



Leiden University
Medical Center

Hb variant rondzending en standaardizatie HbA₂

Kees Hartevelde

Klinische Genetica/Genoom Diagnostiek

LUMC



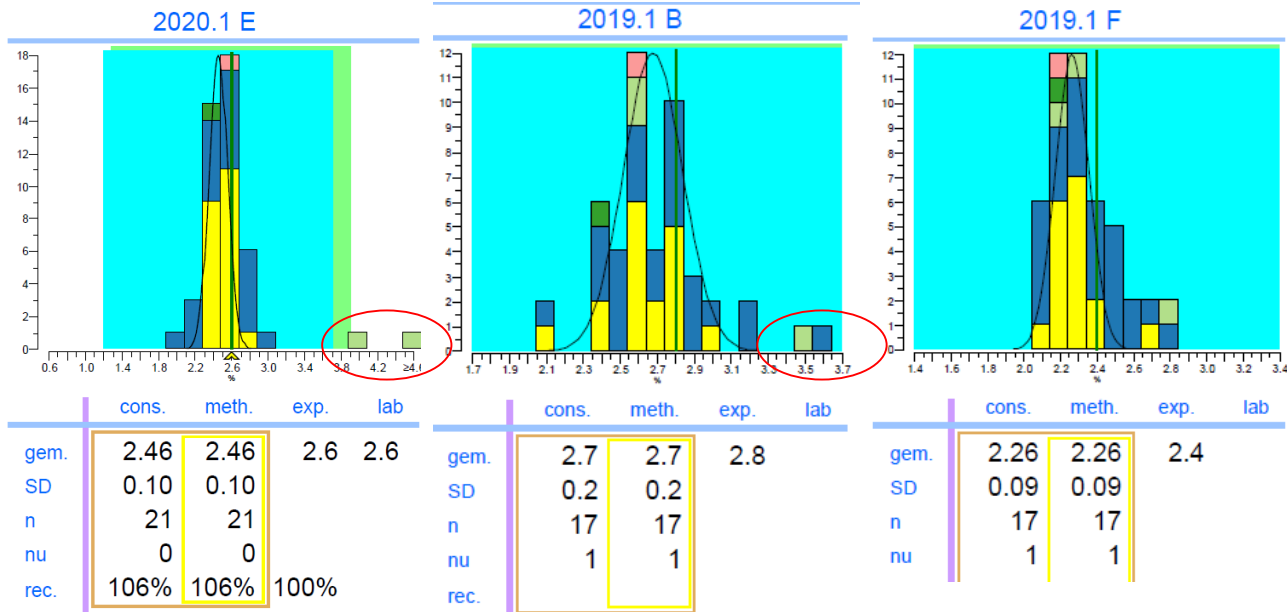
SKML Hb varianten rondzending

- Twee rondes per jaar (.1 en .2), ieder 6 monsters, 12 in totaal
- Casusomschrijving per monster
- Analyse HPLC/CE (in Hb of A1c modus) of Alkaline Electroforese
- Afwijkend/normale scheiding?
- Percentages Hb fracties (HbA, HbA₂, HbF, HbX1, HbX2)
- Eventuele verdenking alfa-thalassemie
- Advies familie/partner onderzoek



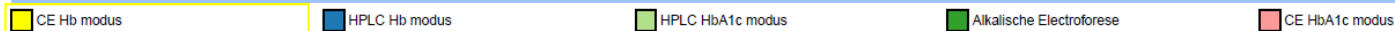
Een verhoogde HbA₂ is indicatief voor een beta-thalassemie drager

Normale HbA₂ waarden (2.2-3.2%)



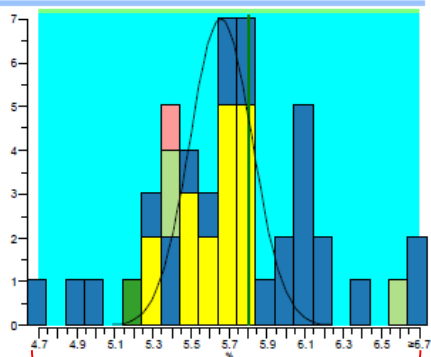
- CE Hb modus
- HPLC Hb modus
- HPLC HbA1c modus
- Alkalische electroforese
- CE HbA1c modus

