containers. Any changes in the distribution lanes used for transport of blood product or shipping duration may impact the temperature performance of the shipment conditions. Therefore, re-qualification should be considered.

Temperature monitoring

The purpose of temperature monitoring is to provide documented evidence that a specific piece of cold chain equipment operated continually within a specified range. These data are not only essential for compliance with legislation but are basic quality requirements.

Responsibility

Temperature monitoring of cold chain equipment is an ongoing task that requires the clear designation of responsibility to individuals. Some technologists may be designated with the task of manually checking and recording temperatures at documented time intervals. These individuals should be trained to detect error trends, and how to react should temperatures approach or exceed allowable limits.

The recording process should be reviewed daily by the individual in charge of cold chain maintenance in the work area. Each reviewed record should be signed off and stored systematically for ready accessibility. These records provide an ongoing history of equipment performance.

Temperature measuring points

A refrigerator is designed to keep the blood and components within a specified temperature range. When recording temperature, it is important to reflect the temperature of the product (liquid) and not the environment (air) in the cabinet. The thermostat triggers the refrigeration motor to switch on or off, depending on air temperature variations within the cabinet. Changes in air temperature occur more rapidly than changes in product temperature. It is the more stable liquid (blood component) temperature readings that are required to accurately reflect product temperature.

To monitor the product temperature, the probe or thermometer bulb should be immersed in a volume of liquid not greater than the volume of the smallest component stored (e.g. for RCCs, this would be a volume of 200–300 ml). The liquid used may be water, but glycerol is frequently added to increase viscosity and to prevent freezing (at temperatures below 0°C). Glass or plastic bottles filled with liquid are usually placed on a high shelf

in the unit as this is likely to be the warmest part in the refrigerator or freezer (warm air rises). Sometimes multiple probes are located at different points in the refrigerator or freezer to monitor the effectiveness of air circulation (such as the highest and lowest shelf of a refrigerator). Temperature distribution should be uniform within the unit.

Temperature monitoring devices

Various measuring devices may be used to monitor and record temperatures of the refrigerators, freezers and platelet incubators as follows:

- Conventional thermometers.
- Chart recorders.
- Electronic data loggers.
- Computer-linked software.

Manual recording requires a trained operator to physically check each piece of equipment at regular intervals (every 4 h) every day (seven days per week). Manual recordings are time consuming and require that the door of the equipment is opened to read the thermometer, resulting in the loss of cool air. This is problematic when checking temperatures on a piece of equipment that is suspected of being faulty. Alternatively, refrigerators, freezers, and platelet incubators shall have their temperatures continuously monitored using chart recorders or electronic data loggers.

Temperature monitoring devices must be checked to ensure that temperature is recorded accurately, and that alarms are activated when expected. This type of validation is time consuming and requires special equipment and skills. Some organisations prefer to have an external agency assist with this.

All temperature measuring devices should be recalibrated, at least annually, by checking performance against a temperature measuring device certified accurate by an approved external agency. A variation of <2°C should be noted in readings when probes are placed together in the same liquid, at various temperatures within the reading range of the thermometers, for the devices to be considered reliable. Contingency plans should be in place for manual recording of temperatures in the event of a system failure.

Thermometers

Mercury or alcohol thermometers

- Relatively inexpensive.
- Usually made of glass and are prone to breakage.
- They offer no electronic record, and all readings must be recorded manually.
- Can be difficult to read correctly particularly if they are badly placed and/or the lighting is poor.

- They are often stored permanently in liquid within the refrigerator or freezer, and the temperature can be read immediately.
- Can be transferred from one piece of equipment to the next. In this setting, the operator must allow the temperature to stabilise before noting the reading on the second piece of equipment.

Maximum/minimum thermometers

- They have two temperature bulbs and reset buttons, one on the minimum temperature side, and the other on the maximum temperature side. By pressing the reset button, a marker settles on the current temperature. As temperature varies, the marker moves and remains at the new temperature.
- They offer no electronic record, and all readings must be recorded manually.
- The maximum and minimum temperatures can be recorded at specified time points. The thermometer should be placed between a sandwich of two packs that have been rubber-banded together during packing of the box at the mobile session.
- As the markers can move if accidentally bumped, they should be used in a vibration free environment. This precludes their use for transportation of units of blood over rough ground.
- They can be used to measure air temperature, such as in controlled room temperature environments, as the probes cannot be placed in liquid.

Digital thermometers

- May be portable or fitted permanently (built into cold chain equipment).
- Fitted units have probes permanently located in a container of liquid inside the refrigerator/freezer. The temperature is constantly visible to personnel responsible for recording the reading, so no delay to allow for temperature stabilisation is needed.
- Display the temperature digitally on an LED (light emitting diode) display.
- Portable units consist of a battery-powered unit with the LED display and probes that can be transferred from one piece of equipment to the next to monitor the temperature of each. There is a waiting period as the probe stabilises to the temperature being tested.
- Even if a continuous recording and alarm system is in place, a manual check and record of temperature should be performed every 24 h.

Chart recorders

- Simple and effective and provides continuous measurement of temperature.
- Temperature is plotted on a rotating graph paper disc (thermograph chart) by a 'pen' that moves up or

down, according to the refrigerator/ freezer temperature. The chart completes one rotation in a week and records temperatures continuously for that period.

- Each week the used chart is replaced with a new one, suitably labelled with equipment identification number and relevant dates.
- The historic charts should be checked, signed and stored to provide a permanent record of equipment performance.
- Some chart recorders have an audible high/low alarm feature to alert personnel when out of range conditions occur.
- The device requires weekly checking of batteries that operate the alarm and clock mechanism. Some older clock mechanisms may require winding.
- The major drawback of a chart recorder is the requisite consumables such as ink, chart paper and pens, so that recording is not interrupted.

Electronic data loggers

- Offers effective electronic capture of data.
- At specific intervals, the device is retrieved and plugged into a computer, using appropriate supported software to download the temperature recordings made of the refrigerator or freezer for that time period.
- Often used to provide a back-up control of the temperature monitoring devices used on the equipment.
- Can be useful in temperature monitoring during blood transportation, to provide a permanent record of temperature for cold chain devices.
- As temperatures can only be noted once downloaded, a manual check and record of temperature should be performed at least once per day.

Computer-linked software

- Systems are available to link probes located in several items of cold chain equipment to a central computer. All temperatures are read at intervals decided by the user (e.g. every minute, hourly). Maximum and minimum temperatures for each piece of equipment are fed into the system so that it will notify users in good time when alarm conditions are reached.
- Some systems can be set to send messages to the telephones of responsible individuals if storage conditions go out of range when the facility is unattended (e.g. outside of normal working hours).
- Records are stored electronically. Documentation should prove that the individual responsible for the cold chain equipment has reviewed the data at least weekly.

- Even with an electronic system as described, a manual check and record of temperature should be carried out at least once per day.

Alarms

All storage devices for blood and blood components shall have alarms that are set to activate under conditions that will allow timely action to be taken before blood or blood components reach unacceptable temperatures out of the range (outside the maximum or minimal range), such as if a door of the refrigerator or freezer is left open or if there is a power failure. These alarms can be audible, or visual, or both (preferable) and shall be regularly tested. Some alarms can be linked to computer or telephone messaging services to alert responsible personnel wherever they may be.

When choosing alarm settings, the user should select temperatures that trigger the alarm a few degrees before the temperature gets too cold or too hot. This will allow time to take corrective action before the product temperature moves out of the required storage temperature range. For refrigerators, the set temperatures are 1.5°C and 5.5°C. For freezers, the alarm is triggered when the temperature rises above -20°C. The alarm system in liquid nitrogen freezers shall be activated before the contained liquid nitrogen reaches a level that could result in the thawing of the stored blood components.

Activation of the alarm shall initiate a process for immediate investigation and appropriate corrective actions. Alarm signals (visual and/or audible) shall be placed in an area that has adequate personnel coverage, 24 h a day. When activated, the responsible individual shall record the time and action taken to resolve the problem. There should be a log of alarm activation events that describes the corrective actions taken. Alarms should have an option to silence them once the problem has been noted. The silenced alarm must automatically sound again after a short period if the alarm condition was not resolved.

To ensure that an alarm can be relied on during a power outage, which is probably the most common cause of temperature moving out of acceptable range, they shall operate off battery or some other form of an uninterruptible power supply (UPS) such as an independent electrical circuit served by an emergency generator. It is important that temperature alarms are checked regularly for alarm functionality and for any defects (e.g. at least weekly). These checks shall be recorded and documented. The alarm should be serviced at least every six months or according to the manufacturer's instructions by a suitably trained maintenance officer (preferably an accredited service agent). During servicing, the accuracy of the alarm

points should be validated. Documented proof of the service and validation should be kept.

Installation and maintenance

Storage of blood and components can be successfully done only if:

- The equipment is properly installed.
- The equipment is regularly monitored to check performance.
- Regular preventive maintenance is performed.
- Repairs are carried out without delay when required.

The blood bank should develop SOPs for the installation usage, and maintenance of blood bank refrigerators, freezers, transport boxes, platelet incubators and electricity generators. A maintenance schedule should be developed and followed for all equipment. Service and maintenance should be performed at least annually. See also *Section 17: Equipment and materials management*.

Installation

When a new cold chain device is installed it is critical to the proper operation of the equipment, that it is correctly sited. Installation of the equipment is determined by the location of the facilities where the equipment will be used. The installation qualification of the equipment should provide objective evidence that the equipment has been installed according to the manufacturer's instructions and meets all specifications. It must document all checks and tests performed to ensure that new equipment meets all manufacturers' specifications, and that it performs according to user's requirements.

The installation should be performed by a trained technician, preferably a representative of the supplying company. Critical factors in selecting the right area for installation are to:

- Avoid heat and direct sunlight.
- Ensure adequate ventilation and good air circulation around the equipment.
- Ensure adequate distance between items of the equipment.
- Ensure it is a suitable distance from the water supply such as taps to avoid the possibility of electric shock.

Once installation in the right area is complete, the technician should supply a signed protocol confirming all checks were passed during the installation. This document should be stored as part of the installation qualification record. Tests then need to be performed to validate the actual performance of the new piece of equipment to ensure that it will operate according to the user's requirements. Documentation of this initial validation is an essential part of the operational qualification procedure.

Each item of cold chain equipment should have a file that contains (at very least) information such as:

- Equipment number.
- Make and model of equipment installed.
- Serial number of equipment installed.
- Specifications.
- Manufacturer's handbook.
- Date of purchase.
- Date of installation and period of guarantee.
- Installation qualification documentation.
- SOP for use and maintenance of the machine.
- Name, address and contact number of technician responsible for servicing and repair.
- Records of services.
- Records of repairs.
- General notes pertaining to performance of the device.
- Names of blood bank staff trained to use the equipment, and those able to carry out routine preventive maintenance.

This information assists in evaluating the performance of the piece of equipment at any time. It also provides a comprehensive record for quality auditing purposes.

Preventive maintenance

The purpose of preventive maintenance is to keep the cold chain equipment in good working order and to reduce the frequency of downtime and breakdowns, ensure its safety, reduce its operating and repair costs and extend its life. SOPs must be developed based on the manufacturer's recommendations. These should include frequency of checks, methods to be used for checking, acceptance criteria and actions to be taken for unsatisfactory results. Examples include:

- Cleaning and blowing out dust from the motor/compressor.
- Cleaning the interior of the piece of equipment where blood and components are stored.
- Replacing worn or corroded parts.
- Replacing filters.
- Checking coolant levels.
- Performing validation and calibration of temperature monitoring devices.
- Performing tasks specific to the manufacturer's maintenance handbook.

The individual responsible should prepare a routine maintenance schedule for each cold chain device operating in the area under control. This should set out a schedule that ensures that each device is serviced, and preventive maintenance is performed as specified by the manufacturer (at least once per year). The schedule should be realistic, and make provision to suit the service agents,

as well as ensuring minimum disruption to blood bank operations. If possible, servicing should be performed in the same month each year to maximise the time between services (i.e. indirectly control costs). Records of all preventative maintenance, including the dates and the identity of the individuals who carried out these operations, should be stored in the record file of each piece of cold chain equipment.

Repairs

Even with the best preventative maintenance it is inevitable that at some stage an unplanned breakdown of cold chain equipment will occur. In this event the organisation should have an arrangement with a service provider to do the necessary repairs as a priority to ensure that the equipment is repaired in a timely and proper fashion.

If the device can be fixed before the temperature rises out of range, then there is no need to move the product. Senior personnel are responsible for making decisions to relocate blood products when equipment cannot be repaired in time, and such decisions should be made before products reach the limits of their storage temperature range and are adversely affected during relocation.

Records of repairs performed, including the dates and the identity of the individuals who carried out these operations and decisions made regarding relocation, need to be stored in the equipment file. Repaired equipment must meet qualification requirements and must be authorised before use.

Contingency measures

A strategy to preserve blood products at the time of equipment or power failure must be developed and documented to prevent loss of components in such instances. The facility should have SOPs to maintain blood and blood components at the required temperature, in the event of power failure of the equipment.

Prevention of product loss from equipment failure

- Effective preventative maintenance minimises equipment failure.
- Replacing old equipment before it become unserviceable helps minimise failures.
- Early detection of temperature moving out of range provides more time for the problem to be corrected before the temperature goes out of range.
- Rapid response by a service agent to repair a device can solve problems before temperature moves out of range.
- Back-up capacity (such as spare equipment, spare transport boxes and coolants, space in another storage refrigerator) means that components can be

transferred from faulty equipment before temperatures move out of range.

Power failure

Power outages cause major temperature containment problems, especially if they are prolonged over many hours.

- The first strategy is to keep all cold chain equipment doors closed. An open door allows cold air to escape and warm air to enter the refrigerator/freezer. With no means of cooling after the door is closed again, the hold-over time of the device is greatly reduced. Procedures should state clearly that doors must remain closed during power outages.
- Alarms attached to refrigerators/freezers should have an alternative power source so that they are able to function during power failure. This alternative power source is usually provided by batteries, or by connection to a UPS.
- Blood services may make the decision to install emergency stand-by power generators to operate when electrical power fails. Installation of generators is a costly option but ensures the continued cold chain maintenance of stored blood and plasma.
- Generators vary in size depending on the energy requirements of the organisation. Some will start up automatically when the mains power is cut, whereas others require manual start up and switch over.
- Generators should be included in the maintenance programme. It is important that they are always fully fuelled and ready to operate. Care should be taken to ensure that non-essential items that unnecessarily drain power from the generator are not attached to the emergency supply.
- If power cuts are a chronic problem, it is important to review alternative options such as the use of solar- or gas-powered equipment.

Links in the blood cold chain

The blood cold chain is a series of interconnected activities that are critical for the safe storage and transportation of blood from the time of donation to the time of transfusion. It should be viewed as a chain of temperature controls for each step (or link) to maintain the blood donation and the components prepared from it, at the prescribed temperature. One may also consider that related to the blood cold chain, is the temperature control of reagent storage, and temperature control of laboratory testing, all of which contribute equally to the issue of safe blood to recipients.

The blood cold chain begins prior to the time of collection, as it is important to store stocks of blood bags at the correct temperature (usually below 25°C) before taking the donation. At the time of donation, whole blood

obtained is immediately placed in a temperature-controlled environment, and from that point on, should not move outside of the set temperature parameters until it is transfused to the patient.

There are many links in the blood cold chain, as shown in Figure 4, and each link should be carefully controlled to ensure that the final product transfused is of the highest quality. Breaks in the cold chain happen for many reasons such as equipment failure. Breaks in the cold chain could lead to the transfusion of substandard or potentially lethal blood products and components.

Records of all the equipment used in maintaining and monitoring cold chain conditions during processing and transportation should be maintained. These records should form an 'audit trail' for any donation passing through all stages and allow for tracking the temperature and storage conditions for individual components, to assess whether they were continually stored, processed and transported under required controlled conditions.

SOPs on installation, operation and maintenance of blood cold chain equipment should be available to all personnel in the specific work area. All blood processing personnel should be trained in the preparation and handling of blood components while working within cold chain parameters and should be able to detect cold chain problems and refer them immediately for attention.

Collection

Blood bag supplies should be kept in a cool place and must never be left in a hot vehicle during mobile blood

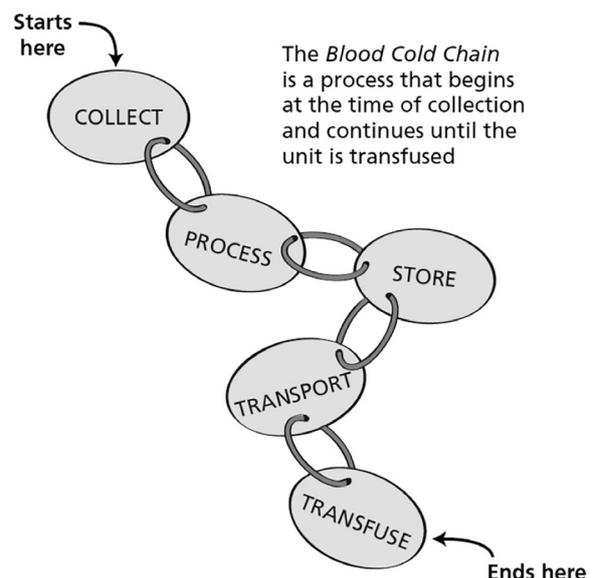


Figure 4 Links in the blood cold chain

drives. The first link in the cold chain is the handling of freshly donated blood at the collection centre or mobile blood drive. Freshly donated units should be placed as soon as possible, but not longer than 30 min after collection, into a temperature-controlled environment to prevent damage as a result of ambient temperature at the venue or in a pick-up vehicle. The temperature-controlled environment for the first 24 h after collection can be either refrigeration temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$) or controlled room temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$).

At fixed sites, refrigeration temperature can best be provided by a suitable blood storage refrigerator. Alternatively, particularly at mobile collection venues, blood may be placed directly into an insulated transport container, with sufficient and appropriate coolant, validated to cool the blood to below 10°C in less than 8 h, and thereafter maintain the temperature below 10°C for a maximum transit time of 24 h.

Controlled room temperature is best provided by placing the collected blood into an insulated transport container, with sufficient and appropriate coolant, validated to cool the blood to $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ within 4 hours of collection, and thereafter maintain the temperature range for a maximum transit time of 24 h.

Maintaining the controlled temperature from collection to processing centre ensures that red cells, clotting factors and platelets are not damaged by excessively high (or low) temperatures during transportation. Blood collection personnel should ensure that delivery to the processing centre is made soon enough to allow processing of the blood and storage of red cells at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ within specified time frames.

Each transport container dispatched from a collection centre to a processing centre should be accompanied with documentation relating to:

- Name/code number of collection area.
- ID number relating to transport container.
- Donation number for each bag loaded into the transport box.
- Time first unit was donated and loaded.
- Time last unit was donated and loaded.
- Contact details of individual responsible for despatching the consignment.

This information is used in the processing laboratory to assess transportation (transit) time and temperature of units received.

Blood collection personnel are also responsible for ensuring that specimens taken at the time of donation, for blood grouping and testing for transfusion transmissible infections, are stored and transported under controlled temperature conditions that comply with manufacturer's instructions related to laboratory testing.

Processing

The processing stage is the next important link in the cold chain as the whole blood is subjected to several processes in its conversion into components. These processes include checking, sorting, weighing, centrifuging, separating, sealing, data capture and storage. If personnel carrying out these tasks are not aware of temperature and time constraints, and/or management has not instituted systems to ensure that cold chain requirements are maintained, then this may be overlooked to the detriment of components produced.

Receipt of whole blood units from the collection centre is the first link in the cold chain for blood processing personnel. The list below includes steps that should be taken to maintain the control of temperature:

- Deal with each insulated transport container individually to avoid mix-ups.
- Locate the relevant documentation for the container (see Collection in previous discussions).
- Record the time of receipt and check that the time from collection to receipt has not exceeded the maximum transit time stipulated by the organisation (should not be more than 24 h).
- Open the lid and record the inside temperature.
- Record the temperature on the documentation.
- Check if the temperature falls within the expected range.
- Perform visual assessment of the units.
- Units within the expected range (or cleared as suitable by the individual in charge) are then checked against documentation before processing begins.

It should be noted that freshly donated whole blood is warm (body temperature) when taken from the donor and placed in the insulated container. If the transportation time from collection venue to processing centre is short, the blood may not have had sufficient time to cool to the range required. In these cases, authority from the individual in charge is required to authorise use. Validation records need to be consulted to determine the 'normal' cooling period for the transport container in use and whether the recorded 'warm' temperature was within the cooling range. If this is found to be the case, then it can be concluded that the whole blood from the collection area was transported within the correct temperature environment.

Blood processing personnel must plan their work carefully so that all blood donations are able to be processed and stored within the time constraints for the components prepared. For example, if a controlled room temperature programme is used, then, before 24 h have elapsed from the time of donation, red cells must be stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, plasma must be frozen and stored colder than

–18°C and platelet concentrates must be stored at 22°C ± 2°C. Sufficient time should be available from time of receipt of donations for processing to be carried out within these time limits. For example, if processing only starts at 23 h, component processing cannot be completed in time. Processing steps should be documented so that records may be consulted to assess whether components complied with time and temperature requirements. Blood unit centrifuges must be refrigerated to provide the correct range of temperature control during centrifugation. Without this control, centrifuges could generate high temperatures that would be detrimental to components.

Red cells must be stored at 4°C ± 2°C as soon as possible after processing and allowed to cool to refrigeration temperature, before further handling. Time on the work bench, out of the 4°C ± 2°C range must be limited and not exceed a cumulative total of 1 h, for additional processing, labelling, packing and testing purposes.

The area where receipt of donations and processing done should, under ideal circumstances, be a controlled room temperature of 22°C ± 2°C. If this is not possible then handling time must be reduced to a minimum, with less units at a time being handled on the bench before replacing them back in the controlled temperature environment before removing the next small batch for handling.

Storage

Once blood has been received from the collection site and processed under the right temperature conditions, it then becomes important that the storage conditions for each component produced be maintained exactly as specified for the entire shelf life. Surplus storage space should be available so that units can be stored in specific demarcated areas and are easy to inspect, select and count. There should be segregated areas for storing blood or plasma of different categories. For example:

- Units for issue, by blood group and in expiry date order.
- Units awaiting completion of testing and labelling.
- Embargoed units for discard.
- Other categories as required.

The storage area shelving should be clearly marked with the category of product stored. Signage on the door should indicate the purpose of the storage facility (e.g. red cells for issue).

Transportation

This important link in the cold chain is to ensure that blood and components are maintained at specified

temperatures during transportation between centres. Each component must be loaded into an insulated container/coolant pack combination designed specifically for the transportation of that component (See *Blood/Blood component transport containers*). When a request is received to send a blood or component order to another venue, personnel should ensure that the products selected for transfer are stabilised within the correct temperature range, within the storage environment (such as a refrigerator at 4°C ± 2°C). This means, for example, that they should not have just been placed in the refrigerator after processing.

A suitable insulated transport container is prepared, using appropriate coolant packs that are not excessively cold. Ideally, they should be frozen solid, but close to melting point at time of use. (See *Transportation of stored red cells or whole blood units from one storage venue to another* earlier in this section, for more detail.) Component details are recorded (or captured into a computer) and selected units are transferred to the prepared insulated container as quickly as possible to avoid unnecessary warming. Transport containers should be appropriately labelled. A delivery note listing the individual donation numbers of each component is prepared and details of the consignment recorded, including delivery note number, date and time of issue, identification number of containers, transportation waybill number (if applicable) and contact details and signature of individuals responsible for the issue. The consignee should be notified of the despatch and when to expect it, so that if not received on time, investigations can be initiated to locate it as soon as possible.

Temperature monitoring on receipt is essential and can be done using a thermometer. Some devices are available to continuously monitor temperature during transportation and provide a complete record of the consignment temperature if used (see *Temperature monitoring* earlier in this section for more detail). On receipt, all details regarding the consignment should be recorded in a register, including date and time, container identification number, delivery note number, transportation waybill number (if applicable), temperature of components on receipt, time in transit and signature of responsible individual. Once checked in, the blood/components received in the consignment must be stored in the appropriate cold chain device, within the correct temperature range, as quickly as possible. Action should be taken to remove components from use if the temperature on receipt was out of range or the maximum transit time allowed was exceeded. The individual in charge should be immediately notified of problems of this nature.

A rigorous cleaning schedule should be part of the blood transportation system so that all containers are kept

in hygienic condition and project a good quality standard. Coolants should also be kept clean and in good order. Sufficient space for orderly storage of blood transport containers in a cool place, should be allocated. Dedicated refrigerator/freezer space should be allocated for storage of coolant packs at the correct temperature.

Transfusion

The blood cold chain should extend to the point of transfusion. A blood service has an obligation to ensure that end users comply with good cold chain management practices. Blood components for individual patients should not be issued in advance and remain stockpiled needlessly and uncontrolled, in the hospital ward, waiting to be used.

Blood issued from the blood bank is sometimes stored in a ward/emergency room blood refrigerator prior to use. These refrigerators must meet specifications for blood storage and be controlled and monitored in the same way as units used in a blood bank. The refrigerator must maintain a temperature of $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and be fitted with an appropriate temperature alarm. Storage of blood in domestic type refrigerators together with other medicines and maybe even food should not occur. The blood service should offer guidelines to the hospital for ward refrigerators and should audit the ward facilities from time to time, to ensure that they comply with cold chain standards. If these blood refrigerators are not available, units should be transfused as soon as they arrive the ward.

Blood issued to the hospital is frequently transferred using small cooler boxes. Care should be taken to ensure that these comply with the requirements of standard size transport containers. In some areas blood is sealed inside the cooler box, which is also sealed closed. If the blood is not used, and the container is returned to the blood bank with the lid still sealed, and the blood pack seals have not been broken, then the blood may safely be returned to stock provided that there is proof that the temperature within the container remained at 1°C – 10°C and that the maximum time for safe storage within the transport box was not exceeded.

Blood warming prior to transfusion should only be performed when absolutely necessary (i.e. for the rapid transfusion of large volumes of blood in trauma) and by using a validated device designed for this purpose. Other practices that are not validated can overheat red cells and lead to haemolysis. If haemolysed red cells are transfused, they will lead to a serious reaction in the recipient. The blood service should provide guidelines on the use of blood warming devices. At the time of request of plasma and cryoprecipitate, thawing should be performed by trained blood bank personnel. Careful control of the method and validation of the equipment used is essential.

Inventory management and control

The purpose of inventory management is to procure sufficient blood donations to meet the needs of patients. If too many donations are collected, some units will reach their expiry date before they are able to be used. Although expiries are inevitable, an excessive number is a bad situation. Firstly, donors expect their donations to be used and secondly, time and money is wasted in collecting, processing and testing blood that will be discarded.

Insufficient blood collections to cater for the needs of all patients cause an even worse scenario. Patients may be severely compromised if blood is not available. When stocks are low, a lot of extra effort is required to manage the small amount of blood available and continue to keep blood banks supplied with minimum numbers of components. Therefore, the organisation should develop, implement and maintain an inventory management system.

Baseline usage figures

The first step in managing inventory is to establish the average baseline usage for each blood bank. Only components that meet the satisfactory requirements for issue, should be held in stock. From this data, a minimum and maximum stock level, by blood group, can be established for each user. This is usually estimated using the projected requirements for 7 days. Once agreed, the stock held at each blood bank should not fall below minimum, or exceed maximum, unless extenuating circumstances exist.

From these baselines the total blood usage, by blood group, may be estimated for the organisation. These figures may be further expanded to estimate the number of collections needed daily for each blood type, to match demand. It should be noted that group O blood is used in greater proportions than its frequency amongst donors since it can be used for recipients of other groups. Blood collection estimates should take anomalies such as this into account.

The figures estimated should be used to plan collections at fixed site centres and mobile venues. Collections from new donors can be estimated to be in the same proportions as the prevalence of blood groups in the population. The plan should include a strategy to procure extra group O donations based on use. Typically, an organisation would aim to keep enough blood for 7 days in stock at each blood bank. If the plan is successfully followed, then the number of units collected each day should match the number being used and the stock should remain constant for most days.

Daily stock count

On a daily basis, a designated stock controller should collect stock figures from each location where blood is stored.

From these figures the total stock for the organisation can be compared with the total stock requirement figure (calculated from usage figures). Action can be taken to collect more donations if stock is low, or to reduce collections if stock is high. The daily stock count also allows the stock controller to prepare top-up orders for each location according to their individual maximum and minimum requirements.

Inventory management presumes that all donations are used strictly in expiry date order. The technologists in charge of storage locations are required to monitor the rotation of blood and components to enable their use before expiry. Blood components returned to the blood bank should be recorded in the “returned to stock” or “discard” register depending on whether they meet the set criteria or not, respectively.

Another important aspect of good inventory control is to ensure that no blood or components are mislaid. Every component produced should have a unique identification number and product code, so that when it is issued, used, or discarded, some record (usually computer based) will show that the component was accounted for and its fate. Investigations should follow up on any unit that does not have a final destination on the check system so that the reason can be identified and corrected.

Key points

- Anticoagulant, preservative and additive solutions prevent blood from clotting and provide nutrients to maintain red cell viability and function throughout storage.
- Whole blood after collection can be immediately placed at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ or it may be placed in a controlled room temperature environment of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for the first 24 h after collection.
- Red cells separated from whole blood collected into an anticoagulant preservative solution (such as CPD) are suspended in additive solution (such as SAGM or AS-3) for extended storage shelf life (up to 42 days).
- RCCs should be stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ immediately after processing.
- Shelf life of platelet components in most blood services is 5 days to minimise the risk of bacterial contamination. Platelets are stored at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with continuous agitation.
- Plasma components are stored frozen at a temperature of -18°C or colder for up to 12 months from collection or -65°C or colder for up to 7 years from collection.
- Design features of blood storage refrigerators and plasma storage freezers include insulation, blower fan, visual temperature display, automatic temperature recording, audio-visual alarm, interior and exterior surfaces that are easy to clean, a well-lit storage area and glass doors.
- Walk-in cold rooms and freezer rooms have insulated walls/door/floor/ceiling, alarms, operating plant, emergency power supply, continuous temperature monitoring, a well-lit interior and should provide easy access for trolleys loaded with components.
- Insulated blood/component transport containers provide a controlled temperature environment for products being moved from one location to another.
- Blood/component transport containers are reusable or disposable, robust, contain spills (i.e. are leak proof), handle easily, are tamper proof, and clearly labelled for identification.
- Each blood transport box requires frozen coolants or ice packs in order to maintain the temperature of blood components within the acceptable range. The quantity and type of coolants to be used will depend on the blood components to be transported and the distance/time expected for transportation
- Blood bankers need to conduct onsite evaluations of the shipping conditions of containers and coolants to ensure the cold life during transportation will be maintained.
- Temperature monitoring for a specific piece of cold chain equipment and shipped components is essential to comply with legislation and is a basic requirement to provide quality products and requires clear designation of responsibility to individuals.
- Measuring points (e.g. probe, thermometer bulb) within a refrigerator or freezer are used to reflect the temperature of the stored components.
- Various measuring devices may be used to monitor and record temperatures of refrigerators, freezers and platelet incubators.
- Different types of thermometers are available, and these can be portable or built into the cold chain equipment (such as digital thermometers). Even with those offering continuous recordings, with an alarm system also in place, a manual check of the temperature is required.
- Chart recorders plot temperature on rotating graph paper, called a thermograph chart, and record temperatures continuously for up to a week at a time, creating a permanent record that can be stored as a record.
- Electronic data loggers can be placed in a location and will record the temperature at define intervals for days if required. When attached to a computer, the temperatures can be downloaded for the period.
- Computer-linked software systems are available that link probes located in several items of cold chain

equipment to a central computer. All temperature readings are carried out at intervals decided by the user, and maximum and minimum temperatures can be fed into the system so that it will alert the users in good time when alarm conditions are reached.

- Different types of alarms that are audible and/or visual are fitted to cold chain equipment that alert the user to an impending problem with the device, such as when temperature is going out of range, if there is a power failure and in some cases, if a door is left open.
- Temperature monitoring devices and alarms must be checked to ensure that the temperature is recorded accurately, and that alarms are activated when expected.
- Installation of cold chain equipment must be done according to manufacturer's instructions and must meet all specifications. Documentation of the initial validation is an essential part of the installation qualification procedure.
- Regular maintenance keeps cold chain equipment in good working order and minimises breakdowns.
- The organisation should have an arrangement with a service provider to do the necessary repairs as a priority to ensure that all cold chain equipment is repaired in a timely and proper fashion.
- A strategy to cover equipment during power failure should be developed and documented including keeping all cold chain equipment doors closed to increase hold-over time, having alarms and equipment powered by a source other than the mains power or a generator as an alternative power source.
- The blood cold chain begins at the time of donation and ensures that the whole blood obtained is immediately placed in a temperature-controlled environment, and from that point on should not move outside of the set temperature parameters until it is transfused to the patient.
- Records of all equipment used in maintaining and monitoring cold chain conditions during processing and transportation should be maintained.
- Hospital ward refrigerators should meet specifications for blood storage and be controlled and monitored in the same way as for units stored in a blood bank.
- The blood service should provide recommendations of when blood warming is necessary at the time of transfusion, and suggest safe methods and equipment for warming blood.
- The purpose of inventory management is to procure enough whole blood donations to meet the needs of the patients served by the organisation. Baseline blood usage figures can be used to estimate how much blood should be collected daily and set maximum and minimum stock figures for each storage location.
- A daily stock count allows the stock controller to prepare top-up orders for each location according to their individual maximum and minimum stock figures.
- An effective system should be in place to account for the end use/disposal of every component made.

Compatibility testing

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Introduction

The laboratory is a vital part of the transfusion chain, and successful pre-transfusion testing is dependent on staff in clinical areas providing timely requests for blood with adequate clinical details, accompanied by correctly labelled samples. The primary aim of pre-transfusion testing is to provide red cells that will have the maximum survival following the transfusion and/or blood components that most effectively improve haemostasis, without causing an adverse reaction in the patient. This requires correct identification of patients 'vein to vein' and provision of ABO compatible blood that is antigen negative for any clinically significant red cell antibodies present in the patient's serum/plasma. A secondary aim is that blood selected for transfusion should not expose the patient to future risks, e.g. sensitisation to the D antigen in females with childbearing potential or the alloimmunisation of patients who, because of their medical condition, will require ongoing transfusion support.

Although the principles of pre-transfusion testing are universal, practice will vary depending on resources available, such as availability of blood components (vs. whole blood), reagents (for ABO/D typing, crossmatching, antibody screening and antibody identification), information technology (IT) and automation, and staff with specialist transfusion training. Therefore, different strategies will be required to achieve the aims. A full understanding of the process is required to be able to design the best system possible in any given circumstances. It is advisable to identify the critical points and carry out a risk assessment, bearing in mind that misidentification of the patient or sample is a major risk and that effective procedures and policies can do as much to improve safety as accurate and sensitive testing.

It is important to achieve a balance between providing blood components of the ideal specification and ensuring timely life-saving transfusions; this is a clinical decision made on a case by case basis by the medical team with input from the laboratory.

All laboratory testing and processes should be subject to good laboratory practice and operate within a quality management system (see *Section 16: Quality*). Effective communication between all staff groups involved in the transfusion chain is essential to ensure patient safety. Practice in clinical areas such as appropriate prescribing, sample collection, administration of blood and

monitoring of transfusions in progress can be influenced and improved by the establishment of a hospital transfusion committee. See *Section 14: Transfusion risks and haemovigilance*.

Learning objectives

By the end of this section, the reader should be able to describe the critical points in provision of compatible blood for transfusion and have gained an understanding of the principles and practice of the following:

- Concept of pre-transfusion compatibility testing
 - Avoiding identification errors
- Patient identification and sample collection in clinical areas
 - Ensuring sample quality
 - Patients with 'high-risk' infections
- Sample and request acceptance
 - Policy for sample acceptance
 - Booking in new requests
 - Request for crossmatch or 'type and screen'
 - Use of stored samples
- Creation and use of patient records
 - Patient records
 - Checking historical records on patient
 - Transplantation leading to change in blood type
- Patient testing
 - ABO/D typing
 - Cold agglutinins
 - Anti-IgG vs. broad spectrum Anti-human Globulin (AHG) reagent
 - Patient antibody screening
- Antibody identification
 - Clinical significance
 - Pan-reactivity
- Methodology at IAT stage of crossmatching
- Selecting blood for crossmatch and managing stock
 - ABO identical or compatible components
 - Use of D negative blood
 - ABO/D check on blood donations
- Additional considerations for whole blood
- Special requirements for blood
 - Transfusion dependent patients
 - Patients with sickle cell disease (SCD)
 - Neonates <4 months old
 - Recipients of BMT or HSCT

- Confirming compatibility (serological crossmatch or emergency issue)
 - Approaches to confirming compatibility
 - Electronic Issue ‘computer crossmatch’
 - Serological crossmatch by direct agglutination ‘immediate spin’
 - Serological crossmatch by Indirect AHG Test
 - Direct agglutination ‘immediate spin’ abridged crossmatch
 - Serological crossmatching procedure
 - Additional techniques for serological crossmatching
- Dealing with unexpected incompatibility
 - Immediate actions
 - Testing on the donation
- Provision of blood in an emergency
 - Standard and emergency situations
 - Massive transfusion
 - Changing blood type during one transfusion episode
 - Internal quality control (IQC) for pre-transfusion laboratory tests
 - ABO/D and phenotyping reagents
 - Polyclonal reagents
 - AHG
 - Screening cells
 - Labelling and Issue
- Administration of blood in clinical areas
 - Bedside checks on the patient and blood component
 - Administration sets
 - Information provided to staff administering blood in clinical areas
- Transfusion reactions and haemovigilance
 - Retention of samples and transfused units
 - Immediate actions in the clinical area
 - Transfusion reaction investigation
 - Haemovigilance

Concept of pre-transfusion compatibility testing

Pre-transfusion compatibility testing in the laboratory must detect ABO/D incompatibility between the patient and donor that can cause serious and even fatal haemolytic transfusion reactions. This requires accurate ABO/D grouping of the patient and donor and an additional test (serological or electronic) to confirm ABO compatibility before blood is issued.

