

# External Quality Assessment (EQA) for cfDNA in pregnancy

June 23, 2019

11.00 – 12.30

ISBT Basel

WP cfDNA subsection

W A Flegel MD

Chief, Laboratory Services Section, Department of Transfusion Medicine,  
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# Presented by: Behnaz Bayat

JUSTUS - LIEBIG -



UNIVERSITÄT  
GIESSEN

FACHBEREICH  
MEDIZIN



- **Sunday 23<sup>rd</sup> June** meeting for the cfDNA subsection-
  - Venue is Boston 3, Basel Convention Centre
  - Meeting time **11 to 12.30 am.**

# Disclaimer & Disclosure

## Disclaimer

- The views expressed do not necessarily represent the view of the National Institutes of Health, Department of Health and Human Services, or U.S. Federal Government.

## Disclosure

- No conflicts of interest.

## Off-Label Usage

- Assays for cfDNA are not FDA approved in the US.



# Topics

- What INSTAND stands for.
  - 4 facts sheets
- What we are doing with EQA since the 2000s.
  - 2 slides
- What we are planning to do.
  - 2 statement slides and 1 picture of the evaluation documents
  
  - Keep it short: 2 – 4 slides per topic.



# What **INSTAND e.V.** stands for

Scientific society for promoting quality assurance  
in medical laboratories e.V

INSTAND e. V.

- is a **non-profit, interdisciplinary scientific medical society** and
- cooperates with a unique and large network of scientists,
- who are also serving as advisers and ring test leaders for External Quality Assessment schemes (EQA schemes = interlaboratory comparison = proficiency testing = ring trial = round robin test) in all essential fields of laboratory medicine **since 1968**.

INSTAND e.V.

- Is accredited according to DIN/ISO17043 for reference institutions



**The certificates are valid for laboratory accreditation processes.**

## Facts about INSTAND e.V.

Scientific society for promoting quality assurance  
in medical laboratories e.V

The results of these EQA schemes

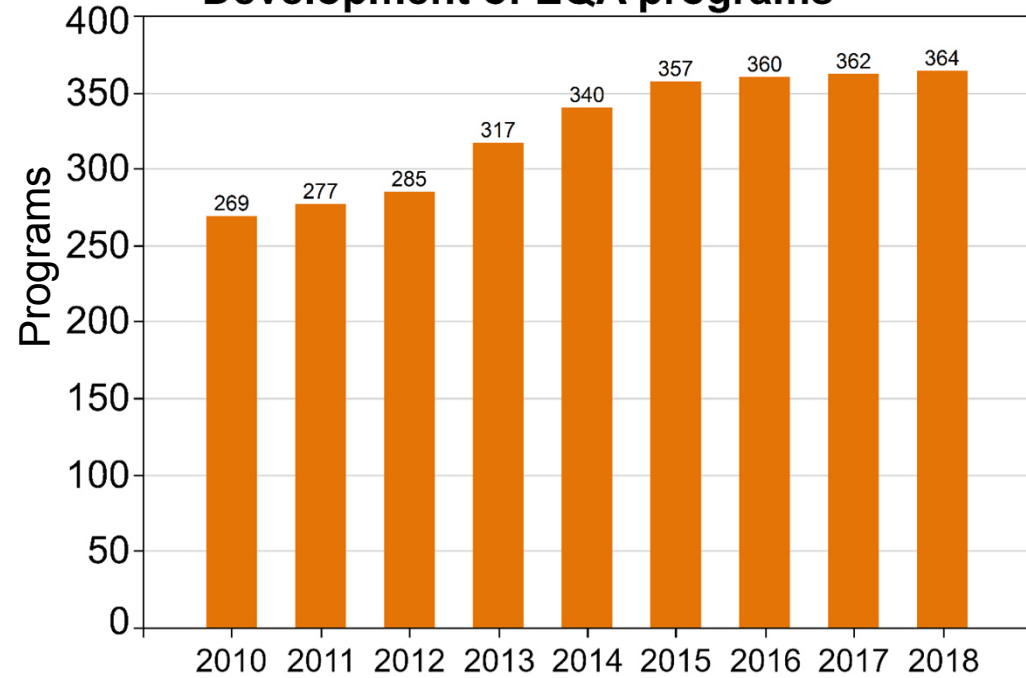
- are scientifically evaluated
- and are basis for surveilling the competence of medical diagnostic laboratories as well as the performance of *in vitro* diagnostic medical devices.

