"Laboratory medicine: Preparing for the 2020's"

IFCC GENERAL CONFERENCE
BUDAPEST NOVEMBER 9-11, 2018

* Standardization of HbA_2: a long way to succeed

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- Why HbA_2 is important
- State of the art
- Activities of the IFCC WG-HbA_2
- Reducing inter-laboratory variability
- Future perspectives

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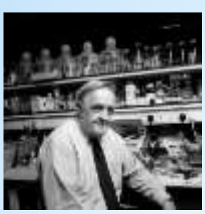

World Distribution, Population Genetics, and Health Burden of the Hemoglobinopathies

Thomas N. Williams[1] and David J. Weatherall[2]

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[2]Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX39DU, United Kingdom

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Table 1. A breakdown of the annual number of births with the different hemoglobin disorders

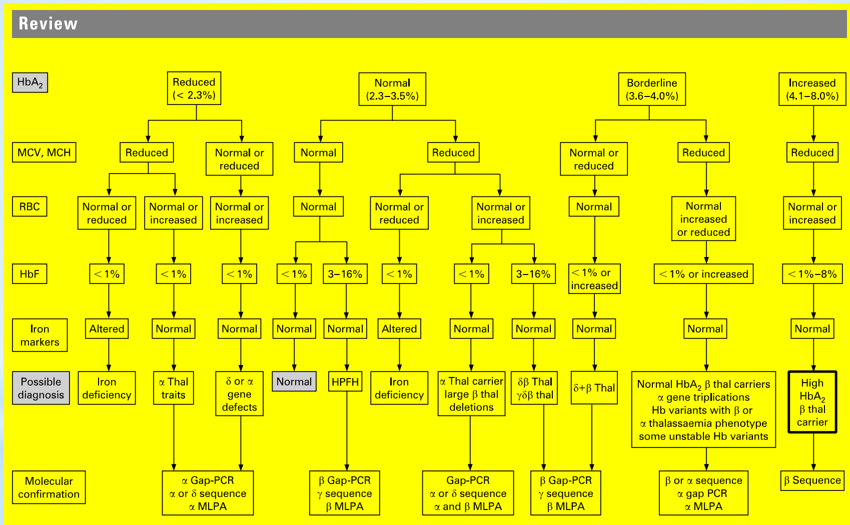
Annual births with major hemoglobin disorders	
β-thalassemia major	22,989
HbE β thalassemia	19,128
HbH disease	9568
Hb Bart's hydrops (α^0/α^0)	5183
SS disease	217,331
S β thalassemia	11,074
SC disease	54,736

From available data (Modell and Darlison 2008; Weatherall 2010).

Figure 1. The world distribution of the origins of the α and β thalassemias. (From Weatherall and Clegg 2001; reprinted, with permission, from the author.)

The role of haemoglobin A_2 testing in the diagnosis of thalassaemias and related haemoglobinopathies

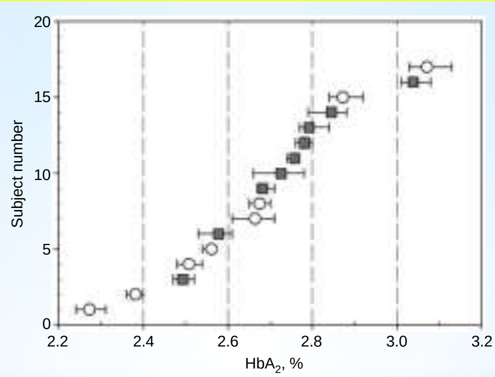
A Mosca,[1] R Paleari,[1] G Ivaldi,[2] R Galanello,[3] P C Giordano[4]



Mosca et al, J Clin Lab 2009;62:13-7

Andrea Mosca*, Renata Paleari, Barbara Wild, on behalf of the IFCC Working Group on Standardization of HbA_2

Analytical goals for the determination of HbA_2



Quality level	Imprecision, %	Bias, %	Total error, %
Optimal	0.2	1.0	1.5
Desirable	0.3	1.9	3.0
Minimal	0.5	2.9	4.5

Table 1 Analytical goals for HbA_2 measurement derived from data on biologic variation.