Testing should also detect clinically significant red cell antibodies that could cause a haemolytic transfusion reaction or reduce red cell survival. This is so that antigen negative blood may be selected for crossmatch. However,

the extent to which the latter is achievable will depend on whether it is possible to screen for, and identify, atypical red cell antibodies in a patient, and obtain suitable antigen negative blood donations for crossmatch. Establishing compatibility and suitability of blood for a patient, in the laboratory, is a complex procedure involving more than serological testing. Critical points must be identified so that appropriate barriers to errors are put into place and check points are part of the procedure, to maximise blood safety. Critical points include:

- Sample and request acceptance.
- Checking historical records.
- Patient testing for ABO/D, antibody screening.
- Establishing compatibility (serologically or electronically).
- Selecting blood components of the correct group and specification.
- Labelling and issue.

Avoiding identification errors

The two most common causes of error in transfusion are misidentification of the patient, or the sample. This can happen either at the bedside when the sample is drawn from the wrong patient, or in the laboratory when the wrong sample is selected for testing. Studies have shown that mistakes are more likely to occur during emergency situations when personnel are working under extra pressure and time constraints.

Systems to avoid patient misidentification are usually distinct and separate in clinical and laboratory areas. Therefore, good laboratory practice will not be able to prevent the consequences of patient misidentification at the bedside. Likewise, good bedside identification will not prevent identification errors in the laboratory. One of the important functions of a hospital transfusion committee is to address such issues. The role of the hospital transfusion committee is described in *Section 14: Transfusion risks and haemovigilance*.

Reasons for patient/sample misidentification include the following:

- Sample taken from the wrong patient (hospital error at the bedside).
- Sample labelled with incorrect patient information (hospital error at the bedside).
- Wrong sample used for testing (laboratory error).
- Wrong blood unit selected for labelling (laboratory error).
- Wrong unit of blood issued (laboratory error).
- Transfusion of blood into the wrong patient (hospital error at the bedside).

It is important that standard operating procedures (SOPs) are in place for all critical tasks related to the transfusion

process. All personnel authorised to carry out these tasks should be trained and able to demonstrate competency. SOPs should stress the importance of checking and rechecking to ensure that patient and unit misidentification does not occur at any stage in the process.

Patient identification and sample collection in clinical areas

Patient identification and sample collection

The first critical step is taking the sample from the correct patient. Whilst this is the responsibility of the clinician at the hospital, the laboratory should be aware of the good practice points below:

- Ask the patient to state his/her full name, rather than stating the name and asking the patient to confirm it.
- Check the patient's wristband identification ID, if available, to confirm identity details.
- Complete or assign the sample tube label at the bedside at the time the sample is collected; do not pre-label sample collection tubes. If pre-printed name/address labels are used, these must be carefully checked at the time the sample tubes are labelled.
- Check that the name and all other details entered onto the sample tube label and the blood request form are the same as those relating to the patient and his/her case notes.
- Check the social security/ID number or the hospital number of the patient.

A system should be in place to link the patient to the correct case notes in the event of the patient being unconscious or unable to confirm their identity for any other reason. There should be no interruptions or distractions when taking the sample from the patient, recording the details on the label and request form, and checking for errors or omissions. If there is an interruption, the process of checking should be repeated and if there is any doubt, a fresh sample should be drawn.

Ensuring sample quality

- The label must be firmly attached to the tube; if the label is separated from the tube during handling, then the blood in the tube cannot be guaranteed as being that of the patient and may not be used for testing.
- The attending clinician should ensure that when collecting the sample, it does not become diluted or contaminated with intravenous fluid being administered to the patient at that time.
- The sample should be taken into the correct tube, according to local requirements (dry tube or

anticoagulated), be correctly labelled, and be of sufficient volume. It should reach the laboratory without delay and show no sign of haemolysis.

For manual compatibility testing, dry tubes are suitable for the collection of blood samples from patients. However, if any part of the testing is automated, anticoagulated samples are required. If a sample is anticoagulated with a substance that chelates calcium, such as EDTA, then complement-dependent antibodies will not be detected during crossmatching or antibody screening. The benefits of automation, in terms of standardisation and security of sample identification, outweigh the risk of failing to detect a complement dependent antibody.

Patients with 'high-risk' infections

Whilst every sample received by the laboratory should be treated as potentially infectious and appropriate care taken during handling, samples from patients with haemorrhagic diseases such as Marburg fever or Ebola fever carry a greater risk, as the viruses responsible for these infections are very easily spread by blood and other body fluids. Samples from these patients should not be submitted to the laboratory, and arrangements should be made by the medical officer of the blood service, with the prescribing clinician, to use uncrossmatched Group O red cell concentrate for such patients.

Sample and request acceptance

Policy for sample acceptance

When several requests reach the laboratory at the same time, they should be handled and registered separately, one at a time.

Each laboratory should have a policy for sample acceptance that is known to staff in the laboratory and clearly communicated to staff in clinical areas. If all patient demographics do not correspond, or if any information deemed necessary by local policy is missing, the request should not be accepted. The requesting clinician should be contacted immediately and asked to submit a new sample. The original sample should be discarded.

Details on the sample label and request form should be checked to ascertain that they correspond exactly, including the following:

- Last name (family name).
- First name.
- Unique number (hospital number and/or social security/ID number).
- Ward (if applicable).
- Date of birth (or age if date of birth is not available).
- Gender.
- Date the sample was taken.

- Signature of requesting clinician.

The request form should include a clinical diagnosis and state the blood components required, the reason for the transfusion and the urgency of the request. If this information is not present, it should be obtained from the requesting clinician, as it may influence the laboratory's decisions regarding the pre-transfusion tests required, priority of testing and specifications for components to be prepared. Provision should be made on the request form, for the clinician to provide information on a history of transfusion, so that laboratory records may be consulted to determine ABO/D blood type, whether irregular antibodies were detected, or whether there was an adverse reaction to a previous transfusion.

All communication with clinical areas regarding patients should be clearly documented on the crossmatch request form, together with dates and times, by the blood bank technologist receiving the message. Any information regarding patients and their records is confidential and should not be divulged to anyone not in authority to receive it, which includes family members.

Booking in new requests

On acceptance, every request should be assigned a unique laboratory number to identify the sample and the respective documentation. This number should appear on the sample tube, the request form and in the crossmatch register if a paper system is used. If a laboratory IT system is used, this number should be in the form of a barcode as well as an eye readable version. Numbering samples for processing in the laboratory is prone to transposition/transcription error and checks should be in place at every stage in the process to ensure that results are attributed to the correct patient.

The patient and request details should be recorded, either in a laboratory IT system capable of recording and retrieving the information below, or in a paper crossmatch register, or in a combination of both.

Unique number allocated by the laboratory.

- Last name.
- First name.
- Gender.
- Date of birth.
- Unique number (hospital number/social security/ID number).
- Hospital ward (if applicable).
- Date and time sample taken.
- Type of request (crossmatch - emergency or standard/type and screen/screen/another request, e.g. for FFP)
- Prescribing clinician.

If the sample was taken into a dry tube, it should be fully clotted before use to avoid interference by fibrin in the tests.

Request for crossmatch or 'type and screen'

Requests may be received for issue for a specific number of red cell units to be crossmatched or for 'type and screen'.

The option of 'type and screen', is useful when it is not certain that the patient will need a transfusion, but it is only possible where there is access to reliable antibody screening.

The attending clinician submits a sample and request form, with the understanding that blood will only be crossmatched if needed, usually over the next few days (or 72 h maximum if the patient has been recently transfused). During this time, blood may be ordered by telephone and can be provided more rapidly than usual as the sample is already checked for labelling and the patient has already been ABO/D typed and screened for irregular, clinically significant red cell antibodies.

Determination of the ABO/D type of the patient before blood is needed provides time to resolve any potential grouping anomalies, check that group compatible units are available in stock and, if necessary, to arrange to obtain such units from another area. Antibody screening in advance allows time for a positive antibody screen to be investigated, atypical antibodies to be identified (or referred for investigation) and compatible blood to be sourced by screening donations for antigen negative blood. In this situation, it would be advisable to undertake the crossmatch as soon as suitable units are found, especially where reliable antibody identification cannot be guaranteed due to lack of resources. If a transfusion is subsequently required, delays due to unexpected incompatibility can then be avoided. The prescribing clinician should be notified at the outset of expected delays in the provision of compatible blood.

It is useful to develop a Maximum Surgical Blood Ordering Schedule (MSBOS) negotiated with clinicians through the hospital transfusion committee, where either a 'type and screen' is performed (with blood available within an agreed timescale), or a set number of units are made available for a list of common surgical procedures.

Where there is an effective MSBOS, most type and screen requests are not converted into crossmatches, reducing demand on the laboratory and preserving reagents and consumables. This system also prevents units of blood being reserved for cases where they will not be used, facilitating better stock control and reducing blood wastage.

Use of stored samples

During a single transfusion episode, requests for additional units to be crossmatched for a patient are sometimes received by the blood bank. The same crossmatch sample may continue to be used, provided that there is sufficient sample remaining for the testing required. However, the sample used should not be more than 72 h old, since the first unit of blood was transfused, in this or any other recent episode of transfusion; in this situation, a fresh sample should be requested if additional units are required to be crossmatched, as it is possible that antibodies may have been produced in response to donations transfused, after the original specimen was taken.

All crossmatch specimens drawn from patients should be stored for at least a week at $4 \pm 2^\circ\text{C}$ in case of an untoward reaction being reported from the clinical area, in which case the specimen must be available for transfusion reaction/investigative testing in the laboratory, to determine if possible, the cause of the reaction.

Creating and using patient records

Patient records

Patient records are a vital resource in the process of pre-transfusion testing/checking and great care and consideration should be given to how they are created and maintained so that they are accurate, complete and easily accessible when required.

If a laboratory IT system is used the patient can be electronically linked to previous records (laboratory results, donations issued, comments) to form a cumulative transfusion record that facilitates checking vs. previous laboratory results and transfusion history. Wherever possible information should be entered into IT systems in the form of 'coded comments' so that it is more easily searchable and retrievable.

If a manual system is used, then this can be indexed by patient, e.g. on cards kept in alphabetical order, with a similar cumulative record relating to each sample and episode of transfusion. This can be cross referenced to the crossmatch register to find details of units transfused. This record should include:

- ABO/D type.
- Comments on difficulties in providing compatible blood and how these were resolved.
- Presence and detail of atypical red cell antibodies.
- Special requirements, e.g. cytomegalovirus (CMV) negative blood.
- Date and reason for request for blood.

Secure systems should be in place to check that the patient's first name, last name and date of birth match before linking electronic records or updating paper records.

Patient and donor records are useful when it is necessary to trace the patient into whom a donation was transfused, so that in the event of a recall, e.g. where the donor is subsequently found to have a transfusion transmissible infection, action may be taken according to the policy of the blood service.

Checking historical patient records

This is an essential part of pre-transfusion testing and should be performed as soon as possible after receipt of the request for blood, as it gives an opportunity to detect errors in sample labelling. Where there is a discrepancy between current and historical blood groups, assumptions should not be made as to which is correct; repeat samples should be requested. The patient's transfusion history, previous special requirements and key laboratory results, i.e. ABO/D type and any atypical antibodies can alert the laboratory to potential delays in obtaining compatible/suitable blood as soon as the request is received (booked in).

- When the blood type of the patient is determined, it must always be compared with the one on record (manually or electronically).
- If there is a discrepancy in the ABO/D type, the details on the sample should be checked and the blood group repeated by going back to the sample rather than any cell suspension already prepared.
- If the ABO/D discrepancy is confirmed, the prescribing clinician should be informed immediately, as it may be that either the previous group is incorrect, or the current sample was taken from the wrong patient. Another sample must be taken from the patient to confirm the blood group.
- If it transpires that the sample of blood was taken from the incorrect patient, then the cause should immediately be investigated, as a second corresponding error is possible if the identity of two patients was muddled up on the ward; the other patient may not have a previous blood group on record.

Checking historical records can also help to prevent delayed transfusion reactions when antibodies found in the past are no longer detectable but have the potential to be boosted by another transfusion; to prevent this, antigen negative blood must be crossmatched in such a situation. Some clinically significant antibodies are often transient in nature, such as anti-Jk^a. When the record states that the patient has anti-Jk^a, Jk(a-) donations

should be selected for the IAT crossmatch, even if the antibody is not detectable in the current sample.

If paper records are kept, the items below should be noted at this stage in the crossmatch register and the current crossmatch form:

- Previous ABO/D type and presence of red cell antibodies previously identified.
- History of transfusions including details on issue of ABO non-identical blood, if applicable.

Transplantation leading to change in blood type

Where the ABO/D type appears to have changed on a patient, once the identity has been confirmed, it is possible that the patient may have undergone bone marrow transplantation (BMT) or haematopoietic stem cell transplantation (HSCT) for a condition such as leukaemia. Blood bank personnel may not always be informed when such a patient receives a BMT or HSCT, especially if this occurred at a referral facility. If the transplanted material was from a donor with a different blood type, then the blood type of the recipient will change to that of the donor, once the transplant is fully engrafted. Whilst the transplant is not fully engrafted or is starting to reject, anomalous ABO/D typing results will be obtained, with mixed field reactions vs. anti-A, anti-B or anti-D.

- Should a post-transplant blood sample be received by the laboratory, it may no longer reflect the blood group on record. Once established that the reason for this is due to a transplant being of a different blood group, this should be recorded in the patient's record.
- If mixed field reactions are observed in any blood group determination with no apparent explanation (e.g. recent transfusion of compatible but non-identical blood), then the possibility that the patient has been transplanted should be considered.

Patient ABO/D typing and antibody screening

In a routine situation these tests are performed on patient samples for 'type and screen' and for crossmatching. This testing takes place as soon as possible after the samples are received and before the final check to confirm compatibility (usually a serological crossmatch). The reliability of results will depend on whether the testing is manual where the risk of procedural error is high or whether automation is used allowing standardisation of testing, electronic recognition of samples/reagents and secure transfer of data to IT systems. The use of previous groups in the testing algorithm will depend on the level of confidence in historical records. The protocol for repeating tests before issue and checking procedures at each stage should be designed to reflect these differences.

ABO/D typing

ABO grouping is the most important element of pre-transfusion testing, since consequences of error can be fatal, and therefore, priority must be given to ensuring the sensitivity and security of the testing system.

For all patient samples, testing should include a 'forward' (red cell) group using anti-A and anti-B reagents (monoclonal where possible, with an anti-B that does not detect acquired B antigens), and a 'reverse' group using A cells and B cells at a minimum.

Before blood is issued a comparison must be made with a second group; if there is a reliable historical group on record, this can be used for this purpose. If there is no such previous group on record, a repeat group (minimum of cell group using anti-A and anti-B) is required before blood is issued. If testing manually, the repeat group should be performed if possible, by a second technologist without sight of the original result. For a repeat group, it is important to return to the original sample, and not to use an existing cell suspension or aliquot, otherwise any sampling error that may have occurred the first time will be replicated, and also the opportunity to re-check the sample identification will be missed.

The prominence given to D typing will depend on the frequency of the D antigen in the population. However, it is especially important not to stimulate the production of immune anti-D in females with childbearing potential, since anti-D can cause severe haemolytic disease of the fetus and newborn (HDFN). Therefore, the reagents, test systems and controls used for patient pre-transfusion testing should be designed to minimise the risk of a false-positive result. Monoclonal saline reacting IgM anti-D reagents are recommended. It is not advisable to confirm D negative results using an anti-D reagent by IAT, or to use potentiated reagents without a suitable control reagent, as false-positive results can be obtained if patient red cells are DAT positive. Rh system reagents other than anti-D are not advised for pre-transfusion patient testing since these add no value to the testing, and problems can arise in interpretation.

Neonates must be tested for ABO/D with a forward (cell) group. Reverse or serum/plasma grouping may not be useful as isohaemagglutinins are not usually developed at this stage. The ABO-D forward grouping should be repeated (going back to the original sample) if there is no historical group. A direct antiglobulin test (DAT) should also be performed on the neonate's red cells.

Where anomalous, i.e. irregular/atypical, results are obtained for ABO grouping and transfusion cannot be avoided, Group O red cell concentrate should be given

until the patient's group is confirmed. Where equivocal, i.e. uncertain, results are obtained for D typing, patients should be treated as D negative.

Cold agglutinins

If the patient has cold haemagglutinin disease (CHAD) with haemagglutinins reactive above 30°C, the sample may be auto-agglutinated on arrival at the laboratory. A free suspension of cells for ABO grouping can usually be obtained by washing the patient's cells in saline warmed to 37°C. In severe cases, it may be necessary to obtain a sample from the patient and keep it at 37°C continuously until the serum/plasma and cells have been separated in the laboratory. The prescribing clinician should be informed of the presence of cold agglutinins in the patient, as it may be necessary to warm the blood when it is transfused, using a validated blood warmer specifically designed for that purpose.

Anti-IgG vs. broad spectrum antihuman globulin (AHG) reagent

The laboratory will have to decide whether to use anti-IgG or broad spectrum AHG for IAT testing as part of antibody screening, identification and crossmatching. A broad spectrum AHG (anti-IgG and anti-C3d) will detect both IgG and complement bound on the red cells, but only in samples that have been taken into a dry tube, so this is not an advantage if EDTA tubes are used to facilitate automation. Use of anti-IgG AHG avoids numerous false positives/cold/apparent non-specific antibodies, especially when testing with polyethylene glycol (PEG).

Patient antibody screening

In a laboratory where antibody screening is not available, pre-transfusion testing proceeds immediately to the serological crossmatch.

Where reagent screening cells are available, an IAT antibody screen should be performed on all patients with requests for blood and/or type and screen, and on mothers of neonates (usually applies to infants <4 months of age) requiring transfusion. Excluding emergencies, this should always take place before blood is issued. The antibody screen is more sensitive than the crossmatch in detecting antibodies since the cells are selected for apparent homozygous expression and are kept in conditions that will better preserve red cell antigens. The screen may detect weak antibodies that could be missed by the crossmatch alone.

The aim is to detect only clinically significant antibodies, and where the IAT is sensitive and reliable there is no

need to screen by other methods, such as using enzyme treated cells.

Screening cells should be Group O and have expression of antigens to which clinically significant antibodies can be formed, i.e. C, c, D, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, and M. Screening cells should be selected to reflect the antigen frequency of the population. In parts of the world where there is a high incidence of clinically significant antibodies to other antigens e.g. Mi^a, then cells carrying these antigens should be included.

To maximise sensitivity, cells from different donations should not be pooled, and cells with homozygous expression of antigens whose corresponding antibodies are clinically significant and show 'dosage' should be included i.e. Jk^a, Jk^b, Fy^a, Fy^b, S and s. The reagent screening cells should also lack low frequency antigens where the corresponding antibodies are not of clinical significance e.g. Bg^a.

If an antibody is detected it should be identified (or referred for identification) prior to crossmatching wherever possible.

If antibody identification is not able to be carried out, it may be possible to reduce the number of possible specificities of the antibody using the antigen profile of the screening cells. Further testing the screening cells against the patient serum/plasma by methods other than IAT that do not require additional resources, can help with this. For example, testing by direct agglutination at room temperature may help to exclude or confirm some antibody specificities such as anti-M, anti-N, anti-Le^a, and anti-Le^b. Even a limited investigation like this may give information on clinical significance and thermal range of the antibodies detected (see Table 1) and can also give an indication of the likelihood of finding compatible blood by crossmatching multiple donations.

If all the screening cells are positive by IAT, it is useful to set up an 'auto' by IAT, i.e. patient serum/plasma vs. patient cells to investigate the possibility of an autoantibody (see *Antibody identification* below).

Antibody identification

The antibody identification process

An initial antibody identification panel can be set up by IAT and it is advisable to include an 'auto' test (patient serum/plasma vs. patient red cells) as this will indicate whether an autoantibody is present. A systematic process for inclusion and exclusion of the common antibody specificities should be undertaken; additional panels and techniques may be used to aid with this, as indicated by the results of the initial panel. The reactions obtained

Table 1 Examples of the clinical significance of antibodies and selection of blood, based on British Society for Haematology guidelines.

Antibody (anti-)	HTR	Suggested selection of donor blood: ABO/D group compatible units in stock, or antigen negative units to be crossmatched by IAT
A1	Rare (if active at 37°C)	Group compatible in stock
M	Rare (if active at 37°C)	Antigen negative if active at 37°C
N	Rare (if active at 37°C)	Group compatible in stock
S	Yes	Antigen negative
s	Yes	Antigen negative
U	Yes	Antigen negative
P1	Rare (if active at 37°C)	Group compatible in stock
D, C, c, E, e, f	Yes	Antigen negative
Cw	No	Group compatible in stock
Lu ^a	Mild (delayed)	Group compatible in stock
Lu ^b	Mild (delayed)	Antigen negative
K	Yes	Antigen negative
k	Yes	Antigen negative
Kp ^a	No	Group compatible in stock
Le ^a	Rare (if active at 37°C)	Group compatible in stock
Le ^b	No	Group compatible in stock
Fy ^a	Yes	Antigen negative
Fy ^b	Yes	Antigen negative
Jk ^a	Yes	Antigen negative
Jk ^b	Yes	Antigen negative
Vel	Yes	Antigen negative
Wr ^a	Yes	Group compatible in stock

with the screening cells should also be taken into consideration when interpreting antibody identification results, and the patient's phenotype, if known.

Clinical significance

Identification of the antibody present will give some clues to the probable clinical significance. Evidence from the literature that some antibody specificities cause haemolytic transfusion reactions (HTR), e.g. most Rh antibodies, is strong. For other specificities, the evidence is not as clear, e.g. anti-M and may depend on the thermal range. Apart from ABO antibodies, those non-reactive above 30°C are rarely clinically significant. Pre-warming techniques are useful to determine the thermal range but should be used with caution where antibody specificities have not been fully identified, as weak examples of common clinically significant antibodies, e.g. anti-Jk^a and/or rare but significant antibodies with variable reactivity at 37°C, e.g. anti-Vel, could be overlooked.

Pan-reactivity

Antibody identification is not always straightforward and pan-reactivity in the antibody screen and identification panel is one of the most difficult problems to resolve when providing blood for transfusion. In this situation the patient's medical history, including the history of previous transfusions and the results of serological testing, should always be sought. Some of the more common causes of pan-reactivity are as follows:

Pan-reactivity due to autoimmune haemolytic anaemia (AIHA)

If the patient is known to suffer from an AIHA, or in the absence of a previous diagnosis, where warm reacting autoantibodies are detected and the DAT is positive, it can be anticipated that all blood crossmatched will be incompatible by IAT. A referral should be made for autoadsorption or alloadsorption techniques to determine whether the patient's autoantibodies are masking any clinically significant alloantibodies. If the patient's Rh phenotype (D, C, c, E, e) is known or the patient's red cells can be typed using saline monoclonal reagents, it is preferable to transfuse Rh phenotype matched blood.

Pan-reactivity due to antibodies to a high incidence antigen or mixture of more common antibodies

Where there is pan-reactivity by IAT but the DAT and 'auto' are negative, the possibility is that the reactions are due to an antibody to a high incidence antigen or to a mixture of more common antibody specificities. The degree of agglutination and the techniques by which the incompatibilities were detected are significant in resolving a case like this. However, identification is difficult, as is exclusion of other antibody specificities that may be 'masked' and it is likely that referral will be required to resolve these cases. Units that are negative for a combination of antigens may be difficult to source, and it may be necessary to obtain blood from a rare donor registry where blood donations from individuals with rare antigen negative blood types may be stored frozen. Units are frozen and stored using specialised techniques, at extremely low temperatures for a greatly extended shelf life whilst frozen. This process takes time and once thawed the shelf life is short. (see *Section 11: Blood processing* for more details).

Pan-reactivity due to 'non-specific' reactions

Some IAT pan-reacting antibodies, non-specific for blood group antigens, can be technology/reagent related, such as those directed against low ionic strength diluents ('LISS only' antibodies), where the solution is to perform

tube IAT using a normal ionic strength saline solution (NISS).

Methodology at IAT stage of crossmatching

Once the specificity of the antibody is known and is classified as clinically significant, various options are available:

- Antigen negative blood may be selected from the stock of phenotyped blood and crossmatched by IAT.
- Commercial reagents may be required to screen units in stock for antigen negative blood that can then be crossmatched by IAT.
- The patient's serum/plasma may be used to screen for blood units. Those that test negative should, if possible, be typed to confirm that they do not have the antigen towards which the patient's antibody is directed.

Table 1 shows some examples of antibodies that may be encountered and their probable clinical significance in terms of ability to cause HTR. The table also suggests whether antigen negative blood must be selected for IAT crossmatch or whether compatibility can be established by a negative reaction in the IAT crossmatch alone.

Selecting blood for crossmatch and managing stock

Stock management

As a general rule, donations that are nearest to their expiry date should be used first. It is standard practice to use stock blood in expiry date order for routine transfusion requests so that there is less likelihood of blood reaching its expiry date and having to be discarded.

In settings where there are difficulties in obtaining sufficient donations, stock control is more difficult. When selecting blood for crossmatch, consideration should be given to the patient's clinical condition, the urgency of the request and the potential risk to the patient of alloimmunisation impacting on future treatment and/or pregnancies.

The final fate of every donation that passes through the laboratory should be recorded i.e. transfused, expired or discarded for another reason. The laboratory should have an arrangement in place with clinical areas to ensure that the final fate of each donation is known, so that the crossmatch register, or IT stock control program can be updated to record that the blood was, or was not, transfused to the patient for whom it was issued.

ABO identical or compatible components

Blood selected for crossmatch should be ABO blood type identical or if this is not available, group compatible. For

example, a Group A patient should be given Group A blood. If Group A blood is not available, then Group O red cells could be selected instead. Red cell concentrates will contain very little anti-A or anti-B, but with whole blood there is a risk of the plasma containing haemolyzing anti-A and/or anti-B and donations tested and found negative for high titre anti-A and anti-B should be selected to avoid haemolytic reactions in recipients with the corresponding ABO antigens. Consideration should also be given to this risk even when transfusing red cell concentrates if the patient is especially vulnerable, e.g. a neonate. (see *Section 10: Donation testing* for more details).

If a patient is Group AB, and Group AB donor blood is not available, then low titre Group A or B blood may be selected instead, depending on which is more readily available (which usually depends on the frequency of the blood group in the donor population). Patients who type as very weak subgroups of A should receive Group O blood. Patients who type AB with a very weak A antigen should receive Group B blood.

Table 2 shows options where group identical blood is not available or where stock management requires use of ABO non-identical units to prevent blood wastage.

Table 3 shows ABO compatible options for the transfusion of red cells, plasma or platelets, into recipients of the different ABO blood groups.

Use of D negative blood

D negative donations are often in short supply and wherever possible, should be conserved for D negative individuals. D positive individuals should not be given D negative blood unless there are extenuating circumstances, such as emergency transfusion or where the D negative donation(s) are about to expire and will be otherwise wasted.

D negative individuals should be given D negative blood wherever possible, with priority given to patients with immune anti-D, who should never be transfused with D positive blood, in order to avoid transfusion reactions, and to females with childbearing potential (i.e. all females aged < 50) to avoid stimulation of immune anti-D that could cause HDFN in future pregnancies. If necessary, D positive blood can be given to other D negative patients, but this must be recorded in their patient records to make the laboratory aware of the potential for immune anti-D in such recipients, if transfusion is required in future.

Should D positive blood be given to D negative females of childbearing potential, as a life-saving last resort, the attending clinician should take responsibility for the decision to do so. In this situation, or where D positive blood

Table 2 Selection of donor blood by ABO and D type for crossmatch

ABO group and D type of patient	First choice ABO group and D type of donation	Group compatible blood in order of selection
A D positive	A D positive	(1) A D negative (2) O D positive (3) O D negative
A D negative	A D negative	O D negative
B D positive	B D positive	(1) B D negative (2) O D positive (3) O D negative
B D negative	B D negative	O D negative
O D positive	O D positive	O D negative
O D negative	O D negative	No other suitable
AB D positive	AB D positive	(1) AB D negative (2) A or B D positive (3) A or B D negative (4) O D positive (5) O D negative
AB D negative	AB D negative	(1) A or B D negative (2) O D negative

was transfused in error, anti-D immunoglobulin (anti-D Ig) should be given immediately to prevent stimulation of immune anti-D in such recipients.

Additional considerations when transfusing whole blood and plasma

Where whole blood is to be transfused, donations tested and found negative for high titre anti-A and anti-B should be selected for ABO non-identical transfusions, to avoid haemolytic reactions in recipients with the corresponding ABO antigens. (see *Section 10: Donation testing* for more details).

Transfused plasma that is ABO incompatible is rapidly diluted in the circulation of the recipient, and low titre anti-A and/or anti-B may also be neutralised by ABH antigens present on a recipient's tissues, so is

unlikely to cause a serious reaction. This may not be the case following HSCT, as ABH antigens are not expressed in soluble form in the recipient's plasma and tissues and are only present on the red cells. Hence neutralisation of transfused anti-A/anti-B does not take place to the same extent and the incompatibility may be of concern.

Transfused plasma may cause reactions in the following circumstances:

- Donor plasma with haemolysing or high titre ABO antibodies may cause a haemolytic reaction. For example, potent anti-A,B in Group O donor blood may haemolyse the red cells of a Group A or B recipient.
- Donor plasma containing leucocyte antibodies to recipient white cell antigens may cause transfusion related acute lung injury (TRALI). This adverse effect of transfusion is discussed in *Section 14: Transfusion risks and haemovigilance*.
- If a non-Group O patient is transfused with large volumes of Group O whole blood in an emergency (e.g. Group O blood given to a Group A patient) and ABO identical blood subsequently becomes available, it may not now be compatible with this patient. A crossmatch should be performed using a fresh sample taken from the patient, to check whether the presence of ABO antibodies (in this case, donor-derived anti-A) in this sample causes blood of the same ABO group as the patient (i.e. Group A) to now be incompatible. If this happens, then Group O blood should continue to be used. Problems like this are more likely to arise when non-identical whole blood is transfused instead of red cell concentrates, because of the volume of donor plasma involved. If the donor ABO antibodies passively transferred to the patient and circulating in the recipient are not of sufficient quantity to react with red cells of the same ABO group as the patient, then blood of the patient's own ABO group can be given. Laboratory protocols should be available to determine the maximum number of Group O units that can be transfused prior to reverting to the group of the patient.

Table 3 Non-identical but ABO compatible red cell and plasma components

Patient's blood group	Patient's plasma contains	Compatible donor red cells	Compatible donor plasma	Platelets in order of preference
O	Anti-A, Anti-B	O	A, B, AB, O	O, AB, A, B
A	Anti-B	A, O	A, AB	A, AB, B, O
B	Anti-A	B, O	B, AB	B, AB, A, O
AB	None	A, B, AB, O	AB	AB, A, B, O

The 'minor crossmatch'

As opposed to the major crossmatch, which is routinely performed and involves testing the serum/plasma of the intended recipient with the red cells of the intended donor units, a minor-side crossmatch is concerned with testing the red cells of the intended recipient with the plasma from each unit of blood selected for transfusion.

The minor crossmatch is seldom performed unless investigating an adverse reaction to the transfusion of whole blood or plasma products, to determine whether donor plasma contained antibodies directed towards a red cell antigen in the patient. Transfused plasma that is ABO incompatible and of low titre or free of haemolysins, is rapidly diluted in the circulation of the recipient, and may also be neutralized by ABH substances present in a recipient's tissues, so is unlikely to cause a serious reaction. In the case of a red cell concentrate, very little plasma remains in the blood product that is supplied.

ABO/D check on blood donations

The ABO type of each donation should be checked by testing vs. anti-A and anti-B particularly if a serological crossmatch is not being performed (i.e. an electronic crossmatch alone is being performed). In some countries, the D type is also checked on D negative units.

Special requirements for blood

Transfusion dependent patients

A side effect of repeated transfusion of red cells is the accumulation of iron in the body. Iron overload can affect organs such as the pituitary gland, thyroid, heart and/or liver, leading to serious health problems. To enable excess iron to be excreted, ongoing chelation therapy using special drugs prescribed by a clinician, may be required.

Some patients, such as those with SCD are at a higher risk of producing red cell alloantibodies, making the provision of compatible blood difficult.

Patients with SCD

- These patients should be phenotyped prior to the first transfusion for D, C, c, E, e and K to allow for the selection of blood 'matched', i.e. lacking the antigens that the patient lacks, to reduce the risk of alloimmunisation, that would complicate the finding of compatible blood for future transfusions. If the patient has been transfused within the last 3 months, it will not be possible to determine the phenotype;

some blood services offer a service to genotype these patients. Where possible, an extended phenotype (or genotype) to include k, Jk^a, Jk^b, Fy^a, Fy^b, S, s is useful to facilitate further matching and to help with antibody identification should alloantibodies be formed.

- Red cells for transfusion into recipients with SCD must not be selected from donors with sickle cell trait. Blood for transfusion should be Haemoglobin S (HbS) negative where possible, especially for exchange transfusion where the aim is to reduce the burden of HbS.
- Where there is no routine access to fully phenotyped red cell donations, a special panel of designated, phenotyped blood donors may be put in place for patients starting a chronic transfusion regime, to minimise the chances of development of clinically significant antibodies, which would make the finding of compatible blood increasingly difficult. Donors, whose red cell antigens match those of the recipient, not only for ABO and Rh, but for other major immunogens such as K, should be selected for such a panel.

Neonates <4 months old

Blood for transfusion to neonates <4 months old requires special consideration and a specialist haematologist may need to be consulted in some cases. It is rare for a patient who is <4 months old to produce red cell antibodies, and any atypical red cell antibodies are likely to have been passively transferred via the placenta, from the mother. Some important points in respect of blood for neonates <4 months old are as follows:

- Wherever possible, an antibody screen should be performed on the mother. If a sample from the mother is not available the neonate can be screened for passively transferred maternal antibodies.
- If a red cell antibody is detected, blood negative for the corresponding antigen, ABO/D compatible with the neonate and ABO compatible with the mother should be crossmatched against the maternal serum/plasma by IAT. If a maternal sample is not available, then the crossmatch should be performed using the neonatal serum/plasma.
- Blood for transfusion to neonates must be tested to ensure that it does not contain high titre anti-A and anti-B.
- If repeated small volume transfusions are anticipated, blood from the same donation should be divided aseptically (using a closed system) into aliquots and reserved for the neonate to reduce the risk of exposure to transfusion transmissible infection (TTI) and to limit the variety of donor red cell antigens.

- If a large volume is to be transfused, such as in an exchange transfusion, it is preferable to use blood that is comparatively fresh (up to 5 days from the date of collection).
- For large volume transfusions it may be better to use blood collected into CPDA-1, rather than red cells suspended in additive solution. Alternatively, the unit may be centrifuged and some of the supernatant removed prior to transfusion.
- Providing filtered red cells that are leucocyte-depleted minimises the chance of CMV being transfused. Alternatively, donations that test negative for anti-CMV should be used.
- After the age of 4 months, routine crossmatching should be performed on the neonate's sample, but CMV negative blood is advised up to 1 year of age.
- There are additional requirements for fetal intrauterine transfusion (IUT), neonatal exchange transfusion, and top-up following IUT, including the use of irradiated blood. In this situation irradiated blood has a shelf life of 24 h, as irradiation increases the release of potassium from the red cells into the surrounding fluid in the blood bag, which carries a risk of neonatal cardiac arrest.

Recipients of BMT or HSCT

- It is recommended that Group O red cells (in additive solution, or with confirmed low titre isoagglutinins in residual plasma) be selected when the transplant is ABO non-identical, between recipient and BMT/HSCT donor.
- Post engraftment, when ABO antibodies in the patient, to the donor ABO type, are undetectable and the DAT is negative, the donor group may be selected. Where multiple cord donations were used for the transplant, it must be established which of these engrafted.
- When either the recipient or donor is D negative, D negative red cells should be selected.
- If graft rejection occurs, selection of red cells should remain compatible with both the patient and donor until complete reversion to the original recipient ABO and D type.
- All red cells transfused should be irradiated to prevent transfusion associated graft vs. host disease.

Confirming compatibility (serological crossmatch or electronic issue)

Approaches to confirming compatibility

Following ABO/D typing and antibody screening, the crossmatch tests required to confirm compatibility between the patient serum/plasma and the donor red cell units will

depend on the security and reliability of pre-transfusion testing performed so far. Traditionally an IAT crossmatch was always performed (where available) before blood was issued. More recently with the use of more accurate ABO/D typing, sensitive antibody screening and secure transfer of data, the final compatibility check may in some cases be abbreviated to omit the IAT. Where the antibody screen is negative, compatibility may be established to crossmatch by direct agglutination (as a check for ABO compatibility), or to electronic issue, i.e. comparison of ABO/D type without direct testing between donor and recipient. Where antibody screening is not available or where antibodies detected in screening have not been identified, and IAT crossmatch must always be included.