Legenda



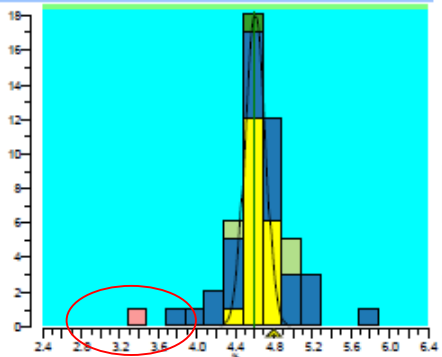
Beta-thalassemie dragers; verhoogde HbA₂ (4-6%)

2019.1 A



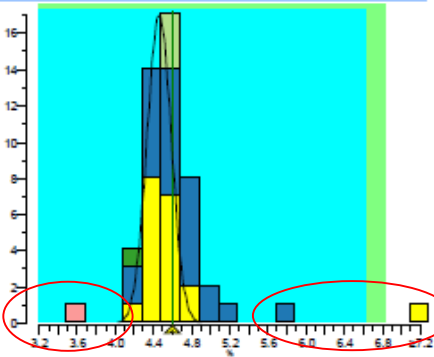
	cons.	meth.	exp.	lab
gem.	5.7	5.7	5.8	
SD	0.2	0.2		
n	17	17		
nu	0	0		
rec.				

2019.2 B



	cons.	meth.	exp.	lab
gem.	4.6	4.6	4.6	4.8
SD	0.1	0.1		
n	19	19		
nu	0	0		
rec.	104%	104%	104%	

2019.2 D



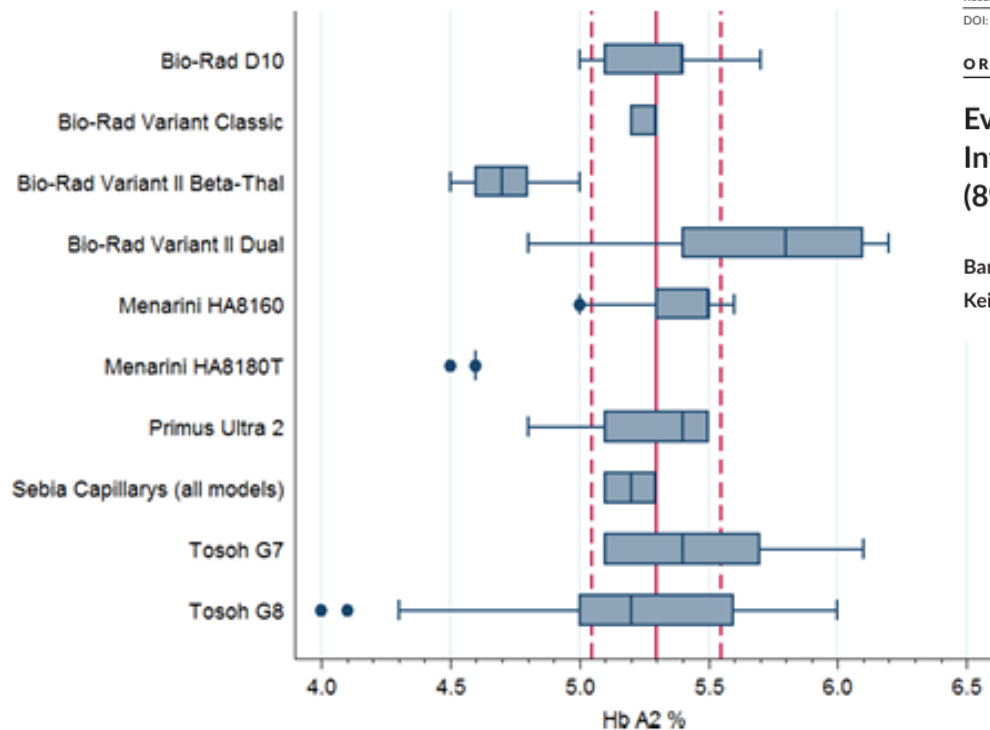
	cons.	meth.	exp.	lab
gem.	4.5	4.5	4.6	4.6
SD	0.1	0.1		
n	19	19		
nu	1	1		
rec.	103%	103%	100%	

- CE HbA1c modus
- HPLC Hb modus
- HPLC HbA1c modus
- Alkalische electroforese
- CE HbA1c modus

Legenda

■ HPLC HbA1c modus
 ■ CE Hb modus
 ■ HPLC HbA1c modus
 ■ Alkalische Electroforese
 ■ CE HbA1c modus