The EQAs follow

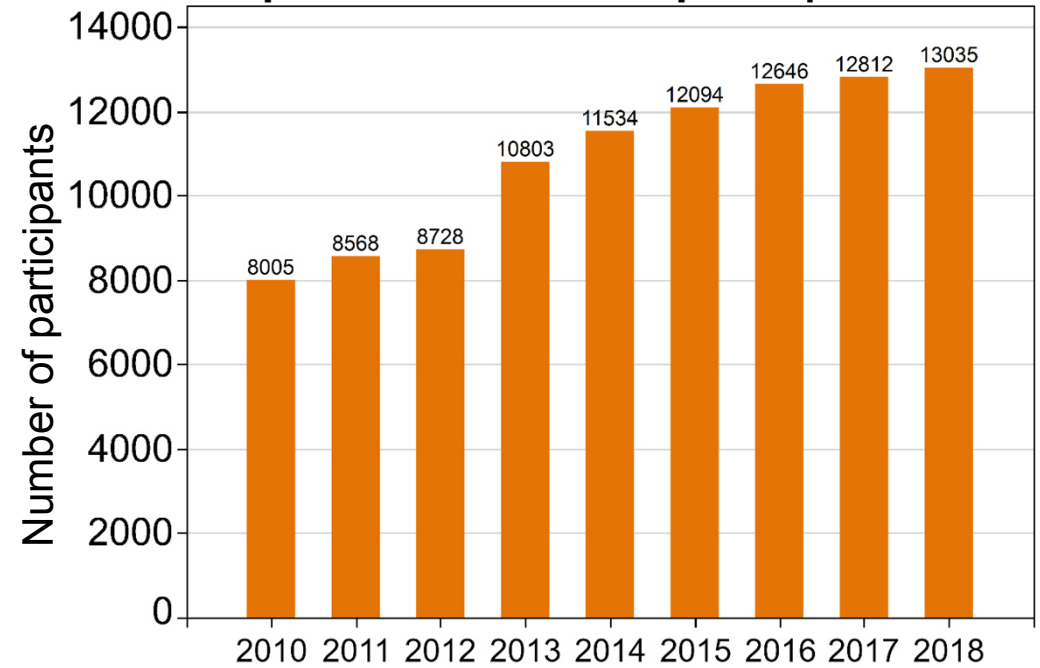
- the requirements laid down in the Directive 98/79/EC of the European Parliament and of the Council of 27<sup>th</sup> October 1998 on *in vitro* diagnostic medical devices (IVD Directive 98/79/EC), the German Medical Devices Act (Medizinproduktegesetz)
- and the Guideline for Quality Assurance of Diagnostic Analyses in Medical Laboratories of the German Medical Association (Rili-BÄK).

# INSTAND e.V. – A few more numbers

## Development of EQA programs



## Development of number of participants



# Facts about INSTAND e.V.

## General EQA procedure

Registration



Shipment of Samples



Analysis in Lab



Reporting results

No.	Description	Standard unit	Method	Reagent	Device	Standard	Unit	Sample S1	Converted S1	Sample S2	Converted S2
1	alpha-1-Fetoprotein	3 - µg/l	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
2	CEA	3 - µg/l	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
3	beta-2-Mikroglobulin	35 - ng/l	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
4	TRF	14 - ng/l	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
5	CA 19-9	16 - U/ml	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
6	CA 19-9	16 - U/ml	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
7	CA 125	16 - U/ml	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
8	HCG intact + beta-HCG free	33 - IU/l	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
10	PSA total	8 - ng/ml	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>



Expert evaluation



**CERTIFICATE**

Survey of 25 January 2017

This certificate expires the last day of the year!