Clin Chem Lab Med 2012

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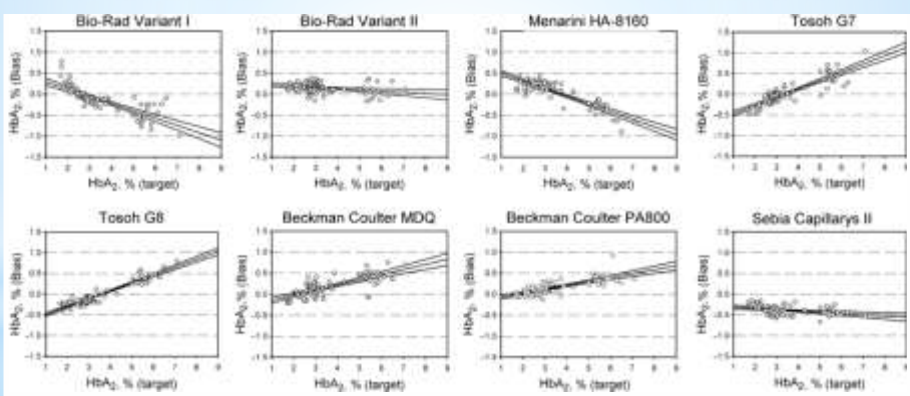
International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology
ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

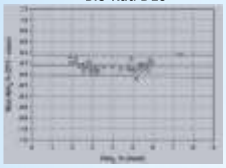
Interlaboratory comparison of current high-performance methods for HbA_2

R. PALEARI*, B. GULBIS[†], F. COTTON[†], A. MOSCA*

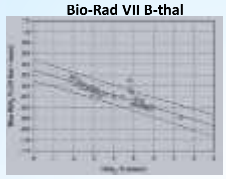


Int. Jnl. Lab. Hem. 2012, 34, 362–368

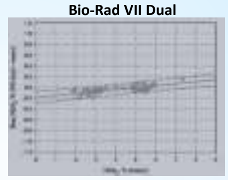
Bio-Rad D10



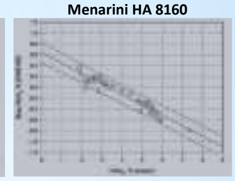
Bio-Rad VII B-thal



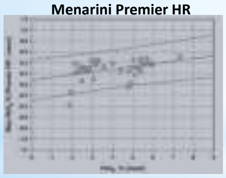
Bio-Rad VII Dual



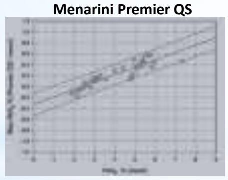
Menarini HA 8160



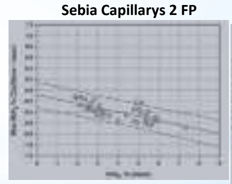
Menarini Premier HR



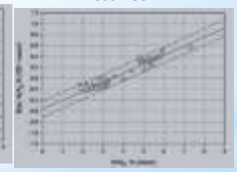
Menarini Premier QS



Sebia Capillarys 2 FP



Tosoh G8



Paleari et al, Clin Chim Acta 2018;477:60-5

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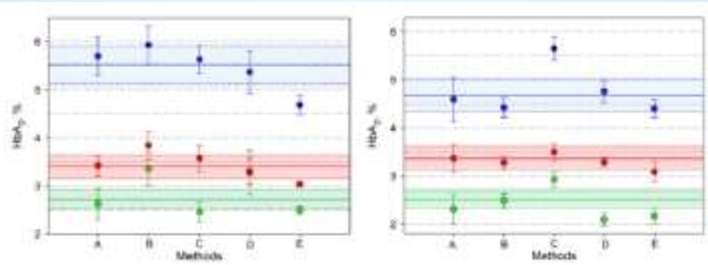

A. Bio-Rad Variant II dual kit (27, 28, 29)
B. Tosoh G7 (10, 10, 8)
C. Tosoh G8 (35, 34, 36)
D. HPLC, others (16, 14, 16)
E. Sebia Capillarys (8, 9, 10)

