

# Disclosure

De spreker heeft

- Geen financiële banden met de IVD industrie
- Geen sponsoring door belanghebbende industrie
- Geen honoraria van belanghebbende industrie
- Geen aandeelhouder van belanghebbende industrie
- Geen andere relaties met belanghebbende industrie die gezien kunnen worden als belangenverstrengeling



# **Rondzending Combi Immunochemie**

**15.15u – 15.35u**

**13 februari 2020**

**De Reehorst**

# The long and winding road naar harmonisatie

## Combi immunochemie:

Inez-Anne Haagen, OLVG Lab BV, Amsterdam

Cas Weykamp, Streekziekenhuis Koningin Beatrix, Winterswijk, chemicus

Eline van der Hagen, Streekziekenhuis Koningin Beatrix, Winterswijk, Deputy Director MCA  
Laboratory / Klinisch Chemicus

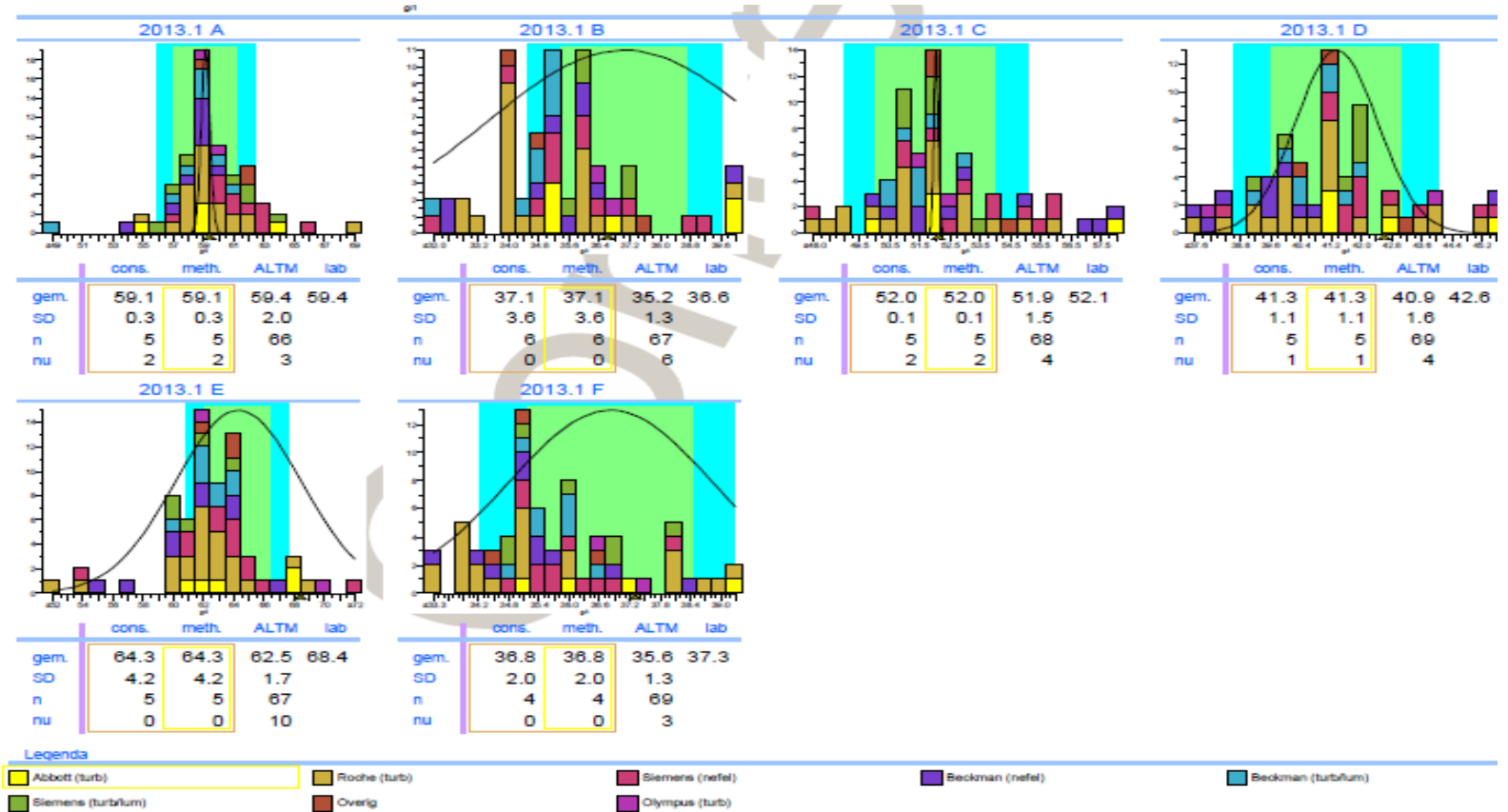
# Eiwitten

Hoe zat het ook al weer....

