Characteristics and Serologic Determination of Antibodies to High Frequency Antigens

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Covering Today.....

- Definition of a High Frequency Antigen (HFA)
- Antibodies to HFAs – why difficult to investigate?
- Show how we use knowledge of antibody characteristics to help determine the specificity of antibodies to HFAs
- Supplementary serological methods
- Case study
- Summary & General Advice
- (Rare Blood Provision)

High Frequency Antigens

- Also referred to as ‘high incidence’, ‘high prevalence’ and ‘public’ antigens
- For HFA classification must have incidence of >90% but majority have an incidence of >99%
- Lack of a HFA = rare phenotype
- ~189 red blood cell antigens classified as HFAs by the ISBT

Some almost ethnically exclusive

Antibodies to HFAs

- Difficult for routine laboratories to investigate
- Invariably referred to a Reference laboratory
- Antibody identification is required to:
  - Assess likely clinical significance
  - exclude underlying alloantibodies
  - to guide decisions regarding suitable blood for transfusion

When to Consider the Possible Presence of an Antibody to a HFA

- Screening cells and all cells of an additional identification cell panel are positive
- Autologous control is negative

1. Antibody to HFA

2. Complex antibody mixture

Ruling Out a Complex Mixture

- Need to know patient's "routine antigen" phenotypes

Important!

ABO, D, C, c, E, e, K, M, N, S, s, Fy^a, Fy^b, Jk^a, Jk^b

- Modes of reactivity – use a range of techniques and temperatures to find the clues

Antibody Characteristics

- Important for all antibody identification
- Essential for determining antibodies to HFAs

➲ Mode of reactivity (technique, temperature)

➲ Reactivity with enzyme treated/chemically modified cells (eg. papain, AET, trypsin)

➲ Strength and consistency of reactivity

➲ Appearance of agglutination

➲ Ability to induce invitro haemolysis

Investigating a Suspected Antibody to a HFA

- All start in the same way

All cells tested are positive

All Cells Positive

Panel Cells	A		B		C		D		
	IAT		IAT		IAT		IAT		18°C
	Unt	Pap	Unt	Pap	Unt	Pap	Unt	Pap	Unt
1	1	0	3	0	4	4	4	H	4
2	3	0	3	0	4	4	4	H	4
3	1	0	3	0	4	4	4	H	4
4	2	0	3	0	4	4	4	H	4
5	1	0	3	0	4	4	4	H	4
6	1	0	3	0	4	4	4	H	4
7	2	0	3	0	4	4	4	H	4
8	3	0	3	0	4	4	4	H	4
9	2	0	3	0	4	4	4	H	4
10	1	0	3	0	4	4	4	H	4
Auto	0	0	0	0	0	0	0	0	0

Anti-Ch/Rg, -Kn^{a}/McC^{a}, -Yk^{a}

All Cells Positive

Panel Cells	A		B		C		D		
	IAT		IAT		IAT		IAT		18°C
	Unt	Pap	Unt	Pap	Unt	Pap	Unt	Pap	Unt
1	1	0	3	0	4	4	4	H	4
2	3	0	3	0	4	4	4	H	4
3	1	0	3	0	4	4	4	H	4
4	2	0	3	0	4	4	4	H	4
5	1	0	3	0	4	4	4	H	4
6	1	0	3	0	4	4	4	H	4
7	2	0	3	0	4	4	4	H	4
8	3	0	3	0	4	4	4	H	4
9	2	0	3	0	4	4	4	H	4
10	1	0	3	0	4	4	4	H	4
Auto	0	0	0	0	0	0	0	0	0

Anti-JMH, -In^{b}, -Ge2, -Yt^{a}

All Cells Positive

Panel Cells	A		B		C		D		
	IAT		IAT		IAT		IAT		18°C
	Unt	Pap	Unt	Pap	Unt	Pap	Unt	Pap	Unt
1	1	0	3	0	4	4	4	H	4
2	3	0	3	0	4	4	4	H	4
3	1	0	3	0	4	4	4	H	4
4	2	0	3	0	4	4	4	H	4
5	1	0	3	0	4	4	4	H	4
6	1	0	3	0	4	4	4	H	4
7	2	0	3	0	4	4	4	H	4
8	3	0	3	0	4	4	4	H	4
9	2	0	3	0	4	4	4	H	4
10	1	0	3	0	4	4	4	H	4
Auto	0	0	0	0	0	0	0	0	0