You have fulfilled the requirements of the External Quality Assessment with the following analysis:

**Tumor Markers (292):**

Validity 6 months:  
 alpha-1-Fetoprotein (R: B14)  
 CA 19-9 (R: B14)  
 CEA (R: B14)  
 HCG intact + beta-HCG free (R: B14)  
 PSA, total (R: B14)

Validity 12 months:  
 beta-2-Mikroglobulin  
 CA 125  
 CA 19-9  
 NCC Intact + beta-HCG free (R: B14)  
 CA 17-4

Cyfla 21-1  
 Immunospecific Endstate  
 PSA, free  
 5100



Publication of results

CEA µg/l	Sample	Target Value	Target Range	Mean	Cv	N	Success Rate (%)	Start Date (%)
25	55.50	42.00	48.00	55.50	53	22	100.00	100.00
10	15.90	12.30	19.70	15.90	44	10	100.00	
20	28.50	20.00	40.00	28.50	27	19	14.29	94.29
20	35.40	27.00	51.00	35.40	41	19	14.29	
10	43.30	32.70	52.70	43.30	41	10	100.00	100.00
20	12.60	9.30	15.60	12.60	81	10	100.00	
10	45.10	34.00	54.00	45.10	23	24	100.00	100.00
10	12.80	9.70	15.90	12.80	83	26	100.00	
10	20.70	15.00	40.00	20.70	44	48	100.00	100.00
10	33.60	25.50	51.00	33.60	44	48	100.00	
10	43.40	40.20	79.40	43.40	93	10	85.00	85.00
10	34.80	14.00	23.00	34.80	104	11	88.00	



cfDNA @ ISBT Basel 2019

Behnaz Bayat PhD  
 Univ Giessen, Germany



# What we are doing since the 2000s

- EQA for molecular immunohematology
- originated 1997 with INSTAND since fall 2006
- organization by Bein, Giessen & Flegel, Bethesda MD
- ~ 100 institutions participating worldwide Oldest and largest EQA available with comprehensive coverage of blood group, platelet and granulocyte antigens



# Published since 2000

*Transfusion Medicine*, 2001, 11, 211–219

## Workshop Report on the Genotyping of Blood Cell Alloantigens

H. Kroll, B. Carl, S. Santoso, J. Bux and G. Bein *Institute for Clinical Immunology and Transfusion Medicine, Justus Liebig University Giessen, Germany*

*Received 20 June 2000; accepted for publication 4 January 2001*

## External quality assessment in molecular immunohematology: the INSTAND proficiency test program

*Willy A. Flegel,<sup>1</sup> Ion Chiosea,<sup>1</sup> Ulrich J. Sachs,<sup>2</sup> and Gregor Bein<sup>2</sup>*



# What we are planning to do.

- Establish an EQA for cfDNA in pregnancy
  - to be organized in collaboration by Flegel/Bein/Clausen
- For worldwide participation
  - logistics managed by INSTAND
- Affordable EQA fee
  - similar to established EQA since 2006, shipment at cost



# Some proposed technical features.

- Real samples of cell free plasma from pregnant women
  - D neg mother with *RHD* gene positive fetus
  - pooled material and comparable control material
  - at least 2 samples per EQA shipment, possibly more.
- Initially planned for one EQA shipment per year.
  - in spring or autumn? Start immediately when logistics established?
- Online result submission
- Certificates in the mail



# Samples sheets for Evaluation Documents, INSTAND style

## Certificates

## Evaluation and statistics



### CERTIFICATE OF PARTICIPATION

Survey of 24 October 2018

You have participated in the External Quality Assessment with the following

#### Clinical Chemistry - Conventional Analysis (100):

Albumin (Electrophoresis)	GGT (R: B1a)	Osmc
Albumin (R: B1a)	GLDH	Pancr
Aldolase	Glucose (R: B1a)	Phosp
alpha-Amylase	GOT (R: B1a)	Protas
AP (R: B1a)	GPT (R: B1a)	Protel
Bilirubin conj.	HBDH	Sodiu
Bilirubin (R: B1a)	IgA (R: B1a)	Trans
Calcium (R: B1a)	IgG (R: B1a)	Trigly
CHE	IgM (R: B1a)	Urea
Chloride (R: B1a)	Iron	Uric A
Cholesterol (R: B1a)	Lactate (R: B1a)	
CK (R: B1a)	LDH (R: B1a)	
Copper	Lipase	
Creatinine (R: B1a)	Lithium (R: B1a)	
gamma-Globulins (Electrophoresis)	Magnesium (R: B1a)	