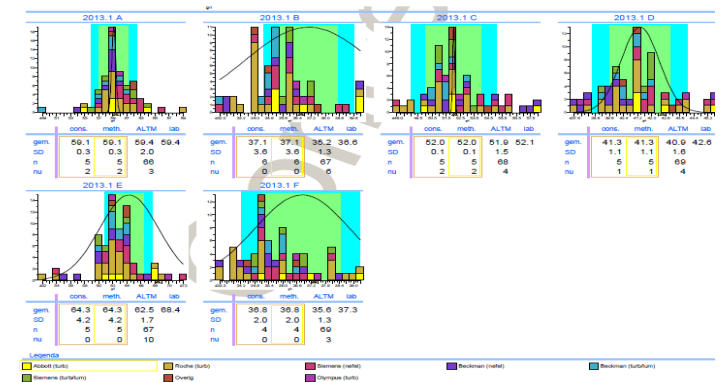
# Albumine 2013

Legenda:

- Abbott
- Roche
- Siemens
- Siemens
- Beckman
- Beckman



# Gebruik van referentie materiaal ondersteund met een doorloper



# Traceability of Laboratory Results

The standard includes 5 categories of reference systems. There are well established procedures to address standardization of measurands in categories 1, 2 and 3. Category 4 includes measurands for which reference materials are available for calibration, but there is no RMP. Category 5 includes measurands for which neither RMPs nor reference materials for calibration are available.

Standardization	Category	Reference measurement procedure	Primary reference material (pure substance)	Secondary reference material (value assigned)	Examples
	1	Yes	Yes	Possible	Electrolytes, glucose, cortisol
	2	Yes	No	Possible	Enzymes
	3	Yes	No	No	Hemostatic factors
	4	No	No	Yes	Proteins, tumor markers, HIV <sup>a</sup>
	5	No	No	No	EBV <sup>b</sup> , VZV <sup>c</sup>

Harmonization

<sup>a</sup> Human Immunodeficiency virus

<sup>b</sup> Epstein Barr virus

<sup>c</sup> Varicella zoster virus

# Characterization of the New Serum Protein Reference Material ERM-DA470k/IFCC: Value Assignment by Immunoassay

Ingrid Zegers,<sup>1\*</sup> Thomas Keller,<sup>2</sup> Wiebke Schreiber,<sup>3</sup> Joanna Sheldon,<sup>4</sup> Riccardo Albertini,<sup>5</sup> Søren Blirup-Jensen,<sup>6</sup> Myron Johnson,<sup>7</sup> Stefanie Trapmann,<sup>1</sup> Hendrik Emons,<sup>1</sup> Giampaolo Merlini,<sup>5</sup> and Heinz Schimmel<sup>1</sup>

**RESULTS:** For 12 proteins [ $\alpha_2$  macroglobulin (A2M),  $\alpha_1$  acid glycoprotein (orosomucoid, AAG),  $\alpha_1$  anti-trypsin ( $\alpha_1$ -protease inhibitor, AAT), albumin (ALB), complement 3c (C3c), complement 4 (C4), haptoglobin (HPT), IgA, IgG, IgM, transferrin (TRF), and transthyretin (TTR)], the results allowed assignment of certified values in ERM-DA470k/IFCC. For CRP, we observed a bias between the lyophilized and liquid frozen materials, and for CER, the distribution of values was too broad. Therefore, these 2 proteins were not certified in the ERM-DA470k/IFCC. Different value transfer procedures were tested (open and closed procedures) and found to provide equivalent results.

We used methods that are validated and well-established routine methods based on turbidimetry, nephelometry, and occasionally visible spectrometry (in the case of ALB). The concentrations of calibrant and reference material dilutions were optimized for each platform/reagent combination.

**Combi immunochemie**  
**Sectie humorale immunologie**

**Certified values for:**  
 $\alpha_2$ macroglobuline  
 $\alpha_1$  acid glycoprotein  
 $\alpha_1$  anti-trypsin  
Albumin  
Complement C3  
Complement C4  
Haptoglobin  
IgA  
IgG  
IgM  
Transferrine  
Transthyretin

**Not Certified for:**  
CRP  
Ceruleplasmin  
 $\beta_2$  microglobulin



# CERTIFICATE OF ANALYSIS

ERM<sup>®</sup> - DA470k/IFCC

*Nr. 10 296*

HUMAN SERUM		
Proteins in the reconstituted material <sup>1)</sup>	Mass concentration	
	Certified value <sup>2)</sup> [g/L]	Uncertainty <sup>3)</sup> [g/L]
α <sub>2</sub> macroglobulin (A2M)	1.43 <sup>4)</sup>	0.06
α <sub>1</sub> acid glycoprotein (AAG)	0.617 <sup>5)</sup>	0.013
α <sub>1</sub> antitrypsin (AAT)	1.12 <sup>5)</sup>	0.03
albumin (ALB)	37.2 <sup>4)</sup>	1.2
complement 3c (C3c)	1.00 <sup>4)</sup>	0.04
complement 4 (C4)	0.162 <sup>4)</sup>	0.007
haptoglobin (HPT)	0.889 <sup>4)</sup>	0.021
immunoglobulin A (IgA)	1.80 <sup>4)</sup>	0.05
immunoglobulin G (IgG)	9.17 <sup>4)</sup>	0.18
immunoglobulin M (IgM)	0.723 <sup>4)</sup>	0.027
transferrin (TRF)	2.36 <sup>5)</sup>	0.08
transthyretin (TTR)	0.220 <sup>5)</sup>	0.018

## ANALYTICAL METHOD USED FOR CERTIFICATION

1. Turbidimetry
2. Nephelometry
3. Visual spectrometry

## Summary

The production and certification of ERM-DA470k/IFCC, a new serum protein reference material intended to replace ERM-DA470, is described.