A. Bio-Rad D10 dual kit (73, 74, 70)
B. Bio-Rad Variant II β-thal short (97, 65, 54)
C. Bio-Rad Variant II dual kit (59, 60, 55)
D. Tosoh G7/G8 (9, 10, 10)
E. Sebia Capillarys (9, 9, 9)

Fig. 1. Inter-laboratories results obtained from two different EQAS providers, at three different levels of HbA_2/total hemoglobin amount of substance fraction, grouped according to the methods used by the participants. The left panel reports the data obtained in 2014 from the Italian Inter-Regional Program. The right panel refers to the data obtained from the international EQAS provided by Bio-Rad Laboratories. The numbers of participants per method are reported in brackets, from the lower to the higher HbA_2 level. Points and error bars represent means and SD, respectively. The dotted lines represent the limits of the allowable total error of ± 7%.

Paleari et al, Clin Chima Acta 2017;467:21-6

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1. Definition of a reference measurement procedure using mass spectrometry associated with proteolytic degradation

Clin Chem Lab Med 2002; 40(1):78–89 © 2002 by Walter de Gruyter · Berlin · New York

IFCC 2002/1

Approved IFCC Reference Method for the Measurement of HbA_{1c} in Human Blood

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[1)2)]

Scientific Division
Working Group on HbA_{1c} Standardisation[3)] and Network of Reference Laboratories for HbA_{1c}[4)]

Prepared for publication[5),6)] *by*

Jan-Olof Jeppsson[1,7)], Uwe Kobold[2], John Barr[3], Andreas Finke[2], Wieland Hoelzel[2], Tadao Hoshino[4], Kor Miedema[5], Andrea Mosca[6], Pierluigi Mauri[7], Rita Paroni[8], Linda Thienpont[9], Masao Umemoto[10] and Cas Weykamp[11]

Figure 1 Flow chart of the reference method procedure.

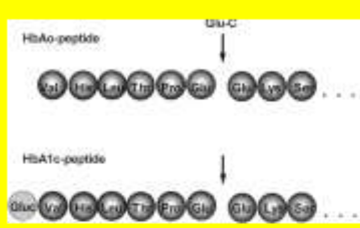

Figure 2 Principle of the proteolytic digestion of haemoglobin chains.

2005 -2009 activities

Development of the methods

- Choise of the proteolitic enzyme (endoproteinase Lys C, Trypsin)
- Definition of digestion protocol (denaturation step with acetonitrile, trifluoroethanol, rapigest, digestion time, temperature, time course)
- Choise of marker peptides (δT2, δT3, δT14, αT4, αT5, αT11)
- Choise of column (Tosoh TSK gel, Zorbax)
- Analytical condition
- ESI-MS detection (double-charge, mono-charge)

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2005 -2009 activities

Interlaboratory exercizes

2006: 6 calibrators, 29 samples

2007: 6 calibrators, 20 samples
(2 digestions, 2 replicates/digested)

2008: 4 calibrators, 3 samples
(3 digestions, 3 replicates/digested)

2009: 1 calibrators, 1 samples
(centralized digestion, measurements over 5 days)

⟹ **Inter-laboratory variability**

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recombinantly expressed, intact

HbA_2 and ^{15}N-labeled HbA_2

HbA_0 and ^{15}N-labeled HbA_0

The metrological traceability of measurement using the HbA_2 and HbA_0 protein standards is ensured by:

1. determination of content of peptide by LC-ID-MS (amino acid analysis)
2. determination of purity by LC-TOF -MS