Electronic issue 'computer crossmatch'

Electronic issue or computer matching of blood has been introduced into busy crossmatching laboratories that have the appropriate infrastructure. This requires patient ABO/D typing and IAT antibody screening to detect IgG antibodies using automated systems, and secure transfer of results to the laboratory IT system with no manual steps to edit results at any stage. The use of an IT system that meets the required specifications, including having algorithms built in to prevent issue of ABO incompatible blood is also required. The donor blood is matched electronically with the patient; no serological crossmatch is performed. Provided that antibody screening tests carried out on the patient are negative and no anomalous results are obtained in the grouping, and there is no history of irregular antibodies, blood of the same ABO/D type can be selected and issued electronically.

This option is not suitable for patients who currently have, or have a history of, clinically significant red cell antibodies, or those who have had a solid organ transplant within the last 3 months, BMT/HSCT recipients, or neonates where the mother has a clinically significant red cell antibody.

Inappropriate use of electronic issue can have serious consequences, and a thorough risk assessment of testing and IT specifications should take place before its introduction, along with extensive validation. Backup systems will be required for occasions when the IT system and/or automation are not functioning. However, when implemented correctly, workload can be significantly reduced, crossmatching errors eliminated and the potential for 'remote issue' made possible.

Serological crossmatch by direct agglutination 'immediate spin'

The direct agglutination crossmatch is performed to detect ABO incompatibility. This is sometimes known as

'immediate spin' crossmatch, but this term is misleading since it is important to incubate the patient's serum/plasma and a 2–3% suspension of donor red cells (ratio 1:1) for two to 5 min before centrifuging and reading, to reduce the risk of false negative results.

Serological crossmatch by IAT

The IAT crossmatch is performed to ensure that the donations selected are compatible with IgG red cell antibodies that may be present in the patient's serum/plasma. An IAT crossmatch is essential where antibody screening and identification is not available. Even when screening and identification are performed, the IAT crossmatch is often still included as it provides an opportunity to detect antibodies missed in the antibody screen due to technical or procedural errors, or because a donation contains a low frequency antigen, and the intended recipient has the corresponding antibody. Such antibodies may occasionally be of clinical significance, e.g. anti-Wr^a.

If clinically significant antibodies are identified, antigen negative units should be selected for the crossmatching by IAT where possible and if appropriate (see Table 1). This gives additional security in the event of the antibody identification being incorrect or incomplete (i.e. an additional antibody is masked by those identified) or units being incorrectly phenotyped; it is especially vital where there are insufficient resources to identify the antibodies present.

Sensitivity of the IAT crossmatch can be controlled by using weak anti-D with D positive cells (or similar) with each set of tests performed, and if using a tube or liquid phase microplate technique, using sensitised (IgG coated) cells to check the washing step. See *Section 4: Principles of Laboratory Techniques* for more details.

Direct agglutination 'immediate spin' abridged crossmatch

Where the antibody screen is considered sensitive and secure, testing can be abridged to omit the IAT and to crossmatch by direct agglutination alone. This approach has value in busy laboratories handling many samples, and the objective is to provide suitable blood quicker, and more cost effectively, without compromising patient safety. This abridged version of the crossmatch is only suitable if no atypical red cell antibodies have been detected in the patient's serum/plasma. When the antibody screen tests are positive or the patient has a history of clinically significant red cell antibodies, then an IAT crossmatch must be performed vs. the donor cells. It is also not suitable for patients who have received solid organ transplants within the last 3 months, as an IAT

crossmatch is required to detect IgG anti-A or anti-B that may have been produced by passenger (donor derived) B lymphocytes.

Serological crossmatching procedure

- Serological crossmatching is a process with many manual steps and carries considerable risk of error.
- Detailed SOPs must be in place and followed carefully.
- The major crossmatch concerns testing the serum/plasma of the patient with the red cells of the donor units.
- Only one crossmatch should be undertaken at a time and, where possible, completed by one technologist.
- The crossmatch sample and request form should travel together through the process of compatibility testing. Every time a sample is handled, the details on the label should be rechecked against the details on the form or an IT generated worklist, to ascertain that they correspond.
- The ABO/D type of the units selected should be compared with the group of the patient to confirm that they are compatible.
- Technical procedures will vary depending on the technology used for IAT crossmatching, and where commercial systems are used, e.g. column agglutination technology (CAT), the manufacturer's instructions should be followed exactly. (see *Section 4: Principles of laboratory techniques*).
- Checks should be made to ensure that all reactants are added. If tubes are used for crossmatching, the addition of patient serum/plasma should be checked before donor red cells are dispensed, and reactant levels checked in CAT cards/cassettes.

Additional techniques for serological crossmatching

Tests other than direct agglutination (to detect ABO antibodies) and IAT (to detect IgG antibodies) are not normally required for compatibility testing, Proteolytic enzymes were historically used in addition to the IAT, but because of their tendency to detect autoantibodies and other antibodies not of clinical significance the enzyme crossmatch has largely been discontinued. Inclusion of this test adds to workload and cost, can delay transfusion unnecessarily, and carries the risk of introducing a culture where it is considered reasonable to ignore equivocal (uncertain) positive reactions.

Other technologies occasionally used include:

- Polybrene: Positively charged polybrene (a polymer of hexadimethrine bromide) enhances the

agglutination of sensitised cells by reducing their negative charge. Polybrene is usually added to cells that have been incubated with serum/plasma in a low ionic strength, low pH medium.

- PEG: PEG is a water-soluble polymer used as an additive to increase antibody uptake. PEG removes water molecules from around red cells, enhancing antibody uptake and reaction strength.

Dealing with unexpected incompatibility

Where an antibody screen is performed, antibodies identified and antigen negative blood selected for crossmatching, it is unlikely but not impossible that the serological crossmatch will be incompatible. Where antibody screening and identification is not possible due to lack of resources or in an emergency, the risk of unexpected incompatibility is higher with a wider range of potential causes.

If donor red cells are sensitised, agglutinated or haemolysed by the patient's serum/plasma during any stage of the cross-matching procedure the incompatibility needs to be investigated as a matter of urgency. The clinician should be notified immediately should there be an anticipated delay in providing compatible blood, checking beforehand that there is enough serum/plasma remaining in the sample from the patient. If not, a further sample should be requested at the same time. Ongoing communication between the laboratory and prescribing clinician is important for optimum patient care. If the incompatibility was detected after the blood was issued on emergency, the clinician should be notified immediately, and the transfusion stopped. All communication between hospital and laboratory staff, regarding the patient, should be documented in writing and kept as a record.

Immediate actions

Every laboratory should have a clear policy on how to deal with incompatibilities systematically so that good patient care is not compromised. The first steps should be:

- Check the ABO group of the patient and the donor blood to ensure that the ABO groups are compatible.
- Check that the correct sample and donation were used for crossmatching

This should be followed by further testing on the patient sample and the donation.

Testing on the donation

Perform a DAT on the donor red cells, and if positive this could indicate that the incompatibility is not due to an

antibody in the patient sample but that the donor's cells have become sensitised prior to the crossmatch, either *in vivo* or *in vitro*.

If the antibody screen on this sample has previously found to be negative, set up a repeat screen by going back to the original sample.

- If still negative, the patient could have an antibody to a low incidence antigen present on the donor red cells but absent from the screening panel cells, e.g. anti-Wr^a. In this case most other donations will be found compatible and once an antibody to a low frequency antigen has been confirmed it is acceptable to issue IAT compatible blood.
- If now positive, there could have been an error in sensitivity of the original test, or the wrong sample may have been used. Proceed to identification as if the antibody had been detected in the original antibody screen.

If antibody screening was not performed, it is possible that there is an IgG antibody or another patient related problem undetected so far:

- The patient could have a clinically significant IgG red cell antibody and the sample should be referred for antibody identification. If blood is required urgently or referral is not possible, then additional donations can be crossmatched by IAT.
- If all units crossmatched are incompatible and the autoantibody test is negative, antibodies to a high incidence antigen or multiple antibodies to more common antigens should be suspected and a referral made for antibody identification.
- The patient may have a non-specific antibody to the LISS medium used in the IAT. The IAT phase of the crossmatch should be repeated without using an additive solution or low ionic strength medium, i.e. a normal ionic strength saline (NISS) IAT.
- If all units crossmatched are incompatible and the autoantibody test is positive, the patient may have AIHA with warm autoantibodies that are interfering with the crossmatch – this will give a positive DAT and the autoantibodies present may be masking underlying isoantibodies. A referral is required for further investigation and if AIHA is confirmed then the clinician should be informed.
 - Consideration should be given to other possible causes of a positive DAT:
 - The patient has been recently transfused and is experiencing a delayed transfusion reaction due to sensitisation of donor red cells from a previously transfused unit.
- During the crossmatch procedure if all units tested are incompatible in the direct agglutination 'immediate spin' phase, the patient may have cold

agglutinins, although this is likely to have caused a problem at an earlier stage with ABO/D typing. Cold agglutinins can cause the direct room temperature crossmatch to be positive and can be excluded by warming the tests to 37°C and rereading. An autoantibody control (patient serum/plasma vs. patient cells) should be set up and read at the same time, and the IAT should be negative before blood is issued as compatible.

If the reason for the incompatibility cannot be established or resolved, a medical decision may be made to transfuse the patient with the least incompatible units if the patient's care will be compromised by withholding or delaying a blood transfusion. This decision is important, and details must be documented.

Provision of blood in an emergency

Standard and emergency situations

Although it may take <30 min to crossmatch blood for a patient using a LISS IAT, logistically, more time is needed, especially in a busy laboratory. Therefore, approximately 2 h is required for a standard crossmatch.

If the emergency is such that the prescribing clinician cannot wait 30 min, he/she may take the responsibility for transfusing blood without an IAT antibody screen or crossmatch, in which case Group O blood (D negative where possible) should be issued (low titre anti-A,B if whole blood is used).

Many hospitals keep small stocks of Group O D negative blood designated for emergency use in an area outside the laboratory, e.g. in a theatre refrigerator. It is important that such stocks are controlled by the blood bank, to ensure that they are kept within the correct temperature range, and that they are not tampered with or removed and then replaced in stock. Records of emergency transfusions are important for traceability, and details of recipients and units transfused must be documented on blood request forms as are routinely used for crossmatch requests.

As soon as the sample is received, and meets the sample acceptance criteria, a 'rapid' ABO/D type should be performed. The rapid group should be a minimum of patient red cells against Anti-A, anti-B and anti-D. Appropriate controls should be included. This rapid group will allow preparation of ABO/D specific blood to begin; however, before group specific (as opposed to Group O) blood is issued, the original sample should be rechecked for labelling and at least a repeat cell group from the original sample, and an 'immediate spin' crossmatch undertaken on the donations to be issued to confirm ABO compatibility. The aim is to conserve Group O D negative

blood whilst ensuring that the patient is not put at risk of ABO incompatibility. In some standards, a second sample must be drawn to confirm ABO/D typing if there are no previous records of the patients' blood group. Where O D negative blood is not available or in short supply, then O D positive blood can be issued for male patients and to females over the age of 50, but in areas where there is no anti-D Ig prophylaxis, considerations should be given to the risk of older women having produced immune anti-D.

Blood that is urgently required may be released for transfusion if suitable at the immediate spin phase, provided that the requesting clinician understands that there has been insufficient time to exclude the presence of IgG red cell antibodies. If an IgG antibody is detected after blood has been issued, the clinician should be requested to discontinue the transfusion immediately. It is therefore important that when blood is issued on emergency, the crossmatch is continued to the IAT phase as soon as possible.

A rural hospital may not have access to a compatibility testing laboratory. Patients may be D typed, either at the bedside or on admission, using 'point-of-care' rapid testing. Those who type as D positive and need transfusion may then be given Group O D positive blood units, reserving the Group O D negative units for D negative patients. Because ABO grouping of recipients is not carried out, it is important that all available units of Group O donor blood is low titre and does not contain haemolyzing isoagglutinins, particularly if whole blood is being used for transfusion. Red cell units stored in additive solutions have considerably less plasma, and therefore are less likely to cause haemolysis. In countries where most of the population is D positive, there would likely be a chronic shortage of D negative donations and this practice prevents the unnecessary use of D negative blood.

Massive transfusion

Massive transfusion is usually defined as the replacement of the patient's blood volume, or more, within a 24-h period, or a 50% blood volume loss within 3 h or a rate of loss of 150 ml/min in an adult. Several factors require consideration in massive transfusions:

- When a massive transfusion has been given, continuing to crossmatch further units requested may not be of value. When authorised by the clinician, further units for transfusion may be retested to check ABO group only and issued without crossmatch.
- Massive transfusion may be associated with the development of coagulation abnormalities. Coagulation factor levels should therefore be monitored, and fresh frozen plasma (FFP) or platelets transfused if

necessary, to correct the situation. (see Table 3 for suitable ABO group choices).

- The rapid infusion of cold blood may cause debilitating hypothermia in the massively transfused patient. To avoid this, blood may be transfused through a blood warming device that meets quality and safety standards.
- There may be toxic effects from citrate in the anticoagulant, which is infused together with whole blood or FFP, and it may become necessary for the clinician to administer calcium to counter these effects. This is usually given in the form of calcium gluconate or calcium chloride, the dose of which should be carefully calculated.
- Microaggregates of white cells and platelets form during the storage of whole blood and if these are not removed by filtration, they may be harmful to the patient.

Changing blood type during one transfusion episode

Patients should ideally receive blood that is ABO group identical. However, available stocks of safe blood may be in short supply. It may be necessary, especially in emergency situations, to change the ABO group of units transfused during the same transfusion episode, for several reasons.

- When stocks of blood that match the ABO group of the patient become depleted before the end of the transfusion episode, it will be necessary to change from ABO group identical to ABO group compatible blood.
- When there is insufficient D negative blood it may be necessary, in an emergency, to transfuse D positive blood into a D negative recipient (this is acceptable for male patients, or female patients more than 50 years old).
- It is important that if the ABO group of consecutive units is different, the administration set is changed before commencing infusion of the second unit. If this is not done, agglutinates of red cells could form beyond the filter and enter the recipient's circulation. This precaution is not necessary if only the D group of the units is different.

Internal quality control (IQC) for pre-transfusion laboratory tests

IQC refers in general to procedures carried out at the time a test is performed and that provides feedback about the reliability of the test. It is essential to record the actual IQC results that are observed and not the results as they

are assumed or expected to be. If any discrepancy is found with the IQC results, the test results to which they apply should be disregarded.

For manual testing, controls should be set up last (so that they have the shortest incubation time in a batch of tests) and read first so that if the control reactions are not as expected the tests are not read. If using automation, the software will not normally allow testing to begin until the IQC results are accepted. Limits should be in place for expected reaction grades for control reagents. If these are not met the tests set up at the same time are invalidated.

ABO/D and phenotyping reagents

The reagents that are in daily use in the compatibility testing laboratory include the following:

- Anti-A and anti-B, anti-D
 - ABO and D typing reagents should be controlled at least daily with the frequency of these IQC tests being determined by whether the testing is automated.
- Phenotyping reagents, e.g. anti-E, anti-K etc.
 - Positive and negative controls should be included each time a phenotyping reagent is used in a batch of tests.

Cells selected as positive controls for blood grouping reagents should express heterozygous 'single dose' or common weak examples of the corresponding antigen, and negative control red cells should not express the corresponding antigen. Table 4 shows suggested positive and negative controls for monoclonal blood typing reagents.

Polyclonal reagents

Where polyclonal reagents are used there is an increased risk that they will be contaminated with other antibodies. In this case, positive control cells with heterozygous expression of the antigen being tested for and negative for antigens corresponding to potential contaminants should be used to avoid false positive reactions. The negative control cells, whilst negative for the antigen being tested for, should express the antigen corresponding to the most likely contaminating antibody. For example, polyclonal anti-D from a human source may be contaminated with anti-C in which case the positive control cells should be D positive, C negative and negative control cells D negative, C positive

Antihuman globulin (AHG)

- The addition and reactivity of the AHG reagent is controlled by the addition of sensitised control cells

Table 4 Example control cells for monoclonal blood grouping reagents

Reagent (monoclonal)	Positive control cells	Negative control cells
Anti-A	A ₂ or A ₂ B	B
Anti-B	B	A
Anti-D	R ₁ r (Cde/cde)	rr (cde/cde)
Anti-C	r'r (Cde/cde)	rr (cde/cde)
Anti-E	r''r (cdE/cde)	rr (cde/cde)
Anti-c	r'r (Cde/cde)	R ₁ R ₁ (CDE/CDE)
Anti-e	r''r (cdE/cde)	R ₂ R ₂ (cDE/cDE)
Anti-K	Kk	K-
Anti-Jk ^a	Jk (a+b+)	Jk (a-b+)
Anti-Jk ^b	Jk (a+b+)	Jk (a+b-)

to all apparent negative IAT tests (see *Section 4: Principles of laboratory techniques* for details).

- In addition, the AHG must be tested with every batch of IAT tests, by including a weakly reacting IgG antibody as a positive control, and inert serum/plasma as a negative control, together with reagent screening cells.

Screening cells

A weak IgG antibody is run as a control on the antibody screening procedure i.e. the weak antibody should be detectable and produce the expected degree of agglutination for the batch of tests to be deemed acceptable. Limits should be set for the expected reaction grade that constitutes a control 'pass'. Reaction grades can be tracked over time to detect any change in sensitivity of the antibody screen over a longer period that may need investigation. A gradual change over time is not acceptable and must be investigated to determine the reason, as it may be that the reagent is deteriorating and becoming unreliable.

Labelling and issue

Once all pre-transfusion testing is complete, results of all tests should be collated, and final checks made, from start to finish, to ensure that there were no errors made from sample used, to tests done, to results obtained, and to transcription of results.

At the time of blood being labelled for a patient, all details should be carefully checked between number of blood unit and number of unit on request form to confirm that they match and that the correct blood, particularly by ABO/D group and donation number, is selected for issue and is compatible. Each unit should be labelled with the patient's first name, surname, identifying number, and blood group, the donation number and blood

group and date crossmatched. At this stage, components should also be checked for signs of any abnormality, such as leakage and haemolysis, that the unit is within date (i.e. not expired) and that any special requirements were met if requested, e.g. CMV negative or irradiated.

A final report may be issued with the first unit for transfusion, listing the donation numbers of the units available, component type, expiry date and blood group of each, and any other pertinent information such as whether the testing was done as an emergency or routine crossmatch, that the units are compatible, and if they are not ABO identical. The report should include all the identification details required for sample acceptance, plus the patient's blood group and antibody status. A better alternative is that all the information related to the blood crossmatched for, and available for the patient is to be found on the crossmatch form used to prepare the blood components for that patient.

The laboratory should have a policy for issuing blood that could include minimum patient details required at collection, checks made on the units, qualifications and training required for hospital staff collecting blood for transfusion.

At the time of issue of blood, the laboratory should check all the details on the request form and on the blood bag label. If the blood is collected directly from the laboratory rather than being taken to an issue refrigerator by laboratory staff, the request slip presented by the hospital representative must also be checked.

Once the blood components have been collected it becomes the responsibility of staff in the clinical areas to follow clearly defined procedures to ensure that the blood is correctly handled, stored and administered to the patient.

Administration of blood in clinical areas

Bedside checks on the patient and blood component

Prior to the administration of the blood, the attending clinician should check the identity of the patient and the blood bag and correlate the following to confirm that they match:

- Verbal verification of the patient's name (ask the patient their name; do not ask the patient to confirm their name).
- All the details on the blood unit label.
- Details on the wristband of the patient, if available.
- Details on the patient's case notes.

In some hospitals the ABO blood group of the patient to be transfused is checked by carrying out a rapid grouping test at the bedside.

Use of portable electronic devices to check patient identification at the bedside, by matching his/her wristband information (which could be barcoded and include a photograph) reduces the chance of misidentification further, provided that the blood bag label is linked into the checking process.

Any errors detected must immediately be brought to the attention of the nursing staff in charge, so that corrective action may be taken.

Administration sets

Blood components should always be administered through an appropriate administration set with an in line 170–200 micron filter chamber. Other intravenous fluids or drugs should not be administered via the blood administration set. For ongoing transfusions, the administration set should be changed every 12 h or after every four units of blood transfused.

Information provided to staff administering blood in clinical areas

Clinicians prescribing transfusion should be provided with information on the correct procedure for administering blood, and handling reactions, for all blood components issued. Besides the checks done to correlate information on the component label, and the identification of the patient, checking procedures should include the following instructions to medical and nursing personnel regarding:

- Checks required on blood components
 - evidence of damage or leakage.
 - evidence of discoloration, contamination or haemolysis of contents.
 - expiry date, which should not have been reached.
 - details on the compatibility label, which should match those of the patient.
- Storage conditions: at $4 \pm 2^\circ\text{C}$ for red cells and FFP, and that FFP and platelets should be transfused on receipt from the blood bank.
- Warming of blood: that this should be performed only when absolutely necessary, such as for exchange or massive transfusion, and then by using an approved blood warmer specifically designed for that purpose.
- Addition of medication and solutions: no additives should be transfused with blood; the only exception being sterile normal saline.
- Blood administration sets: an appropriate administration set fitted with a 170–200 micron filter should be used whenever blood products are transfused.
- Used blood containers: after transfusion, empty containers and administration sets used should be kept at $4 \pm 2^\circ\text{C}$ for a minimum of 24 h, in case of

adverse reaction and subsequent investigations that may be required.

- For at least the first 30 min of any blood transfusion, the patient should be carefully observed for any signs of an adverse reaction. See *Section 14: Transfusion risks and haemovigilance*, for the signs and symptoms of acute reactions.

The blood should be administered slowly at first, if feasible, because the amount of blood transfused is often proportional to the severity of reaction. Observation and monitoring of the patient during a transfusion are essential if adverse reactions are to be quickly identified and managed.

Observations (pulse, blood pressure, temperature and respiratory rate) should be undertaken and documented for every unit transfused. Minimum monitoring of the patient should include the following:

- Pre-transfusion observations taken and recorded no more than 60 min before the start of the component transfusion.
- Observations taken and recorded 15 min after the start of each unit.
- Post-transfusion observations taken and recorded not more than 60 min after the end of the transfusion and regular visual monitoring of the patient throughout the transfusion episode.
- Deterioration of the patient's condition or development of symptoms suggesting a transfusion reaction should prompt more frequent observations and review, dictated by the clinical situation.
- Should the condition of the recipient deteriorate and indicate the start of a transfusion reaction, the infusion must immediately be discontinued, and the clinician notified.

Transfusion reactions and haemovigilance

Retention of samples and transfused units

In case of an adverse transfusion reaction, samples used for crossmatch and units transfused may need to be investigated and tests repeated. It is therefore very important that samples used for crossmatches are retained by the blood bank. It is just as important that the hospital retains the remains of all transfused units. The length of time that samples and transfused units should be retained depends on the available storage space at $4 \pm 2^\circ\text{C}$; this should be for a minimum of 24 h, but preferably longer, up to 1 week.

Immediate actions in the clinical area

If a reaction is observed, the nursing personnel responsible for the observation of the patient should stop the

transfusion immediately and alert the medical personnel. The patient should be immediately treated for the reaction.

Thereafter, the prescribing clinician should complete and submit a reaction form provided by the blood bank, in which the following information is documented:

- Details of the patient (name, hospital, number and ward, diagnosis, state of consciousness).
- Details of the blood component (type, container number, blood group, volume transfused).
- Details of the reaction (time transfusion started, time of start of reaction).
- Clinical signs and symptoms should be indicated, as described in *Section 14: Transfusion risks and haemovigilance*.

Adverse reactions should be investigated to establish the root cause, and all findings documented and analysed to determine corrective and preventive actions to be initiated.

Transfusion reaction investigation

When notified of a transfusion reaction, laboratory personnel should request the return of all blood bags from which blood was transfused during the transfusion episode, together with a post-transfusion blood sample for investigation. Transfusion reaction investigations should always be carried out immediately on receipt and discrepancies detected should immediately be brought to the attention of the transfusion laboratory manager and the consultant haematologist.

Clerical check

On receipt of the reaction report and post-transfusion samples, the first step is a clerical check of all transfusion documentation to ensure that the correct blood was issued and that there was no misinterpretation of the laboratory results or any other errors made. The reaction report form submitted by the clinician should also be checked against the original crossmatch request form to ensure that the patient identification details match. The signs and symptoms noted by the clinician should be checked as they may assist in identifying the type of reaction.

Visual checks

The serum/plasma from the patient's pre- and post-transfusion samples should be examined for any sign of haemolysis or jaundice and evidence of either, documented. If the post-transfusion sample appears haemolysed or jaundiced the clinician should be informed and the reason established. Further testing to determine the bilirubin levels should be requested.

The bags from which blood was transfused are returned and must be examined for haemolysis, discoloration or untoward appearance. This may be helpful in identifying

problems caused by bacterial contamination or freezing or inappropriate heating of red cell components.

ABO group

It is most important to verify that blood of compatible ABO group was transfused. Therefore, the ABO groups of both pre- and post-transfusion samples, plus all units of blood transfused, are tested to confirm ABO compatibility, or discover if there was an ABO mismatched transfusion. It should never be presumed that only the last unit transfused was the cause of the reaction.

Antibody screen

The IAT antibody screen is repeated using the serum/plasma of both pre- and post-transfusion samples and the same batch of screening cells as used in the initial compatibility procedure. This is to determine whether an irregular antibody has become detectable in the post-transfusion sample that was not present during initial testing, or whether the antibody was missed in the original test.

Antibody identification

Antibody identification should be performed if there is any incompatibility between the serum/plasma of the patient and the red cells of transfused blood, or if the antibody screen is positive. If a clinically significant antibody is identified, the red cells of the unit(s) transfused, should be phenotyped to determine whether the corresponding antigen is present, which could therefore have been the cause of the reaction. The patient's pre-transfusion sample should also be typed for the relevant antigen (a negative result helps to confirm the identity of the atypical antibody found in the serum/plasma of the recipient) providing the patient was not transfused within the last 3 months (in which case transfused red cells may still be present).

Immediate 'spin' crossmatch

Direct agglutination crossmatches between the serum/plasma of both pre- and post-transfusion sample and red cell suspensions prepared from the transfused units are carried out. These 'immediate spin' tests are performed with a 5-min incubation period at room temperature. Their main purpose is to check for ABO compatibility but also to detect IgM reacting irregular antibodies in the serum/plasma of the patient.

IAT crossmatch

An IAT crossmatch is performed using both pre- and post-transfusion samples against the red cells of the units transfused. This is to determine whether an irregular antibody against a donor derived antigen has become

detectable in the post transfusion sample, and to check that an incompatibility as a result of an IgG antibody was not present during initial testing – an antibody may have been present but was below the detectable level. Any incompatibility found is investigated to identify the specific cause.

Direct antiglobulin test

A DAT is performed on the red cells of both pre- and post-transfusion samples and on units transfused to detect sensitisation of the recipient cells or the red cells of the units transfused. Where relevant, an EDTA sample may be requested from the recipient to prevent complement becoming cell bound, leading to a false positive result.

Elution

If the DAT is positive an elution can be performed to identify the antibody coating the red cells.

Haemoglobin

A failure of the haemoglobin level to rise post-transfusion may be an indication that transfused cells were coated with antibody and removed from the patient's circulation. The patient may be suffering a delayed transfusion reaction.

Tests on urine

A pooled 24-h urine sample should be collected and then tested for the presence of haemoglobin, as this is an indication of a haemolytic transfusion reaction. This test is not necessarily performed at the blood bank.

Tests for bacteria

A Gram stain and culture may be performed on the blood remaining in the bags of blood transfused. Blood cultures should also be performed on the recipient if symptoms are suggestive of a septic transfusion reaction. It is important to identify the type of micro-organism if it is found that a blood bag is contaminated. If the transfusion reaction was as a result of bacterial contamination of blood transfused, this is extremely serious and life-threatening.

It can be difficult to determine the cause of a transfusion reaction. In a true septic reaction, a bacterially contaminated unit is transfused, resulting in symptoms such as fever and hypotension. However, the unit may have been sterile at the time of transfusion, but may be contaminated when the transfusion is terminated, and during storage on the ward post-transfusion, prior to culture. In general, if a patient develops symptoms compatible with a septic transfusion reaction, it should be assumed that the bag was contaminated pre-transfusion, and components that were prepared from the original donation

should be traced, recalled and also tested for bacterial contamination, and if confirmed, discarded.

Haemovigilance

The process of monitoring and evaluating all incidents and accidents related to the transfusion chain is part of haemovigilance, first discussed in *Section 9: Blood collection*, as haemovigilance relates also to blood donors. Haemovigilance as it relates to transfusion hazards and reactions is described in more detail in *Section 14: Transfusion risks and haemovigilance*.

Key Points

Patient identification/Sample and request acceptance/Patient records

- Pre-transfusion compatibility testing in the laboratory must detect ABO/D incompatibility between the patient and donor that can cause fatal haemolytic transfusion reactions. It should also detect clinically significant red cell antibodies that could cause a haemolytic transfusion reaction or reduce the survival of red cells transfused.
- Effective procedures and policies should be in place in both clinical areas and the laboratory to prevent misidentification of the patient or the sample at any stage in the transfusion process.
- Each laboratory should have a policy for sample acceptance that is known to staff in the laboratory and clearly communicated to staff in clinical areas and samples not meeting the set criteria should be rejected.
- Patient records are a vital resource in the pre-transfusion testing process, and they should be created and maintained so that they are accurate, complete and easily accessible when required. Secure systems should be in place to check that the patient's unique number, first name, last name and date of birth match before linking patients electronically or updating paper records.
- Patient history should be checked as soon as possible after receipt of the request for blood; this is an opportunity to detect errors in sample labelling and potential difficulties in providing compatible blood or blood with special requirements.

Routine laboratory testing

- ABO/D typing, and antibody screening should be performed on all type and screen, or for crossmatch requests, as soon as possible to alert the laboratory

to potential difficulties with crossmatching and to allow comparison with a previous group to detect a possible 'wrong blood in tube' incident.

- Where anomalous results are obtained for ABO grouping and transfusion cannot be avoided, Group O blood should be given until the patient's group is confirmed. Where equivocal (indeterminate) results are obtained for D typing, patients should be treated as D negative. Reagents, testing methods and controls used should be designed to minimise the risk of a false positive D typing result.
- Where the antibody screen is positive, the antibody identification should be undertaken to determine the likely clinical significance, the potential delay to finding compatible blood for transfusion, and to facilitate the provision of antigen negative blood.
- Where antibody screening and identification cannot be undertaken, an IAT crossmatch should always be performed before blood is issued (except where required as an emergency or where it is not possible to perform an IAT). An IAT crossmatch is also required where the patient has red cell antibodies, even where these have been identified and antigen negative blood is selected for crossmatch.
- When providing blood for neonates, a crossmatch should be undertaken using the mother's serum/plasma. If the mother's serum/plasma cannot be obtained, then the neonatal serum/plasma can be used.
- The laboratory should have procedures in place for investigating unexpected incompatibility encountered during serological crossmatching, and good communication with clinicians is vital in this situation, as the provision of compatible blood may be delayed.
- Policies such as type and screen, immediate spin crossmatch and electronic issue save the laboratory time and resources, but abbreviation or omission of the crossmatch carries a high risk if secure supporting systems are not in place. The introduction of electronic crossmatching requires comprehensive risk assessment before implementation.
- The ABO type of each donation should be checked by testing the donor red cells with anti-A, anti-B to ensure that the results match the group assigned. This testing does not necessarily have to take place during the crossmatch procedure. In some countries, the D type of D negative units is also verified.
- Internal quality controls should be in place for all tests, and it is essential to take care to record the IQC results as they are observed and not as they are expected to be. If any discrepancy is found with the IQC results, the test results that they apply to should be disregarded.
- Before blood is issued in routine situations, patients must be fully ABO grouped (forward and reverse) and D typed, and a second ABO/D type done for comparison. The second ABO/D typing can either be historical or obtained by going back to the current sample and re-testing.

Stock management and selection of blood

- It is standard practice to use stock blood in expiry date order for routine transfusion requests to reduce wastage, but consideration should also be given to the patient's clinical condition when selecting donations. Some groups of patients, e.g. those with sickle cell disease, neonates < 4 months old, recipients of BMT or HSCT, may require fresh blood with additional specifications, e.g. phenotyped or irradiated.
- Red cells selected for crossmatch should be ABO identical with the recipient, where possible, otherwise ABO compatible. If whole blood is used, donations negative for haemolysing or high titre anti-A and anti-B should be selected to avoid haemolytic reactions in recipients with the corresponding ABO antigens. Consideration should also be given to this risk, even when transfusing red cell concentrates, if the patient is especially vulnerable, e.g. a neonate.
- D negative patients should receive D negative blood, but where D negative blood is in short supply, priority should be given to patients with immune anti-D and to females with childbearing potential (i.e. all females aged < 50).
- The laboratory should be able to record the final fate of each donation it receives. This information, together with patient records, should allow for full traceability should a lookback be required, such as when a donor who gives a subsequent donation, is reactive for a TTI.
- Alternatives to transfusion should be considered both when planning and undertaking surgery. See *Section 18: Indications for transfusion of blood components*.
- There are additional considerations when transfusing whole blood which is not ABO identical to the recipient. Haemolysing or high titre ABO antibodies present in the donor plasma may cause a haemolytic transfusion reaction.

Emergency and massive transfusion

- Before blood other than Group O is issued in emergency situations (to a non-group O recipient who is bleeding profusely), a minimum of a forward (cell

group) with anti-A, anti-B and anti-D should be performed, followed by a second check for ABO. This can be a forward group of the original sample received by the blood bank or an 'immediate spin' crossmatch on the donations to be issued. If an incompatibility with any of the units issued is subsequently detected (due to an IgG antibody detected in the IAT crossmatch completed only after issue of the blood), the clinician should be informed immediately.

- In an emergency, and where it is necessary to conserve Group O D negative blood, Group O D positive blood may be given to male patients. Group O D positive blood may not be given to D negative female patients with childbearing potential due to the risk of stimulating the production of anti-D antibodies capable of causing HDFN. However, in emergency situations, females over the age of 50, whose serum/plasma is confirmed not to contain anti-D, may be given D positive blood. (as a reminder, the transfusion of D positive blood into a patient with anti-D has the potential to cause a serious haemolytic transfusion reaction.)
- Massive transfusion can lead to coagulopathies and patients may require support with other blood components such as FFP and platelets. The rapid infusion of cold blood should be avoided, as it can be life-threatening. Where the volume of blood transfused within 24 h equals the estimated blood volume of the patient; and when authorised by the clinician, further units may be issued without a crossmatch.
- Should blood stocks become depleted before the end of a transfusion episode, it will be necessary to change from ABO identical to ABO non-identical blood, in which case the administration set must be changed.

Administration of blood/transfusion reactions and Haemovigilance

- The laboratory should have a policy for issuing blood that includes the minimum patient details required when blood is collected for a patient, checks made on blood units to be issued for this patient, and the qualifications and training required for hospital staff collecting this patient's blood from the laboratory.
- Blood administration at the bedside is a critical step carried out by clinical/nursing (non-laboratory) staff, but the laboratory should provide clinical staff with guidance. The hospital transfusion committee is a useful forum to ensure that policies such as the collection of blood for a patient, the storage of blood in the hospital ward, and the checks required before administration, are fully understood and complied with, by all.
- Patients should be carefully monitored during transfusion; any evidence of transfusion reaction should be acted on immediately and the attending clinician and laboratory staff informed.
- Samples used for crossmatch and blood remaining in used blood bags should be kept for at least 24 h (and longer if ward facilities allow) in case an investigation is needed. Transfusion reactions may not be immediate but delayed. Therefore, days may pass before the reaction is noticed and reported to the blood bank and only then will an investigation be initiated.
- The root cause of all transfusion incidents should be determined, and a report made (to the Haemovigilance scheme if one is in place). Corrective and preventive actions should be put in place to reduce the risk of recurrence.

Transfusion risks and haemovigilance

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Introduction

Safe blood transfusions save lives; patients in need would either die if blood was not available, or their recovery from a variety of illnesses or procedures would be prolonged. Many patients on long-term therapy depend on safe blood transfusions for survival. However, although there is a tremendous benefit to receiving blood, transfusion unfortunately also presents risks to patients. The potential risks to the patient include accidental transfusion of the incorrect unit of blood, infection with a transmissible disease carried by the donor, or the patient may suffer any of a variety of other complications. Therefore, blood transfusion should be given only when needed to save a life and reduce morbidity. A quality system should be in place in the blood service (and in the hospital), to standardise the recruitment and selection of safe donors, the safe collection of blood donations, the correct processing and thorough testing of donated blood, the management of blood stocks and its appropriate storage and transportation, the crossmatching of appropriate blood for patients, the correct identification of the recipient and the effective monitoring of the recipient during and after the transfusion. An effective quality system focuses on the critical control points of this transfusion chain, to maximise patient safety.