HbA₂ UKNEQAS QA scheme Hb variants



Received: 15 October 2018 | Accepted: 28 November 2018

DOI: 10.1111/ijlh.12966

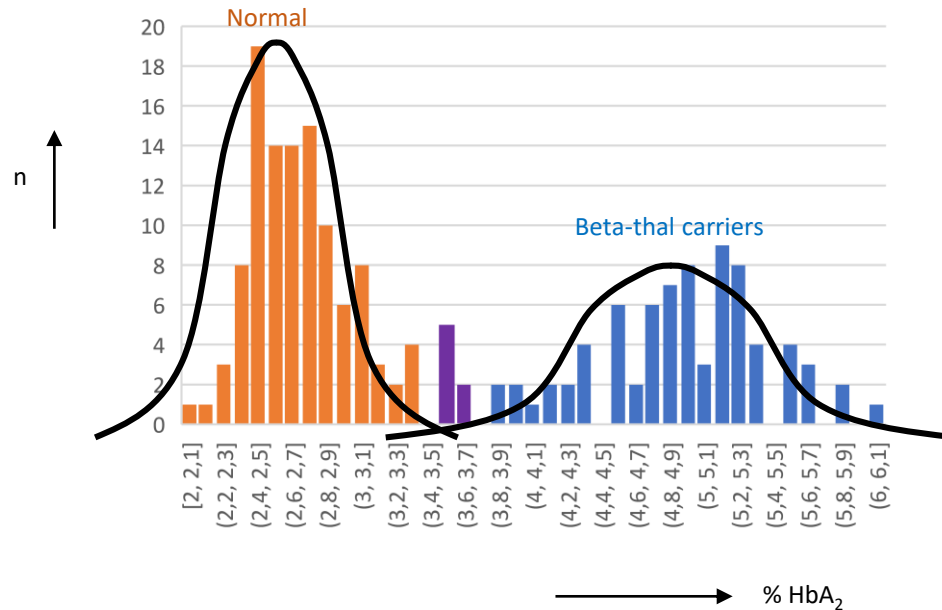
ORIGINAL ARTICLE

WILEY | ISLH International Journal of Laboratory Hematology

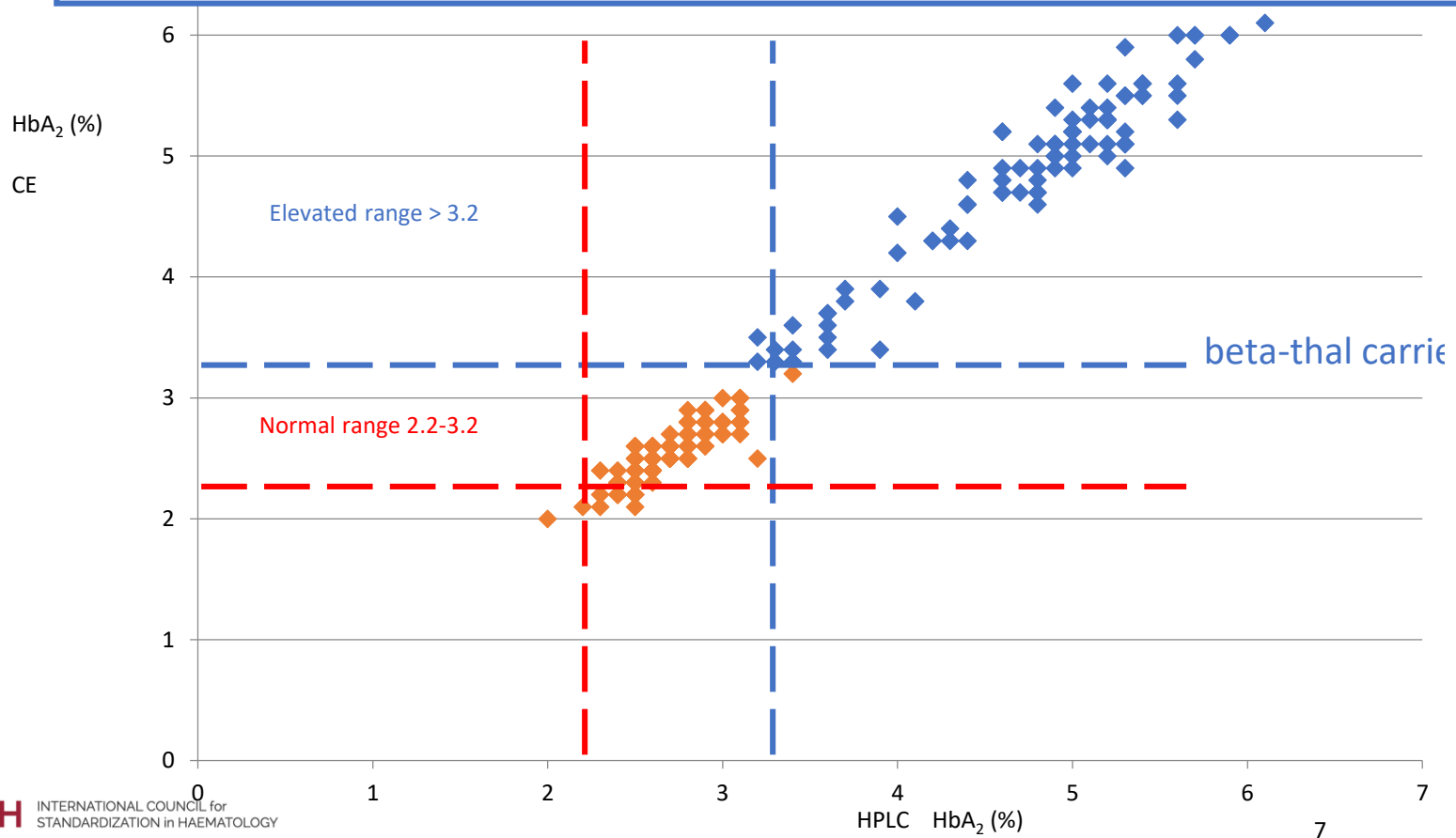
Evaluation of the suitability of the World Health Organization International Reference Reagent for Hb A₂ quantitation (89/666) for continued use