(R) analysis is subject to the RIBÄK

Participant:  
1  
Dr. med. Hans Mustermann  
Musterstr. 21  
47131 Musterstadt

Düsseldorf, 13 November 2018

*Michael Spannagel*  
Prof. Dr. med. Michael Spannagel  
(Head of Reference Institution)



INSTAND  
Gesellschaft zur Förderung der Qualitätssicherung  
in medizinischen Laboratorien e.V.  
Überrstr. 20 | 40223 Düsseldorf



### CERTIFICATE

Survey of 24 October 2018

You have fulfilled the requirements of the External Quality Assessment with the following analysis

#### Clinical Chemistry - Conventional Analysis (100):

Validity 6 months:	Albumin (R: B1a)	IgM (R: B1a)	Albumin (Electrophoresis)
	AP (R: B1a)	Lactate (R: B1a)	alpha-Amylase
	Bilirubin (R: B1a)	LDH (R: B1a)	Bilirubin conj.
	Calcium (R: B1a)	Lithium (R: B1a)	CHE
	Chloride (R: B1a)	Magnesium (R: B1a)	Copper
	Cholesterol (R: B1a)	Phosphate (R: B1a)	gamma-Globulins (Electrophoresis)
	CK (R: B1a)	Potassium (R: B1a)	GLDH
	Creatinine (R: B1a)	Protein (R: B1a)	HBDH
	GGT (R: B1a)	Sodium (R: B1a)	Iron
	Glucose (R: B1a)	Transferferrin (R: B1a)	Lipase
	GOT (R: B1a)	Triglycerides (R: B1a)	Osmolality
	GPT (R: B1a)	Urea (R: B1a)	Pancreas Amylase
	IgA (R: B1a)	Uric Acid (R: B1a)	
	IgG (R: B1a)		

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Überrstr. 20 | 40223 Düsseldorf

1/21



Listing and evaluation of the results

1: Dr. med. Hans Mustermann

Survey of 24 October 2018

Adviser: Dr. rer. nat. Manfred Falck  
Instand e.V.  
Überrstr. 20  
40223 Düsseldorf

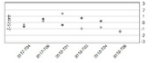
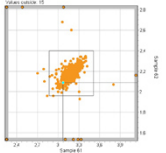
Tel.: +49 211 178 1  
Mail: falck@instand.de