Learning objectives

By the end of this section, the student should be able to describe the risks of transfusion and the basic components of a haemovigilance system. From the viewpoint of the technologist, the student should be able to discuss the following:

- Transfusion reactions
 - Classification of transfusion reactions
 - Signs and symptoms of acute transfusion reactions
- Subtypes of acute transfusion reactions
 - Acute haemolytic transfusion reaction (AHTR)
 - Allergic reaction
 - Anaphylactic reaction
 - Febrile non-haemolytic transfusion reaction (FNHTR)

- Septic transfusion reaction (Transfusion transmitted bacterial infection)
- Transfusion-associated circulatory overload (TACO)
- Transfusion-related acute lung injury (TRALI)
- Subtypes of delayed transfusion reactions
 - Delayed haemolytic transfusion reaction (DHTR)
 - Delayed serologic transfusion reaction (DSTR)
 - Transfusion-associated graft-versus-host disease (TA-GVHD)
 - Transfusion transmitted disease (TTD)
 - Transfusion-related immune modulation
 - Transfusion-associated iron overload (transfusion haemosiderosis)
 - Post-transfusion purpura
- Other risks of transfusion
 - Citrate toxicity
 - Potassium toxicity
 - Hypothermia
 - Haemolysis not related to red cell antibodies
- Reporting transfusion reactions and laboratory investigations
- Haemovigilance
 - Biovigilance
 - Donor haemovigilance
 - Patient haemovigilance
 - Monitoring, evaluation and reporting
- Hospital transfusion committee
- National haemovigilance system

What is 'safe blood'?

Safe blood is blood that benefits the patient and does not have any detrimental effect when transfused. The ongoing challenge for the blood service is to put measures in place and maintain them to ensure that preventable detrimental effects from transfusion – especially those that occur mainly as a result of poor management or process design – are avoided. Examples include transfusion of ABO mismatched or expired blood. The ongoing challenge for the clinical staff in the hospital is to transfuse appropriately, to obtain informed consent for the transfusion and ensure that patients are not misidentified, either when a sample is taken for crossmatch, or when a transfusion is started. It is very important to remain vigilant and monitor the

patient closely during the blood transfusion for signs and symptoms of an adverse reaction so that action can be taken promptly to minimise harm.

Patients' understanding of benefits and risks of transfusion

The prescribing clinician should discuss the need for a transfusion with the patient, so that there is clear understanding of both benefits and risks, that the risks of transfusion are put into perspective with risks of not receiving the transfusion, and that the transfusion when needed is necessary and will be of benefit that outweighs the risks involved. The patient should be provided the opportunity to ask questions and gain clarification so that before the transfusion is undertaken, there is reassurance that he/she has been part of the decision. This process of gaining the patient's approval for transfusion is called 'informed consent'. In some countries a written 'informed consent' form, signed by the patient, is required.

Transfusion reactions

Classification of transfusion reactions

The general classification of transfusion reactions is based on the time between the transfusion and the onset of the reaction. Transfusion reactions can be acute or delayed. Acute transfusion reaction occurs within 24 h of the commencement of the transfusion, while delayed transfusion reaction occurs after 24 h. Table 1 describes the general classification and subtypes of acute transfusion reactions. Table 2 describes the general classification and subtypes of delayed transfusion reactions. The ISBT Haemovigilance Working Party has developed a classification system for transfusion reactions, available on the ISBT website, <https://www.isbtweb.org/working-parties/haemovigilance/>. Use of standardised definitions assists in comparison of reaction rates in each country over time and allows benchmarking with international rates.

Table 1 Subtypes of acute transfusion reactions

Classification of acute transfusion reactions
Acute haemolytic transfusion reaction (AHTR)
Allergic reaction
Anaphylactic reaction
Febrile non-haemolytic transfusion reactions (FNHTR)
Septic reaction (Transfusion transmitted bacterial infection)
Transfusion-associated circulatory overload (TACO)
Transfusion-related acute lung injury (TRALI)

Table 2 Subtypes of delayed transfusion reactions

Classification of delayed transfusion reactions
Delayed haemolytic transfusion reaction (DHTR)
Delayed serologic transfusion reaction (DSTR)
Transfusion-associated graft-versus-host disease (TA-GVHD)
Transfusion transmitted disease (TTD)
Transfusion-related immunomodulation (TRIM)
Transfusion-associated iron overload (transfusion haemosiderosis)
Post-transfusion purpura (PTP)

Signs and symptoms of acute transfusion reactions

Acute reactions can present with a range of signs and symptoms. Many of the signs and symptoms of acute transfusion reactions are similar and overlap, and some features can be obscured by the patient's underlying condition or treatment. Thus, all signs and symptoms should be taken seriously. The facility should educate its health-care workers on the identification, recording, management and reporting of adverse events in blood transfusion recipients.

Note that a 'sign' is evidence of a disease or other ailment that can be identified by individuals other than the patient, such as a cough, or a skin rash. A 'symptom' is evidence of a disease or other ailment that can only be identified by the patient, such as a headache, back pain or fatigue.

The most commonly encountered reactions are febrile and minor allergic reactions, whereas the reactions that cause the most concern are transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), acute haemolytic transfusion reaction (AHTR), septic reaction (transfusion transmitted bacterial infection) and transfusion-associated graft-versus-host disease (TA-GVHD) all of which may be serious and life-threatening.

An important concern is the accidental administration of ABO incompatible red cell units, which is likely to have serious consequences. Every precaution should be taken, from the time of withdrawing the pre-transfusion blood specimen from the patient, during crossmatch procedures, until the time of issue of the unit and the initiation of the transfusion, to ensure that no error in patient identification or unit selection was made. It is in vain that all laboratory protocols are correctly completed, all unit numbers correctly transcribed, all tests carefully done and interpreted, and units selected that are found compatible, if the wrong patient's sample was used.

The most important point to remember about any acute transfusion reaction is that its severity is nearly always related to the volume transfused. Therefore, whenever a

reaction is suspected the transfusion should be stopped immediately. This crucial first step applies to all acute reactions. Visual assessment of the component and a clerical check to ensure compatibility is required. Treatment to control the reaction must be immediate and is the responsibility of the clinician in charge of the patient and is based on the patient's signs and symptoms.

Information on further treatment given in this section is for the general information for the technologist and must not be seen as prescriptive in any way. All transfusion reactions should be reported to the blood service or blood bank in the hospital. Transfusion reactions can affect adults, children and neonates and recognition is more challenging in paediatric patients.

Subtypes of acute transfusion reactions

Acute haemolytic transfusion reaction (AHTR)

Acute haemolytic transfusion reaction occurs secondary to a mismatched transfusion due to ABO incompatibility, when a patient inadvertently receives blood of the incorrect, incompatible ABO blood group. The reaction involves antibodies in the recipient that react with antigens on the incoming red cells. This is termed a major incompatibility. For example, if a Group O patient is transfused with Group A red cells, the patient's anti-A antibodies will immediately react with the transfused red cells, leading to intravascular haemolysis. The complement and coagulation pathways will be activated, and cell lysis will occur. Various cytokines such as interleukins are released during a haemolytic transfusion reaction and are responsible for many of the clinical effects of the reaction. The activation of the coagulation cascade and thrombin generation predisposes the patient to disseminated intravascular coagulation (DIC). When free haemoglobin is released directly into the bloodstream as a result of immune haemolysis, it is likely to cause renal damage.

Acute haemolytic transfusion reaction occurs as the result of clerical error, improper sample labelling or patient misidentification either at time of collection of pre-transfusion sample or before the actual transfusion. Although they are most likely to occur in emergency situations when personnel are under extreme pressure and are more likely to make mistakes, such errors may also occur during other times due to non-adherence to existing policies and procedures. An adverse reaction may also be seen when plasma with high titre or haemolysing ABO antibodies is transfused into a patient of a compatible but non-identical ABO blood group, such as donor Group O plasma with high titre or haemolysing anti-A antibodies being transfused into a Group A recipient. An adverse reaction may also occur when the donor plasma contains

strong acquired antibodies of other specificities, to which the recipient has the corresponding antigen. These adverse reactions are termed minor incompatibilities and are seldom seen.

Acute haemolytic transfusion reaction may also be caused by non-ABO red blood cell antibodies in a patient who is previously immunised. This can occur in a patient with red cell alloantibodies that are below the level detected by the antibody screen, or due to a clerical or technical error during patient's antibody screening or during compatibility testing, or failure to detect red blood cell antibodies due to limitation of the laboratory assay or use of un-crossmatched blood in a patient already alloimmunised.

Signs and symptoms

The signs and symptoms that suggest a haemolytic transfusion reaction include the following:

- Fever.
- Chills, rigors.
- Facial flushing.
- Haemoglobinuria.
- Chest pain.
- Abdominal pain.
- Back/flank pain.
- Nausea or vomiting.
- Burning sensation at the site of infusion.
- Sudden drop in blood pressure.
- Feeling of anxiety.
- Pallor and jaundice.
- Oliguria and/or anuria.
- Diffuse bleeding.
- Dark urine.
- Shock.

Another complication of AHTR is disseminated intravascular coagulation (DIC). It occurs because of the presence of thrombin in the bloodstream, causing fibrin formation in the microcirculation. As a result, clotting factors such as fibrinogen, prothrombin, and FV and FVIII are consumed, as well as platelets. The result is widespread bleeding. The condition can be very serious and may require the transfusion of fresh plasma to replace consumed clotting factors. If the fibrinogen level falls very low, it may be necessary to transfuse cryoprecipitate as a source of concentrated fibrinogen.

Action and treatment.

- The transfusion must be stopped immediately, and the needle withdrawn from the vein of the patient, to prevent any further infusion of cells, and the reaction reported promptly to the attending clinician and the blood bank.

- Intravenous (IV) fluids and inotropes (medication that change the force of heart contraction) to maintain blood pressure and urine output, is often required.
- DIC is managed as clinically indicated.
- Nursing staff must remain constantly at the bedside of the affected patient, who should not be left alone.
- An immediate clerical check must be carried out at the bedside, to confirm a match or to detect a mismatch between component that was being transfused, and recipient, i.e. details on record for the patient, details on the compatibility label of the component and details on the original blood request form.
- Visual inspection of the component should also be done immediately to check for abnormality.
- The transfusion reaction should be reported to the blood bank and blood remaining in the bag and tubing (which must be clamped to prevent leakage) despatched to the blood bank, along with a post-transfusion sample of blood from the recipient.
- The laboratory must carry out an investigation including the following tests:
 - ABO group on pre- and post-transfusion samples.
 - Antibody screening tests and full crossmatch on pre- and post-transfusion samples.
 - Direct antiglobulin test (DAT) on post-transfusion sample.

A post-transfusion urine sample should be sent for urinalysis. See *Section 13: Compatibility testing*, for further information on laboratory investigation of possible AHTR.

Prevention.

- Adherence to policies in patient identification and in labelling tubes during sample collection.
- Adherence to compatibility testing procedures in the laboratory, including the critical step of checking that the correct sample is used and that the correct components are selected and tested.
- Adherence to policies to verify the identity of the patient before transfusion, including asking the patient for his/her name and checking the details on the wristband.
- Adherence to policies related to checking component label against patient details to confirm that the correct unit is going to be transfused.

Allergic reaction

Allergic transfusion reactions are common. The cause is unclear but relates mainly to factors in the plasma portion of the component. For example, if the recipient is

allergic to an allergen present in the donor blood, then an allergic reaction could occur as a result of the transfusion. An allergic reaction could also occur if the donor has the allergy and the donation therefore contains the antibody, and the recipient has the corresponding allergen. Symptoms are caused by mediators such as histamine released on activation of mast cells and basophils. Immune complexes as a result of antigen–antibody reaction initiated either by recipient or donor derived allergens probably stimulate tissue mast cells to release histamine, which results in vasodilatation and skin rash. This usually occurs during, or a few hours after the transfusion and can be minor or severe.

Signs and symptoms

- Signs and symptoms of minor (non-severe) allergic reactions include:
 - Localised itchy red skin rash (urticaria/hives).
 - Mild cough or wheeze.
 - Swelling of the lips and tongue.
 - Itchiness, swelling and redness of the eyes.
- Signs and symptoms of more severe reactions include:
 - Widespread skin rash usually occurring during or very shortly after transfusion.
 - Difficulty in breathing, coughing, wheezing and hypoxaemia.
 - Swelling of the lips and tongue.
 - Tightness in the throat, difficulty in speaking and swallowing.
 - Drop in blood pressure.
 - Nausea and vomiting.
 - Generalised flushing or anxiety.

Action and treatment

- Minor (non-severe) allergic reaction:
 - The transfusion should be paused without removing the needle from the vein of the recipient.
 - Antihistamine (e.g. diphenhydramine) should be administered by the attending clinician.
 - If the hives/rash subside and there are no symptoms suggesting a severe allergic reaction, the transfusion may be cautiously resumed at a slow rate under direct observation. If symptoms recur or if additional symptoms appear, the transfusion should be discontinued.
 - The transfusion reaction should be reported to the blood bank. However, post-transfusion samples and the implicated unit do not need to be submitted to the laboratory for testing.
- Severe allergic reaction:
 - The transfusion should be discontinued.

- Antihistamine should be given. Corticosteroids might also be needed.
- In the case of breathing difficulty or oedema, adrenalin should be administered. Clearing the airway is essential.
- Support measures should be taken as necessary.
- The transfusion reaction should be reported to the blood bank. However, post-transfusion samples and the implicated unit do not need to be submitted to the laboratory for testing.

Prevention

If allergic reactions are recurrent or severe, pre-medication with antihistamine with or without steroids is indicated. Transfusion with washed red cell units can be considered.

Record keeping

The details of the allergic reaction must be documented by the clinician, and by the laboratory to which the reaction was reported, for reference. This record forms part of the history of the recipient and may need to be retrieved and consulted in the future.

Anaphylactic reaction

This is a severe allergic reaction that occurs with subsequent exposure to an immunogen that initially stimulated the production of IgE antibodies. Anaphylactic reactions are rare and sometimes unexplained. Anaphylactoid reactions resemble anaphylactic reactions clinically but are caused by an IgE mediated response. The terms are sometimes used interchangeably.

Individuals with IgA deficiency may develop anti-IgA antibodies (as a result of an earlier/historical transfusion or pregnancy), which can induce complement-mediated anaphylactic reactions to transfusions of even small volumes of plasma or blood containing IgA protein. Similarly, hepcidin-deficient individuals (mainly of Asian origin) may experience anaphylaxis on exposure to hepcidin (a regulator of the entry of iron into the circulation) in transfused blood.

Transfusing blood containing an allergen such as penicillin or aspirin to a sensitised patient may cause anaphylaxis.

Signs and symptoms

Anaphylaxis is a severe, life-threatening reaction. Features include:

- Widespread skin rash, oedema, pruritis and urticaria (hives).

- Angioedema.
- Breathing compromise including tightness in the throat, hoarseness, stridor, wheezing, chest pain and dyspnoea.
- Circulatory compromise, hypotension and syncope/collapse.
- Abdominal pain, nausea and vomiting.
- Death can occur within a short period of time, as breathing becomes impossible.

Action and treatment

- The transfusion must be stopped immediately, and the reaction promptly reported to the attending clinician.
- Airway and blood pressure should be maintained.
- Resuscitation with IV fluids, oxygen, adrenalin (epinephrine), antihistamine, bronchodilators and corticosteroids.
- The transfusion reaction should be reported to the blood bank. However, post-transfusion samples and the implicated unit do not need to be sent.
- Testing for IgA deficiency is to be considered.

Prevention

- Pre-medication with antihistamine and steroids prior to future transfusions.
- Minimise the plasma content in the red cell units by washing, or using platelets stored in additive solutions if available.
- Future transfusions must be directly and closely monitored and performed in clinical areas with resuscitation facilities.
- If the patient was found to be IgA deficient with anti-IgA antibodies, future transfusions are required to be from IgA-deficient donors if available. In the event that such donors are not available, red cell units for further transfusions should be washed prior to use to remove all traces of IgA.

Febrile non-haemolytic transfusion reaction (FNHTR)

In FNHTR, the recipient develops fever compared to baseline pre-transfusion temperature. This finding can be due to the transfusion itself or may be due to the patient's underlying medical condition or treatment. It is important to exclude other more serious transfusion reactions such as AHTR and septic reaction (transfusion transmitted bacterial infection). The pathophysiology is likely to be multifactorial including the patient's reaction to white blood

cells or other substances in the component being transfused.

Signs and symptoms

Clinical features include:

- Fever.
- Chills or rigors – with or without fever.
- Nausea.
- Headache.

Action and treatment

- Transfusion should be stopped.
- Supportive care with paracetamol or acetaminophen.
- The transfusion reaction should be reported to the blood bank.
- Further investigations with repeat antibody screen and crossmatch should be considered.
- If a serious reaction is excluded, and the reaction subsides, transfusion can be resumed slowly. If there is no improvement or worsening of symptoms, transfusion should be discontinued.

Prevention

- Leucoreduction (especially pre-storage) of cellular blood components reduces the rate of FNHTRs. If fever is persistent or leucoreduction is unavailable, washed red cell units may be considered although efficacy has not been proven.
- Patients with severe or recurrent FNHTR may benefit from pre-medication with paracetamol given an hour prior to subsequent transfusions although there is not sufficient evidence to support this practice.

Septic transfusion reaction (Transfusion transmitted bacterial infection)

Blood is an ideal medium for the growth of harmful bacteria. Blood components with the greatest risk of bacterial contamination are platelet concentrates, as platelets are stored at a higher temperature, i.e. $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$, which may promote the more rapid growth of contaminating bacteria.

Septic reactions related to red cell or whole blood transfusions are likely to be caused by bacteria capable of growing at low temperatures. Because platelets are stored at room temperature, they support the growth of many more bacterial species. About two thirds of implicated bacteria are gram-positive, and most often originate from the donor's skin. Gram-negative organisms may also be implicated, such as *Yersinia enterocolitica*, a micro-organism that may be present as a subclinical infection in the

bloodstream of the donor at the time of donation. This organism multiplies readily at low temperatures, and produces endotoxins, i.e. toxins which are released from an organism when it disintegrates. Endotoxins may cause extremely severe reactions and lead to the death of the recipient.

The contamination of a donation with gram-positive *Staphylococcus* or *Streptococcus* from an unclean venepuncture site may lead to a serious or fatal reaction, as may *Pseudomonas*, which is a widespread gram-negative microbe that flourishes at low temperatures.

Blood components may be contaminated by:

- Unrecognised bacteraemia in an apparently healthy donor.
- Introduction of micro-organisms at the time of donation as a result of an inadequately cleaned venepuncture site, or contamination of the phlebotomy needle at the time of venepuncture.
- Contamination of the component during preparation or handling as a result of faulty equipment or blood bags.
- Contamination of the component during storage or transportation as a result of non-adherence to the recommended storage or transportation temperatures.

Although bacterial contamination of a unit of blood may be obvious on inspection, there may be no visible changes. Red cell units may be dark purple to black in appearance and may contain clots or bubbles. Cloudiness or grey discoloration may be a sign of a contaminated unit of platelet concentrate. Plasma units may show increased opacity (cloudiness). It is crucial to routinely inspect all units during processing, before issue from the blood bank and before transfusion to check for discoloration, particles, clumps or signs of leakage. Some blood services have the facility to screen all platelet concentrates for bacteria prior to issue, and discard those that indicate contamination, preventing the transfusion of an infected unit. Other blood services have introduced pathogen inactivation technologies.

Definitive diagnosis of septic transfusion reaction requires isolation of the same organism from the component and the patient.

Signs and symptoms

There may be no immediate signs of bacterial infection of the patient after transfusion, if the bacterial load in the unit is small. However, most septic reactions occur during or within 4 h of transfusion. Gram-negative infections typically present within 15 min of the start of the transfusion. Clinical features include:

- Fever, chills and rigors.

- Tachycardia and tachypnoea (abnormally rapid rate of breathing).
- Nausea and vomiting.
- Dyspnoea (difficulty in breathing).
- Low blood pressure and shock.
- DIC.

Action and treatment

- The transfusion should be stopped immediately, and details of the reaction reported promptly to the attending clinician.
- The transfusion reaction should be reported to the blood bank.
- Blood remaining in the implicated unit must be returned to the blood bank for gram stain and culture (aerobic and non-aerobic).
- After collection of necessary samples, the recipient should be given broad spectrum antibiotics without delay.
- As soon as possible, testing should be carried out by the laboratory, on the remaining blood component, if available, and on the recipient. This may include Gram staining of the remaining blood component, and blood cultures of the recipient and the remaining blood component. Should a clinically significant micro-organism be identified, sensitivity tests may be done to determine the most effective course of antibiotic treatment to clear the infection. Intravenous fluids and inotropes might be required to provide cardiovascular support and maintain urine output.
- The transfusing hospital should inform the blood collection centre, so that any other components from the same donor(s) can be quarantined and discarded. In addition, any recipients of other components need to be identified and followed up.

Prevention

To minimise the risk of bacterial contamination, the following measures are undertaken (see *Section 8: Blood donation*, for more details):

- Donor screening should include questions about recent infections, and donor temperature may be taken.
- Prior to donation, the venepuncture site should be effectively disinfected according to standard operating procedure (SOP), to reduce the risk of bacterial contamination by skin flora.
- The first 10-50 ml of blood drawn from the donor should be diverted into an integrally connected

pouch to minimise the risk of contaminating the blood unit.

- Blood units should be maintained at appropriate storage and transportation temperatures.
- Blood units should be inspected before issue and before transfusion and should not be used if they appear faulty.
- Platelet units may be cultured prior to issue to test for contamination.
- Pathogen reduction systems may be introduced as the most effective way to eliminate the growth of all viruses, bacteria and parasites.

Transfusion-associated circulatory overload (TACO)

Transfusion-associated circulatory overload is characterised by pulmonary oedema due to fluid overload of the recipient. This occurs due to rapid transfusion or a high volume of transfused components. Patients at risk include young children, the elderly and patients who are already in cardiac failure, volume overload or renal dysfunction and have difficulties tolerating an increase in blood volume. Symptoms may occur at any time within the first 12 h of transfusion and may lead to the death of the patient.

Signs and symptoms

Clinical presentations include:

- Acute or worsening respiratory distress.
- Dyspnoea, orthopnoea (difficulty in breathing when in a prone position).
- Cough with frothy sputum (can be pinkish).
- Cyanosis.
- Fever.
- Increased heart rate.
- Increased blood pressure.
- Evidence of volume overload.
- Bilateral pulmonary oedema on chest X-ray.

Action and treatment

- The transfusion should be stopped immediately, and the reaction promptly reported to the attending clinician.
- High-flow oxygen should be initiated.
- Diuretics to be given if the patient is haemodynamically stable and volume overloaded.
- The transfusion reaction should be reported to the blood bank.
- To relieve life-threatening symptoms, it may be necessary to withdraw blood from the recipient.

Prevention

- Pre-transfusion assessment of the patient for clinical features that would predispose to volume overload.
- Close monitoring of vital signs.
- Avoiding transfusion of more than one unit at a time.
- Slowing the rate of transfusion in high risk patients (to a maximum of 4 h for red cell units).
- Consider diuretics prior to transfusion into high risk patients.
- The unit to be transfused may be split - using a closed system, into smaller aliquots (bags) which continue to be stored at the appropriate storage temperature. Each aliquot can be administered slowly over three to 4 h, thereby increasing the time over which the transfusion is administered.

Transfusion-related acute lung injury (TRALI)

Transfusion-related acute lung injury is a non-cardiogenic pulmonary oedema that may occur as a complication following blood transfusion. Although all types of blood components have been implicated, plasma is most commonly the cause. This reaction is caused by anti-human leucocyte antibodies (anti-HLA) or anti-neutrophil antibodies (anti-HNA) in donor's plasma formed as a result of the sensitisation of donors due to previous pregnancy (in multiparous female patients) or blood transfusion. Plasma containing these antibodies may activate neutrophils within the pulmonary microcirculation of the recipient, and cause lung injury as a result. This often occurs in patients with critical underlying illness, surgery or severe sepsis. With TRALI, patients present with new lung injury within 6 h of transfusion, which usually resolves within 96 h of transfusion.

Signs and symptoms

Clinical features include:

- Acute difficulty in breathing.
- Severe hypoxemia.
- Hypotension.
- Tachycardia.
- Fever.
- No evidence of circulatory overload.
- Bilateral interstitial lung infiltrates on chest X-ray.
- No other risk factors that can explain the patient's presentation.

Action and treatment

- The transfusion should be stopped immediately, and the reaction promptly reported to the attending clinician.
- Cardiovascular and airway support is required.
- High-flow oxygen should be initiated.
- Some patients may require mechanical ventilation.
- The transfusion reaction must be reported to the blood bank to quarantine/recall all other components made from the implicated donation and to make decisions on donor deferral.

Prevention

- Because of the severity of TRALI as a reaction, with fatality a real possibility, some blood centres do not produce plasma for transfusion from donors at risk of white cell antibodies, such as multiparous women.
- Donors confirmed to be implicated in an episode of TRALI are permanently deferred.

Subtypes of delayed transfusion reactions

Delayed haemolytic transfusion reaction (DHTR)

Delayed haemolytic transfusion reaction refer to haemolytic anaemia as a result of red cell incompatibility that becomes apparent between 24 h and 28 days post-transfusion. Recipient red cell antibodies may have been too weak for detection during antibody screening or pre-transfusion crossmatching, and incompatible red cell units inadvertently transfused. This causes an immune response in the recipient. Nothing untoward is noted immediately after the transfusion, and the reaction is often suspected when the haemoglobin fails to increase after transfusion.

Kidd antibodies are commonly implicated in DHTR. Kidd antibodies (such as anti-Jk^a) are likely to fall below detectable levels over time, so if a patient with undetectable Kidd antibodies needs a subsequent transfusion, DHTR may occur if the patient is transfused with Kidd positive units. This demonstrates the importance of good laboratory records. Whenever a request for blood is received for a patient who previously had a transfusion, the records should be consulted to determine whether an antibody was previously detected and identified and if so, antigen-negative blood units should be selected for crossmatch even if the antibody is no longer detectable at the time of request. Other antibodies that are commonly implicated in DHTR are anti-E, -C, -Fy^a and -K.

Signs and symptoms

Signs and symptoms of DHTR are similar to AHTR, but less severe. Most DHTR present with hyperbilirubinaemia and inadequate rise of post-transfusion haemoglobin level or unexplained fall in haemoglobin are often the only apparent anomalies after a blood transfusion. However, life-threatening haemolysis with severe anaemia and renal failure may occur.

Clinical features include:

- Anaemia.
- Reticulocytosis.
- Spherocytosis.
- Raised bilirubin.
- Raised lactate dehydrogenase (which is an indicator of tissue damage).
- Positive antibody screen.
- Positive direct antiglobulin test.
- Renal failure if haemolysis is severe.

Treatment

- The untoward reaction should be reported to the blood bank and blood residue remaining in the unit (s) returned to the blood bank along with a post-transfusion sample. Repeat investigations as per SOP, should be done, including blood group, antibody screen, crossmatch and direct antiglobulin test.
- Clerical information must also be checked, related to the patient, compatibility label of the unit(s) transfused, and original blood request form.
- A urine sample from the recipient should be sent for urine analysis.
- In cases with severe haemolysis with renal damage initial treatment should involve the maintenance of urinary output.
- If further transfusion is required, the patient should be transfused only with blood which is antigen-negative for the offending antibody, and which is cross-match compatible.

Prevention

- Maintain and retrieve blood bank records of all red cell antibodies and the patient's extended red blood cell phenotype.
- Use an antibody screening methodology with maximum sensitivity so that weak or developing antibodies that may otherwise be missed are detected. See *Section 13: Compatibility testing*, for more details of how to select appropriate red cells for transfusion.
- Some centres make use of an antibody card system for patients on chronic transfusion support. The card, which the patient keeps as a record to present to

medical authorities, indicates ABO/Rh type, the presence of irregular, clinically significant antibodies, and special blood that may be required for transfusion. This is helpful in the event that such patients require transfusions in centres other than their own.

- Extended red blood cell antigen matching prior to the outset of transfusion therapy, so that the patient receives blood that does not contain immunogenic antigens that he/she lacks, reduces the likelihood of alloimmunisation over time, in chronically transfused patients, e.g. individuals with sickle cell disease.

Delayed serologic transfusion reaction (DSTR)

When a new, clinically significant red cell antibody is detected in a recipient after transfusion, and when there is no clinical evidence of *in vivo* haemolysis, as there is in cases of DHTR, it may be concluded that alloimmunisation has occurred. A DAT carried out on the recipient may be positive, which indicates that the newly formed antibody has reacted with transfused donor red cells. Alloimmunisation to red cells or platelets may complicate further transfusions and make it increasingly difficult to find compatible blood components in the future, for the patient concerned.

Prevention of DSTR, in patients facing long-term transfusion therapy prospective antigen matching so that cells selected for crossmatch and transfusion do not contain antigens which are good immunogens and which the prospective recipient lacks.

Transfusion-associated graft-versus-host disease (TA-GVHD)

Transfusion-associated graft-versus-host disease is a rare complication of transfusion in which donor T-lymphocytes initiate an immune response to recipient lymphoid tissue. Donor lymphocytes are usually recognised as foreign and are therefore destroyed on entering the bloodstream of the recipient. However, if the immune system of the recipient is compromised and lacking functional T cells, such as in patients with congenital immunodeficiency or in patients on cancer treatment, this may not happen. Additionally, immunocompetent patients are also at risk when receiving cellular components from a donor with whom the recipient shares an HLA haplotype and where the donor is HLA homozygous for that haplotype. In this situation, donor white cells are not recognised as foreign and are able to proliferate in the recipient, causing TA-GVHD. This is far more likely to occur when the recipient receives blood components from a first- or

second-degree relative, or in populations where there is limited HLA diversity.

Signs and symptoms

The signs and symptoms occur 5–10 days after transfusion. Although TA-GVHD attacks the liver, skin and/or gastrointestinal tract, the high mortality rate is usually the result of involvement of bone marrow tissue leading to cytopenia and susceptibility to infections. In such instances, the mortality rate is over 90%. Death is often attributed to pancytopenia, i.e. deficiency of all cellular components, and infection.

Clinical features include:

- Fever.
- Skin rash.
- Abdominal pain.
- Severe diarrhoea.
- Nausea and vomiting.
- Liver dysfunction.
- Pancytopenia with bone marrow aplasia.

Action and treatment

- The transfusion reaction should be reported to the blood bank.
- Supportive care.

Prevention

Patients at high risk require irradiated blood components. In this way, donor lymphocytes are prevented from forming antibodies against the host, and from causing immunological destruction of the host. Local procedures should be followed in providing irradiated blood to at-risk patients. These include:

- Patients with congenital immune deficiency.
- Fetal intrauterine transfusion.
- Transfusion of preterm, low birth weight neonates.
- Neonatal exchange transfusion.
- Patients on high dose chemotherapy or radiotherapy.
- Patients undergoing stem cell transplantation.
- Recipients of directed transfusion from first- or second-degree relatives, i.e. family members.
- Patients with advanced Hodgkin's lymphoma.
- Recipients of HLA-matched platelets.
- Cancer patients being treated with purine analogues (e.g. fludarabine), purine antagonists (e.g. bendamustine), alemtuzumab and anti-thymocyte globulin.

Transfusion transmitted disease (TTD)

Should the recipient of a blood transfusion develop an infection which could possibly have been transmitted by the blood transfusion, the attending clinician should

consider that it could be a TTD, and notify the blood service. A look-back investigation should be initiated by the blood service and the donors whose donations were used should be traced and retested for the infectious agent concerned. It is important that such investigations are carried out by authorised personnel only, that they are carried out with discretion, and that confidentiality of all parties is maintained. See *Section 8: Blood donors*, for more information on donor selection steps and *Section 10: Donation testing*, for more information about the testing performed on donor blood to reduce the risk of TTIs.

Micro-organisms with potential to cause TTD

See *Section 10: Donation testing and transfusion transmissible infections* for pathogens that may be transmitted by transfusion.

Transfusion-related immune modulation (TRIM)

Studies have shown that transfusion may enhance the survival of renal allografts, increase the recurrence of cancers and increase the incidence of post-operative infection. Red cell components contain many immunomodulatory mediators that interact with and alter the immune system. These can lead to pro-inflammatory and immunosuppressive effects even in pre-storage leucoreduced blood components. The mechanism of TRIM is unclear and likely to be multifactorial. Some mediators are related to white cells in the unit, while others can be related to other contents such as cytokines, free haem, active lipids and platelet derived factors. Pre-storage leucoreduction to produce leucoreduced components might mitigate TRIM and improve clinical outcome.

Transfusion-associated iron overload (transfusion haemosiderosis)

Transfusion-associated haemosiderosis is caused by excess deposits of iron in the body, arising from the breakdown of haemoglobin. This is a long-term complication of individuals on chronic transfusion support, such as patients with thalassaemia. The body does not excrete iron when aged red cells are removed from circulation but stores it for reuse. Excess iron accumulates around the internal organs (such as the liver and heart) and may become life-threatening. To prevent this, iron-chelating agents may be administered to such patients to promote the excretion of iron.

Post-transfusion purpura (PTP)

Post-transfusion purpura is an uncommon reaction, being the result of recipient antibodies reacting with antigens on transfused platelets. Most cases occur in multiparous women who have developed platelet antibodies as the result of past pregnancies. Alternatively, the transfusion of platelets stimulates an immune response in the recipient and in such cases, the PTP reaction is delayed for about a week before thrombocytopenia develops, with a drop in platelet count. In rare cases, a PTP reaction may be fatal.

A PTP reaction usually occurs in patients whose platelets lack the HPA-1a or HPA-5b antigen and because of pregnancy or transfusion, have developed the corresponding immune antibodies. It is unclear why such alloantibodies attack both donor and self-derived platelets.

A PTP reaction is often sudden in onset and self-limiting, resolving within 2 weeks. Treatment with intravenous immunoglobulin is often the treatment of choice.

Other risks of transfusion

Citrate toxicity

Citrate is the anticoagulant used in blood components. Citrate from transfused blood is rapidly metabolised by the liver of the recipient and excreted in the urine. The ability to metabolise citrate is reduced in liver disease, hypothermia, in neonates, infants and in trauma patients or apheresis donors, where large volumes of citrated blood enter the circulation. Features include hypotension, a taste of bitterness in the mouth, shivering, nausea, perioral (around the mouth) and peripheral numbness, tetany (body stiffening) and arrhythmia. Such reactions may be treated with giving calcium chloride or calcium gluconate. Prevention during apheresis procedures can be achieved by concurrent administration of calcium.

Potassium toxicity

Potassium levels increase in stored blood as potassium from within the red cells diffuses into the surrounding plasma. The transfusion of large volumes of stored blood can cause life-threatening hyperkalaemia, particularly in neonates. In addition, this may become a hazard in patients who are already hyperkalaemic – such as patients with impaired renal function. Contributing risk factors include longer storage age of red cell units, irradiation of red cell units, rate and volume of red cell components transfused, younger patient age and presence of other co-morbidities. Increased potassium levels can

result in cardiac arrest. At-risk patients, such as neonates, should therefore be given fresh blood (<7–10 days old) if they are receiving large volume transfusions. Infusion rates in neonates and children need to be controlled.

Hypothermia

Blood administered rapidly and in large volumes, can lead to hypothermia. Trauma patients and neonates undergoing exchange transfusion are at high risk of hypothermia. Low body temperature may inhibit citrate and lactate metabolism, interfere with normal haemostasis and platelet function, and impair oxygen delivery due to vasoconstriction. Severe hypothermia can result in cardiac arrest.

The core temperature of the patient needs to be monitored and managed in a massively bleeding patient. When a patient becomes hypothermic, several warming devices (e.g. warming blankets) to increase the patient's core temperature should be used. In high risk cases, an approved and properly maintained in-line blood warming device is required.

Haemolysis not related to red cell antibodies

Haemolysis might occur in the following settings:

- Use of hypotonic solution administered with red cell transfusion.
- Malfunction of cell saver or blood warmer.
- Over-heating of red cell units due to inappropriate storage.
- Red cell units which have passed their expiry date at the time of infusion.
- Accidental freezing of red cell units in a freezer or if in direct contact with ice during transportation.

Reporting transfusion reactions and laboratory investigations

The reporting of transfusion reactions to the blood bank is essential. It directs immediate actions when haemolytic transfusion reactions, septic reactions or TRALI are suspected, and also contributes to the haemovigilance system (see *Haemovigilance/biovigilance* information, in a following sub-section). The process of reporting should follow local procedures. There must be an SOP and information document on how to handle transfusion reactions in the laboratory. At minimum, the investigation should include:

- A clerical check of all relevant transfusion records.
- Visual inspection of returned blood or blood component transfused (if available) to check for labelling, integrity and any evidence of haemolysis or bacterial contamination.

- Visual inspection of post-transfusion specimen for haemolysis.
- Determination of ABO and D types of both pre-and post-transfusion specimens and transfused units, if available.
- Compatibility testing using both pre-and post-transfusion specimens.
- Direct antiglobulin test on both pre-and post-transfusion specimens.

If AHTR is suspected, the first voided urine sample obtained from the patient should be assessed for the presence of haemoglobinuria. If there is suspicion of septic reaction (transfusion transmitted bacterial contamination), blood cultures should be obtained from the patient and the implicated unit and a gram stain can be done on the implicated unit. Results of the evaluation should be reported to the treating clinician and recorded in the transfusion record of the patient.

The clinician must be notified as soon as possible of findings, so that appropriate treatment may be initiated without delay.