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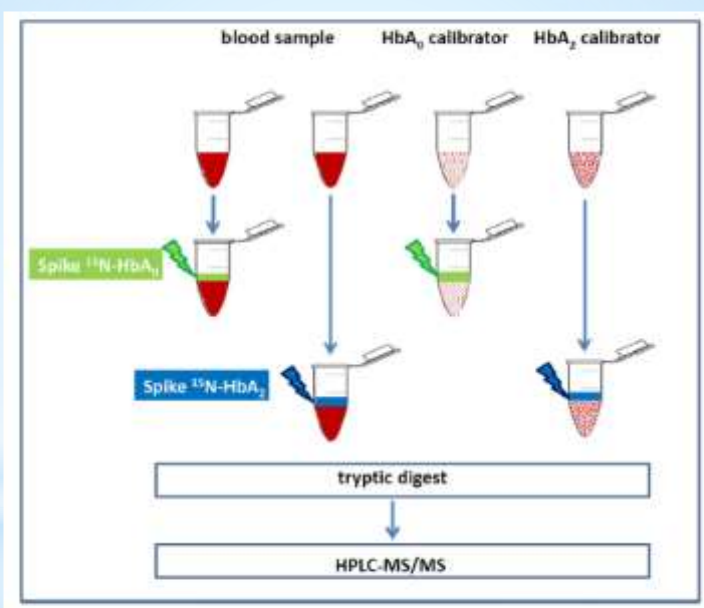

Fig. 4. Workflow for the ID-MS method.

Paleari et al, Clin Chima Acta 2017;467:21-6

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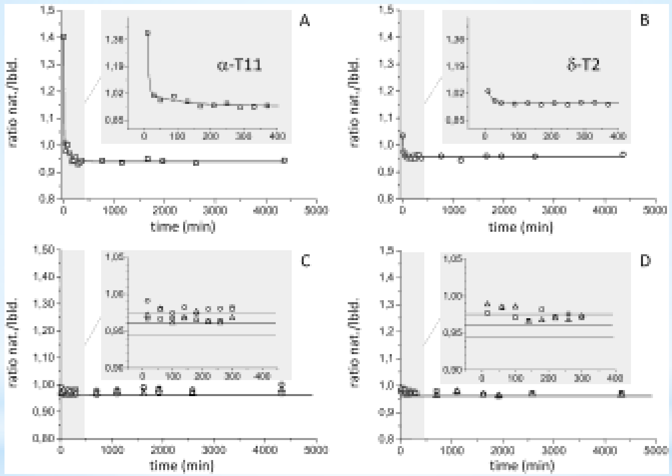


^{15}N-HbA$_2$

Native HbA$_2$ +^{15}N-HbA$_2$

○ α–T4
△ α–T5
□ α–T6

△ δ–T3
□ δ–T2

Arsene et al, Clinica Chimica Acta 2018

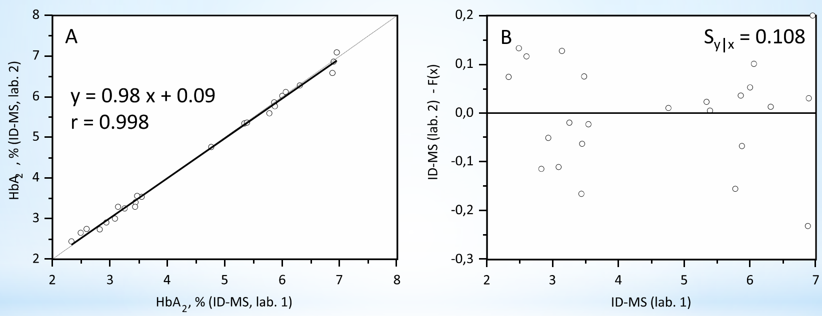
Repeatability and within-lab precision of HbA2 determination using IDMS (EP15-A3)

Table 2 repeatability and within-lab precision of HbA$_2$ determination using IDMS

	day	aliquot 1	aliquot 2	aliquot 3	mean	SD
			HbA$_2$ (%)			(%)
	1	2.95	3.00	2.91	2.95	1.53
	2	2.96	2.95	3.01	2.97	1.08
sample 1	3	2.94	2.91	3.02	2.96	1.92
	4	2.99	2.99	3.07	3.02	1.53
	5	3.03	2.97	3.06	3.02	1.52
repeatability (%)		1.50				
within-lab precision (%)		1.68				