Barbara De la Salle¹ | Adrian D. Stephens¹ | Barbara J. Wild¹ | Cornelis L. Harteveld² | Keith Hyde³

HbA₂ is measured by CE and HPLC to distinguish between carriers and non-carriers of beta-thalassemia



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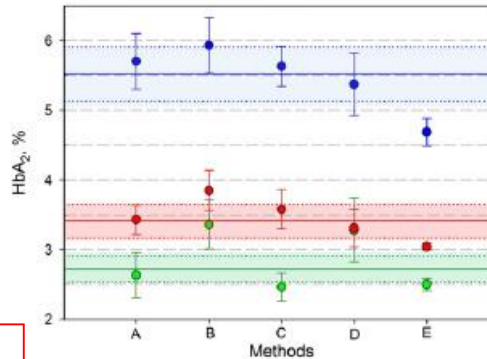


HbA₂ is measured to distinguish between carriers and non-carriers of beta-thalassemia

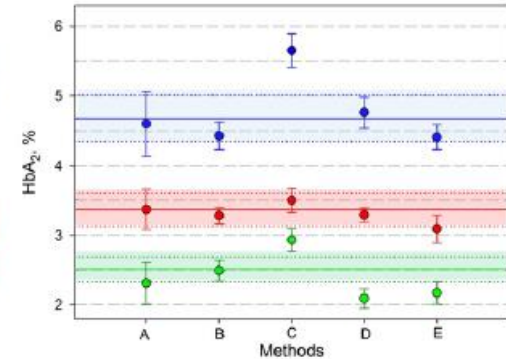
Different separation methods used to quantify HbA₂:

different CE and HPLC devices and programs are being used:

Need for standardization to compare results within and between laboratories



- 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)

From: "Developing a reference system for the IFCC standardization of HbA₂"
Paleari et al. 2017 Clinica Chimica Acta; 467:21-26



Need for Standardization of HbA₂

IFCC/ICSH Joined Working Group for HbA₂ standardization

Members:

Andrea Mosca (IFCC, project leader)

Kees Hartevelde (ICSH-representative)

IFCC members:

**Ian Young (chair IFCC), Ferruccio Ceriotti,
Christa Cobbaert, Sverre Sandberg**

ICSH members:

**Wendy Erber (chair ICSH), Gina Zini, Sara
Trompeter, Vip Viprakasit, Barbara
DelaSalle**

Background:

IFCC working group:

A. Mosca	Chair	IT	1st	2017 01 - 2019 12
C. Arsene	Member	DE		
P. Kaiser	Member	DE		
Q. Liu	Member	SG		
R. Paleari	Member	IT		

Terms of Reference

- To promote the standardisation of hemoglobin A2 measurement through the definition of an international reference system, including a reference measurement procedure and primary and secondary reference materials.

Current Projects

- Definition of a reference measurement procedure using mass spectrometry associated with proteolytic degradation.
- Preparation of a secondary reference material for hemoglobin A2 (in cooperation with JRC).