Haemovigilance/biovigilance

Haemovigilance is a set of surveillance procedures for the assessment of adverse events along the entire transfusion chain, from blood donation, testing, processing, storage, transportation and transfusion into patients. It includes monitoring, investigating, analysing and reporting of adverse events at any link along this chain, and taking preventive action to avoid recurrence.

When the concept of haemovigilance was first introduced, the focus was on transfusion reactions, their classification and cause, with a view to improving transfusion safety. This concept has developed to include all links in the transfusion chain. The goal of haemovigilance is continuous quality improvement through corrective and preventive actions to improve donor and patient safety, improve transfusion appropriateness, and reduce wastage. It includes examining all aspects of the transfusion chain to identify weak links and strengthen them, identify trends in adverse events and reactions to improve donor and patient safety, correct problems, raise awareness and prevent hazards. As part of a haemovigilance programme, a uniform and systematic method for reporting and evaluation of transfusion reactions should be implemented.

Biovigilance is a broader scheme that extends the term haemovigilance beyond blood to describe an integrated national approach to the collection of data on the use and impact of all medical products of human origin (such as tissue, organs and cells of transplantation), their analysis and evaluation, plus comprehensive reporting on outcomes

that would include statistics on organ transplantation and the use and effect of blood components. Pharmacovigilance is the specific term used for the monitoring and assessment of adverse events associated with medication. There may be an overlap between biovigilance and pharmacovigilance (e.g. plasma derivatives may be included in either haemovigilance or pharmacovigilance).

Ideally, the haemovigilance programme should be nationally coordinated and fully representative of all fields related to blood transfusion. There should be a robust, confidential, anonymised, non-punitive approach so that all participants feel free to report incidents and accidents without fear of reprisal. This may be carried out by giving ongoing assurance that the focus of the programme is on the improvement of patient care. Clear and standardised reporting forms and SOPs are required. Education and training of all stakeholders, the mechanism of monitoring the implementation of corrective and preventive actions, and timely reporting of trends with expert analysis and recommendations for improvements, are all part of the programme.

Donor haemovigilance

Incidents and accidents involving donors and donations should be documented. This would include:

- Events occurring immediately before, during and after the blood donation.
- Significant deviation from protocols and SOPs.
- Near misses (where an incident or accident nearly happened and was narrowly avoided).
- Incident at a blood collection centre that could compromise the safety of donors.

All steps that involve the handling of donors and blood donations should be documented in detail, so that a comprehensive analysis may be performed to investigate problems, identify gaps, take corrective action and provide the opportunity for improvement. For example, in incidents related to the release of non-tested blood components, it should be possible to trace the donation back to the collection stage, the processing procedures, the labelling stage and the release of the components, and in so doing, determine the cause of the error and why, when and where it happened. In this way corrective action can be taken to change a faulty process to eliminate the chance of such an error occurring again. See *Section 9: Blood collection*, for more details on donor care.

Patient haemovigilance

An investigation should be performed on all untoward reactions to the transfusion of blood components, the reasons ascertained if possible, and steps taken to resolve the

problem and prevent recurrences in the future. This would include:

- Errors in the collection and testing of blood samples.
- Errors in the identification of patients.
- Inappropriate use of blood components.
- Incorrect blood component transfused.
- Significant deviation from protocols and SOPs.
- Near misses.
- Adverse reactions associated with transfusion of blood components.

Traceability of blood components is an important prerequisite for the investigation of transfusions that result in the transmission of infectious disease. For example, should the recipient become infected with malaria post-transfusion, it should be possible to trace the donation back to the collection stage, to the nurse who carried out the donor interview, to the training of that nurse and the result of that training from the perspective of deferral of donors with the potential risk of malaria. The look-back exercise should also identify gaps in such a process and provide the opportunity for improvement of systems to avoid similar deficiencies. If the blood component originated from a different facility, the facility that transfused the blood shall report that information to the collecting facility. The collecting facility should have procedures for investigating and deferring donors when such reports are received.

Monitoring, evaluation and reporting

Monitoring may be defined as asking the question, 'What is being done?' and documenting all data collected in this regard. Evaluation may be defined as asking the question, 'How well is the job being done, what are the issues and problems, and how might they be resolved, and systems improved?' and assessing the data collected to find the answers.

Reporting should be performed according to an agreed format, so that data collected from different areas may readily be collated and interpreted, with a view to implementing actions for improvement. These should link to clinical audit programmes, so that recommendations arising from haemovigilance reports can be incorporated into standards and guidelines, and compliance further assessed.

There is always the underlying concern that transfusion incidents may go undiagnosed or that there may be under-reporting. This may be the result of ignorance or failure on the part of personnel to recognise reactions that are not life-threatening, or there may be the deliberate non-reporting of accidents related to negligence. If there is fear of legal action, mistakes are likely to be deliberately concealed. To avoid this, there should be a

'no-blame' approach with the focus on problem solving rather than punitive measures.

Readers are encouraged to visit the website of the UK Serious Hazards of Transfusion (SHOT) at the following address: <http://www.shotuk.org>. The SHOT reports analyse reported hazards of transfusion from participating hospitals and blood services in the UK.

Hospital transfusion committee

The hospital transfusion committee is a multidisciplinary group that aims to maintain safe hospital transfusion practices. The formation and active commitment of hospital transfusion committees is required to maintain close links and co-operation between the providers and users of blood; the blood service and the hospitals.

Members of a hospital transfusion committee should be representative of all stakeholders including the following:

- Medical director or transfusion medicine consultant from the blood service.
- Clinicians from all clinical areas where blood is used (e.g. anaesthesia, emergency medicine, surgery, medicine/haematology, paediatrics/neonatology, obstetrics and gynaecology).
- Blood bank administrative manager/ senior scientists/ chief technologist.
- Transfusion practitioner or nurse from a hospital ward.
- Quality manager from the blood service.
- Representative from hospital administration.
- Administrative officer – blood service.
- Administrative officer – hospital.

The role of the hospital transfusion committee is to improve patient safety through multidisciplinary collaboration in the development of evidence-based and patient-centred guidelines, monitoring and evaluating blood transfusion and its impact on patient safety, and to participate in transfusion-related education and training. There should be agreed responsibilities for each committee member, and active participation to reach defined goals.

The goals of the committee should include:

- Proper and effective implementation of national blood policy.
- Implementation of national guidelines on the use of blood.
- Development and implementation of standardised transfusion guidelines, procedures and policies for the administration of blood and blood components.
- Oversight of the development of transfusion-related education modules and their implementation across all stakeholders.
- Development of a uniform approach to monitor blood transfusion and to measure component usage

vs indications and compare/ benchmark outcomes against other institutions.

- Provision of blood utilisation reports, analysis of data and recommendations for corrective action as needed.
- Introduction of measures to reduce blood wastage.
- Monitoring, reporting and investigation of transfusion adverse events and near misses along the transfusion chain to undertake steps for corrective action and prevention in the future.
- Ensure a programme of clinical audits of transfusion practice, transfusion safety and compliance with national requirements.
- Implementation of patient blood management initiatives, and the promotion of transfusion alternatives where appropriate.
- Assessment of the development of a patient blood management programme within the institution.

A facility that collects and issues blood for transfusion shall perform at least annual evaluations of blood need, blood supply and blood usage, and shall use the information gained for continuous improvement and planning.

National haemovigilance system

A national haemovigilance scheme may be managed directly by the Ministry of Health. Within a national framework, the responsibility of a national haemovigilance committee/office includes:

- Putting in place mechanisms for data collection, analysis, publication, dissemination of reports and development of recommendations.
- Receiving adverse event reports from blood services and hospitals.
- Reviewing reports to ensure the quality of reporting and identifying trends.
- Investigating underlying causes and providing expert non-judgmental guidance to address root causes and improve safety.
- Identifying improvements in processes along the transfusion chain.
- Provision of clear recommendations for improvement.
- Improving education in haemovigilance.
- Producing an annual national haemovigilance report.
- Conducting periodic review, monitoring and evaluation of the haemovigilance system.

Key points

- Safe blood may be defined as blood that benefits the recipient and does not have any detrimental effect.
- Patients requiring a blood transfusion should be given the opportunity to understand the benefits and risks related to blood transfusion and agree that it is necessary for them to be given blood (being informed and therefore able to give consent to having a blood transfusion).
- Transfusion reactions can be acute or delayed. Diagnosis is made by correlating signs and symptoms with laboratory investigations.
- Whenever a transfusion reaction is suspected, the transfusion should be stopped immediately, and the reaction reported promptly to the attending clinician and blood bank.
- Acute haemolytic transfusion reaction is life-threatening and can be minimised by stringent applications of patient identification policies and procedures and eliminated by thorough testing, including antibody screening and crossmatching according to SOPs.
- The most common causes of transfusion reaction are allergic and febrile non-haemolytic, which are not likely to be life-threatening.
- Severe and potentially fatal adverse reactions to transfusion are caused by ABO mismatch, TACO, TRALI, anaphylaxis, and sepsis as a result of receiving a bacterially contaminated blood component.
- Red cell or platelet units may be filtered to reduce the risk of febrile reactions.
- Red cell concentrates may be washed to eliminate IgA from a unit to be transfused, for patients with recurrent or severe allergic reactions due to anti-IgA alloantibodies.
- Delayed haemolytic reactions may be the result of recipient antibodies too weak for detection at the time of crossmatching. To ensure that this is avoided, blood bank records of transfusion history should be kept up-to-date and consulted at the outset, when a request for blood is received, to ensure that clinically significant antibodies on record for that patient, are taken into account and antigen-negative units selected.
- Risk of TA-GVHD is minimised by irradiation of cellular components for high risk patients, i.e. those who are immunocompromised, or those who are to receive blood from first degree relatives.
- Alloimmunisation is a long-term hazard because transfusion recipients may develop antibodies to donor red cells, and this may complicate compatibility testing and the provision of safe blood in future.

- Citrate and potassium toxicity and hypothermia are also hazards of transfusion.
- All transfusion reactions should be reported to the blood bank. The remnant contents of the blood component bag and its connected tubing (clamped) should be sent for all but allergic reactions. Post-transfusion recipient samples and urine samples also need to be submitted for investigation.
- Haemovigilance is a set of surveillance procedures covering the entire transfusion chain. Donor haemovigilance involves all steps in the handling of donors and their blood donations, while patient haemovigilance involves all reactions involving transfusion.
- A record system shall make it possible to trace blood or blood components from source to final disposition, including all screening and testing and the investigation of adverse reactions.
- Details on transfusion incidents and accidents should be documented, and data should be regularly analysed and reported on, with a view to taking action to prevent similar problems recurring and in so doing, improving patient safety.
- Hospital transfusion committees should be formed to include representatives from all stakeholders from the hospital and blood service. The role of the hospital transfusion committee is to improve patient safety through multidisciplinary collaboration in the development of transfusion-related education and training, evidence-based and patient-centered guidelines, monitoring and evaluating blood transfusion and its impact on patient safety.
- A haemovigilance programme should be nationally coordinated and be fully representative of all stakeholders in the transfusion chain.

Safety, health and the environment

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Introduction

Safety is everybody's responsibility.

Blood service personnel are at risk of all the usual hazards to be found in any working environment such as electrical and fire hazards and risk of accidental injury, but they are also exposed to risk from contaminated blood or blood products; needlestick injuries, cuts from sharp objects, hazardous chemicals and to the extremely low temperatures found in freezer rooms.

In order to protect themselves from hazards, personnel should be trained in, and fully understand, health and safety principles and the importance of following basic safety practices such as handwashing, using protective equipment, and wearing protective clothing when appropriate.

Learning objectives

By the end of this section, the student should be able to describe the general safety requirements of the blood service, and give a clear account of details related to the following:

- Organisational safety policy
- Safety Committee
- Safety Manual which includes the requirements of the safety programme such as:
- Training identification of risks
 - Work areas
 - Work practices
 - Personal protective equipment
 - Reporting incidents, near misses and risks
 - Bloodborne pathogens
 - Handling of blood spills
 - Decontamination of work surfaces and equipment
 - Electrical hazards
 - Fire prevention
 - Waste management

Organisational safety policy

The safety policy should include a statement of the chief executive officer's (CEO) commitment to ensuring a healthy and safe workplace. This policy must be kept up to date and communicated to every employee soon after

their employment starts. It should be displayed for all to see in the workplace. Although it should be quite short, the policy statement should include the following:

- The commitment of management to protect the health and safety of all employees.
- The responsibilities of the employees for their own health and safety.
- A statement of who is responsible and accountable for the health and safety programme.
- The fact that deviations from the health and safety programme will result in disciplinary action.

It is the responsibility of every individual to follow safety rules and apply good judgement to reduce the risk of accidents and injury in the workplace.

Senior management of the organisation has overall responsibility for safety, health and the environment and should set aside a dedicated safety budget to ensure that the requirements of the safety policy are achieved and maintained.

Many countries have legislation in place that lists the minimum requirements that must be met in providing a safe work environment. Management should regard compliance with legal requirements as the lowest acceptable standard for the organisation and should have as its aim, the development of 'best practice' in health and safety, with the health and safety of personnel as a core management function.

The safety policy should refer directly to the safety manual, which in turn should set out the elements of the safety programme.

Safety committee

The organisation should have an elected safety committee comprised of representatives from each work area. The safety committee should be chaired by a trained safety officer appointed by, and acting on behalf of, the CEO. The committee should be responsible for monitoring health and safety statistics, conducting routine safety inspections and making recommendations regarding safety issues to senior management, in writing. If senior management does not act on recommendations, the reasons should be recorded by the safety committee for future reference. The committee should also monitor the effectiveness of any corrective or preventive actions.

Safety manual

Each area should have a safety procedure manual that adds specific detail to the safety policy of the organisation. It should include the specific safety requirements of the area concerned, the standard operating procedures (SOPs) that each individual working in the area is expected to follow, as they relate to equipment and procedures performed in the laboratory. It should also include standard risk assessments for commonly performed tasks, a register of equipment, chemical and biological agents, material data safety sheets (MDS), emergency procedures, and waste management and disposal procedures.

The safety manual should be compulsory reading for all personnel. Some organisations require, as a condition of employment, that employees sign a statement that they have read, understood and will abide by the requirements of the safety manual.

The safety manual must detail the elements of the safety programme applicable to the work environment as well as the following:

- Organisational safety policy.
- Responsibilities of management and staff for the safety of themselves and their co-workers and the provision of a safe working environment.
- An explanation of the roles and responsibilities of the safety committee.
- The requirement for compulsory safety training during the orientation period for new personnel, and refresher/update training for all personnel.
- The frequency and format of workplace safety inspections.
- Emergency procedures, including evacuation procedures.
- An explanation of how incidents, such as fire, will be communicated to personnel in their work areas.
- The use of protective clothing, such as gloves, eye protection and masks.
- Information on health and safety programmes, including immunisation programmes if available.
- Information on bloodborne pathogens.
- Information on radiation hazards.
- Information on chemical hazards.
- Information of basic first aid (where to go and what to do).

Elements of the safety programme

Good judgement and every individual's personal interest in the safety of themselves and their colleagues are the greatest guarantees of safety at work. The organisation

should take the safety of all personnel seriously and any wilful violation of safety rules could be a cause for disciplinary action.

An organisation-wide safety programme should embrace all of the foregoing and should include at least the elements that follow in this section.

Training

All personnel should be trained to protect themselves from injury in the workplace. This means that each individual should clearly understand potential hazards to which they may be exposed, and the precautions to be taken to manage risks. After health and safety training, a clear understanding should be demonstrated by the trainee, before they are assigned to a task or work area where the risks are present.

Personnel should be advised of organisational standards for personal hygiene and given training in the correct handwashing technique, with emphasis on the need to maintain a clean work environment, especially when managing donors and during processing and testing of blood.

Fire safety training should also be provided. It should include the location and use of fire control equipment such as hoses and extinguishers; emergency fire alarms, smoke detectors and fire exits, as well as evacuation procedures. Plans should be developed, and appropriate training given for dealing with emergencies such as fire, explosion, chemical spills, electric shock, and personal contamination. Fire drills should be practiced at least annually.

Identification of risks

The safety of the workplace relies on personnel being trained to recognise and report hazards as soon as they are noticed. Faulty electrical equipment with a risk of electrical shock or of fire should be labelled 'out of order', taken out of use and reported immediately to the responsible member of the safety committee. SOPs should be available that define how various hazards (such as blood spills and exposure to hazardous chemicals) must be handled.

Each member of the safety committee should be required to make formal inspections of the work area that they represent, using an approved checklist, to ensure that all hazards are reported and appropriately dealt with to prevent possible accidents or injury. These inspections are usually conducted once a month.

All accidents and incidents, or 'near misses' that do not result in accidents but could have, if the circumstances had been slightly different, should also be reported and evaluated by the safety committee, which should

then make recommendations regarding corrective and preventive action.

Safety hazards (defined as objects or events that may cause injury or damage) are many and varied, and include the following:

- Biological hazards, including pathogenic organisms such as bacteria and viruses that may be present in blood.
- Chemical hazards, including corrosive acids and alkalis and flammable agents such as alcohol.
- Environmental hazards, including noise, heat and cold.
- Electrical and mechanical hazards, including equipment with moving parts such as centrifuges.

Warning signs should be displayed in work areas to alert personnel and visitors of any specific hazard that exists in that area, such as a slippery floor in a freezer room.

Work areas

The design and maintenance of work areas and of the premises in general are important in ensuring that the overall environment is safe for personnel or visitors to the area. The first step in an effective safety programme is the provision of a workplace designed to eliminate, as far as possible, all risks to health and safety. The design should include provision of adequate fire control equipment, chemical fume cupboards and safe storage areas for flammable liquids and hazardous chemicals, adequate storage areas to avoid clutter, proper signage, fire exits, the provision of adequate electrical outlets and wash stations for handwashing. Thereafter, good housekeeping should be practised, including:

Emergency telephone numbers, including those of the fire department, ambulance service, the police, and medical director should be prominently displayed in each work area to avoid unnecessary delay in an emergency.

- Emergency numbers should be verified and updated if necessary, every month.
- The name of the 'on-duty' safety officer should be displayed in each work area.
- Work areas should be kept free of clutter.
- Exits and doorways should be kept clear of any obstruction.
- Access to fire control equipment should always be kept clear.
- Fire control and other safety equipment should be provided, serviced and checked regularly. This includes fire extinguishers, safety drench showers, eye-wash fountains, first aid kits, fire blankets and fume cabinets.

- Personnel should familiarise themselves with the use and location of fire extinguishers and fire hoses, and the location of the nearest exits. This should form part of safety training.
- Work surfaces should be regularly cleaned and disinfected to avoid the risk of contamination.
- Compressed gas cylinders should be stored in accordance with the supplier's recommendations and secured so that they cannot be accidentally knocked over.
- Hazardous chemicals and flammable liquids should be stored in an appropriately constructed flammable liquids storage cabinet and only minimal amounts kept in the work area.
- Laboratories in which hazardous chemicals are used should be equipped with eye-wash facilities (fountains) and personnel should be trained in their use.
- Depending on the nature of the work carried out in the work area, it may be necessary to provide drench showers for the rapid decontamination or treatment of personnel. Drench showers may be required in areas where caustic, corrosive or toxic chemicals are used.
- Lifting devices or trolleys should be available for movement of heavy objects.
- Biohazardous waste containers and sharps containers should be available where required, and properly labelled.
- Entry to any work area should be restricted to only those personnel authorised to enter. Those entering the area should receive appropriate information regarding safety measures to be observed and be provided with the necessary protective clothing even if they plan to remain in the area for a limited time. Children should never be allowed to enter a laboratory even if they are under the supervision of an adult.

Work practices

Part of the validation procedure for SOPs should be an evaluation of the risks to which personnel could be exposed when following the procedure. Before undertaking a procedure, personnel should understand the risks to which they may be exposed and how to manage them. For example, if hazardous chemicals are used in the procedure, personnel should know how to handle and dispose of these chemicals in order to reduce personal risk and risk to others.

When conducting procedures that involve hazards or potential hazards, the following questions should be asked when designing the procedure and writing the SOP:

- What are the hazards and the potential hazards? Also, consider the impact of non-standard events such as power outages and interruptions to the water supply.
- What could go wrong during the procedure, and how must the individual deal with these problems?
- What aspects of good laboratory or clinical practice are required, such as protective clothing and equipment, to minimise the risks?
- Is special training required before the task can be undertaken safely?

Other general considerations when reviewing work practices:

- The following should be prohibited in the laboratory or when attending to blood donors:
 - Smoking and vaping.
 - Eating and drinking or chewing gum.
 - Application of cosmetics.
 - Insertion or manipulation of contact lenses.
 - Placing any objects, such as pencils, in the mouth.
 - Mouth pipetting.
- Uncovered cuts and grazes on exposed parts of the body.
- The storage of food and drink in the laboratory or blood collection area, or in a refrigerator belonging to either area. Laboratory glassware should never be used for food or drink.
- Eating or drinking utensils should never be washed in the workstation sink.
- Water for filling a kettle should never be drawn from the workstation sink.
- Long hair should be tied back e.g. out of the way of moving machinery.

Work practices should be designed so that ergonomic hazards such as repetitive movement, manual handling requiring exertion and repetitive or sustained awkward postures are eliminated or reduced as much as possible.

Personal protective equipment

Personal protective equipment (PPE) may be defined as any specialised clothing worn, either in place of or over personal clothing, for protection against hazards such as exposure to blood, extreme heat or cold, and noxious fumes, and includes items such as gloves, boots, warm clothing, hearing protection, eye protection (face shields, safety glasses), closed shoes and laboratory coats/gowns. Appropriate PPE should be worn whenever its use will provide additional safety to the wearer. In general, the use of PPE should not be optional, nor left to the discretion of the individual involved, but should be compulsory under defined conditions. For example, the wearing of

disposable gloves and closed laboratory coats or aprons should be compulsory when working with blood samples from patients. Hearing protection must be worn when using noisy equipment. The use of PPE also applies to visitors to a work area, and particularly to contractors who are working on equipment or performing any other maintenance.

By the nature of the work in which they are involved, personnel in many areas of the blood service are exposed to blood in various ways, necessitating the use of PPE. Personal clothing for work should also comply with the standards of the organisation. For example, the wearing of shoes should be mandatory, and in some instances, closed shoes should be worn rather than sandals.

Personal protective equipment should be worn to prevent:

- Exposure to blood when collecting donor samples.
- Exposure to blood when testing patient and donor samples.
- Accidental injury with sharps (needles, lancets and contaminated glassware).
- Splashes of blood onto mucous membranes, such as the eyes.
- Exposure to noisy equipment.
- Exposure to extreme temperatures.
- Exposure to harmful chemicals.
- Accidental injury including cuts, crushing injuries and exposure to corrosive chemicals.

Personal protective equipment should be removed at the time of leaving the work area and should never be worn in rest areas or tearooms. Visibly contaminated clothing should be removed promptly and placed in a container reserved for soiled clothing. Individuals responsible for laundering potentially contaminated clothing must be made aware of the hazards and must employ methods that will decontaminate all soiled items. Personnel should not take soiled clothing home for laundering as this may spread the contamination and the home laundering may not be effective in decontaminating the garments.

Reporting incidents, near misses and risks

When an incident or a near miss occurs, it is important that it is reported to the designated member of the safety committee or the safety officer as soon as possible. Processes should be in place to identify, track and reduce incidents/near misses. Reporting procedures and report forms (electronic or paper) should be available to ensure that as much information as possible is recorded soon after the event. This is necessary for a full root (primary) cause investigation into the incident/near miss with the purpose of preventing a recurrence by introducing

preventive measures, where necessary. The nature of these preventive measures should be recorded, and follow-up conducted to ensure that the measures successfully address the problem. In addition, incidents, particularly those that result in personal injury, may result in insurance and/or workers compensation claims and the insurers will require full details if the claims are to be honoured.

Bloodborne pathogens

The organisation should have measures in place that minimise the risk of exposure to bloodborne pathogens. These measures should include the following work practices:

- Treating all blood samples if they are infectious. This is a necessary precaution, as very often it is not known in advance which sample may be contaminated with bacteria or viruses.
- The wearing of gloves, masks, eye protection and laboratory coats as appropriate when carrying out tasks that may result in blood splashes.
- Frequent handwashing, but particularly after touching blood or any items that may be contaminated with blood.
- Regular decontamination of work surfaces and equipment.
- Technologists should be aware of the dangers of creating aerosols, for example by centrifuging uncapped / de-bunged blood sample or lifting the lid of the centrifuge before it has stopped rotating.
- Method for the proper disposal of sharps.
- Provision of spill kits with all necessary items and instructions for the cleaning up of blood and chemical spills.

Special precautions may be required if the facility handles samples from patients with haemorrhagic diseases such as Marburg fever or Ebola fever, as the viruses responsible for these infections are very easily spread by blood and other body fluids. Precautions may include notification of laboratory staff and special sample collection procedures, quarantine of samples, special decontamination and cleaning procedures and secure transportation methods to minimise the risk of transmission. Local regulations may require that all such samples, and the individuals working with the samples, be retained in quarantine until the situation is under control.

Blood samples received for crossmatch should not be handled until the clinical diagnosis provided on the request form has been noted. If the diagnosis indicates that the patient may have a highly infectious haemorrhagic disease, a senior member of staff is immediately notified to provide guidance on managing the situation.

In this case, it is preferable to use un-crossmatched Group O D negative blood in order to avoid the risks of de-bunging the sample.

Many blood services offer hepatitis B vaccination to employees who are exposed to blood while performing their routine duties. Depending on the level of exposure in the general population, individual members of staff may be screened for anti-HBs as an initial step, and the vaccine offered to those who do not have the antibodies, or who have them only at a very low level. Participation in this vaccination programme should be voluntary, but if an individual declines the vaccine, this should be documented.

An individual exposed to blood through a needlestick or similar accident should receive appropriate counselling and be monitored for hepatitis B, hepatitis C and human immunodeficiency virus (HIV). If possible, the source material should also be tested, and procedures should be in place for offering prophylaxis such as hepatitis B immunoglobulin or antiretroviral therapy should this be indicated. It is important that a written agreement of understanding is signed by the individual (informed consent) before any tests are carried out. If an individual declines the recommended testing or the offered prophylaxis, this should also be documented.

Handling of blood spills

Blood spills in a blood service are almost inevitable and can occur as a result of e.g. samples being dropped or blood bags breaking while being centrifuged, and the facility should be prepared for such an event.

Main steps in the clean-up of a spill

- Contain the spill to prevent it spreading.
- Keep the area clear. This is particularly important if the spill is in a public place such as a blood collection area.
- Wear appropriate PPE for the clean-up operation, including gloves. If the spill is likely to include sharps, such as broken glass, extra precautions will be necessary to avoid injury.
- Use absorbent material such as paper towels to mop up as much of the spill as possible and then clean the area with detergent.
- Disinfect the area, using an approved disinfectant according to the manufacturer's instructions.
- Dispose of all contaminated cleaning materials according to the organisation's guidelines on the disposal of contaminated waste.
- Arrange to have the spill kit replenished without delay. The contents of spill kits should be checked periodically to confirm that they are not expired.

Decontamination of work surfaces and equipment

Laboratory benches and other work surfaces such as the phlebotomist's table that may become contaminated with blood should be thoroughly cleaned and decontaminated at least once a day. Any obvious spills that occur during the day should be cleaned up and the area decontaminated immediately. At a standard convenient time, usually the end of the shift, all work surfaces should be thoroughly cleaned before being wiped down with an approved disinfectant. Equipment that may have been exposed to blood should also be decontaminated each day and should be specifically decontaminated before being repaired or serviced.

The choice of the disinfectant used for decontamination of work surfaces will influence the contact time that is required for effective decontamination and the concentration at which the disinfectant should be used for best results, and this information should be available to personnel in the form of an SOP that explains the decontamination process.

General cleaning of the office, blood collection area or laboratory should also be carried out according to a schedule, to ensure that all areas of the facility remain clean and orderly.

Electrical hazards

The proper use of electrical equipment such as centrifuges, water baths and microscopes, together with proper maintenance and the prompt recognition of hazards, is essential in preventing accidents that could result in electric shock or even electrocution.

Personnel should be trained in the proper use of such equipment:

- Personnel should report faulty connections, frayed electrical cords, damaged switches and other potential hazards promptly to the safety officer and should refrain from using any faulty equipment until it has been repaired.
- Electrical extension cords are intended for temporary use only and should not be used as a substitute for permanent wiring.
- Flexible electrical cords should not be allowed to trail loosely across the floor. They should be protected from accidental damage and secured in a way that will prevent anyone tripping over them.
- Faulty or damaged equipment should be labelled as such and removed from the work area.
- Personnel should not plug in, or unplug, any electrical equipment if their hands are wet.
- Personnel should not tamper with any appliance while it is plugged into the circuit.

- Care should be taken not to plug too many electrical items into the same circuit, as overloaded circuits may constitute a fire hazard.
- Basic training should be provided to all personnel on the treatment of electric shock.

Fire prevention

Awareness of potential fire hazard is important. All staff should become familiar with fire safety procedures, including the assembly point in the grounds of the organisation, should the building need to be evacuated, and the location of fire equipment in the laboratory and surrounding areas.

- Fire escapes and pathways must always be kept clear.
- Electrical equipment should be checked regularly for faults and when found, must be repaired promptly.
- Checking of all other equipment must also be regularly undertaken, and faults found must be rectified promptly.
- Smoking / vaping is not allowed in laboratories and work areas; this is only permissible in areas designated by the organisation.
- If open flames are required, such as for Bunsen burners, they must never be left unattended.

Waste management

The management of waste, and of biohazardous waste in particular, is an important part of the safety programme. All waste should be stored away from public access and in a manner that prevents tampering with the contents. Many organisations have waste minimisation programmes that address methods of reducing the generation of waste through effective planning, linked to recycling programmes for non-contaminated material such as glass, paper, plastic and metal. Waste minimisation should not focus only on 'physical' waste, such as programmes for saving paper, e.g. the unnecessary printing of emails, but should include efforts to minimise the use of electricity, water and non-renewable resources such as petrol, diesel and coal.

Work practices should also be designed to reduce any possible waste of blood products due to prolonged time out of correct storage or poor handling or storage of products.

Wastage because of rework

Another important element of waste management is the reduction of 'rework'; work that must be repeated because it was not carried out properly in the first instance, or because the equipment used was not properly maintained

or calibrated. Rework leads to a waste of time as well as reagents and other disposables, and under some circumstances can result in delays in the delivery of products and services.

Disposal of confidential waste

Records are maintained for a specified length of time, according to regulations imposed by the blood service. Those which reach their disposal time may contain confidential information and must be disposed of in a manner that prevents disclosure to third parties. The waste management system should be designed to ensure that records with confidential information are not accessible to any third party including personnel of the paper recycling company or waste disposal team. Confidential records including donor or patient records should be shredded or otherwise rendered unreadable so as to prevent disclosure to any unauthorised personnel.

Separation of waste by category

Attention should be paid to the separation of normal or household waste, such as uncontaminated paper, metal and plastic, and biohazardous or contaminated waste. If an item could be infectious, was in contact with blood, or came from a contaminated environment or surgical procedure it should be considered biohazardous waste. Separation of waste should occur at the point where the waste is generated. Most commonly this is performed using colour-coded garbage bags, e.g. using red bags for biohazardous waste and black bags for household waste. This colour coding may be extended to ensure the separation of items for recycling by providing different colour containers for paper, metal, and plastic. Biohazard warning labels should be affixed to waste containers that carry biohazardous waste.

Biohazardous waste disposal

Biohazardous waste requires special handling, preferably using red bags to make them easily identifiable. The system should ensure that individuals handling these bags during the disposal process are not exposed to contaminated material, and this may require double bagging of waste, or packing the bags into a secondary outer container. If the waste is not removed from the work area to the disposal site immediately, it should be stored securely so that it is not accessible to any individual without the required authority. Depending on the nature of the waste, the bags may require refrigeration while awaiting disposal. Biohazardous waste should never be compacted prior to disposal because of the risk of exposing personnel to the waste. Waste should be stored in a way that will not attract vermin, such as rats and mice, or be accessible to the public.

Disposal of sharps

Another category of waste that requires special attention is 'sharps': needles, lancets, broken glass and other items that can stab or cut if handled inappropriately. A system should be in place for the safe disposal of sharps, and this usually requires that the sharps are placed in a special container immediately after use. This container should have a wide opening through which sharps can be dropped, be impenetrable to sharps once they are inside, and be easily and securely closed when full. The container should also be clearly labelled as biohazardous. These sharps will almost invariably be contaminated with blood and therefore the sharps container is also disposed of, along with the sharps it contains, into the bag designated for biohazardous waste.

Chemical waste disposal

The disposal of hazardous chemicals can be problematic and is best contracted to be done by 'experts in that field, who can also provide information on the storage of the material prior to disposal as this will differ, depending on the type of chemicals involved.

Methods of disposal

Domestic waste should be disposed of according to local regulations and should be kept covered in such a way, while awaiting collection, that flies and other pests are not able to reach it.

Biohazardous waste should be disposed of by a method that has the approval of the local authorities for the type of material. This is usually by incineration or by autoclaving followed by disposal at an approved landfill site. The recommended temperature for the incineration of medical waste is 800°C to 1000°C or higher. This will result in complete incineration of the material with a minimum of resulting ash and will also limit the production of pollutants.

If waste disposal is contracted to a third party, a certificate of disposal should be provided by the waste management company, as a record that the waste disposal was correctly carried out.

Key Points

- Safety is everyone's responsibility.
- Senior management of the organisation has a responsibility for safety, health and the environment.
- Management's policy on safety should be formally documented and should be compulsory reading for all personnel.
- Each individual's personal interest in his/her own safety is the greatest guarantee of safety at work.

- The organisation should have an elected safety committee responsible for maintaining a high level of safety in the workplace.
- Personnel should be trained in all aspects of the safety programme and on how to keep themselves safe in the work environment.
- PPE includes specialised clothing and/or accessories that must be worn for protection against hazards such as exposure to blood, extreme heat or cold and noxious fumes. PPE should be used whenever this will reduce the risk of harm.
- The safety of the workplace is best maintained when personnel are trained to recognise and report hazards as soon as they are noticed.
- The validation procedure for SOPs should include an evaluation of the risks to which personnel will be exposed when following the procedure.
- When an incident/near miss occurs, it should be reported to the designated member of the safety committee or the safety officer as soon as possible and steps taken to avoid recurrence.
- The organisation should have measures in place that minimise the risk of exposure to bloodborne pathogens.
- Procedures should be available and used for the decontamination of blood spills.
- Procedures for the management and disposal of waste, and particularly of biohazardous waste, form an important part of the safety programme.

Quality

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Reviewer for Second Edition: Leslie Bust

Introduction

There is a thread of quality running through all sections of this publication. This is because the quality system in a blood service includes all aspects of transfusion practice; including the education, identification, screening and selection of blood donors; the actual collection of blood; the preparation of components; appropriate and accurate laboratory testing, including the storage and transportation of blood products; the identification and testing of recipients; and the documentation of all results. It also includes the selection, validation and maintenance of equipment used in these processes; the design and provision of a suitable working environment; and the appropriate storage and maintenance of laboratory and other records.

A commonly quoted definition of quality is 'fitness for purpose'. This is an appropriate definition to use in the blood service, where the main objective of the quality system is to ensure the transfusion of safe and efficacious blood and blood components, i.e. components that are fit for the purpose for which they are intended. The quality system should be designed to deliver a sufficient supply of blood and blood components with maximum efficacy and with minimum risk to donors and recipients alike, and to ensure that the components and services delivered meet the expected standard.

Effective quality management is achieved by adopting good manufacturing practice (GMP), good laboratory practice (GLP) and good hospital and clinical practice. Personnel involved at any stage of the blood transfusion chain should be aware of the importance of quality management and their role in achieving it successfully. Good record keeping, the adherence to well-designed standard operating procedures (SOPs) and laboratory worksheets and following well-conceived safety guidelines will further improve the quality of the work produced.

Learning objectives

By the end of this section the student should be able to describe the quality management system in a blood service, with the focus on the following elements:

- Quality management system
- Organisational structure

- Premises
- Equipment and materials
- Management of processes and procedures
- Documentation and record keeping
- Quality assurance and quality control
- Management of non-conforming product or material
- Waste management – overview
- Monitoring and evaluation of the quality system
- Process improvement
- Ethical guidelines.

Quality management system

A quality management system may be defined as the set of policies and procedures for establishing and implementing the intentions of the organisation with respect to quality. The factors that can affect the quality of the products and services delivered by the blood service are numerous, yet all must be considered in the development of the quality management system. They include the following:

- Equipment and reagents used in the various processes.
- Personnel carrying out these processes.
- Environment in which personnel work.
- Environment to which blood products are exposed during testing, storage and transportation.
- Methods/ techniques and equipment used to carry out the numerous procedures and the environment in which work is done. The management of each of these aspects is important if quality requirements are to be met. The principle elements of a quality management system include:
 - Organisational structure.
 - Premises.
 - Equipment and materials.
 - Management of processes and procedures.
 - Documentation and record keeping.
 - Quality assurance and quality control.
 - Management of non-conforming product.
 - Waste management.
 - Monitoring and evaluation of the quality system.
 - Process improvement.
 - Ethical principles.