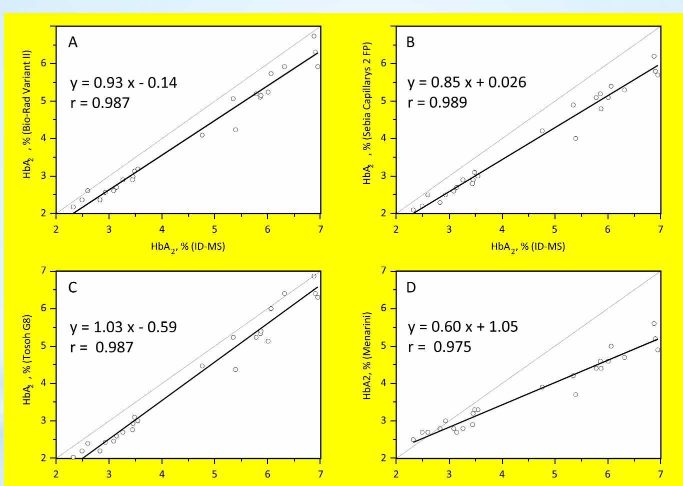
Arsene et al, Clinica Chimica Acta 2018

Correlation between IDMS-results obtained in two laboratories



Arsene et al, Clinica Chimica Acta 2018

Correlation between IDMS and routine methods



Arsene et al, Clinica Chimica Acta 2018

2. Preparation of a secondary reference material for hemoglobin A_2 (in cooperation of IRMM)

Development of a candidate certified refernce material (CRM)

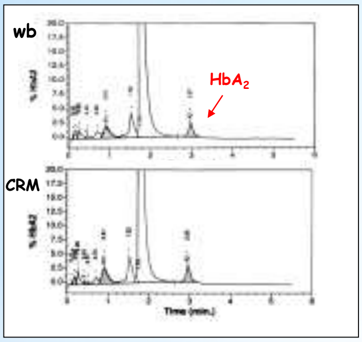
- Lyophilized material

First pilot batch (April 2008)

- homogeneity
- total Hb content
- MetHb
- stability at +4° /-20 ° C
- commutability

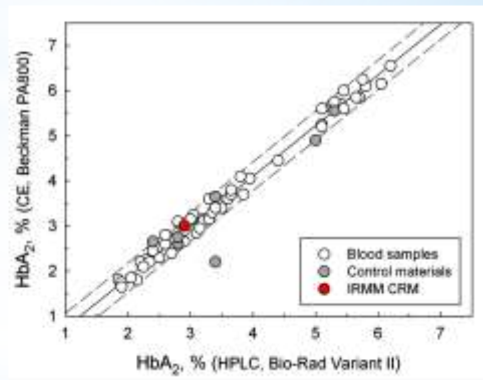
Second batch (November 2010)

-Storage without O_2 to limit oxydation
- accelerated degradation experiments
-Long term stability

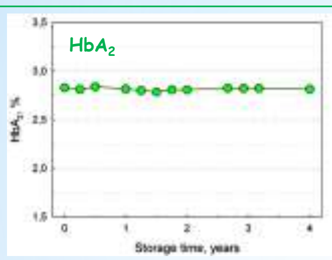


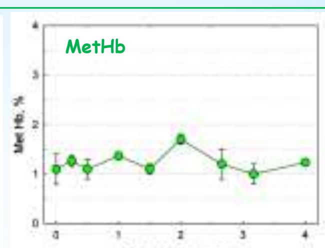
No unexpected peaks due to preparation/lyophilization process

Good commutability

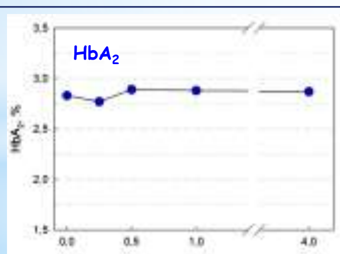


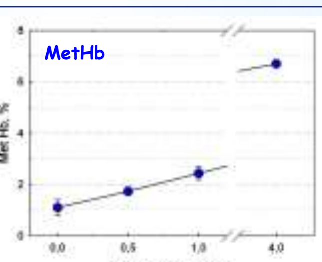
Stability of the lyophilized material





Storage Temp = -20 ° C





Storage Temp = +4 ° C

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Clinica Chimica Acta 477 (2018) 60–65