IFCC and ICSH Joint Working Group (Paris 2015):

Sam Machint
Adrian Stephenst

Barbara Wild
Barbara De La Salle

Andrea Mosca IFCC
Graham Beastall IFCC

Kees Hartevelde

De CRM (certified reference material) bestaat uit een normale HbA₂ waarde (mix van normaal donorbloed) en een verhoogde HbA₂ waarde (mix van beta-thalassemie donoren) (Palaria et al. 2010 Clin Chem Lab Med; 48:1611-1618)

Om de HbA₂ waarde toe te kennen aan de CRM is een isotoop gelabelde spiking methode ontwikkeld door twee Metrologische Laboratoria:

- INSTAND e.V., Düsseldorf, Germany (Patricia Kaiser)
- Physikalisch-Technische Bundesanstalt, Braunschweig, Germany (Cristian Arsene).

Reference measurement procedure

“Developing a reference system for the IFCC standardization of HbA₂”

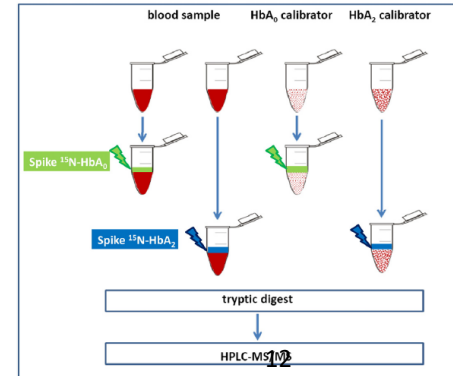
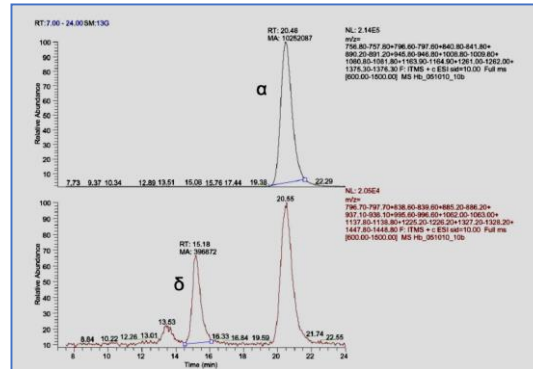
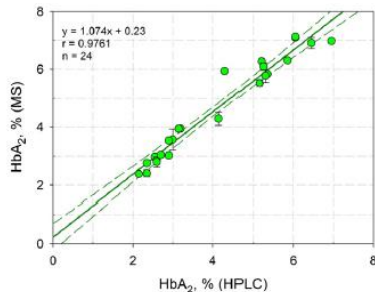
(Paleari et al. 2017 Clinica Chimica Acta; 467:21-26)

Reference measurement procedure (RMP):

Two principles to determine δ/α ratio (as representative for HbA₂):

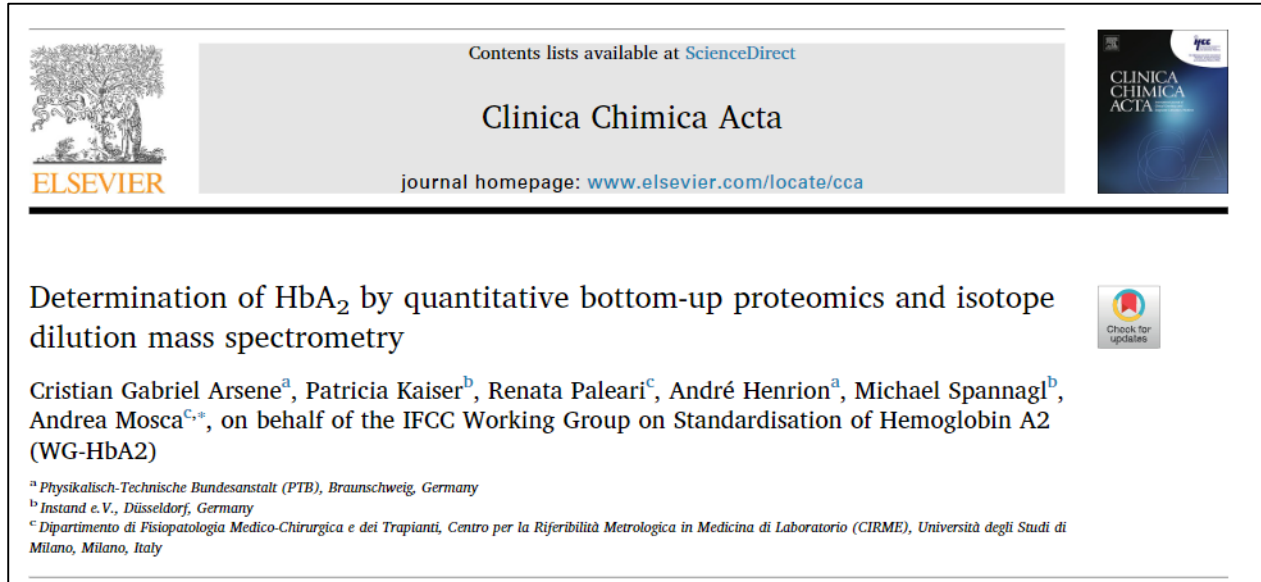
1-Separation of *INTACT* δ - and α -globin chains by Liquid Chrom-ElectroSpray Ionization–tandem Mass Spec (LC-ESI/MS)