The general elements constituting a quality system are known as 'GLP' or 'GMP' and these terms are often

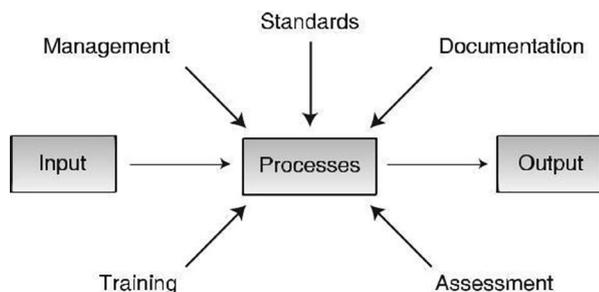


Fig. 1 Elements of the quality system.

used when referring to overall quality within an organisation. Figure 1 shows the elements of the quality system.

Organisational structure

The blood service should have a clearly defined mission statement that sets out the organisation's core purpose and focus. The mission statement should be supported by a declaration of organisational values or principles that guide the organisation's internal conduct as well as its interactions with customers, partners and other stakeholders. The mission statement should be further supported by a corporate code of ethics designed to guide management and staff in conducting their activities in accordance with the organisation's primary values and ethical standards. The code of ethics shall be supported by processes to identify and resolve ethical dilemmas in a timely way.

The organisation should develop and maintain a strategic plan that clearly defines its long-term objectives and its strategy for the allocation of resources to pursue those objectives as well as addressing the major changes and improvements required to better realise its mission. The strategic plan should be supported by an organisational plan that identifies the expected milestones in the strategic plan, provides guidance and an action plan, and enables progress towards achieving the milestones to be measured.

The blood service must be organised and structured in a way that will maximise the effective implementation of the quality system. Senior management, and in particular the individual in overall charge, such as the medical director (MD) or chief executive officer (CEO), are responsible for the implementation of the quality system. They are also responsible for documenting the organisational structure and defining the roles and responsibilities of each individual within the organisation with respect to quality. Ideally, this structure would include one individual, independent of all production activities, and reporting directly to the CEO or MD, with the overall responsibility for the management of the quality system.

In smaller organisations, it may not be feasible to dedicate one individual to this function. In such cases, care should be taken to arrange the duties of the designated individual so that he/she has sufficient time to devote to the required quality activities. If this is not done, then quality may be the first aspect of the job to suffer should workload increase.

The organisational structure is usually documented in the form of an organisational chart or organogram. This shows the reporting lines within the organisation and suggests the following:

- Personnel should be available in sufficient numbers.
- Personnel should have the appropriate education and training to perform the tasks to which they are assigned.
- Job descriptions should be available for all personnel and should clearly define responsibilities with respect to quality as well as required qualifications and experience, general responsibilities and reporting relationships of the position.
- Personnel records should be maintained to include staff qualifications, training and experience, competency assessments, ongoing education and appropriate certification or licence.

Figure 2 gives just one example of the many ways in which a blood service could be set up, in terms of its organisational structure.

A concerted effort should be made to create a 'quality consciousness' within the organisation and to build an environment that encourages practices relating to quality. Although it may be difficult to achieve, having an environment where personnel have quality in the forefront of their thinking at all times is the most effective way of ensuring that the programme is successful. It costs the same and takes very little time and effort to ensure that labels are carefully affixed to blood bags in the correct places and that they are stuck on straight and not carelessly at an angle; that documents for photocopying are squarely positioned on the platform before copying, and clearly legible by being copied from the original and not from a fading copy, and so on. These simple actions contribute greatly to quality consciousness. Accepting shoddy work, even when it involves a relatively minor task, can

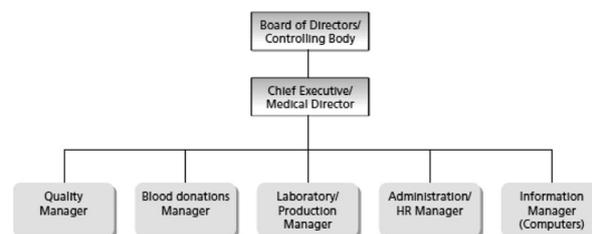


Fig. 2 Example of organisational chart for blood service.

very quickly become the norm and must therefore be promptly stopped.

In the effort to build a quality organisation, senior management should take the lead by formulating a policy statement that clearly shows their total commitment to quality, and this statement should be displayed in all work areas. Senior management should also formulate a more detailed quality policy that includes a description of how the quality policy will be implemented within the organisation.

There should be an organisational training policy and programme. The training programme should include ongoing education and training for all personnel as necessary for them to acquire and maintain the required level of competence in the performance of their assigned activities. There should be a system for competency assessment of individual personnel after initial training and at least once every 12 months thereafter.

Specific training on the quality management system should be provided for all personnel at all levels within the organisation and such training should be compulsory. For example, the CEO/MD should be trained to support the system, as should junior personnel responsible for the transport of blood, waste management, handling of blood samples and other tasks that can have a profound effect on the quality of the final products and services.

Ongoing awareness may be created throughout the organisation using posters with slogans such as:

'Quality is everybody's responsibility: Quality starts with me'.

It is important that all personnel understand the quality system and acknowledge their role in delivering a quality product. The quality system, however well-designed, is really only as good as those who implement it.

Premises

It is the responsibility of the organisation to provide a safe and healthy workplace for all employees, and for visitors such as donors, patients and others.

All premises, including laboratories, should be constructed and maintained in a way that suits the activities that are carried out. They must be designed to allow effective cleaning to minimise the risk of contamination and to provide a safe working environment. It is important to consider the flow of work, arranging procedures in a logical sequence in order to reduce congestion and the risk of errors. Work areas should provide adequate space and be well lit, well ventilated, kept at a comfortable temperature, and be free of excessive noise. The

provision of adequate water, toilet and washing facilities is, of course, essential.

Procedures should be in place to address general safety, such as those relating to electrical hazards, fire safety and preparedness to deal with disasters. Laboratories may require that attention be paid to more specific issues such as biological safety, particularly with respect to blood-borne pathogens, chemical safety and possibly radiation safety, and the management of biohazardous waste.

Entry to storage areas for blood and blood products should be limited and controlled, and these areas should be used only for their intended purpose. Conditions in these storage areas should be controlled and monitored. High- and low-temperature alarms, preferably both audible and visual, should be fitted and checked regularly to ensure that they are functioning correctly. Alarms should be set at temperatures that will alert personnel in time to carry out appropriate corrective action before the temperature of the products reaches an unacceptable level. SOPs should describe the action to take if the temperature goes out of the acceptable range.

Equipment and materials

Equipment should be adequate in number to carry out procedures in required time frames, placed in a location suitable for optimal operation and used in accordance with manufacturer's instructions.

Equipment should be selected and validated to suit its intended purpose. Maintenance, cleaning and calibration should be done regularly and recorded. SOPs should be written and always followed, describing the correct use, cleaning and servicing of equipment, and describing action to take when equipment malfunctions or fails. Properly cleaned and maintained equipment should last longer and operate more reliably than equipment that is neglected.

Each piece of equipment should be uniquely identifiable. The organisation should maintain a list of all critical equipment, its location, date of purchase, and service and performance record.

For materials, the organisation should develop, implement and maintain an inventory management system. Prior to being taken into routine use, incoming reagents and other materials should be evaluated thoroughly to ensure that, under the conditions of the laboratory, they perform satisfactorily. Materials that could directly affect the quality or safety of products or services (critical materials) should be obtained only from approved suppliers. Certificates of compliance or certificates of analysis for each batch of materials should be requested from suppliers. Reagents that are prepared by the facility should be

standardised to meet or exceed performance specifications of commercial reagents.

A blood service that does procurement should have procedures to evaluate the ability of suppliers of critical materials, equipment and services to consistently meet specified requirements. Records of suppliers' performance should be maintained. Agreements with suppliers or subcontractors should be reviewed at least every two years and prior to any extension or renewal.

Management of processes and procedures

The blood service should manage risk through the development and implementation of a risk management framework to address potential business, operational and safety risks. The risk management framework should be supported by:

- A risk management plan.
- Relevant policies and procedures.
- The maintenance of a risk register.
- Annual risk assessments to identify potential hazards in the workplace, to the environment, to staff and to clients.

All new procedures and the equipment used in the procedure should be validated before being adopted for routine use. This validation should be carefully designed and carried out to ensure that the procedure is capable of reliably and consistently delivering expected results.

Laboratories should have documented SOPs for all procedures that may affect the health or safety of donors and recipients of blood products and also personnel handling products or associated samples. Written procedures are essential to prevent errors that may arise from verbal instruction and to limit unintentional variation. SOPs should include an accurate description of each step in the method, considering any local constraints and limitations. Generally, the responsibility for the preparation and authorisation of SOPs lies with the head of the work area in which the SOP is to be used. SOPs should also be approved by the quality manager and, when appropriate, by the MD or CEO.

It is important that SOPs are kept up-to-date and a procedure should be in place for the regular review (and revision if necessary) of each procedure, preferably by management level personnel with direct authority over the process. It is also good practice for an appropriate member of the quality department to provide input before the SOP is finalised.

An important step before an SOP is released for use, is the validation of the process, i.e. carrying out the procedure exactly as documented to check that in doing so, the correct result is obtained. If this is not done, the SOP is very likely to contain errors or steps that are not in the

correct sequence or that have been omitted. It is particularly important to validate procedures when it is difficult to inspect the finished product in order to confirm conformance with specifications, such as the procedures for the preparation of blood components. Process validation also provides an important opportunity to optimise the performance of each process, e.g. in establishing ideal centrifugation speeds and times in procedures for the preparation of blood products of consistently high quality.

When a procedure is changed, or when a major piece of equipment or a reagent that is used in a procedure is changed, it is necessary to revalidate the procedure to ensure that the change does not cause a drop in quality, i.e. an outcome which is below standard. If the validation process shows that the procedure does not produce the expected result, the procedure should be corrected and this corrective action documented, after which the validation procedure should be repeated.

When a procedure has been validated, finalised and released, personnel required to perform the procedure should be trained to the SOP and allowed to perform the procedure without supervision only when found to be competent to do so.

The validation of computer systems is especially challenging. Computer systems are often critical components of the quality system and are useful in the management of donor and laboratory records. They are useful for record keeping, and in the analysis and interpretation of data. The use of bar-coded identification labels on blood units and their related samples, together with the use of barcode scanners to read these labels electronically, virtually eliminates transcription errors and provides a fast and accurate method for dealing with large numbers of samples in a busy work area. Most modern sample-handling equipment, such as automated pipetting systems and laboratory analysers, have integrated barcode scanners and data management software to provide fast and accurate linkage of samples and results.

It is very important that any computer system is properly evaluated and validated within the laboratory in which it is to be used to ensure that it delivers expected results and integrates data in an expected way. This is especially important when linking equipment or systems from different suppliers, because data are not always recorded or handled in the same way. For example, if data originating from an enzyme-linked immuno-sorbent assay (ELISA) system are incorporated into the main database, care should be taken to ensure that the data are interpreted by the main database as expected and that the results are assigned to the correct sample or donor records.

All computer, i.e. information technology (IT) data, should be backed-up in a systematic and organised way to prevent loss of critical information in the event of

equipment failure. With all IT equipment, it is not so much a question of *if* it will fail, but more a question of *when* it will fail, and a good back-up system will ensure that a minimum amount of information is lost when this happens. Back-up systems, preferably off site, should be validated and tested regularly. If an automated system of testing or data management is used, it is important to have an alternative system, usually manual, that can be used in the event of the main (IT) system failing. This alternative system should be tested regularly to ensure that personnel know how to use it effectively and without increasing the risk of errors.

The organisation should ensure that the hardware, software and peripheral devices such as printers and barcode scanners function reliably and accurately in the environment in which they are to be used. Validation should include verification of data capture and manipulation, security and safety. If the computer system is used in the final release of product, this feature should be thoroughly tested and challenged to ensure that under all conditions and when presented with any permutation of results, the system will respond as expected. For example, a system designed to prevent the labelling of blood units with a reactive marker for a transfusion transmissible infection (TTI) may work perfectly on the original whole blood unit, but may not prevent the labelling of processed or pooled units when the original pack identification number has been changed by the addition of a suffix or the assignment of a pool number.

It is also important to establish that personnel who use the computer system can do so accurately and reliably in the context of their normal workload. Problems are often experienced with the entry of free text, such as donor or patient names and phone numbers, where it is not practical for the computer to perform extensive checks that the information entered is correct. Significant problems can arise when the data are retrieved, often months later, and found to be inaccurate. This can result in a great deal of confusion and frustration; a waste of time and effort in tracing the correct information, which could lead, for example, to the loss of contact with valuable donors. Also, attention should be paid to the operator's ability to respond to messages and warnings generated by the computer system, and not to accept whatever message is presented without due consideration of the implications. An operator may often be requested to confirm a procedural step such as 'Please confirm the discard of RCC no. 123456'. If the 'Confirm' choice is selected without the information being properly checked, and it is later found that it was FFP no. 123456 (not RCC no. 123456) that should have been discarded, it may be extremely difficult to 'undo' the incorrect step. Worse still, if such an error

is not detected, serious problems of traceability could result.

Documentation and record keeping

Quality documentation should provide a framework for the effective communication of the quality management system throughout the organisation, ensuring proper understanding and effective implementation at all levels. There should be policies and procedures in place that define clearly and unambiguously how various processes are intended to work, the relationships between them and how, and by whom, they are to be implemented. Written policies, SOPs or work instructions, laboratory worksheets and forms, records and labels are all important parts of the documentation system.

- SOPs, in particular, are often supported through the use of flowcharts that show, in a simplified way, the major steps in the procedure.
- Flowcharts are also very useful in the design of procedures and, if used correctly, will assist in highlighting ambiguities and missed steps in the procedure. It is common, for example, for an SOP to include a step such as 'Check that the patient's details on the pack label and the blood requisition form correspond' followed by the instruction to 'Issue the unit'. The critical step that is omitted is what to do if the patient's details do not correspond. A carefully compiled flowchart will highlight this omission.
- Policies and procedures generally provide a description of what is intended.
- Records provide the evidence of what actually happened.
- Worksheets and forms must be designed to ensure that all essential information is recorded.

Examples of important quality principles that emphasise the need for accurate documentation, and that may be used in the form of displayed posters in the workplace, include:

If it isn't written down, it didn't happen!

Do what you document, and document what you do.

In many quality systems, documentation is assigned to different 'levels' in order to accentuate the relationships between the various documents and to emphasise the importance of each.

Figure 3 shows the suggested levels of documentation in a quality system. Policies are the highest-level documents that define overall quality policy. Procedures are the next level, documenting 'how things will be carried

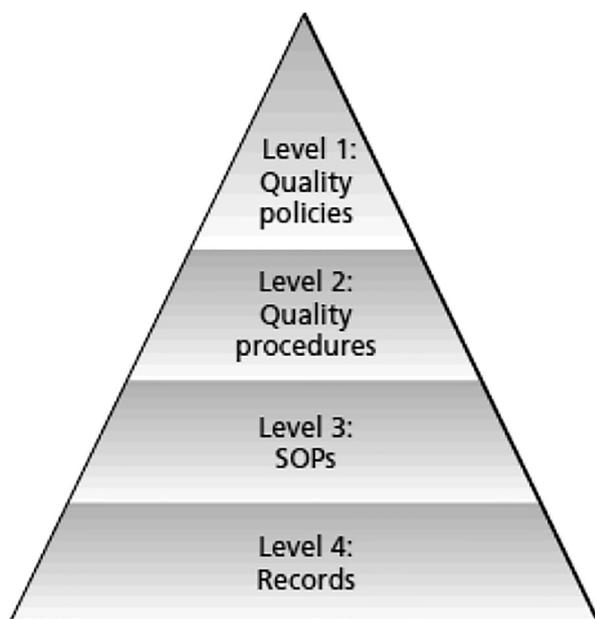


Fig. 3 Levels of documentation

out'. SOPs are the work instructions that provide a step-by-step account of work that was done and results that are achieved. Records are the forms and worksheets on which the work done, and results achieved are documented and retained. Organisations may choose to simplify the levels by reducing them to three levels whereby procedures and SOPs are combined.

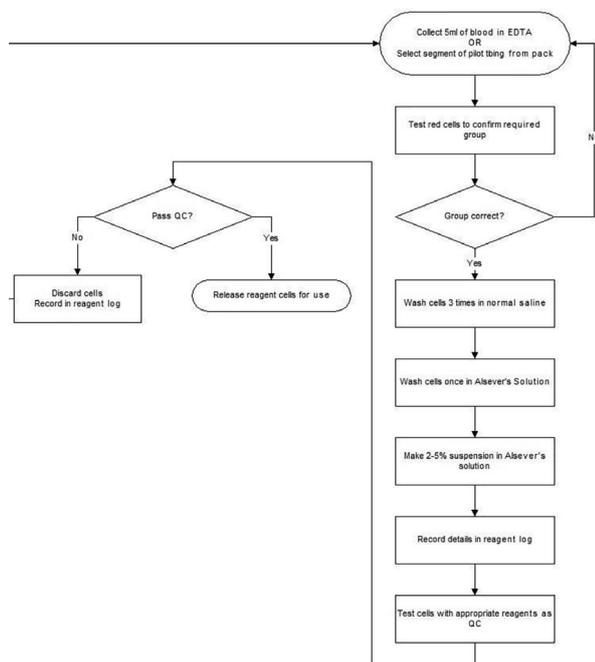


Fig. 4 Example of a flowchart that may be used to support an SOP

Figure 4 provides a flowchart of the main steps in a procedure for the preparation of reagent red cells.

At policy level, a quality manual should be compiled and implemented. The manual must explain how the elements of the system apply and relate to each other.

Accurate and comprehensive records enable each step in a procedure to be traced, providing evidence on how the procedure was carried out. This is necessary for monitoring that SOPs are followed correctly and that they produce the intended results, especially for complex processes such as component preparation and laboratory testing. By examination of the laboratory and other records, it should be possible to trace the sequence of events from the collection of a unit of blood, through all the processes until it is issued, revealing who was responsible for each step and gaining the assurance that each step was properly controlled. This audit trail should allow for the traceability of each unit from the donor to the recipient or recipients. Accurate records can also help to identify possible sources of error and enable appropriate corrective and preventive action to be taken to prevent a recurrence.

When a donor presents at a collection centre, it is important that he/she is correctly identified and that the results of the health check are accurately recorded at the time the checks are carried out. Even in a busy collection area, recording the results of these checks should never be deferred until personnel have more time, as this will increase the chance of error. The records, even if reviewed years after the event, should clearly identify the donor of each particular unit, and should reveal all the information related to the donation, including the date, time and venue of the collection, the personnel involved in the evaluation and collection of blood from the donor, and the type and lot number of the blood pack used.

In the laboratory, recording test results accurately and immediately the test is read is important to avoid errors, and to ensure that results are linked to the correct sample. The results of tests performed manually should be recorded in a format that shows the results in a clear and understandable way, preferably on a standard laboratory worksheet, and both the observed laboratory result and the interpretation of the results should be recorded whenever applicable. When performing an ABO group on a red cell sample, for example, the results of the anti-A, anti-B and (if used) anti-A,B should be recorded as well as the group interpreted from those results. The identity of individuals performing the work together with the date and time it was carried out should also be recorded on the worksheet. It needs to be emphasised that technologists must record the results produced by the actual tests that they have carried out and must under no circumstances record 'expected' results. This is particularly important in

a test such as an ABO group, where a particular pattern of results is expected for a particular blood group.

Records of blood collections, including the records of health checks and the record of the donor's agreement (informed consent) to donate and be tested, and laboratory records, particularly the records of tests carried out on donors and recipients, should be filed and retained in a document archives for a period of time determined by the organisation, but usually not less than 10 years. In view of this length of time and because many records are paper records, the archives area should be dry, dustproof, free of rodents and other harmful pests, and protected from fire. Access should be limited, and procedures should be in place for the management of documents entering the archives and for the temporary retrieval of documents that may be required for reference purposes. Due to the confidential nature of many records, particularly the results of testing for TTIs, only personnel with authority to study records, should be given access to do so. If records are converted to any other medium, particularly to an electronic storage system such as one that stores scanned documents on optical disks, care must be taken to ensure that such records remain legible as technology changes over the storage time of records.

Archiving systems should allow for the efficient retrieval of documents within time frames established by the organisation. These time frames should be determined by the urgency and frequency with which information is likely to be required.

The management of written policies and procedures is an important aspect of the quality system, and it is essential that all documentation is controlled in such a way that the documents are available where and when they are required. All older versions should be replaced with updated versions when documents are revised. The distribution of documents should allow time for personnel required to use the them, to become familiar with the changes before the changed procedure becomes effective.

Document management systems should include at least the following:

- The verification and validation of documents before approval and release.
- A system for the regular review of documents that includes a record of changes made in the latest version and the revision status (i.e. an indication of whether this is the first version or a subsequent version of the document).
- Confirmation that all documents are distributed to those areas that may require them.
- A signing system for users to record that all relevant new or updated documents have been read and understood and that training as required has been received, so that only those with demonstrated

competency carry out procedures without supervision.

- A system to control the distribution and retrieval of documents that prevents the use of obsolete versions.
- The archiving of one copy of outdated and original policies or SOPs.

Quality assurance and quality control

Quality assurance (QA) is not linked directly to the performance of a particular process but deals more with the maintenance of a system to ensure that the overall performance is of the required quality and to detect shifts or trends in the quality of products or services that may require attention.

Quality control (QC) refers to a procedure that is carried out at the time a test is performed and that provides feedback to the blood collection or laboratory personnel about the state of the test. QC should be designed to test the materials, equipment and technique used in the procedure so that the individual carrying it out can determine that everything is as expected, and that the test result can be accepted. If something is not functioning or behaving as it should, the test result should be disregarded until the matter has been resolved. For example, when carrying out ABO groups, the anti-A, anti-B and anti-A,B should be tested against the appropriate cells of known ABO groups to check that the reagents are both sensitive and specific.

Acceptable results for QC should be defined during the process validation and should be available to personnel performing the procedure so that unacceptable results are immediately apparent to them. QC results should be recorded at the same time as test results whenever possible, although the frequency with which QC is performed will depend to some extent on the type of assay being carried out. For example, QC samples should be assayed with every batch of ELISA or nucleic acid test (NAT) assays. Indirect and direct antiglobulin tests (IATs and DATs) are controlled by batch, and all negative test results are further controlled by the addition of sensitised cells to confirm that the AHG used, was active. Under most circumstances, it is acceptable to QC ABO test reagents only once a day, unless a new reagent bottle is opened for use, as the nature of ABO grouping is such that problems with the reagents are readily apparent.

Random sampling and quality control testing should be performed on blood and blood components. A minimum of 1% of the total number of each component routinely prepared or four units per month, whichever is the higher, should be tested for compliance with specifications.

All QC should be carried out according to a defined plan and results should be subject to periodic review. If QC results do not satisfy predefined criteria, the product or test results should not be released until the problem has been satisfactorily identified and resolved.

A QA programme involves sampling techniques, specifications and testing, as well as organisational, documentation and release procedures that together ensure the satisfactory quality of the process. For example, the accuracy of any test on a blood sample can be assured by a quality programme that:

- Ensures accurate identification of the patient or donor.
- Assesses the quality and suitability of the specimen for the assay to be carried out.
- Documents the validity of the test method, equipment, and reagents.
- Monitors the performance of the test method, equipment, reagents, and personnel.
- Reviews test results and QC results for errors prior to release. An effective QA programme is dependent on a continuous process of assessment and improvement.

It is also important that the performance of tests in the laboratories and blood collection areas be regularly assessed by participation in a formal system of proficiency testing or external quality assessment (EQA). EQA is a system whereby test performance is assessed by an outside agency. Usually, a set of unknown samples is sent by the external agency to the laboratory or blood collection area for testing. When the tests have been carried out, the results are returned to the external agency and are compared with those of an approved reference laboratory, and often with other participants in the programme. In this way, the laboratory or collection centre is assured of the accuracy (or otherwise!) of the results that are routinely released. If the feedback shows that the results are not within acceptable limits, the cause of the deviation should be established so that corrective action can be implemented immediately. The corrective action should be monitored carefully to ensure that the problem is effectively and reliably corrected. If the facility is not able to participate in an EQA system, then an inter-laboratory testing programme should be defined and documented and initiated to compare results of laboratory testing in a similar way as for EQA.

Management of non-conforming product or material

The quality system should include a procedure for dealing with products or services that do not conform to the documented requirements of the organisation. This procedure

should include the identification and handling of non-conforming products before or after the release of product as well as the response to reported adverse reactions to blood donation and to transfusion.

It is important that personnel are trained to recognise and report such events promptly if the occurrences are to be handled effectively. Examples of non-conforming product include a unit of platelet concentrate that contains less than the specified number of platelets or a unit of whole blood that exceeds the maximum allowable volume.

The way in which non-conformances are handled will depend on the nature and severity of the event and the likelihood of a recurrence. In each case, the occurrence should be documented and investigated. Attention should be paid to the possible impact on related activities and other products as it may be necessary to embargo or recall the balance of a batch of product until the investigation has been completed and satisfactorily resolved. The event must be analysed to establish the root cause of the problem, as this may not be obvious (equipment out of calibration, badly designed procedure, poorly trained personnel). Corrective and preventive action based on the root cause analysis (i.e. the investigation that is designed to establish the primary cause of the problem) should be recorded and implemented, after which the effectiveness of the changes should be monitored.

In some rare circumstances, it may be necessary to recall a batch of products as a result of a quality defect being identified after the products have been released, and a documented procedure should be in place to describe such a procedure. For example, if it is found that the anticoagulant has leaked from some blood bags belonging to a particular batch, and some bags from that batch have already been used for the collection of blood, it may be necessary to recall the units of blood immediately so that they can be checked for possible clotting, leakage or any other defect.

On occasion, it may be necessary for the organisation to deviate from its own approved procedures. This may be necessary for a variety of reasons, for example to meet the particular needs of a specific patient, but in each case the deviation should be documented. The nature of the deviation and the reason for the deviation should be recorded and approved by a senior person such as the quality manager. If applicable, the period for which the deviation is valid should also be recorded. By way of illustration, the ABO reagents in a laboratory may have reached their expiry date, but the new batch of reagents has not arrived from the supplier (which, of course, should not happen!). After confirming that the expired reagent meets the sensitivity and specificity

requirements of the laboratory, a deviation could be issued that allows the reagent to be used for a further set period of time, perhaps including the requirement for more frequent controls to be carried out. The primary (root) cause of this problem was that fresh reagents did not arrive from the supplier in time. This needs to be investigated and plans put in place to minimise the chance of recurrence.

Waste management – overview

The management of waste is detailed in *Section 15: Safety, health and the environment*.

For the effective disposal of waste, the separation of normal, or household waste, such as paper, tin and plastic, and biohazardous or contaminated waste such as used needles, and blood-soiled materials, is essential, and is best carried out at the point where the waste is generated. This is commonly performed using colour-coded garbage bags, e.g. using red bags for biohazardous waste and black bags for household waste.

The system for the disposal of biohazardous waste should ensure that the individuals handling the bags during the course of the disposal process will not be exposed to contaminated material and this may require double-bagging of waste, or the packing of red bags into a secondary outer container.

Another category of waste that requires special attention is that of 'sharps'; needles, lancets, broken glass and other items that can stab or cut if handled inappropriately. A system should be in place for the safe disposal of sharps and this usually requires that all sharps be placed in a special container immediately after use. The biohazardous waste must be disposed of by a method that has the approval of the local authorities for this type of material. This is usually by incineration or by autoclaving followed by disposal at an approved landfill site.

Monitoring and evaluation of the quality system

The quality management system should be examined regularly to monitor its effectiveness and the level of compliance with the documented system. This is accomplished through two mechanisms:

- Formal management review.
- Audits.
 - Internal audits conducted by trained and competent personnel from within the organisation.
 - External audits carried out by individuals from outside the organisation.

Once or twice a year a management review should be carried out. This provides an opportunity for senior management to review the activities of the quality programme to check that the system delivers useful information that is used productively to improve quality. Without management review, the organisation runs the risk of having a quality programme that delivers information that is ignored by the operational divisions and is therefore largely a waste of time and money.

The type of information presented to the management review meeting, usually by the quality manager, includes the following:

- Number of blood units collected, and number issued, which provides a measure of over/under collections.
- Prevalence of TTI markers in the donor population.
- Quality reports on components produced.
- Amount of rework in the laboratory resulting, e.g. from failed QC or failed equipment.
- Wastage of blood products as a result of failed storage conditions.
- General non-conformances identified and the effectiveness of resulting corrective and preventive actions.
- Frequency and nature of adverse reactions in donors.
- Frequency and nature of adverse reactions in recipients.
- Customer complaints received and the corrective actions put into place.
- Findings of internal and external audits.

Internal audits on an annual basis are usually carried out by members of the quality department, although they may be assisted by other personnel. Using a carefully compiled checklist, the auditors will measure the level of compliance with the documented quality system, with the formal legislation controlling blood transfusion activities and with any other legislation such as local fire, waste disposal and health regulations. Corrective actions to rectify any recorded non-conformances are agreed to by the auditors and the laboratory or blood collections manager, documented at the time of the audit and a time period set for rectifying deficiencies.

External audits, usually carried out less frequently than internal audits, are audits carried out by individuals from outside the organisation and should be encouraged to ensure that the highest levels of quality are met and maintained. If these external audits are carried out by trained auditors from an accreditation agency using defined and acceptable quality protocols, the organisation may be 'accredited' by that agency, which will give it additional credibility and lead to a high level of confidence in the eyes of customers and the general public.

Process improvement

Continuous improvement is one of the goals of a quality management system. A quality improvement plan that includes all parts of the organisation should be developed. This plan should allocate responsibilities for effective implementation and should be subject to regular review.

There should be a system in place for recording and investigating complaints received regarding the quality of blood products or services. These complaints should be investigated to determine the cause of the defect and to determine what improvements, or preventive actions, can be put into place to minimise the risk of a recurrence. Likewise, all errors and accidents, including 'near misses' (events that did not result in an error or accident, but that could have done so, had the circumstances been slightly different) should be documented and investigated in order to identify system problems that can be corrected. It is important that all personnel are clearly advised of the focus of an error management programme, which is aimed at developing and improving processes and is not at punishing the individual(s) who made the error. This should encourage prompt reporting of errors and lead to a more effective quality management system.

It is important to stress that quality is an ongoing activity; it is not a goal that can be reached before moving onto the next project. Edward Deming, one of the world authorities on quality, expressed this concept as a cycle, referred to as the Deming cycle, expressed as 'Plan, Do, Check, and Act'. If the quality system does not deliver continuous improvement, it is not functioning correctly. Figure 5 shows the Deming cycle.

Process improvement often occurs because of corrective or preventive action initiated by a complaint or

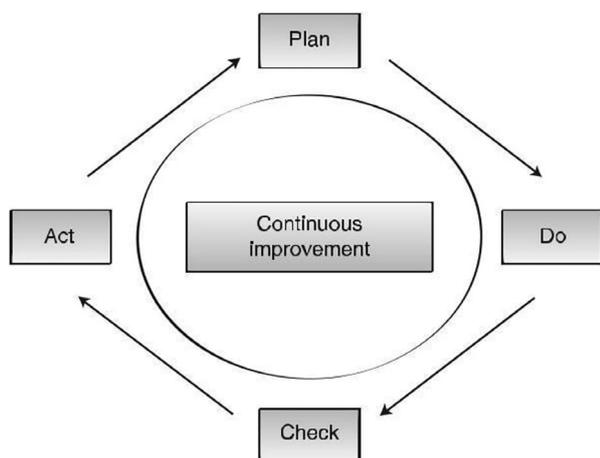


Fig. 5 Deming cycle.

non-conformance. Corrective action is taken when the cause of an existing non-conformance is removed, preventing a recurrence. This is a reactive response. Preventive action is taken when a potential risk of non-conformance is removed, to prevent an occurrence. This is a proactive response.

There are many sources of data that may be used for process improvement, some of which are:

- Analysis of QC on blood products.
- Reports on non-conforming products.
- Customer complaints regarding products and services.
- Analysis of proficiency test results (EQA).
- Internal and external quality audits.

When a problem is identified, it presents an opportunity for improvement. The problem should be thoroughly investigated to determine its root cause, and its extent and frequency of occurrence. Once a good understanding of the situation is gained, corrective action can be designed and implemented. All corrective action taken should be documented and monitored to check whether it was effective.

The quality manager should be concerned with change management, as part of the continuous improvement concept. Characteristically, there is resistance to change, when individuals are reluctant to do their tasks differently from how they did in the past. When technology changes, or when systems change, personnel need to be educated on the change and trained to carry out different tasks related to the change. It is important that the training includes the rationale for the change, and how it will be of benefit in the future.

Ethical guidelines

In order to inspire public confidence in every aspect of its operations, the blood service should conform to the highest ethical standards, and, as with the organisation's quality policy, these ethical principles should be stated by senior management in writing. Some of the principles that could be included in the organisation's 'Code of Ethics' are as follows:

Blood donors

- Blood donation should be voluntary and unpaid.
 - Donors should never be coerced into donating, either directly by the blood service or by teachers at schools, or superiors in the work environment. Patients requiring blood, or their friends and relatives, should not be required to 'replace' the units that have been utilised by the patient.

- Donors should not be paid, either in cash or in kind. Donors may, however, receive refreshments and token awards of recognition, reasonable time off work and reasonable compensation for travel expenses incurred in attending the donor session.
- Donors should be fully advised of the risks of donating blood or blood components (such as the increased risk of fainting) and sign an agreement (give properly informed consent) for the withdrawal of blood or blood components. This agreement to donate blood means that the donor gives permission for the phlebotomy and the withdrawal of blood after fully understanding the risks involved and that tests will be done on his/ her blood to detect transfusion transmissible infections.
 - Care should be taken with those donors who have difficulty in understanding such an agreement, e.g. individuals with diminished responsibility, and such donors shall not give blood.
 - Care should also be taken with those donors who may require educational material to be explained to them, e.g. the visually impaired, or require interpretation resulting from language barriers. Donations may be taken only if collection personnel are confident that all educational material has been fully understood, and that the donor agrees with the need to sign for this, in order to give blood.
- Donors should be informed of the tests that will be carried out on their blood, and of the fact that they will be informed of any reactive results.
 - If blood samples from donors are to be stored and may be used at some future time in surveys for new markers, or using improved testing techniques, the donors should be informed of this fact. Any research projects involving the testing of blood donors should be approved by an ethics committee (e.g. from the local university) before the project is commenced.
 - Donors should be informed that their blood will be used for transfusion and must agree to this.
 - If the blood is not intended for transfusion, e.g. it is intended for laboratory use, this must be explained to the donor who should give consent for this prior to the donation.
- Donors should be fully advised of the risk to a patient of donating infected blood and should be advised not to donate blood if they are motivated by the desire to obtain a TTI test.
- Other than in special cases (e.g. in directed donations where the donor donates for a nominated patient), donors should not be informed of the name of the patient to whom their blood will be transfused.
- Blood donors should not be discriminated against based on gender, race, political affiliation or religion.
- The health and safety of the donor should always be protected.
 - The donor should be assessed before donation to provide reasonable assurance that he/she will not be harmed by the withdrawal of the blood.
 - Only sterile, disposable equipment used once only, should be used for the collection of the blood.
 - The donor should be monitored throughout the donation procedure for any indication that he/she is undergoing an adverse reaction and if so, the donation should be discontinued immediately.

Blood recipients

- Potential recipients should be fully advised of the risks associated with a transfusion and should be asked to note in writing, their agreement to having the transfusion. This means that the benefits and the risks associated with the transfusion were explained and that he/she fully understands these risks and benefits. If a patient is unable to give this agreement, the decision to transfuse should be taken by the clinician in the best interests of the patient, based on his/her clinical requirements.
- Other than in special cases (e.g. in directed donations where the donor donates for a nominated patient), recipients should not be informed of the identity of the donor(s) whose blood they were given.
- Transfusion should only be given under the supervision of the prescribing clinician.
- Patients requiring blood should not be discriminated against on the basis of gender, race, political affiliation, religion, criminal record or their ability to pay for the blood or blood component.
- Recipients should be professionally informed if they have been harmed in any way, such as through the transmission of an infection via the blood transfusion they received.

General considerations

- Confidentiality of patient and donor information: all donor and patient information, including test results, should be regarded as confidential and care should be taken to ensure that records of such information are stored securely.
- Personnel should be trained to treat each other, donors and all other individuals with whom they have contact, with respect and dignity at all times.
- When reading test results, laboratory personnel should maintain a high level of concentration on the

job in hand, be totally honest and show no bias in recording test results. It should be noted that an 'expected' outcome may not be the same as the 'actual' outcome that is observed when the test is read, but it is the 'actual' outcome that must always be the one that is recorded.

- Personnel should not accept gifts from manufacturers or suppliers that could influence decisions on the procurement of equipment and materials for the blood service.