#### 100 Clinical Chemistry - Conventional Analysis

Analyte	Sam- ple	Unit	Your value	Target TV	Type	Upper limit	Deviation
Albumin	61	g/l	6.50	6.51	SV	5.21	7.81 -0.2%
Albumin (Electrophoresis)	61	%	62.3	61.2	SV	52.6	69.8 1.8%
Aldolase	61	U/l	+4.00	2.30	SV	1.51	3.01
alpha-Amylase	61	U/l	+4.00	2.96	SV	1.98	3.94
AP	61	U/l	365	372	SV	269	484 -1.9%
Bilirubin	61	mg/dl	3.82	4.09	SV	3.19	4.99 -6.6%
Bilirubin conj.	61	mg/dl	0.820	0.916	SV	0.714	1.12 -10.5%
Calcium	61	mmol/l	1.06	1.04	SV	0.666	1.41 1.9%
Calcium	61	mmol/l	0.350	0.344	SV	0.220	0.468 1.7%
CHE	61	U/l	3.07	3.19	RHV	2.87	3.51 -3.8%
Chloride	61	mmol/l	2.19	2.18	RHV	1.96	2.40 -0.1%
Chloride	61	mmol/l	9588	10203	SV	8366	12040 -6.0%
Cholesterol	61	mg/dl	221	222	RHV	6376	9176 -7.3%
CK	61	U/l	102	126	RHV	116	136 -0.8%
Copper	61	mg/dl	110	111	MM	102	120 -0.9%
Creatinine	61	mg/dl	219	232	RHV	202	262 -5.6%
Gamma-Globulins (Electrophoresis)	61	%	137	152	RHV	132	112 -0.1%
GLDH	61	U/l	266	294	RHV	235	353 -9.5%
Glucose	61	mmol/l	89.0	107	RHV	85.6	128 -16.8%
GOT	61	U/l	30.0	33.0	SV	24.3	39.7 -6.2%
GPT	61	U/l	21.1	19.4	SV	14.7	24.1 8.7%
Iron	61	mg/dl	3.16	2.97	RHV	2.38	3.56 6.4%
IgA	61	mg/dl	10.2	0.966	RHV	0.733	1.10 11.4%
IgG	61	%	14.5	14.8	SV	10.1	19.5 -2.0%
IgM	61	%	14.1	14.5	SV	9.86	19.1 -2.8%
Lipase	61	U/l	156	159	RHV	126	162 -1.9%
Lithium	61	mg/dl	36.0	39.1	RHV	30.9	47.3 -7.9%
Lithium	61	U/l	8.80	9.92	SV	6.94	12.9 -11.3%
Magnesium	61	mg/dl	179	189	RHV	161	217 -5.2%
Magnesium	61	U/l	81.1	76.2	RHV	64.8	87.6 6.4%
Protein	61	U/l	251	252	RHV	181	281 8.2%
Protein	61	U/l	41.9	41.6	RHV	32.9	50.3 0.7%
Urea	61	U/l	155	152	RHV	120	184 1.8%
Urea	61	U/l	40.7	40.3	RHV	31.8	48.8 1.0%
Urea	61	U/l	310	303	SV	239	347 2.3%
Urea	61	mg/dl	124	124	SV	98.0	150 0.0%
Urea	61	mg/dl	276	275	SV	220	330 0.4%
Urea	61	mg/dl	204	203	SV	162	244 0.5%
Urea	61	mg/dl	1510	1452	SV	1191	1713 4.0%
Urea	61	mg/dl	1070	1032	SV	846	1218 3.7%
Urea	61	mg/dl	152	150	SV	111	189 1.3%
Urea	61	mg/dl	104	103	SV	76.2	130 1.0%

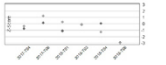
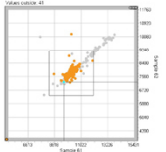
Collective	Sam- ple	Target value	Target range	Participants collective AVG CV Num.	Rate (%) Sam. total
Ionized Calcium	61	Sample not evaluated Sample not evaluated		3	96.4
RMV	61	3.19 2.87 - 3.51	3.18 2.70 3.70	670 97.8	96.4
	62	2.18 1.96 - 2.40	2.18 2.80 670	97.6	

Rate of success: 96%



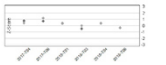
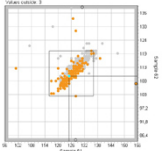
Collective	Sam- ple	Target value	Target range	Participants collective AVG CV Num.	Rate (%) Sam. total
Albumin	61	6.50	6.50 - 6.50	1.52 1.52 25	96.0
Beckman	61	9897	8110 - 11678	9897 3.42 14	100
Beckman	62	7552	6993 - 8911	7552 3.24 14	100
Siemens	61	12525	10271 - 14780	12525 7.95 32	100
Siemens	61	9335	7655 - 11015	9335 8.17 32	100
Siemens	61	16225	13318 - 19157	16225 5.69 40	92.5
Olympus	61	9964	8170 - 11958	9964 3.73 58	98.3
Other provider (1)	61	3922	6225 - 8959	7502 4.06 58	98.3
Roche	61	10203	8366 - 12040	10203 2.08 273	99.6
Roche	62	7776	6376 - 9176	7776 2.35 273	99.3
Other provider (1)	61	9527	7812 - 11242	9527 11.2 7	
Other provider (1)	62	7277	5967 - 8587	7277 8.22 7	

Rate of success: 98.4%



Collective	Sam- ple	Target value	Target range	Participants collective AVG CV Num.	Rate (%) Sam. total
Siemens	61	124	114 - 134	124 1.67 62	96.8
Siemens	62	110	101 - 119	110 1.76 62	96.8
Roche	61	126	116 - 136	126 2.52 231	97.8
Roche	62	111	102 - 120	111 2.57 231	99.1
Other (RMV)	61	134	123 - 145	131 2.29 227	95.0
Other (RMV)	62	120	110 - 130	116 2.09 227	97.4

Rate of success: 96.5%



cfDNA @ ISBT Basel 2019

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