2- Separation of *TRYPTIC DIGEST* of δ - and α -globin chain fragments by Isotope Dilution Mass Spectrometry (ID-MS) using recombinant ¹⁵N-HbA₀ and ¹⁵N-HbA₂ as calibrator (Arsene et al. 2018 Clinica Chimica Acta; 487:318-324)



Een gepubliceerde referentie-methode is een vereiste voor de productie van een CRM

(Arsene et al. 2018 Clinica Chimica Acta; 487:318-324)



Contents lists available at [ScienceDirect](#)

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Determination of HbA₂ by quantitative bottom-up proteomics and isotope dilution mass spectrometry

Cristian Gabriel Arsene^a, Patricia Kaiser^b, Renata Paleari^c, André Henrion^a, Michael Spannagl^b, Andrea Mosca^{c,*}, on behalf of the IFCC Working Group on Standardisation of Hemoglobin A2 (WG-HbA2)

^aPhysikalisch-Technische Bundesanstalt (PTB), Braunschweig, Germany
^bInstand e.V., Düsseldorf, Germany
^cDipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Centro per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME), Università degli Studi di Milano, Milano, Italy

Vervolgens produceert EU-JRC dit CRM

Reduction of Inter-method differences

“Calibration by commutable control materials is able to reduce inter-method differences of current high-performance methods for HbA₂”
(Paleari et al. 2018 Clinica Chimica Acta; 477:60-65)

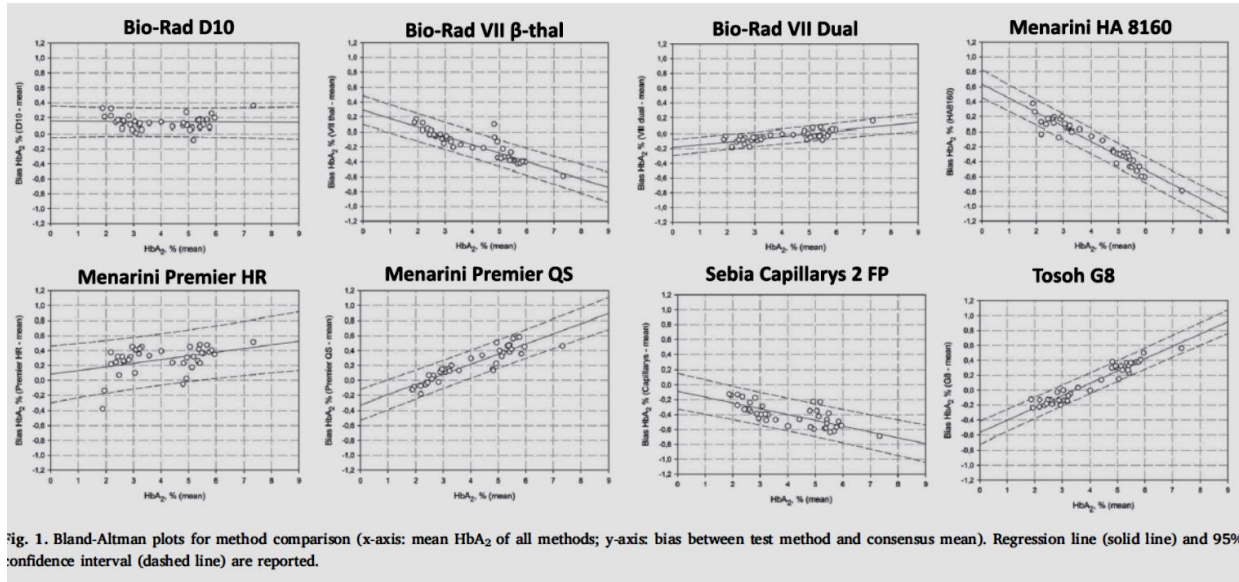
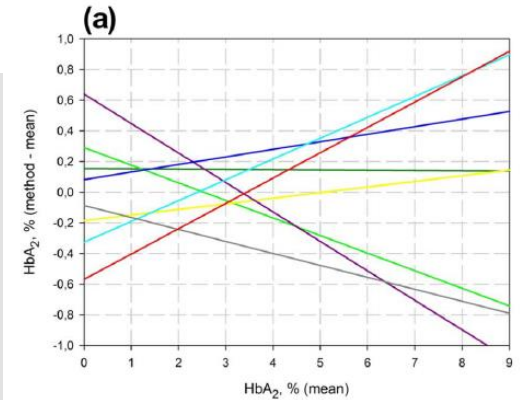