Key points

- The blood service should have a clearly defined mission statement supported by a code of ethics.
- A strategic plan should be developed that defines the objectives of the organisation.
- The blood service should be organised and structured in a way that maximises the effective implementation of the quality system.
- Senior management and the CEO are accountable for and bear the overall responsibility for quality.
- In general terms, quality is everybody's responsibility.
- It is the accountability of the organisation to provide a safe and clean workplace for all employees, and also for visitors such as donors, patients and others.
- A risk management framework should be developed to address potential business and safety risks.
- Equipment has a major influence on the quality of products, test results and services, and should be carefully selected and validated to test that it suits its intended purpose.
- Maintenance, cleaning and calibration of equipment should be performed regularly, and the activities documented.
- All new procedures and the equipment to be used in the procedure should be validated before being adopted for routine use. This validation should be carefully designed and carried out to ensure that the procedure will reliably and consistently deliver the results that are expected.
- Laboratories should have documented SOPs for each procedure that may affect the health or safety of donors and recipients of blood products.
- Personnel who are expected to perform the procedure should be trained to the SOP beforehand, and only when found competent, be allowed to perform the procedure without supervision.
- Computer systems should be evaluated and validated within the area in which they are to be used to ensure that they can manage data correctly and can deliver results in the expected way.
- Quality documentation should provide a framework for the communication of the quality system throughout the organisation, ensuring proper understanding and effective implementation at all levels.
- The QA programme includes sampling techniques, specifications and testing, as well as the organisational, documentation and release procedures that together ensure the satisfactory quality of the process.
- QC refers to a procedure that is carried out at the time a test is performed and that provides information regarding the validity of the test outcome.
- The quality system should include a procedure for dealing with products or services that do not conform with the documented requirements of the organisation (non-conforming products).
- Household waste, such as paper, tin and plastic, and biohazardous or contaminated waste should be separated at the point where the waste is generated.
- Biohazardous waste should be disposed of by a method such as incineration or by autoclaving followed by disposal at an approved landfill site.
- The quality system should be evaluated regularly. This is accomplished through formal management review and by means of internal and external audits.
- Continuous improvement is one of the main goals of the quality system.
- In order to inspire public confidence in every aspect of its operations, the blood service should conform to the highest ethical standards.

Equipment and materials management

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Reviewer for Second Edition: Judith Chapman

Introduction

In this section, equipment and materials management will refer to all activities needed for the efficient selection, procurement, distribution and maintenance of items in operation within the blood service. As it is not possible to include details of all such equipment and materials, examples will be given to illustrate activities described.

The goal of equipment and materials management is to provide the blood service with all the resources required to produce high-quality components, as expected by the medical community. Efficient planning is needed to avoid shortages of critical items such as blood bags and blood grouping reagents, and that there is always sufficient equipment to maintain testing and component manufacture.

The range of equipment and materials used within a blood service is large, and many items have a direct impact on the quality and/or safety of the end product or service. Many items are specialised and in some countries the number of vendors capable of supplying a particular item may be very limited. In addition, there may be strict storage requirements; the supply chain must be reliable if shortages or quality failures of received items are to be avoided.

Equipment and materials management in the context of this section refers to the acquisition of equipment, reagents, disposables and all other materials required for the efficient and effective operation of a blood service, including the acquisition of spare parts and replacements; and the validation, calibration and maintenance of equipment and materials as appropriate.

Materials that could directly affect the quality or safety of products or services are referred to as critical materials and should be obtained only from suppliers that have been shown to meet the organisation's requirements in terms of service, quality and cost. Examples of critical materials are blood bags and blood grouping reagents. Some equipment used in the blood service is also critical to the quality of the end product, e.g. scales or balances used in the collection of blood units, and should be obtained from, and serviced by, vendors approved by the organisation.

Many of the observations regarding equipment and material validation and supplier approval, made in this section, will also apply to other facilities such as the waste disposal company, or the company providing transportation of blood and blood products. The comments

and observations should therefore be seen in this broader context.

Learning objectives

By the end of this section, the student should be able to describe the following in simple terms:

- Procurement of equipment and materials
- Suppliers and supplies
 - meeting quality standards
 - periodic evaluation
- Acceptance of equipment and materials
 - incoming materials
- equipment qualifications
- Refrigerated centrifuge for component production
 - Servicing
 - Recalibration
 - Training
 - Records
- Materials management
 - antiseptic and virucidal agents
 - blood grouping reagents
 - reagent red cells
 - low ionic strength saline solutions
 - blood bags

Procurement of equipment and materials

Before initiating the purchase of any item of critical equipment, or any other critical material, it is important to define the functional and operational specifications for the item and the criteria that will be used in the selection of the supplier. This will give the reassurance that the selected equipment or material will satisfy the requirements of the end user and will deliver the expected results when used in the local environment. In quality terms, this initial process of documenting the functional requirements is sometimes referred to as the 'Design qualification'.

For example, if the equipment to be purchased is a benchtop centrifuge to be used in the serology laboratory, it would be important to determine the following before seeking a supplier:

- How many tubes must be centrifuged at one time?
- What size are these tubes?
- What maximum gravitational force (-force) will be required?

- What range of time must be covered by the automatic timer?
- What level of braking is required? Must this be variable?
- Is the user protected against aerosols? Is it possible to operate the centrifuge while the lid is open?
- What level of service and support will be required from the supplier?
- What is the budget?

Different types of equipment will obviously have different requirements and the extent of the application and the impact of this process will depend on the type and nature of the equipment under consideration.

Some of the general elements that may need to be considered during the design qualification include:

- Possible effect of temperature, humidity and lighting conditions in the working environment.
- Fluctuations in the electricity supply: requirement for an uninterruptible power supply (UPS).
- Purity requirements for the water supply: requirement for distilled or deionized water.
- Contamination of the environment by the process (e.g. generation of biohazardous or chemical waste).
- Noise created by the equipment.
- Service and backup requirements.
- Safety of the operator: requirement for special protective clothing or other personal protective equipment.
- Training requirements for the operator.

Attention should be paid to the elimination of all controllable environmental variables, such as temperature, humidity, light and extent of operator training. Eliminating these variables will reduce the variation in the overall process and make it more likely that the equipment will consistently deliver the expected performance.

Thoughtful consideration of the design qualifications for critical equipment and materials before purchase will help to minimize problems and frustrations later and it is important to invest adequate time and resources in this process.

Suppliers and supplies

Once a potential supplier (or suppliers) has been identified for the critical item, the supplier should be 'qualified' by the organisation to ensure that they are able to meet the required standards of service. This is the only way in which reliable and ongoing delivery of critical items can be established. The quality standards for the materials and the organisation's expectations from the supplier should be clearly defined, preferably in a written agreement between the two parties. This is particularly

important for items such as blood bags, where running out of supplies is life-threatening, and another source of supply may not be readily available.

Meeting quality standards

Some of the factors to consider when approving a supplier are:

- Is the supplier certified or accredited by an accreditation agency? This would provide a measure of assurance that the supplier meets established quality management criteria, but does not necessarily guarantee that they, or their products, will meet all stipulated requirements.
- Is continuity of supply assured? Does the company hold stock, or do they only order items when they receive the order?
- Is it possible to audit the facility? This may be important even if the facility only stores and reships critical items, such as test kits, which are carefully quality controlled after production, but which can be rendered ineffective if not subsequently stored and shipped at the correct temperature.
- Are the results of the supplier's own quality audits available, including customer complaints that have been received?
- Is the supplier financially sound? This could be important in terms of the ongoing supply of the items under consideration.

Periodic evaluation

A list of approved suppliers should be maintained for all critical items, and, if feasible, should include an approved alternative supplier. All critical items should be obtained only from the approved supplier. Suppliers should be re-evaluated periodically, and the frequency of the re-evaluations will depend on the nature of the critical item and the organisation's experience with the quality of the items and service delivered by the supplier. For example, the failure of an item, or the supplier, to meet the agreed specifications or standard of service should result in immediate corrective action if the impact on the quality and safety of the organisation's products is to be minimized.

Acceptance of equipment and materials

Incoming materials

Although materials may have been acquired from an approved supplier that has met all the stipulated requirements of the organisation, critical materials should still be inspected and, if necessary tested, before

being taken into use. The acceptance criteria for critical supplies, and any tests that are to form part of the acceptance criteria should be documented and should include the action needed if the material is shown to be unacceptable. This action may involve returning the material to the vendor for replacement, but, depending on the circumstances, the material may be destroyed with the vendor's approval.

Incoming materials that are awaiting testing, inspection or approval should be kept separate from approved materials so that they cannot inadvertently be taken into use. Likewise, any incoming materials that are rejected must be immediately placed in a designated and locked area and clearly labelled as unsuitable for use.

Equipment qualifications

All qualification testing that is done, must be documented and stored as a record.

Installation qualification

When equipment is received and prepared for commissioning, the process of receiving the equipment and installing it correctly may be referred to as the 'Installation Qualification' (IQ). IQ involves establishing that the key aspects of the equipment, including any ancillary equipment such as power supplies or purified water supplies, are installed strictly according to the manufacturer's specifications, and that any additional recommendations have been addressed. IQ will include steps to confirm that the equipment is received as specified, that it is undamaged, that it is installed correctly and that the environment into which it is installed meets the requirements of that equipment.

Operational qualification

'Operational qualification' (OQ), which follows IQ, is the name that is given to the process of calibrating, challenging, testing and evaluating the equipment after installation to ensure that it functions as expected in the selected environment. OQ should include a review of the standard operating procedures (SOPs) for the start-up, operation, maintenance, and cleaning of the equipment.

Performance qualification

Performance qualification (PQ) refers to the process of ensuring that the instrument consistently and reliably performs according to the defined specifications on an ongoing basis in the environment in which it will be used routinely. This will involve carrying out a series of tests using the equipment and following the relevant SOPs. All test results must be evaluated to determine whether they meet the requirements of the organisation.

The life cycle approach to equipment validation can be summarised as follows:

- (1) Design qualification (or functional requirements).
- (2) Installation qualification.
- (3) Operational qualification.
- (4) Performance qualification.
- (5) Evaluation of data and acceptance (or rejection – return to point 1).
- (6) Ongoing/routine Quality Control.
- (7) Ongoing/routine cleaning and maintenance.

Figure 1 illustrates the life cycle of equipment in the form of a flowchart.

If the design, installation, operational and performance qualifications are correctly drawn up and followed, and routine calibration, cleaning and maintenance are carried out as stipulated, the equipment is likely to perform as expected and deliver results that are within specification over a long period of time.

Refrigerated centrifuge for component production

A refrigerated centrifuge is used to illustrate some of the factors that should be considered in the selection and commissioning of an item of laboratory equipment.

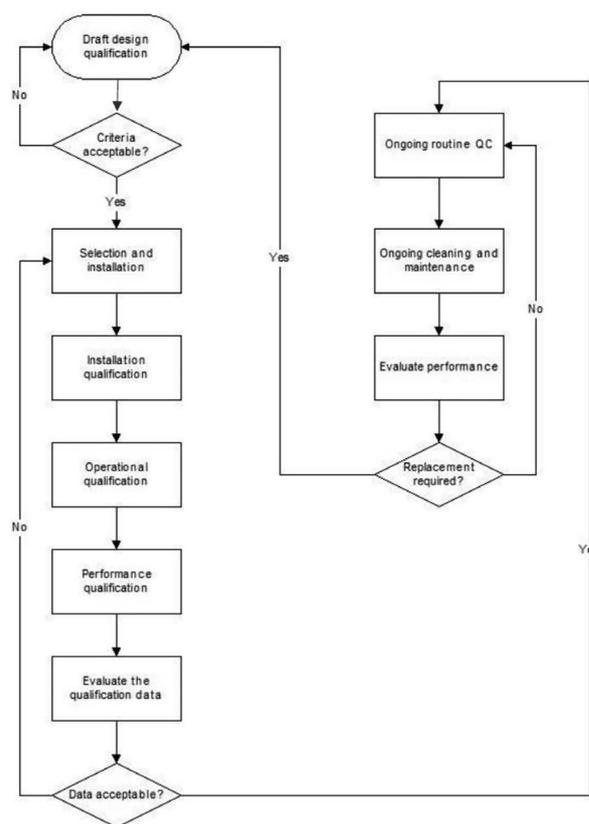


Fig. 1 Equipment life cycle.

Although each piece of equipment will present its own considerations, the basic principles remain the same.

Red cells, platelets, white cells and plasma all have differing specific gravities and are separated by the process of differential centrifugation using a refrigerated centrifuge (See *Section 11: Blood processing and components*). The spinning speed and time, the temperature, as well as the rotor radius, are some of the critical variables in the successful separation of these components. The centrifuge to be used in this process therefore plays an important role in the quality of the final product and should be chosen with appropriate care and properly validated before use.

Although the protocols for centrifugation often specify rpm (revolutions per minute) of the rotor, the separation of the constituents in the blood bag being spun depends more specifically on the relative centrifugal force (rcf), measured according to gravitational force (g), with one g being equivalent to the force exerted by gravity at the earth's surface, as applied during the centrifugation. Although rcf and rpm are related, rpm is strictly a measure of how fast the rotor is turning, whereas rcf takes into account the speed of rotation as well as the radius of the rotor. This is important because the greater the radius of the rotor, the greater will be the force applied to the blood bag for any given rotational speed. Thus, if a procedure stipulates the centrifugation as rpm, it will be necessary to convert this to the rcf or g for the particular centrifuge being used before the method can be applied. For more information, see *Section 11: Blood processing and components*.

The IQ, OQ and PQ protocols will often be outlined in a single master validation plan but will be dealt with separately here.

Some factors to consider in the selection of a centrifuge:

- How many blood bags are to be centrifuged (spun) at one time?
- What size bags are to be spun? The space required in the centrifuge buckets must be sufficient for the additional satellite bags, one of which may contain red cell preservative solution.
- Will the centrifuge and the proposed rotor provide sufficient g to effect/ produce the separation required?
- What temperature range and level of control is required?
- Does the centrifuge provide for control over acceleration and braking?
- Is there provision for the various centrifugation programmes that will be required to produce various components?

The information gathered by answering these questions will assist in compiling the design qualifications and

identifying the types of available equipment that will meet these requirements. The selection of a supplier will include an evaluation of the various suppliers of the equipment in the area, the availability of spares and servicing, and an evaluation of the initial as well as the long-term operating costs.

The design and installation qualification will involve determining at least the following when the equipment specifications are available:

- Is an appropriate location for the centrifuge available? Is it convenient to the work area where the centrifuged bags will be processed? Is the floor strong enough to support the equipment? Is the floor level?
- Is the required electrical power (voltage, amperage) available? Is a backup power supply required? If so, is it available?

An IQ protocol is then compiled which details the checks to be performed on the equipment and the tests that need to be carried out to ensure that the equipment is installed correctly. This could include determining the following:

- Does the lid of the centrifuge lock correctly when the centrifuge is operating?
- Can the lid be opened in the event of a power interruption during a spin cycle?
- Is the speed measurement within acceptable limits?
- Is the time measurement within acceptable limit?
- Is the acceleration and braking within acceptable limits?
- Is the refrigeration functional even after extensive use? Does it operate correctly at all required temperatures?

The IQ protocol should include the acceptable specification determined by the tests that are required i.e. What variation in the temperature during a spin cycle is acceptable?

When the IQ protocol has been completed, and this should be done by the supplier providing the equipment, the results obtained are compared to the expected results recorded in the IQ protocol. If the requirements are met, this will provide the necessary evidence that the equipment is installed as intended. If the requirements are not met, then the equipment cannot be used.

The next step is the operational qualification. The objective of OQ is to clearly define the process and the controls that will be required to ensure that the equipment and method will consistently result in an acceptable output. The OQ protocol will therefore include the required specifications for each blood component to be produced using the centrifuge and will define the tests to be carried out to ensure that these specifications are met. The tests should be designed to deliver sufficient data to enable the spinning speed, spinning time, and the rate of

acceleration and deceleration to be selected. SOPs for the various components produced using this centrifuge can then be written and must include what action to take when specifications are not met and the blood product produced fails.

The objective of the next step, PQ, is to demonstrate that the SOP will produce acceptable components consistently. It is usual to process several batches (three is usually deemed sufficient) as part of PQ. As with the qualifications described above, a protocol should be compiled before PQ is commenced, in which the number of components to be produced and how these will be evaluated is clearly defined.

Planned equipment maintenance

Once the process of selecting, evaluating and installing the equipment has been completed, a programme of planned (or preventive) maintenance should be started. All equipment needs to be properly maintained if it is always to work efficiently. In addition, properly maintained equipment will generally have a longer lifespan than equipment that is poorly maintained. The type of maintenance that is required and the intervals at which the maintenance needs to be carried out will depend on the type of equipment, the frequency of use and the environment in which the equipment operates. Some types of equipment, e.g. refrigerators used for the storage of blood, may require more regular attention because of their importance in maintaining the quality and safety of the stored products. Some equipment, for example the centrifuges used in the preparation of blood products, require regular calibration and this will form part of the planned maintenance programme.

Servicing

Planned maintenance may be regarded as a proactive series of regular checks that will ensure the ongoing accurate functioning of the equipment. It will also extend the life of the equipment and minimise the chances of equipment failure, thus reducing the overall cost of ownership. After the guarantee period, minor servicing requirements may sometimes be met by trained blood service personnel; major servicing should be carried out by the supplier, so this is a consideration when procurement is done.

Recalibration

Calibration, which often forms part of planned maintenance, involves the comparison of actual performance against required performance and should include

adjustments to ensure that the result falls within acceptable limits. If the equipment fails the calibration, it should be so labelled, locked and removed from use until the cause of the problem has been rectified and recalibration has shown performance to fall within the acceptable limits.

Training

Training of personnel in the proper use and cleaning of equipment is an essential part of the maintenance process. Equipment may require a schedule of daily, weekly and monthly cleaning and maintenance, often carried out during the start-up or shut-down procedures, in order to retain optimum performance. This maintenance should routinely be carried out by the operator and recorded in the equipment file. Training of personnel should also address safety issues that may relate to the operation of the equipment, such as the correct handling and disposal of dangerous chemicals and biological waste, and safety of electrical connections.

Records

Efficient documentation of all planned and unplanned maintenance (when there are unexpected problems and breakdowns) is important so that each piece of equipment has a maintenance history, which should be easily accessible when needed for reference. Records with dates, should also be kept of regular personnel training and retraining, to show that all those who operate the equipment are qualified to do so, and have been given all the instructions required, and have demonstrated that they know how to use the machine correctly.

Essential steps in establishing a planned maintenance programme:

- Create a register of all equipment that will require maintenance and calibration. This register should include the type of equipment; the make, model and serial number; the date of purchase; the name of the supplier; a unique equipment identifier and the location of the equipment.
- Establish the acceptable performance specifications for each type of equipment. For example, what is an acceptable temperature variation in the blood storage refrigerator? The temperature will, of necessity, fluctuate around the set temperature, but the acceptable fluctuation should be defined as part of the performance specifications.
- Determine the maintenance and calibration intervals for each type of equipment, what type of maintenance is required and who will be responsible for

the maintenance. Very often the suppliers of the equipment will assist with the provision of this information.

- If maintenance is to be carried out by an outside facility, it is often advisable to have a maintenance agreement in place that details the service and maintenance requirements.
- Establish the documentation requirements. Each time an item of equipment is serviced or repaired, the date, responsible individual and the nature of the repair or service should be recorded in the equipment register. It is also important that any breakdowns in the equipment, or error messages generated by electronic equipment should be recorded. Accurate record keeping will provide a history of the equipment's performance and assist in making decisions regarding replacement.

Materials management

Because of the wide range of materials used by the blood service, it is impossible to document the specifications and quality requirements for every item in this publication. A few items have been selected to provide examples from which an overview can be obtained for designing the requirements for any other items.

Antiseptic and virucidal agents

Antiseptic and virucidal agents are used extensively in the blood service for a variety of processes ranging from the preparation of the donor's venepuncture site prior to phlebotomy in a way that will provide reasonable assurance that viable micro-organisms will not enter the donation, to maintaining a workplace relatively free of infectious agents. Many different disinfectants are available and the choice of which should be used for a particular application is not a simple one. There is probably no single agent that is suitable for all applications. Some of the factors that should be considered before selecting a particular product are the following:

- The type of surface (topical, i.e. applied to a particular part of the body, or hard surface) and the material (such as skin, metal, glass, plastic) to be disinfected.
- The type of micro-organisms that may be present.
- Any hazardous or unwanted properties of the antiseptic or virucidal agent, e.g. corrosiveness or the depositing of stains.
- The organic load, i.e. the degree of microbial hazard, that may be present in the area to be disinfected, e.g. an area covered by a blood spillage.
- The temperature at which the process is carried out.

- The dilution of the disinfectant.
- The time over which the antiseptic or virucide is to remain in contact with the surface before the process is considered complete and disinfection achieved.

After the 'ideal' disinfectant has been selected for a particular application, procedures should be written that will ensure the practice is effective consistently. Procedures should cover:

- The storage of the agent (temperature and expiry).
- Preparation of the agent prior to use (e.g. dilution) and the change in expiry time, if any (and labelling of the container used).
- Steps to be taken before a particular disinfection procedure (e.g. if treating a blood spill, time should be allowed for the aerosol i.e. any fine blood particles that may be suspended in the air to settle, and absorbent material should be used to remove as much of the spill as possible).
- The method of application of the disinfectant and the contact time that should be allowed.
- Final disposal of the spill and cleaning materials.
- Steps that are taken to ensure that sufficient cleaning materials are in place for the next spill.

As always, when a disinfectant has been selected for a particular application, procedures should be validated to ensure that the expected results will be obtained.

Blood grouping reagents

Most antigens are made up of multiple epitopes, and therefore induce a response from multiple B cell clones, each producing antibodies against one specific epitope (See *Section 2: Immunology* for further details). The result is a mixture of antibodies, each reacting with a different epitope on the same antigen. These are referred to as polyclonal antibodies. *In vivo*, this mixture of specificities is very effective in facilitating phagocytosis and the complement-mediated destruction of the antigen, but in the laboratory the diverse specificity reduces the efficacy of the reagent.

Monoclonal antibodies are derived from a single B cell clone, and therefore all antibodies are directed against the same epitope. The clone is carefully selected by the manufacturer of the reagent to provide maximum efficacy. Thus, for most routine blood grouping purposes, monoclonal antibodies are the reagents of choice.

When selecting a blood grouping reagent, samples of the available reagents should be obtained and appropriately validated to ensure that the expected results will be obtained in the local circumstances under which they will be used routinely. If the reagent is to be produced in-house, extensive testing is required to ensure that it meets requirements with respect to specificity and

sensitivity. Procedures should be written for the use and quality control of the reagent before it is taken into routine use.

Reagent red cells

Reagent red cells used for ABO grouping, detection of irregular alloantibodies and in the control of blood grouping reagents, are prepared from human blood and that introduces additional requirements. The donors of reagent red cells should be thoroughly tested and investigated to ensure that the reagents are free of known viruses and other infectious agents that could place the user at risk.

The requirements for the preparation of reagent red cells are quite extensive and may be beyond the capabilities of many blood bank laboratories. Reagent red cells are available commercially and because of their short lifespan, usually four to 6 weeks, are often supplied on contract only. Because of the sensitive nature of the product, it is important to pay specific attention to the storage and supply chain of the cells. Red cells that are inappropriately stored or transported cannot be relied upon to produce the expected results in laboratory tests.

Some factors that should be considered in the production of reagent red cells:

- The presence or absence of the expected antigen should be confirmed using a minimum of two different reagents (e.g. if the K antigen is required to be present on the red cells, this should be demonstrated by positive reactions using two different examples of anti-K).
- Red cells should be washed beforehand, so that suspensions are free of ABO and other antibodies, and free of soluble ABH substances.
- Red cells should be suspended in a solution that will ensure the stability of the red cell antigens throughout the storage period.
- The expiry date and storage temperature of the red cells should be clearly stated on the reagent container.
- The concentration of the red cells in solution should be accurately prepared and should be suitable for the method by which the reagent will be used.
- Unless intentionally coated with IgG antibodies or components of complement, the red cells should give a negative result in the direct antihuman globulin test with anti-IgG, anti-complement and also polyspecific antihuman globulin.
- Red cells should not produce unexpectedly positive results, i.e. when tested against an inert reagent.

- Red cells sets that are to be used to screen patients for the presence of irregular alloantibodies should be from single donors, i.e. they should not be pooled, and the cells should contain specified blood group antigens, relevant to the population where testing is done.

Low ionic strength saline solutions

Low ionic strength saline (LISS) is used for the detection of irregular red cell alloantibodies using the indirect antiglobulin technique (IAT). LISS functions by increasing the rate at which the antibody combines with the corresponding antigen when compared with cells in normal saline, thus enabling the incubation time to be reduced substantially. LISS methods include suspending the test cells in LISS or, perhaps more commonly, adding LISS to the serum/cell mixture before incubation. Whichever method is used, the uptake of antibody is influenced by the proportion of serum/plasma to LISS and therefore care should be taken to ensure that the manufacturer's instructions are followed.

LISS can be obtained commercially or produced locally. In either case, it is important that the solution is carefully validated to show that the reduction in the incubation time that accompanies the use of the reagent will not result in a loss of sensitivity.

LISS should be controlled by testing a weak alloantibody by IAT. An antibody that reacts in a LISS test, but which fails to react, or reacts only very weakly with red cells suspended in normal saline after a 10-min period of incubation, should be selected for this control so that the increased rate of antibody uptake onto red cells suspended in LISS, leading to a clearly positive result, can be clearly shown.

Blood bags

In many countries, blood bags are required to be approved or licensed by the medicines control authorities. This can be a lengthy procedure, the responsibility for which generally falls to the manufacturer or agent for the bags. The first step in identifying the supplier of the bags for the organisation therefore is to establish that the bags on offer meet with the local licensing requirements.

Blood bags are one of the single most important items used by the blood service and they have a profound effect on the quality and safety of the final product. Containers must be sterile and pyrogen free and must contain sufficient anticoagulant for the volume of blood that is to be collected. Bags from a

reputable manufacturer can be expected to meet these basic requirements. Blood bags also make up a sizable portion of the budget and it is therefore important that due consideration be given to the selection of the product and the vendor.

Some requirements for consideration of a blood bag manufacturer and supplier are as follows:

- Is the full range of bags available to meet requirements? The type of blood bags and satellite bags needed depends on the blood components processed in the facility.
- Are the bags available with a diversion pouch, if required?
- If an additive solution for the preservation of red cell concentrate is required, is this available in the formulation needed?
- Are the bags designed for the volume of blood that it is planned to collect?
- Will blood bags be supplied with a sufficiently long expiry date?
- Can storage requirements for stocks of blood bags be adequately met?
- Will the vendor be able to satisfy ongoing delivery and supply requirements?
- Will the bags, anticoagulants and preservative solutions provide the storage times for blood donation that are required? Are there studies available that successfully demonstrate satisfactory red cell survival following the maximum period of storage?
- Are the bags robust enough to withstand normal handling, including centrifugation?
- Do the bore, sharpness and bevel of the venepuncture needles match requirements?
- Are blood bags supplied with a needle safety guard?
- Can the vendor provide additional products and services that may be required, such as tube sealers, tube strippers, infusion sets, filters and assistance with the training of personnel?

Key Points

- Before initiating the purchase of equipment, or any other critical material, it is important to define the functional and operational specifications for the item and the criteria that will be utilised in the selection of the supplier.
- The initial process of documenting the equipment requirements is referred to as the design qualification.
- Suppliers and supplies should meet quality standards as defined by the blood service.
- Periodic evaluation should be performed on suppliers to ensure that their supplies continue to meet these quality standards.
- Incoming materials should be inspected on arrival and not accepted unless they meet quality standards.
- Equipment qualifications include installation qualification, operational qualification and performance qualification.
- The selection criteria for an appropriate blood bag centrifuge for the preparation of components is used as a practical example in this section.
- A planned maintenance programme should be in place for all pieces of equipment, including servicing and recalibration.
- Personnel should receive training on preventative maintenance and this training, together with all servicing and calibration activities should be documented to compile a history for each piece of equipment in service.
- Materials management is also important, as their use affects the quality of the services provided by the blood service, and in the case of blood grouping reagents and supplies, the quality of the results obtained in laboratory tests.
- Blood bags are a critical component of a safe blood supply, and their control and quality must receive ongoing attention.

Indications for transfusion of blood components

Authors: Miquel Lozano & Maha Badawi

Introduction

Around the world, millions of blood donations are collected annually. Whereas most donations from countries with a high or medium development index (HDI or MDI respectively) are processed into components, this may not yet be the case in low development index (LDI) countries where transfusions of whole blood are common.

As with all therapeutic interventions, blood transfusions should only be prescribed when clinically indicated. Careful consideration of expected benefits and potential risks should take place before every transfusion order. Although transfusions save many lives, they may conversely be associated with serious adverse events, including infectious and non-infectious complications. However, when the most sensitive means of testing of donations is included, and pathogen inactivation technologies are used during the preparation of components, the risks of transmission of infectious agents is greatly reduced, although never eliminated. For further information see *Section 14: Transfusion risks and haemovigilance*.

Additionally, studies have shown that a liberal transfusion strategy may be harmful when compared with a restrictive transfusion strategy, in specific patient populations.

Careful management of limited resources available for the collection, testing and component preparation of safe blood for patients highlights the need to follow the specific indications for each component before transfusion.

Learning objectives

By the end of this section, the student should be able to describe the various components that may be prepared from blood donations, and their specific uses, including the following:

- Evaluation of the indications for transfusions based on currently available scientific evidence
- Red cell components
 - Indications for red cell transfusion
 - Contraindications for red cell transfusion
- Platelet components
 - Indications for platelet transfusion
 - Contraindications for platelet transfusion
 - Dosage and administration for platelet transfusion

- Plasma components
 - Indications for plasma transfusion
 - Contraindications for plasma transfusion
 - Dosage and administration for plasma transfusions
- Cryoprecipitate
 - Indications for cryoprecipitate transfusion
 - Dosage and administration for cryoprecipitate transfusions
- Alternatives to the transfusion of donor blood
 - Patient blood management
 - Replacement fluids to maintain blood volume
 - Crystalloids
 - Colloids
 - Recombinant plasma protein products
- Alternatives to transfusion under development
 - Red cell substitutes
 - Modified red cells
 - Cultured red cells

Transfusion of red blood cell components (including whole blood)

Red cells contain haemoglobin and serve as the primary agent for the transport of oxygen to the tissues. The primary red-cell-containing transfusion component is red cell concentrate (RCC). This component is prepared by centrifugation or sedimentation of whole blood and the removal of much of the plasma. RCC components can also be prepared by apheresis. For further information see *Section 9: Blood collection* and *Section 11: Blood processing and components*.

Processing and/or storage deplete RCCs of virtually all potential therapeutic benefit attributable to the functions of white cells and platelets. These inactive cellular elements remain in the RCC and may cause adverse immunologic or physiologic consequences. Depending on the method of production, RCCs may contain approximately 20 to 100 ml residual plasma. RCCs prepared with additive solutions have limited residual plasma and are the most commonly used red cell products.

All components containing red cells increase the recipient's oxygen-carrying capacity by increasing the mass of circulating red cells.

Whole blood units are prepared in an aseptic manner in a ratio of 14 ml anticoagulant-preservative solution per 100 ml whole blood targeted for collection. In areas where components are available, there are few indications for the use of unprocessed whole blood. These include:

- Massive haemorrhage, where there is a possibility that this will be ongoing or will recur.
- Exchange transfusion in both adults and children.

In recent years there has been an increased use of whole blood for trauma resuscitation in the battlefield but also in civilian trauma patients, not only at hospital level but also in air and ground ambulances. If whole blood is used because RCC is not available, the indications and guidelines as described for RCC (below) would apply.

Autologous whole blood is collected from patients who anticipate requiring blood transfusions. Donor-safety screening criteria and testing procedures applicable to collection from allogeneic donors do not always apply to these components. All units intended for transfusion to the donor/patient must be labelled 'AUTOLOGOUS DONOR'. In recent years, mainly due to the introduction of patient blood management programmes, the use of autologous whole blood has decreased significantly. For further information see *Section 9: Blood collection*.

Types of RCC

- **RCC:** Red cells are prepared from blood collected into an anticoagulant-preservative solution and separated from the plasma by centrifugation or sedimentation. RCC may contain from 160 to 275 ml red cells (50–80 g haemoglobin) suspended in varying quantities of residual plasma (20–100 ml).
- **Red cells in additive solution** are prepared by centrifuging whole blood to remove as much plasma as possible and replacing the plasma with 100 to 110 ml of an additive solution that containing dextrose, adenine, sodium chloride, and either monobasic sodium phosphate or mannitol. The haematocrit is usually between 55% and 65%. RCC in an additive solution has a lower viscosity than RCC suspended in plasma and flows through administration systems in a manner more comparable to that of whole blood. RCC stored with an additive solution have a shelf life of 42 days at $4 \pm 2^\circ\text{C}$.
- **RCC and RCC in additive solution leucocyte reduced (RCC leucocyte reduced)** is prepared from a unit of whole blood (collected in anticoagulant-preservative solution as noted above) initially containing 1 to 10×10^9 white cells per unit. Leucocyte reduction is achieved by filtration: either soon after collection (pre-storage) or after storage, sometimes at the bedside of the patient at the time of administration. Filtration will decrease the cellular content and volume of blood according to characteristics of the filter system used. RCC leucocyte reduced should have a residual content of leucocytes of 1.0 to 5.0×10^6 per

unit, depending on the standards in a given jurisdiction. Leucocyte reduction filters variably remove other cellular elements in addition to white cells, although at least 85% of the pre-filtration red cell content should be retained.

Indications for RCC transfusion

Components containing red cells are indicated for treatment of a symptomatic deficit of oxygen-carrying capacity. They are also indicated for red cell exchange transfusion.

Transfusion of adult and paediatric patients in intensive care should be considered at haemoglobin concentrations of 70 g/L or less. In post-operative surgical patients, or in hospitalised, haemodynamically stable patients with pre-existing cardiovascular disease, transfusion should be considered at a haemoglobin concentration of 80 g/L or less or if the patient displays symptoms such as chest pain, orthostatic hypotension (i.e. low blood pressure when standing), or tachycardia unresponsive to fluid resuscitation, or congestive heart failure.

In general, the decision to transfuse should be influenced by symptoms as well as haemoglobin concentration.

Recent evidence suggests that for patients of 65 years or more, the haemoglobin transfusion threshold might need to be higher; approximately 10 g/L to decrease morbidity and maintain quality of life.

Contraindications for RCC transfusion

Components containing red cells should not be used to treat anaemias that can be corrected with specific haematinic medications such as iron, vitamin B12, folic acid, or erythropoietin. RCC or whole blood should not be used solely for volume expansion or to increase oncotic pressure of circulating blood.

Dosage and administration for RCC transfusion

Each unit of RCC or whole blood contains enough haemoglobin to increase the haemoglobin concentration in an average-sized adult (70–75 kg) by approximately 10 g/L (and to raise the haematocrit by 3%). Smaller aliquots can be made available for use with neonatal or paediatric patients, or adults with special transfusion needs.

The ABO group of all red cell components must be compatible with ABO antibodies in the recipient's plasma. Whole blood should be ABO identical with the recipient; RCC, which has a reduced volume of antibody-containing plasma, need not be ABO identical. Serologic compatibility between recipient and donor must be established

before any red cell component is transfused. In cases when delaying a transfusion to complete laboratory testing would be life-threatening, uncrossmatched Group O RCC or ABO group specific RCC may be transfused before completion of pre-transfusion compatibility testing. For further information see *Section 13: Compatibility testing*.

The initial portion of each unit transfused should be infused cautiously and with sufficient observation to detect the onset of an acute reaction. Thereafter, the rate of infusion can be more rapid, as tolerated by the patient's circulatory system. It is undesirable for components that contain red cells to remain at room temperature longer than 4 h. If the anticipated infusion rate must be so slow that the entire unit cannot be infused within 4 h, it is appropriate to order smaller aliquots for transfusion.

Transfusion of platelet components

Platelet components are platelet concentrates resuspended in an appropriate volume of plasma or a mixture of plasma and platelet additive solutions (PAS) that are stored with continuous agitation at a temperature of $22 \pm 2^\circ\text{C}$ in a plastic container suitable for the exchange of oxygen and carbon dioxide. Platelets may be provided either singly or as a pool of four to six units, the general therapeutic dose for an adult patient. Platelet concentrates can also be prepared by apheresis, in which case they should contain over 3.0×10^{11} platelets, the equivalent to four to six units of platelets separated from whole blood donations.

Platelet components may contain a varying number of leucocytes depending upon the technique used in preparation, and as with red cell components, may be leucocyte reduced by filtration. Some units may contain more than a trace of red cells and will appear pink to salmon in colour. This occurs more frequently with whole blood-derived platelets than apheresis platelets.

Platelets play a key role in haemostasis. They circulate in the periphery of the blood flow in the vessel and adhere when sub-endothelium is exposed at sites of vessel wall injury. Upon adhesion and activation, platelets release the contents of their granules. This serves to recruit new platelets in the area to form a platelet aggregate and will serve as a support for the activation of coagulation complexes and result in the formation of thrombin and a blood clot. In order to remain in the circulation platelets should retain the biconvex disc shape typical of the resting platelets. Any form of activation during preparation or storage (including exposure to temperatures of approximately 4°C) will decrease platelet viability and survival after being transfused, as well as their ability to circulate in the recipient.