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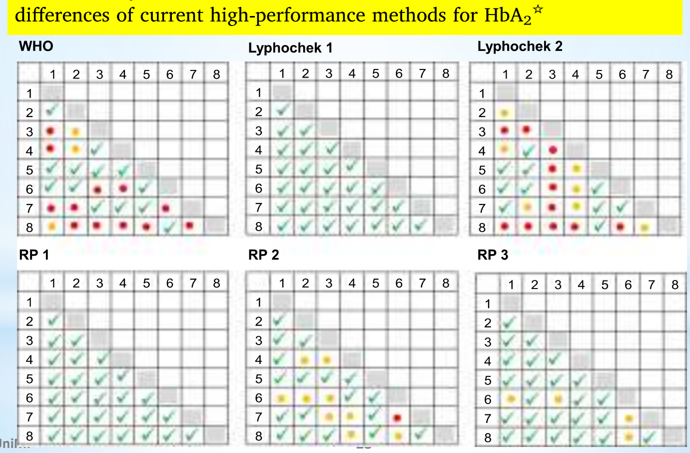
Calibration by commutable control materials is able to reduce inter-method differences of current high-performance methods for HbA_2☆



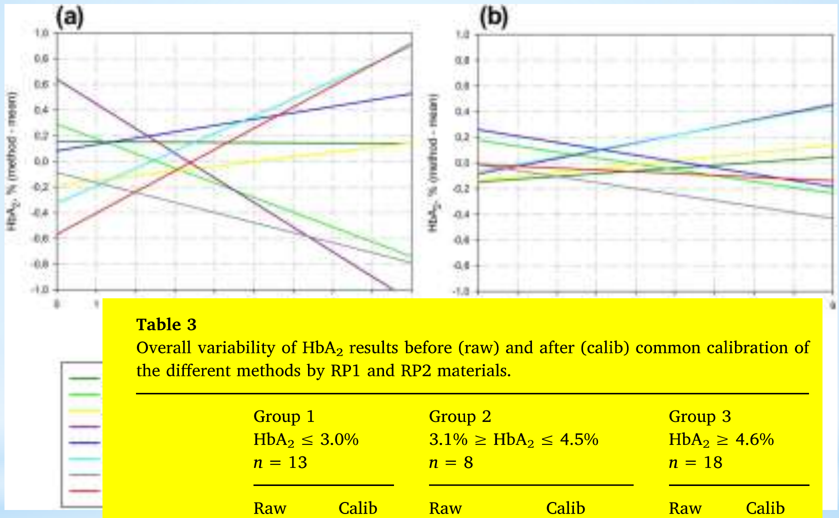


Table 3
Overall variability of HbA$_2$ results before (raw) and after (calib) common calibration of the different methods by RP1 and RP2 materials.

	Group 1 HbA$_2$ ≤ 3.0% n = 13		Group 2 3.1% ≥ HbA$_2$ ≤ 4.5% n = 8		Group 3 HbA$_2$ ≥ 4.6% n = 18	
	Raw	Calib	Raw	Calib	Raw	Calib
HbA$_2$, %	2.46	2.46	3.48	3.48	5.44	5.45
SD	0.17	0.08	0.23	0.16	0.36	0.16
CV, %	6.8	3.4	6.6	4.6	6.7	3.0

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* Reference measurement procedure: to be approved by IFCC (ballot)
* Certified reference material
  * to be prepared in at least one large batch
  * to be distributed and used (manufacturers)
* Definition of new reference intervals for HbA_2 (?)
* State-of-the-art: to be monitored on a regular base by adequate EQAS studies and/or surveys
* Outcome: screening procedures to be optimized, more careful requirements for molecular analysis

Next steps

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* IFCC WG members (R. Paleari, C, Arsene, P, Kaiser)
* C. Hartefeld (Leiden University, NL)
* I. Zegers, H. Schimmel (JRC, BE)

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