Rh, Kell, Jk, Scianna, Colton, Dombrock, Diego, Cromer

All Cells Positive

Panel Cells	A		B		C		D		
	IAT		IAT		IAT		IAT		18°C
	Unt	Pap	Unt	Pap	Unt	Pap	Unt	Pap	Unt
1	1	0	3	0	4	4	4	H	4
2	3	0	3	0	4	4	4	H	4
3	1	0	3	0	4	4	4	H	4
4	2	0	3	0	4	4	4	H	4
5	1	0	3	0	4	4	4	H	4
6	1	0	3	0	4	4	4	H	4
7	2	0	3	0	4	4	4	H	4
8	3	0	3	0	4	4	4	H	4
9	2	0	3	0	4	4	4	H	4
10	1	0	3	0	4	4	4	H	4
Auto	0	0	0	0	0	0	0	0	0

Anti-Vel, -PP1P^{k}, -H (made in O$_{h}$)

What Next?

Options

1. Screen the patient's cells for **selected** HFAs
2. Match **selected** rare phenotype & null cells against patient's plasma

selection based on antibody characteristics observed in initial panels and any information regarding the patient's ethnicity

Option 2

Matching rare phenotype & null cells

Caution needed

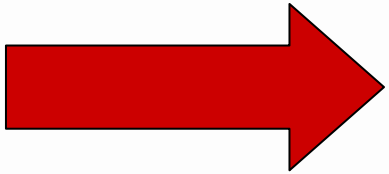
- Underlying antibodies may be present
- Beware ABO!

Option 1 & 2

Negative Found!

- Type patient's cells for relevant antigen(s)
- Match further examples (if possible) in order to exclude underlying antibodies

All positive



Supplementary Tests

Eluate

- Make an eluate from Gp O ‘antigen matched’ cells
  - eliminates ABO incompatibility issues
  - isolates antibody to HFA
  - can match rare phenotype cells of any ABO group and without worry of contaminating antibodies to ‘common antigens’

Supplementary Tests

Enzymes

	Papain	Trypsin	Chymotrypsin	Pronase	AET
Knops	+/-	-	-	+	-
Ch/Rg	-	-	-	-	+
Cromer	+	+	-	+	(+)
Vel	+	+	+	+	+
Lan	+	+	+	+	+
Kell	+	+	+	+	-
JMH	-	-	-	-	-
LW	+	+	+	-	-

Examples of effect of enzyme treatment/chemical modification

Supplementary Tests

Panel Cells	A	
	IAT	
	Unt	Pap
1	1	0
2	3	0
3	1	0
4	2	0
5	1	0
6	1	0
7	2	0
8	3	0
9	2	0
10	1	0
Auto	0	0

C4 coated cells

- Ch/Rg are plasma antigens, located on complement receptor C4
- C4 coat cells in vitro → increased amounts of Ch/Rg
- Test in parallel with uncoated cells
- Strong reaction = instant indication of anti-Ch/Rg specificity