Types of platelet components

- A **single platelet unit** is a concentrate of platelets separated from a single unit of whole blood. One unit of platelets should contain $>5.5 \times 10^{10}$ platelets suspended in 40 to 70 ml of plasma. This component is usually provided as a pool of four to six units. See *Section 11: Blood processing and components* for further details on methods of platelet production.
- **Pooled platelets** are composed of individual platelet units combined using a sterile connecting device (SCD). Alternatively, individual buffy coats are pooled using a SCD and then centrifugated to separate off residual plasma, leaving behind the platelet concentrates. Shelf life varies between five and 7 days depending on suspending fluid used. The number of units of platelets or buffy coats combined in the pool varies between four to six and the platelets can be suspended in 100% plasma or a combination of plasma and PAS. The final volume of a typical platelet pool is between 300–350 ml.
- **Apheresis platelets** is a platelet concentrate collected from a single donor using a blood separator. Apheresis platelets contain approximately 3.0×10^{11} platelets, equivalent to four to six units of platelets prepared from whole blood donations. The number of leucocytes contained in this component varies depending upon the blood cell separator and protocol used for collection.
- **Platelets leucocyte reduced** is a pooled platelet or an apheresis platelet product with a leucocyte content of <1 to 5×10^6 leucocytes. The product can be prepared at the time of preparation (by filtration in the case of platelets prepared from whole blood donations or by using a blood separator in the case of apheresis platelets) or at the bedside using a filter, at the time that the product is administered.
- **Pathogen reduced platelets** are prepared from pooled or apheresis platelets, and in general are leucoreduced and suspended in PAS that is treated by one of the pathogen inactivation technologies currently licensed. In general, pathogen reduced platelets have a shelf-life of 7 days.

Indications for platelet transfusions

Platelet transfusions may be given to patients with thrombocytopenia or dysfunctional platelet disorders (congenital, metabolic, or medication-induced), in situations of either active platelet-related bleeding or for prophylaxis of bleeding.

Prophylactic platelet transfusion is indicated when the platelet count of the patient is of $10 \times 10^9/L$ or less to reduce the risk for spontaneous bleeding. Platelet transfusion is also indicated in cases of invasive or surgical procedures with a platelet count of $<50 \times 10^9/L$.

In case of bleeding and a platelet count of $<50 \times 10^9/L$ platelet transfusion is indicated. Higher platelet levels ($>100 \times 10^9/L$) may be required in clinical settings involving critical bleeding risks (e.g. neurosurgical and intraocular procedures).

Prophylactic platelet transfusion may not be of therapeutic benefit when thrombocytopenia is related to destruction of circulating platelets secondary to autoimmune disorders [e.g. immune thrombocytopenic purpura (ITP)]; however, transfusion may be indicated for active bleeding in these patients.

Platelets which are leucoreduced are indicated to decrease the frequency of recurrent febrile, non-haemolytic transfusion reactions, to prevent alloimmunisation against human leucocyte antigens (HLA), and to avoid transfusion-transmitted cytomegalovirus (CMV) infection.

Contraindications for platelet transfusions

Platelets should not be transfused if bleeding is unrelated to decreased numbers of, or abnormally functioning, platelets. Platelets should also not be transfused when the platelet count is $>100,000/\mu L$, unless there is documented or suspected abnormal function due to the pharmacologic effect of treatment. Patients with congenital surface glycoprotein defects should be transfused conservatively to reduce the possibility for alloimmunisation to the missing protein(s).

Dosage and administration for platelet transfusions

Compatibility testing is not necessary in routine platelet transfusion. Except in unusual circumstances, the donor plasma should be ABO compatible with the recipient's red cells when this component is to be transfused to infants, or when large volumes are to be transfused. The therapeutic adult dose usually contains 3.0×10^{11} platelets (one unit of apheresis platelets or four to six units of whole blood-derived platelets). One unit of platelets would be expected to increase the platelet count of a 70 kg adult by 30 to $40 \times 10^9/L$. For prophylaxis, this dose may need to be repeated in 1–3 days because of the short lifespan of transfused platelets.

Platelet components must be examined before administration for abnormal appearance such as a lack of swirling or the presence of aggregates. Units with excessive aggregates should not be administered. Transfusion may

proceed as quickly as tolerated and should not take more than 4 h. Platelet products must not be refrigerated during storage or prior to use.

The corrected count increment (CCI) is a calculated measure of patient response to platelet transfusion that adjusts for the number of platelets infused and the body mass of the recipient, based upon body surface area (BSA). BSA is measured in m^2 and is calculated by the formula:

$$BSA = \sqrt{\frac{H \times W}{3600}}$$

Where BSA is the body surface area in square metres, H is the recipient's height in centimetres and W is the recipient's weight (body mass) in kilograms. 3600 is a constant.

The CCI can then be calculated using the formula –

$$CCI = \frac{(\text{post - count} - \text{pre - count}) \times BSA}{\text{platelets transfused}}$$

Where post-count and pre-count are platelet counts ($\times 10^9/L$) after and before transfusion, respectively; BSA is the patient body surface area; and platelets transfused is the number of administered platelets ($\times 10^{11}$). The CCI is usually determined measuring the post-transfusion platelet count between 10 to 60 min after transfusion (1-h CCI) or 24 h after transfusion (24-h CCI).

In the clinically stable patient, the 1-h CCI is typically >7.5 and remains above 4.5 at 24 h. Both immune and non-immune mechanisms may contribute to reduced platelet recovery and survival. Along with supportive serologic test results, a 1-h CCI of less than five may indicate an immune-mediated refractory state to platelet therapy. With non-immune mechanisms of platelet consumption, the 1-h CCI is within the expected range, while the 24-h CCI is lower than expected.

Transfusion of plasma components

Plasma is the clear fluid that separates from whole blood after centrifugation. Fresh plasma contains all coagulation factors in addition to other plasma proteins. Plasma can be prepared from whole blood donations or through plasmapheresis. The shelf life of frozen plasma depends on storage temperature. Plasma may be stored for 12, 36 months, or 7 years if stored at $-18^\circ C$, $-25^\circ C$, and $-65^\circ C$ respectively. Blood services and blood suppliers need to verify the relevant regulatory recommendations in this regard.

When frozen plasma is ordered for transfusion, it is thawed by the blood service using either dry heat,

microwave, or waterbath methodology. Care must be taken to avoid contamination or over-heating during the thawing process. After thawing, plasma should be infused as soon as possible, but may be stored at $4 \pm 2^\circ\text{C}$ for up to 24 h.

Types of plasma components

- **Fresh frozen plasma (FFP):** plasma separated and frozen within 8 h of collection.
- **Plasma frozen within 24 h of phlebotomy (PF24):** PF24 contains lower levels of factors V and VIII than FFP and is therefore not the product of choice for the replacement of these factors.
- **Liquid plasma** may be separated from whole blood at any time during storage and may be stored at $4 \pm 2^\circ\text{C}$ for up to 5 days after the expiration date of whole blood from which it was separated. This type of plasma is never frozen and may contain viable white cells and requires irradiation if it is to be transfused to patients at risk of transfusion-associated graft vs. host disease. This type of plasma has less factor V and factor VIII than FFP. It can be utilised for all indications for FFP except for replacement of factors V and VIII.
- **Thawed plasma:** After thawing plasma from a frozen state, it may be stored at $4 \pm 2^\circ\text{C}$ for 24 h. After the initial 24 h post-thaw, plasma may be relabelled as thawed plasma and stored for four additional days. Levels of labile coagulation factors (V, VII, VIII, and protein S) show significant reduction in comparison with levels in the first 24 h post-thaw. It may be utilised to replace multiple coagulation factors in patients with peri-operative bleeding or those patients who have had massive transfusions.
- **Cryo-supernatant plasma:** This is the product that remains after removing cryoprecipitate from FFP. This product contains lower levels of factors VIII, XIII, fibrinogen, and Von Willebrand Factor (VWF) compared with FFP. It is typically utilised as a replacement fluid during plasma exchange in patients with thrombotic thrombocytopenic purpura, either during initial sessions or as salvage therapy for an unsatisfactory response post plasma exchange using FFP.

Indications for plasma transfusion

In general, plasma is utilised to replace coagulation factors in bleeding patients (treatment) or in patients who may bleed during an invasive procedure (prophylaxis). Coagulation factors may be reduced in patients with

inherited conditions, liver disease or disseminated intravascular coagulation. Specific situations include the following as adapted from Recommendations for the transfusion of plasma and platelets, Italian Society of Transfusion Medicine, 2009:

- Correction of congenital or acquired deficiencies of clotting factors (for which there is not a specific concentrate), when the activated partial thromboplastin time (aPTT) ratio or international normalised ratio (INR) is >1.5 :
 - Liver disease:
 - Active bleeding.
 - Prevention of bleeding in the case of surgery or invasive procedures.
 - During treatment with vitamin K antagonists (if prothrombin complex concentrate, which is the first-choice treatment, is not available):
 - In the presence of major or intracranial haemorrhage.
 - In preparation for surgery than cannot be delayed.
- Acute disseminated intravascular coagulation (DIC) with active bleeding, in association with correction of the underlying cause.
- Microvascular bleeding during massive transfusion (>1 blood volume), even before the results of prothrombin time (PT) and aPTT are known.
- Deficiencies of single clotting factors, in the absence of specific concentrates (e.g. of factor V), in the presence of active bleeding or to prevent bleeding during an invasive procedure.

As the replacement fluid (i.e. to replace the removed plasma) when therapeutic plasmapheresis is used to treat condition such as thrombotic thrombocytopenic purpura (TTP) or haemolytic uraemic syndrome (HUS).

Reconstitution of whole blood using a unit of RCC, for exchange transfusion.

For the treatment of hereditary angioedema if C1-esterase inhibitor is not available.

Contraindications for plasma transfusion

Plasma should not be used for volume replacement in patients who are hypovolaemic but not bleeding and have no coagulation abnormalities. It should not be used to replace albumin in patients with hypoalbuminaemia as albumin is available as a pharmaceutical product. In patients with deficiency of specific factors, specific products to replace those factors should be the first line management, with plasma being ordered only if these specific factors are unavailable. These specific factors include factor VIII (plasma derived or recombinant) in patients with haemophilia A, factor IX (plasma derived or recombinant) in patients with haemophilia B, and prothrombin complex

concentrate for vitamin K antagonists (e.g. warfarin or coumadin) reversal.

Dosage and administration for plasma transfusion

A dose of 15 ml/kg of body mass is usually ordered to raise coagulation factors to haemostatic levels in a bleeding patient.

The average unit of plasma derived from whole blood is 200–250 ml. Multiple units are required for adult patients. FFP may be associated with transfusion reactions, including transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO). TRALI mitigation measures introduced in the last few years were successful in reducing the risk of TRALI from FFP. These measures include manufacturing plasma from donors who do not have white cell antibodies, such as male donors or female donors who have not been pregnant.

Patients should receive FFP from donors of the same ABO group if possible, to avoid transfusion reactions due to high titre or haemolysing anti-A and/or anti-B. Group AB plasma does not contain ABO group isoagglutinins and can therefore be safely used in patients of all ABO groups. If group AB plasma is not available, then Group A, B or O plasma will need to be used, and only if it does not contain high titre/ haemolysing isoagglutinins.

Cryoprecipitate

Cryoprecipitate is obtained by thawing whole blood derived or apheresis FFP at $4 \pm 2^\circ\text{C}$, and then centrifuging and resuspending the precipitated proteins in a small volume of plasma and refreezing within 1 h. Cryoprecipitate contains fibrinogen, factor VIII, factor XIII, VWF, and fibronectin.

Cryoprecipitate may be made available as individual units (from a single whole blood donor) or as pools (typically five units). Pooling of individual units usually takes place using a closed system, before freezing, and is seldom done at the time of issue.

Cryoprecipitate is stored frozen at -18°C for 12 months or -25°C for 24 months. After thawing, the product should be transfused as soon as possible, but may be held at $4 \pm 2^\circ\text{C}$, for up to 6 h if manufactured using a closed system. If an open system is used to pool thawed individual units, then the product may be held at $4 \pm 2^\circ\text{C}$ for up to 4 h if necessary.

Indications for cryoprecipitate transfusion

Cryoprecipitate may be used to replace fibrinogen, factor XIII, factor VIII, and VWF, provided that a specific alternative is not available. Pharmaceutical concentrates of all

these factors are available, and utilisation of these over cryoprecipitate is encouraged due to the lower risk of transfusion transmissible infections and the provision of a more accurate dose of factor.

Dosage and administration for cryoprecipitate transfusion

A typical adult dose is 10 individual units (or two pools of five units each). This volume is expected to increase the fibrinogen in an average-sized adult by approximately 1 g/L.

Table 1 summarises the use of the various blood components, and Table 2 summarises the use of plasma derivatives. More detailed information on individual components or products may be found in other sections of this publication.

Alternatives to the transfusion of donor blood

Patient blood management (PBM)

The principles of PBM should be applied wherever possible to ensure that unwarranted transfusions are avoided. PBM refers to a global approach to optimise the care of patients who may require a transfusion and requires proper planning and a multidisciplinary approach. Education of prescribing physicians, adoption of hospital transfusion guidelines, and audits of blood utilisation are all building blocks of PBM.

For surgical procedures, different strategies are combined and employed in the preoperative, intra-operative, and post-operative setting to optimise care for an individual patient.

Pre-operative measures

Pre-operative measures include screening and treating patients for anaemia, ideally about a month before elective surgery. Oral iron, intravenous iron, folate, and Vitamin B12 may be administered to correct nutritional deficiencies. In certain situations, Erythropoiesis-stimulating agents (ESAs), such as epoetin alfa, are used in combination with iron. Patients are also screened for bleeding risks, and anticoagulant and antiplatelet drugs may be discontinued prior to surgery. Patients may also participate in a pre-operative autologous blood program (PAD); see *Section 8: Blood donors* and *Section 9: Blood collection* for further details about PAD.

Intra-operative measures

Intra-operative measures include optimisation of surgical techniques, topical haemostatic agents such as fibrin

Table 1 Use of blood components

Blood component	Notes on clinical use
Whole blood	Generally used in the absence of a component processing facility for patients requiring red cell transfusions.
Fresh whole blood	This may be required for: (1) Neonatal and adult exchange transfusion. (2) Massive transfusion (defined as volume of blood transfused within 24 h equal to estimated blood volume of patients).
Red cell concentrate (RCC)	Given to correct severe anaemia as a result of disease or blood loss.
Fresh RCC	Depending on local guidelines, fresh RCCs may be preferred for newborns and infants up to 4 months of age.
Leucocyte-depleted RCC	Universal leucodepletion is routine practice in some services and countries. Selected units may be leucodepleted to reduce the risk of transmission of CMV for patients such as: (1) Newborn infants (2) Intrauterine and neonatal exchange transfusions (3) Patients scheduled for stem cell transplantation They may also be used to prevent febrile non-haemolytic transfusion reactions attributable to white cell antibodies.
Irradiated RCCs	Units are irradiated to inactivate viable lymphocytes, to prevent transfusion associated graft versus host disease (TA-GVHD) in situations including: (1) Blood from a first degree relative of the patient (2) Immunodeficient patients (3) Transplant recipients on immunosuppressive medications (4) Fetal intrauterine transfusion or neonatal exchange transfusion
Washed RCC	Used if patient has had an anaphylactic transfusion reaction and may have antibodies to plasma proteins such as IgA, IgA deficient products may also be used if available. May also be used for patients with severe febrile non-haemolytic transfusion reactions
Cryopreserved RCC	Stored for patients requiring extremely rare blood types, or rare combinations of antigens. Units must be thawed and deglycerolized before use and will have a reduced shelf life depending on methods used.
RCC – paediatric	A single donation of red cells from a safe donor may be divided into four paediatric aliquots, since paediatric patients usually require smaller volumes of blood. If more than one transfusion is needed then additional aliquots from the same donor may be transfused, with the following advantages: (1) The red cells from one donation may be used more economically (2) The patient's exposure to donor antigens is reduced (3) Patient exposure to the risk of transmissible infection is limited
Platelet concentrate	Transfused to correct thrombocytopenia caused by defective platelet production, rather than by immune destruction, such as immune thrombocytopenic purpura (ITP). Platelets may be leucocyte-depleted and/or irradiated before use, for the same indications as red cells. HLA matched platelets collected by apheresis may be needed for patients with anti-HLA antibodies who are refractory to transfusion.
Fresh frozen plasma (FFP)	Used to promote haemostasis when multiple clotting factors are needed or when the specific clotting factor product is not available in situations such as: (1) Disseminated intravascular coagulation (DIC) (2) In conjunction with massive transfusions of red cells in trauma or surgery (3) In some cases of liver disease (4) Correction of bleeding associated with warfarin effect (Vitamin K, and if available, appropriate clotting factor concentrates are preferable) Used for exchange transfusions in Thrombotic thrombocytopenic purpura (TTP), to replace the metalloproteinase ADAMTS13
Cryoprecipitate	Thawed just before issue and is transfused to patients with: (1) Haemophilia A, either therapeutically to stop bleeding or prophylactically to prevent bleeding if FVIII concentrate is not available. (2) Fibrinolysis associated with DIC (3) Hypofibrinogenaemia in surgery or trauma (lack of fibrinogen) (4) FXIII deficiency

sealant, use of crystalloid or colloid replacement fluids, and administration of antifibrinolytic agents, such as tranexamic acid, to reduce bleeding. Use of transfusion

algorithms, based on laboratory or point of care testing may assist in optimising transfusion of various components. Point of care testing (POCT) uses devices such as

Table 2 Use of plasma derivatives

Plasma derivative	Notes on clinical use
Albumin – large pool, pasteurized	Albumin is supplied in concentrations of 4% and 20% and may be used to treat: <ol style="list-style-type: none"> (1) Extensive burns (2) Traumatic shock (3) Hypoproteinaemia (4) Hypovolaemia, although in many situations synthetic colloids may be preferred
Immunoglobulin – specific	Short-term passive immunity is provided using specific immunoglobulins prepared from the plasma of selected hyperimmune blood donors <ol style="list-style-type: none"> (1) Used to treat patients after exposure to or infection by infectious agents such as: <ul style="list-style-type: none"> – Hepatitis B – <i>Varicella zoster</i> – Tetanus – Rabies <p>Anti-D is used to prevent sensitisation to the D antigen in patients during a D positive pregnancy, after delivery of a D positive infant, or for female patients of child-bearing potential, following accidental transfusion with D positive blood.</p>
Immunoglobulin for intramuscular use	Contain a broad spectrum of antibodies to various infectious agents. A plasma derivative prepared for intramuscular use should never be given intravenously, as it could cause anaphylaxis. <ol style="list-style-type: none"> (1) Used prophylactically in patients after contact with infections such as measles and hepatitis A. (2) Used prophylactically in patients with congenital immunoglobulin deficiencies or transient hypogammaglobulinaemia, if no intravenous or subcutaneous formulation is available
Immunoglobulin –for intravenous use	Used for: <ol style="list-style-type: none"> (1) Replacement therapy in primary antibody deficiency syndromes (2) Myeloma or chronic lymphocytic leukaemia with severe hypogammaglobulinaemia and recurrent infections (3) Children with congenital AIDS and recurrent infections, in the absence of effective antiviral treatment <p>Used in autoimmune conditions such as:</p> <ol style="list-style-type: none"> (1) Idiopathic or immune thrombocytopenic purpura (ITP) (2) Kawasaki disease (an uncommon illness in children that usually resolves over several months). A rare and serious complication is the formation of blood clots within arteries around the heart. The illness may be treated by giving immunoglobulin, aspirin or carrying out plasma exchange. (3) Guillain Barré Syndrome (an autoimmune disease of the nervous system that usually responds to treatment with immunoglobulin or plasma exchange. Although most patients recover if treatment is initiated without delay, patients may be left with some degree of paralysis). (4) Other neuropathies.
Immunoglobulin –for subcutaneous use	Used for same indications as immunoglobulins for intravenous use, particularly for patients on chronic therapy.
Factor VIII, Factor IX, and Factor IX complex	The fractionation process is used to isolate FVIII, FIX and FIX complex (prothrombin complex) from plasma pools. FVIII and FIX may also be recombinant rather than plasma-derived. Used for treatment in: <ol style="list-style-type: none"> (1) Haemophilia A (FVIII) (2) von Willebrand's disease – patients benefit from an intermediate purity product which contains FVIII and von Willebrand factor (3) Haemophilia B (FIX) (4) Deficiency of FII, FVII, FIX and FX, such as patients bleeding and on warfarin (Factor IX complex)
FFP – large pool, freeze-dried	FFP may be pooled and solvent/detergent treated. Subsequent freeze-drying provides a stable product with an extended shelf life at ambient temperatures (below 25°C). Clinical uses include the following: <ol style="list-style-type: none"> (1) Plasma replacement (2) Replacement of clotting factors as required

thromboelastography (TEG) or rotational thromboelastography (ROTEM) to perform testing rapidly in the operating room using a small volume of whole blood. The tests measure many aspects of coagulation, including platelet function and clot stability, to guide treatment with platelets, plasma, and fibrinogen.

Intraoperative blood salvage involves using a special apparatus to collect blood aseptically from where it is shed, within the surgical area. It is then anticoagulated, filtered and re-infused either during the operation or soon after surgery. This process is not suitable for procedures involving bacterial contamination of the surgical field.

Acute normovolaemic haemodilution by perioperative blood donation involves collecting blood from the patient immediately before surgery and replacing it with fluids to maintain blood volume, which is important for cardiac function and the delivery of oxygen to the tissues. The patient becomes anaemic but remains normovolaemic. After surgery, when bleeding stops, the blood collected is returned to the patient. Because it is fresh, it contains viable platelets and clotting factors. This strategy is more likely to be beneficial in patients undergoing surgeries with high anticipated blood loss.

Post-operative measures

Post-operative measures include blood salvage, where blood lost after surgery is aseptically collected from surgical drains or wounds using a special apparatus, anticoagulated and filtered and then re-infused to the patient. It is used mainly when the shed volume of blood is likely to be large. Limiting phlebotomy for laboratory testing and using appropriate transfusion thresholds are also important for post-operative blood management.

Replacement fluids to maintain blood volume

Replacement fluids may be used for resuscitation, to limit blood transfusion, or when blood is not readily available. Replacement fluids may be needed when a large volume of blood has been lost, such as immediately after trauma, during surgery or in the treatment of extensive burns. The most important treatment for hypovolaemia is to restore the circulating blood volume in order to maintain tissue perfusion and oxygenation.

Replacement fluids are usually crystalloids or colloids

- Crystalloids are aqueous (water-based) solutions of low molecular weight salts. Glucose may also be added to these solutions. Crystalloid solutions are isotonic or slightly hypotonic. They cross the capillary membrane from the bloodstream into the interstitial spaces and are rapidly distributed within the extracellular compartment.
- Colloids contain larger molecular weight molecules in suspension, such as proteins or large glucose polymers. Colloids do not pass through the capillary membranes into the tissues and therefore raise the oncotic pressure of blood.

Crystalloids and colloids used as replacement fluids should ideally:

- Be widely available and cost effective.
- Be non-toxic, and unlikely to cause infection or allergic reactions.

- Remain in the intravascular compartment for a sufficient length of time.
- Assist with blood volume expansion.
- Be metabolised or eliminated in due course from the body.

The benefit of crystalloids is short-lived as they are redistributed extravascularly. Therefore, about three times the volume of blood that was lost is needed for replacement. Crystalloids accumulate in the extravascular space, which may cause interstitial/pulmonary oedema.

Colloids have a longer duration of action but are more expensive and may cause volume overload. They may interfere with haemostasis, cause allergic reactions, risk renal dysfunction and provide no clinical evidence of being more effective than crystalloids for resuscitation.

Crystalloids

- Normal saline (0.9%).
- Other crystalloids are commercially available, such as Ringer's Lactate and Plasmalyte B.
- Dextrose-containing crystalloids are only used for maintenance, not for volume replacement.

Colloids

- Human serum albumin – fractionated from large pool plasma, and pasteurised.
- Gelatines.
- Hydroxyethyl starches.

Recombinant plasma proteins

For details on Haemophilia and other bleeding disorders, see *Section 1: Haematology*.

- Recombinant clotting factors are genetically engineered forms of the clotting factors FVIII, FIX and FVIIa. As they are not derived from human blood, they do not carry the same risk of TTIs. Even though human-derived plasma derivatives are made viral-safe, concern remains about the risk of transmission of as yet unidentified infectious agents. As part of the manufacturing process for recombinants, a copy of the human gene for FVIII, FIX or FVII is inserted into a host cell, where copies of the gene continue to be produced. This host cell came originally from a hamster, but now large volumes of the designated human clotting factor are produced in manufacturing laboratories.
- Recombinant products perform in the same way as human plasma derivatives and replace the clotting factor lacking in the patient, in order to stop or prevent bleeding.
- A small minority of patients develop inhibitors to FVIII or FIX, which complicates the efficacy of the

product, whether of human origin or a recombinant. Patients who develop inhibitors to FVIII or FIX may be treated with recombinant FVIIa. This product can also be used to treat patients with a FVII deficiency. At this stage, patients with von Willebrand disease continue to be treated, when supplementation of von Willebrand factor is specifically indicated, with plasma-derived FVIII (which contains von Willebrand factor).

- Recombinant clotting factors are provided in powdered form, for reconstitution with sterile water immediately before use. The products should be stored in powdered form, according to manufacturer's instructions, which is usually for up to 6 months at room temperature (maximum of 25°C).

Blood substitutes under development

Red cell substitutes (oxygen therapeutics)

Alternatives to the red blood cell as a vehicle for the delivery of oxygen have been under development for decades, but in most countries, none are currently licensed and available for transfusion. It is recognised that the availability of a red cell substitute would present many advantages, such as universal usage without the risk of ABO mismatch. However, to find a suitable alternative as the carrier and distributor of oxygen has proved an enormous challenge.

Red cell substitutes cannot be used in a country without appropriate licensing. Those that are currently available can at best be seen as a bridge to transfusion. When blood is not available, they may be used primarily to treat severe anaemia and resuscitate patients with hypovolaemia.

They fall into two main categories:

Haemoglobin solutions

The haemoglobin used for these solutions is either of human, animal (usually bovine) or microbial origin. Although solutions may be pasteurised, there are still concerns regarding safety.

Perfluorocarbons

These are synthetic compounds that are solvents for oxygen. They are produced as emulsions and when infused, increase the transportation of gases *in vivo*.

Possible clinical uses of red cell substitutes are:

- Replacement at the time of preoperative haemodilution.
- Resuscitation after massive blood loss.
- Extracorporeal use, such as in bypass or haemodialysis machines.

- Substitute for blood for patients with multiple antibodies.

Advantages of red cell substitutes

- Reduction or avoidance of human blood use.
- Immediate benefit for trauma patients before blood is available.
- Preservation of organs for transplantation (perfusion after harvesting).
- Compatible with patients of all blood groups.
- Storage at room temperature.
- Non-immunogenic.

Disadvantages of red cell substitutes

- Short half-life *in vivo*.
- Costly to produce and provide.
- May be toxic.
- Not available most countries where they are not licensed.

Modified red cells

Research has been ongoing on the conversion of Group A (or B) blood to Group O. This has been performed by using bacterial enzymes that are able to remove sugar molecules from the red cell surface, thus removing the A (or B) terminal sugars that determine blood group. The conversion of Group B to Group O has also been achieved by using recombinant coffee bean alpha-galactosidase. If research of this nature proves successful, it may eventually lead to the availability of universal red cells that may be transfused into patients of any ABO group. This research does not include the D antigen, so D negative blood would still be required for D negative recipients.

Cultured red cells

Cord cell-derived CD34+ stem cells are used as the starting material to culture and develop mature red blood cells that function normally. During the process of conversion, CD34+ cells are mixed with erythropoietin and interleukin 3. Thereafter, the stem cells are cultured on cell stroma extracted from bone marrow or fetal liver with the addition of cytokines. The mature red cells that are produced have the same lifespan as red cells *in vivo* (120 days), which is a major advantage.

Cultured red cells carry the blood group antigens of the selected stem cells and therefore need to be ABO and D compatible with the patient for transfusion. When multiple units are transfused, the patient would be exposed to fewer antigens than would be the case with donated units, as all the cultured units would be antigenically the same.

The biggest disadvantage is the cost of producing these 'designer' red cells, and the size of the facility that would be needed to produce enough stocks.

Key points

- In general, the decision to transfuse should be taken after careful consideration of expected benefits and potential risks.
- The main indication for red cell transfusion is to increase the transportation of oxygen to the tissues.
- The decision to transfuse should be influenced by symptoms as well as the haemoglobin level in the patient.
- The transfusion threshold for haemoglobin in adults and paediatric intensive care unit patients is 70 g/L.
- In the case of pre-existing cardiovascular disease, red cell transfusion should be considered at a haemoglobin level of 80 g/L.
- In order to decrease the likelihood of morbidity and maintain a better quality of life, patients of 65 or more years of age may need a higher haemoglobin trigger for transfusion.
- Red cell transfusion should not be used to treat anaemias that can be corrected with specific haematinic medications (iron, vitamin B12, folic acid...)
- The indication for platelet transfusions is to treat quantitative and/or qualitative platelet disorders.
- Platelet transfusion is indicated when the platelet count of the patient is $10 \times 10^9/L$ or less to reduce the risk of spontaneous bleeding.
- Platelet transfusion is also indicated in case of invasive or surgical procedures with a platelet count $<50 \times 10^9/L$.
- One unit of platelets would be expected to increase the platelet count of a 70-kg adult by 30 to $40 \times 10^9/L$.
- Transfusion of plasma is indicated for replacement of single or multiple coagulation factor deficiencies in patients who are bleeding or when an invasive procedure is planned, and the specific plasma product is not readily available.
- Transfusion of cryoprecipitate is indicated for replacement of VWF, Factor VIII, Factor XIII, or fibrinogen, in patients who are bleeding or when an invasive procedure is planned.
- Plasma and cryoprecipitate should only be used in the absence of a specific alternative providing the same constituent.
- Patient blood management (PBM) refers to a global approach to optimise the care of patients who may require transfusion.
- PBM includes pre-operative, operative, and post-operative strategies to minimise blood usage.
- Replacement fluids, such as crystalloid and colloid solutions, may be used for resuscitation, to limit blood transfusion or to stabilise a patient as soon as possible, before blood becomes available.
- Recombinant plasma proteins such as FVIII, FIX, and FVIIa are available to treat coagulopathies, are not derived from human plasma, and therefore do not have risks of infectious disease transmission.
- Various red cell substitutes, such as haemoglobin solutions and perfluorocarbons are under investigation, but are not commonly available at this time.
- The enzymatic removal of A and/or B antigens from red cells, and the culturing of red cells are also being investigated with the intention of improving access to safe transfusions.

REFERENCES

- 1 ISBT website and education portal, and educational app, some information is open access, while some is available only to ISBT members. Available from: <https://www.isbt.org/>.
 - 2 WHO, Global Status Report on Blood Safety and Availability. Geneva, WHO 2017. Available from: www.who.int/health-topics/blood.
 - 3 Council of Europe, European Guide to the Preparation, Use and Quality Assurance of Blood Components, EDQM, 19th edition. Council of Europe, 2017. Available from: [www.edqm.eu/Transfusion et Transplantation/publications](http://www.edqm.eu/Transfusion-et-Transplantation/publications).
 - 4 Joint United Kingdom Blood Transfusion and Tissue Transplantation Service Professional Advisory Committee (JPAC): Guidelines for the blood transfusion services, and donor selection guidelines. Available from: www.transfusionguidelines.org/dsg.
 - 5 Clinical Guide to Transfusion, Canadian Blood Services. Editors: G Clarke, S Chargé. Available from: [https://professionaleducation.blood.ca/Transfusion/Clinical Guide](https://professionaleducation.blood.ca/Transfusion/Clinical-Guide).
 - 6 Bloody Easy e-Tools & Publications:
Bloody Easy 4: Blood Transfusions, Blood Alternatives and Transfusion Reactions. JL Callum, PH Pinkerton, A Lima, *et al.* Published by Ontario Regional Blood Coordinating Network (ORBCON). Available from: www.transfusionontario.org/bloodyeasy.
 - 7 *AABB Technical Manual*, 19th edition. Editors: Fung MK, Eder AE, Spitalnik SL, *et al.* 2017, AABB Press, Bethesda, Maryland, USA.
 - 8 *AABB Standards for Blood Banks and Transfusion Services, 31st edition*: AABB Press. Maryland, USA, Bethesda, 2018
 - 9 AABB website. Available from: www.aabb.org.
 - 10 FDA website, guidance documents. Available from: <https://www.fda.gov/cber/guidelines.htm>.
- Immunohaematology**
- 11 ISBT Working Party in Red Cell Immunogenetics and Blood Group Terminology. Available from <https://www.isbt.org/working-parties> → Red Cell Immunogenetics and Blood Group Terminology.
 - 12 *Essential Guide to Blood Groups*, 3rd edition. Editors: Daniels G, Bromilow I, 2014, John Wiley & Sons.
 - 13 *The Blood Group Antigen Facts Book*, 3rd edition. Editors: Reid ME, Lomas-Francis C, Olsson M. 2012, Academic Press, Elsevier.
 - 14 *Blood Group Antigens & Antibodies: A Guide to Clinical Relevance & Technical Tips*. Editors: Lomas C, Reid ME. 2007, Star Bright Books, New York, NY, USA.
 - 15 De Haas M, Thurik FF, Koelewijn JM, *et al.*: Haemolytic disease of the fetus and newborn. *Vox Sang* 2015; 109:99–113
 - 16 White J, Qureshi H, Massey E, *et al.*: Guideline for blood grouping and red cell antibody testing in pregnancy. *Transfus Med* 2016; 26:246–263. Available from: (<https://b-s-h.org.uk/guidelines/guidelines/blood-grouping-and-antibody-testing-in-pregnancy/>).
- 17 Barros MM, Langhi DM Jr, Bordin JO: Autoimmune hemolytic anemia: transfusion challenges and solutions. *International Journal of Clinical. Transfus Med* 2017; 5:9–18. Available from: <https://www.dovepress.com/autoimmune-hemolytic-anemia-transfusion-challenges-and-solutions-peer-reviewed-fulltext-article-IJCTM#F1>
 - 18 National Organization for rare diseases; paroxysmal cold hemoglobinuria. Garratty, G. Available from: <https://rarediseases.org/rare-diseases/paroxysmal-cold-hemoglobinuria/#related-disorders>.
- Blood donation**
- 19 Donor Management Manual, Domaine. Available from: www.sanquin.org/research/donor-studies-projects → domain.
 - 20 ISBT Haemovigilance Working Party, ISBT Standard Definitions for Surveillance of Complications Related to Blood Donation. Available from: http://www.isbtweb.org/fileadmin/user_upload/Donor-Standard-Definitions.pdf.
 - 21 Goldman M, Land K, Robillard P, *et al.*: Development of standard definitions for surveillance of complications related to blood donation. *Vox Sang* 2016; 110:185–188
 - 22 Blood Centre websites with detailed information about blood donor eligibility and transfusion medicine education. Available from:
 - American Red Cross: www.redcrossblood.org
 - Australian Red Cross Blood Services: www.donateblood.com.au (donor eligibility) and www.transfusion.com.au → education.
 - Canadian Blood Services: www.blood.ca (donor eligibility) and <https://professionaleducation.blood.ca> (education).
 - Héma-Québec, Québec, Canada: www.hema-quebec.qc.ca, professionnels de la santé, formation, formation en immunologie érythrocytaire (immunohaematology course in French, English, or Spanish).
 - Sanquin, Netherlands: www.sanquin.org.
 - NHS Blood and Transplant, UK: www.nhsbt.nhs.uk, and www.transfusionguidelines.org/dsg (guidelines and education).
- Transfusion Guidelines and Patient Blood Management**
- 23 ISBT Clinical Transfusion Working Party, resources to support the implementation of patient blood management. Available from: [https://www.isbt.org/working-parties/clinical-transfusion/](https://www.isbt.org/working-parties/clinical-transfusion/patient-blood-management-resource) → Patient Blood Management Resource.
 - 24 Society for the Advancement of Blood Management (SABM). Available from: <https://www.sabm.org/who-we-are/>.
 - 25 Estcourt LJ, Birchall J, Allard S, *et al.*: British Committee for Standards in Haematology. Guidelines for the use of platelet transfusions. *Br J Haematol* 2017; 176: 365–394

- 26 Kaufman RM, Djulbegovic B, Gernsheimer T, *et al.*: Platelet transfusion: a clinical practice guideline from the AABB. *Ann Intern Med* 2015; 162:205–213
- 27 Liunbruno G, Bennardello F, Lattanzio A, *et al.*: Italian Society of Transfusion Medicine Immunohaematology Working Group. Recommendations for the transfusion of plasma and platelets. *Blood Transfus* 2009; 7:132–150
- 28 Simon GI, Craswell A, Thom O, *et al.*: Impacts of aging on anemia tolerance, transfusion thresholds, and patient blood management. *Transfus Med Rev* 2019; 33:154–161
- 29 Yazer MH, Spinella PC, Allard S, *et al.*: Vox Sanguinis International Forum on the use of prehospital blood products and pharmaceuticals in the treatment of patients with traumatic haemorrhage. *Vox Sang* 2018; 113:701–706
- 30 Muñoz M, Stensballe J, Ducloy-Bouthors AS, *et al.*: Patient blood management in obstetrics: prevention and treatment of postpartum hemorrhage. A NATA consensus statement. *Blood Transfus* 2019; 17:112–136
- 31 Desai N, Schofield N, Richards T: Perioperative patient blood management to improve outcomes. *Anesthesia Anlg* 2018; 127(5):1211–1220
- 32 Mueller MM, Van Remoortel H, Meybohm P, *et al.*: Patient blood management: recommendations from the 2018 Frankfurt Consensus Conference. *JAMA* 2019; 321:983–997