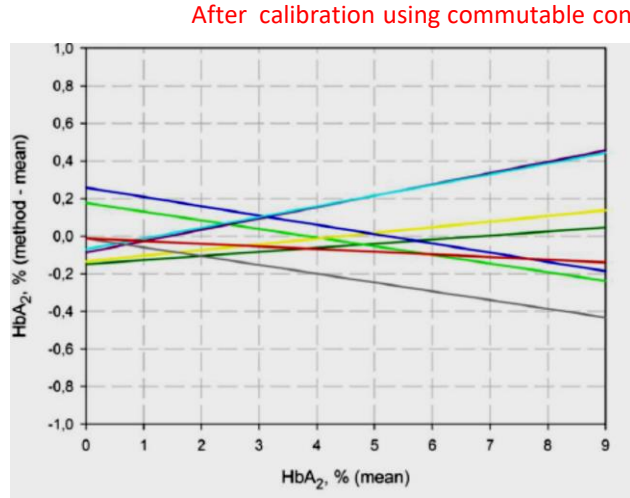
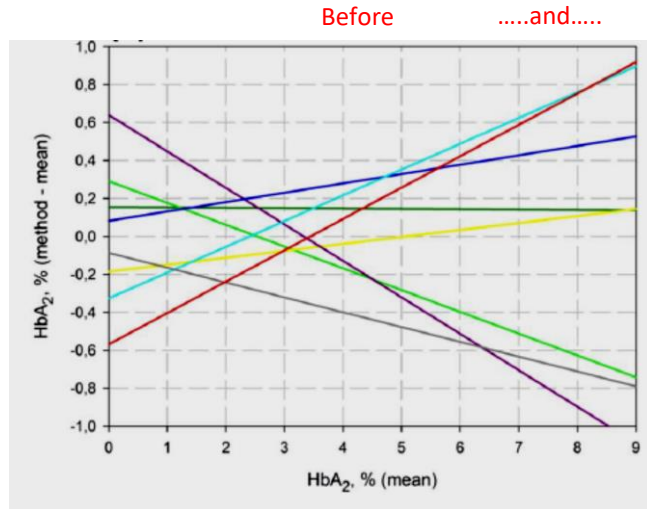
Fig. 1. Bland-Altman plots for method comparison (x-axis: mean HbA₂ of all methods; y-axis: bias between test method and consensus mean). Regression line (solid line) and 95% confidence interval (dashed line) are reported.



Bland-Altman plots
Total of all 8 method comparisons
including 6 different devices and 8 running
modes

Reduction of Inter-method differences

“Calibration by commutable control materials is able to reduce inter-method differences of current high-performance methods for HbA₂”
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Bland-Altman plots
Total of all 8 method comparisons
including 6 different devices and 8 running
modes

Manufacturing the reference materials at the
Joint Research Center (EU-JRC, Geel, BE)