Uncoated	C4 coated
	5

Supplementary Tests

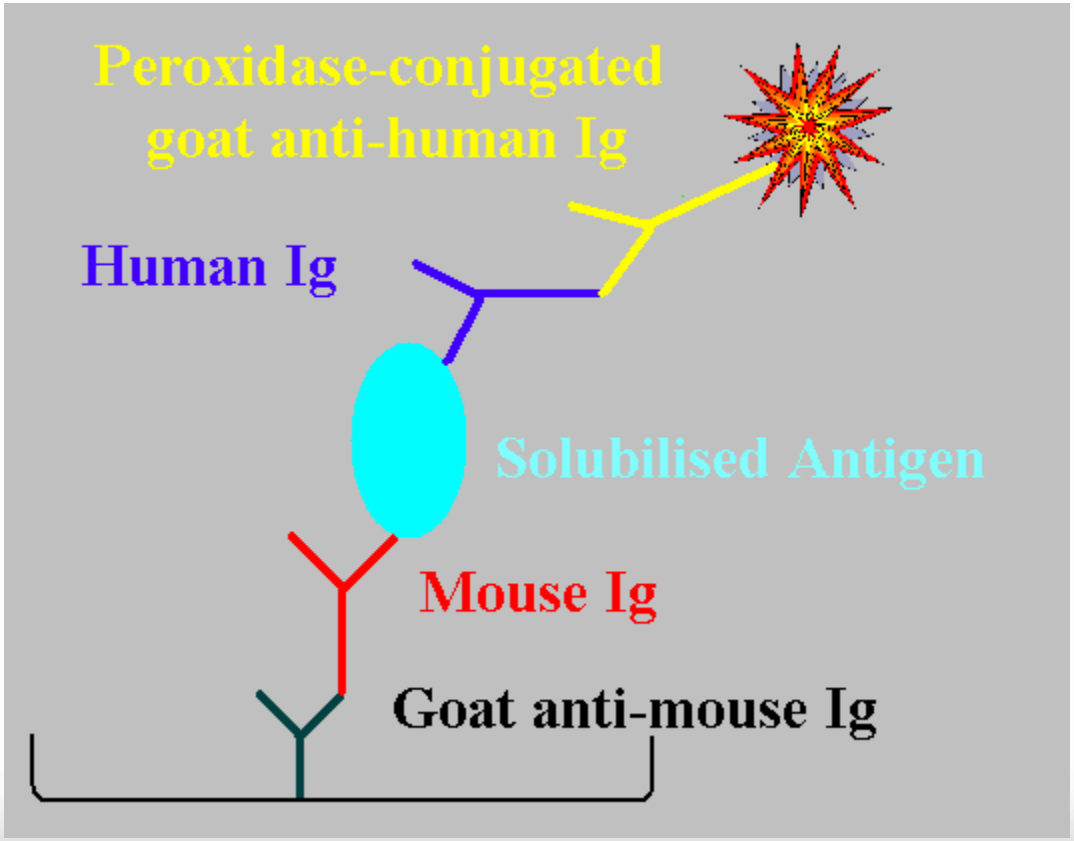
Inhibitions

- Anti-Ch/Rg can be inhibited by C4 in plasma
- Soluble recombinant blood group proteins (sRGB) are another way of determining if a supected blood group protein is the culprit
  - Incubate patient's plasma with sRBG in parallel with a diluent control
  - Test both with known positive cells and if reactivity is diminished or eliminated in the presence of a positive diluent control, indicates antibody has been inhibited
  - particularly useful for CR1-related antibodies

Supplementary Tests

MAIEA assay

- Monoclonal Antibody Immobilisation of Erythrocyte Antigens



- Mabs specific for suspected RBC membrane protein
- Positive result indicates human antibody has bound to targeted protein
- Can also be used as competitive binding assay to map epitopes