Preparation of large batch of CRM for value assignment

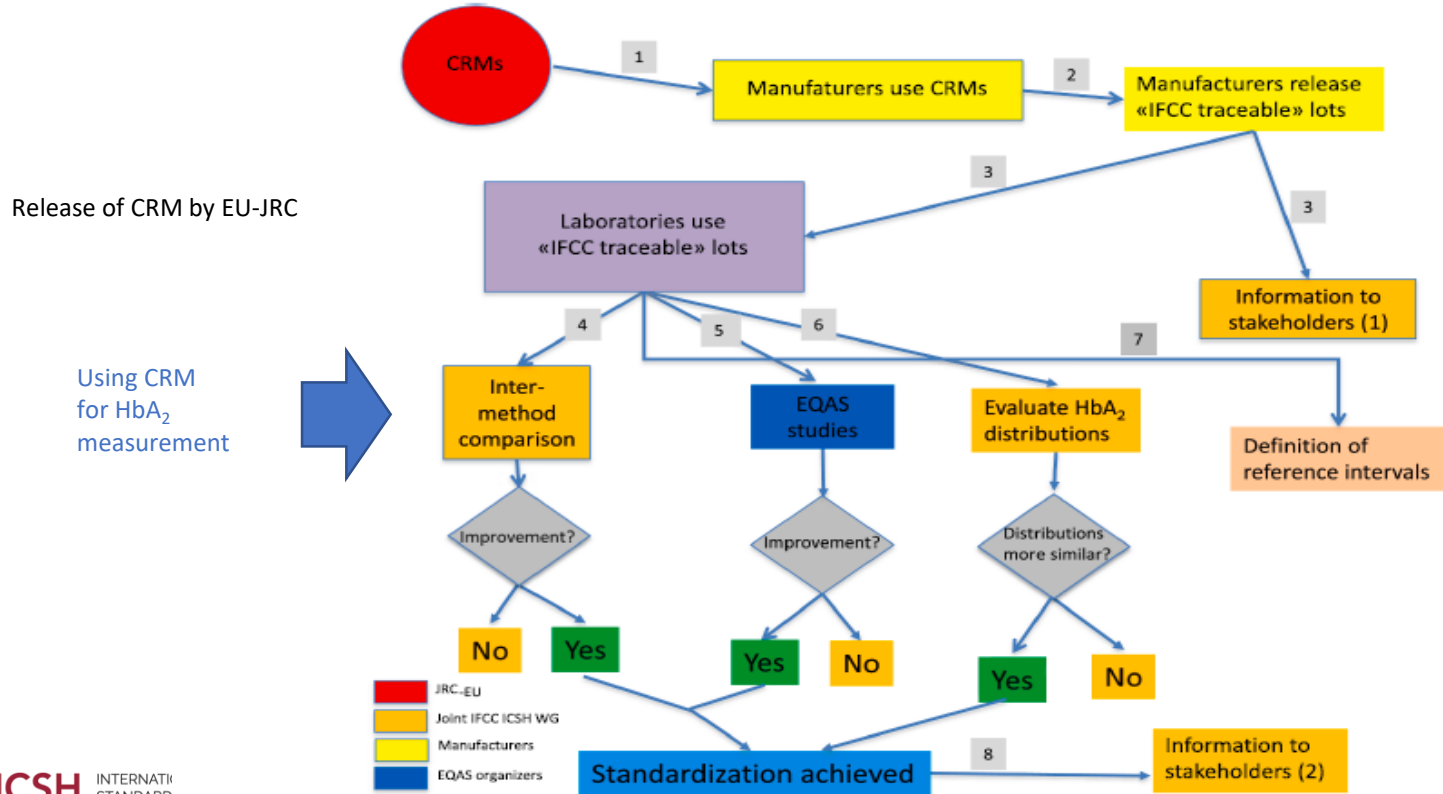
- **EU-JRC (European-Joined Research Center, Geel Belgium)**
 - Preparation of two values CRM (normal 2.5% and elevated 5.4% HbA₂), 3500 ampoules freeze-dried material of pooled donors.
- **Homogeneity and short-term stability (accelerated degradation tests) completed**
- **Long-term stability completed**
- **Commutability tests completed 2022**

Data collected on 9 methods (Bio-Rad Variant II dual kit and beta-thal mode, Bio-Rad D10, Trinity Premier HR, Helena CE V8 Nexus, Menarini Hb Next, Tosoh G11, Arkray Adams HA-8180T and Sebia CE)

- **Final value assignment with uncertainties**
 - Value assignment using the Reference Measurement Procedure in two separate rounds in INSTAND e.V., Düsseldorf, Germany (Patricia Kaiser) and Physikalisch-Technische Bundesanstalt, Braunschweig, Germany (Cristian Arsene) is now ready (summer 2024).
 - CRM will be available from EU-JRC by the end of this year

Roadmap Certified Reference Material

(Mosca et al. 2021 Clinica Chimica Acta; 512:185-190)



- De CRMs in twee waarden komen eind 2024 beschikbaar
- Firma's hebben toegezegd als stakeholders dat zij CRM als kalibratoren zullen gebruiken om hun instrumenten te ijken.
- Feedback aan de Joined Working Group door test-labs/gebruikers
- Continue monitoring in external quality assessment schemes
- Is standardisatie bereikt? (dat zal moeten blijken)

Acknowledgements

Andrea Mosca (IFCC, project leader)

Ian Young (chair IFCC)

Ferruccio Ceriotti

Christa Cobbaert

Sverre Sandberg (IFCC)

Renata Paleari

Patricia Kaiser

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Wendy Erber (chair ICSH)

Gina Zini

Sara Trompeter

Vip Viprakasit

Barbara De la Salle (ICSH)

Adrian Stephens †

Barbara Wild

Sam Machin †

Terry Fawcett