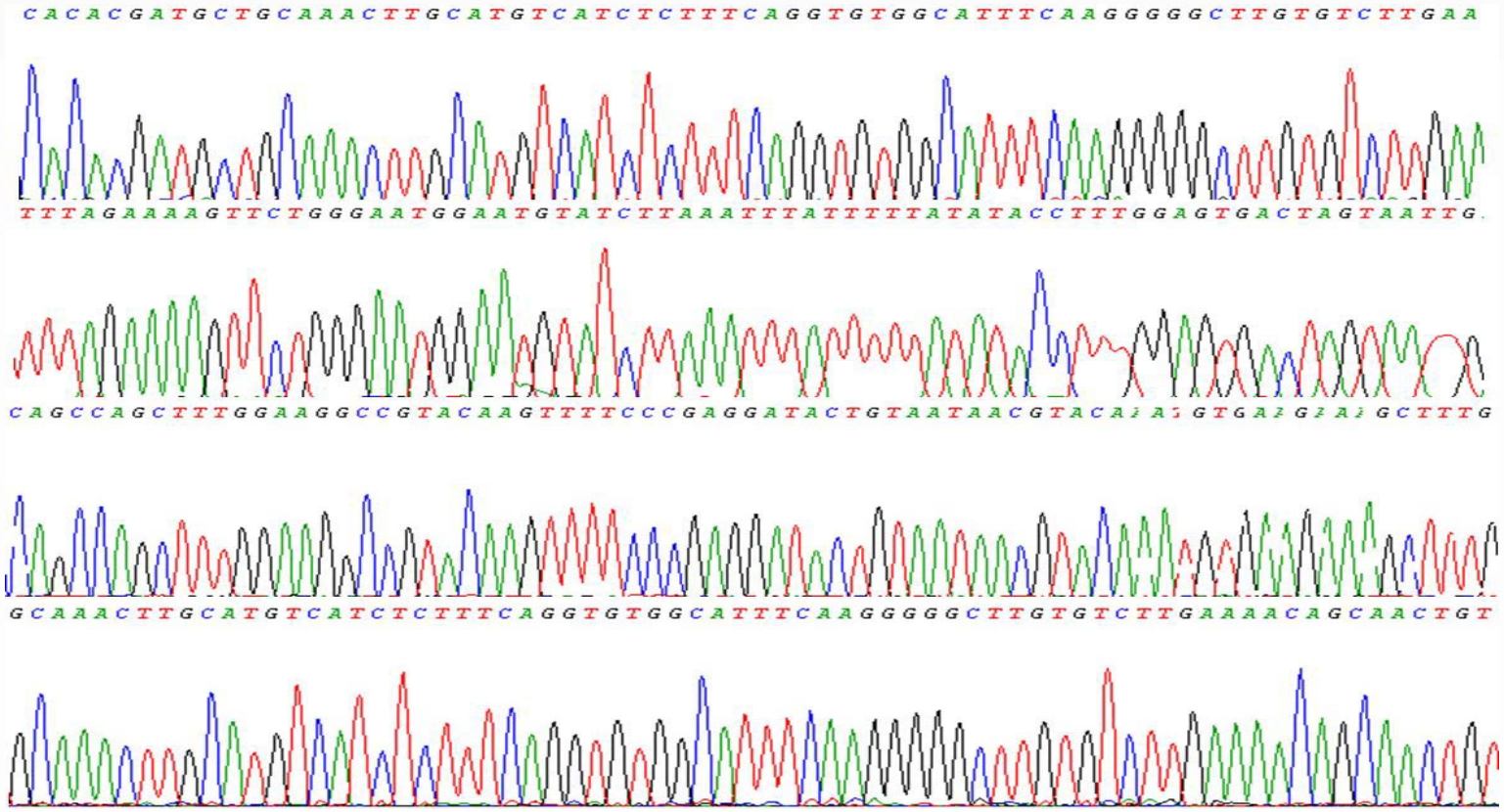
Supplementary Tests

MAIEA assay

- Useful when particular blood group system is suspected (usually based on enzyme studies)
- Economical on plasma
- Particularly effective for identifying CR1-related antibodies and for helping to assign novel specificities
- We currently use MAIEA for: Knops, Cromer, Lutheran, Kell, Yt and the Indian system
- Lu21, INFI & INJA HFA's discovered with help from MAIEA

Supplementary Tests

Gene sequencing



- Clues from serology → which gene to target

Case Study

Panel Cells	IAT	
	Unt	Pap
1	3	3
2	3	3
3	3	3
4	3	3
5	3	3
6	3	3
7	3	3
8	3	3
9	3	3
10	3	3
Auto	0	0

- Chinese patient, samples referred from Australia
- All cells positive
- Typed cells for HFAs, all positive
- Matched selected null cells, all positive

Case Study

	Papain	Trypsin	Chymotrypsin	Pronase	AET
Knops	+/-	-	-	+	-
Ch/Rg	-	-	-	-	+
Cromer	+	+	-	+	(+)
Vel	+	+	+	+	+
Lan	+	+	+	+	+
Kell	+	+	+	+	-
JMH	-	-	-	-	-
Patient	+	+	-	+	(+)

Clue!

Case Study

- Soluble recombinant DAF protein – antibody inhibited!
- Typed patient's cells for Cromer HFAs (in small batches based on rarity of antibody)

UMC-

- No UMC- cells for matching
- One IFC- compatible, one Dr(a-) weakly incompatible
- *DAF* sequencing revealed homozygous mutation in exon 6, 749C>T encoding Thr250Met in DAF protein. Known to be associated with UMC- phenotype
- Mother and only sibling both heterozygous

Take Home Points

- Identification of antibodies to HFAs is time consuming and complex→ delay in patient care
- Observing the clues is essential to a timely resolution
- Knowledge of different antibody characteristics is key to recognising the clues – get to know them!
- The “gut feeling” of an experienced serologist is invaluable

General Advice

- Occasionally antibodies do not do as expected!
- Very rare specificities – the described characteristics are based on limited observations
- Use the expected characteristics as a GUIDE not an absolute!

Finally……

- Successful determination of antibodies to HFAs requires competency in manual serological techniques. A dying art.

Thank You