Serum was produced from blood collected in 6 blood collection centres according to a procedure ensuring that it was obtained from healthy donors, and that the lipid content of the serum was low.

The serum was processed in 5 batches, and then pooled, spiked with B2M and CRP and filled into vials (1 mL serum per vial). The serum was lyophilised in the vials, afterwards closed with rubber stoppers and screw caps and stored at -70 °C. The stability and homogeneity of the material were assessed for 14 proteins, including CER and B2M.

ERM-DA470k/IFCC was characterised for 12 proteins using the reference material ERM-DA470 as calibrant. This was achieved using a value transfer protocol that can be considered as reference procedure.

The results show that open and closed value transfer procedures give very similar results, and lead to robust values for A2M, AAG, AAT, ALB, C3c, C4, HPT, IgA, IgG, IgM, TRF, and TTR.

2015

## CERTIFICATION REPORT

### The Certification of the Mass Concentration of C-Reactive Protein in Human Serum

#### Certified Reference Material ERM<sup>®</sup>-DA474/IFCC

The CRM has been accepted as European Reference Material (ERM<sup>®</sup>) after peer evaluation by the partners of the European Reference Materials consortium.

The following value was assigned:

	Mass concentration	
	Certified value <sup>2)</sup> [mg/L]	Uncertainty <sup>3)</sup> [mg/L]
C-reactive protein (CRP) <sup>1)</sup>	41.2	2.5

1) CRP as measured by immunonephelometry and immunoturbidimetry using ERM-DA470 as calibrant (Baudner et al., EUR reports 15423 and 16882 European Communities, Luxembourg (1993)), applying the procedures described for the certification of ERM-DA472/IFCC, ERM-DA470 and 1<sup>st</sup> Int. St. for CRP Code 85/506.

JCTLM 2015  
ERM-DA470k/IFCC, human serum

albumin (ALB)  
alpha1 acidglycoprotein (AAG)  
alpha1 antitrypsin (AAT)  
alpha2macroglobulin (A2M)  
complement 3c (C3c)  
complement 4 (C4)  
haptoglobin (HPT)  
immunoglobulin A (IgA)  
immunoglobulin G (IgG)  
immunoglobulin M (IgM)  
**C-reactive protein (CRP)**  
transferrin (TRF)  
transthyretin (TTR)

Greyed out rows indicate the (Certified) Reference Materials reviewed for compliance with ISO 15194:2003 but not reviewed against ISO 15194:2009

beta-2-microglobulin in processed human serum

European Commission, Joint Research Centre (JRC), European Union

Phone : +32 (0) 14 571 705

Email : [jrc-rm-distribution@ec.europa.eu](mailto:jrc-rm-distribution@ec.europa.eu)

Fax : +32 (0) 14 590 406

Web : <https://crm.jrc.ec.europa.eu/>

Name of the reference material	ERM-DA470k/IFCC, human serum
Quantity	Mass concentration
Analyte certified/assigned value	2.17 mg/L
Expanded uncertainty (level of confidence 95 %)	0.07 mg/L
Reference(s) on commutability	Information available in the Certification Report of the certification of the material in human serum : EF
Other relevant publication(s)	Development and production of reference material: for Zegers et al., <i>Clin. C</i>
Comment(s)	Each sample consists of human serum. It contains (4-(2-hydroxyethyl)-1-sodium azide, because the material is kept under
Traceability	traceable to SI
CRM listing	List I

JOINT RESEARCH CENTRE  
Institute for Reference Materials and Measurements

# CERTIFICATE OF ANALYSIS

ERM<sup>®</sup> - DA470k/IFCC

N° 17662, 17663, 17664

## HUMAN SERUM

Proteins in the reconstituted material <sup>1)</sup>	Mass concentration	
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$\alpha_2$ macroglobulin (A2M)	1.43 <sup>4)</sup>	0.06
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$\alpha_1$ antitrypsin (AAT)	1.12 <sup>5)</sup>	0.03
albumin (ALB)	37.2 <sup>4)</sup>	1.2
$\beta$ -2-microglobulin (B2M)	0.00217 <sup>6)</sup>	0.00007
complement 3c (C3c)	1.00 <sup>4)</sup>	0.04
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Aug 2018



Stichting Kwaliteitsbewaking  
Medische Laboratoriumdiagnostiek

Combi immunochemie  
Sectie humorale immunologie

# A1AT

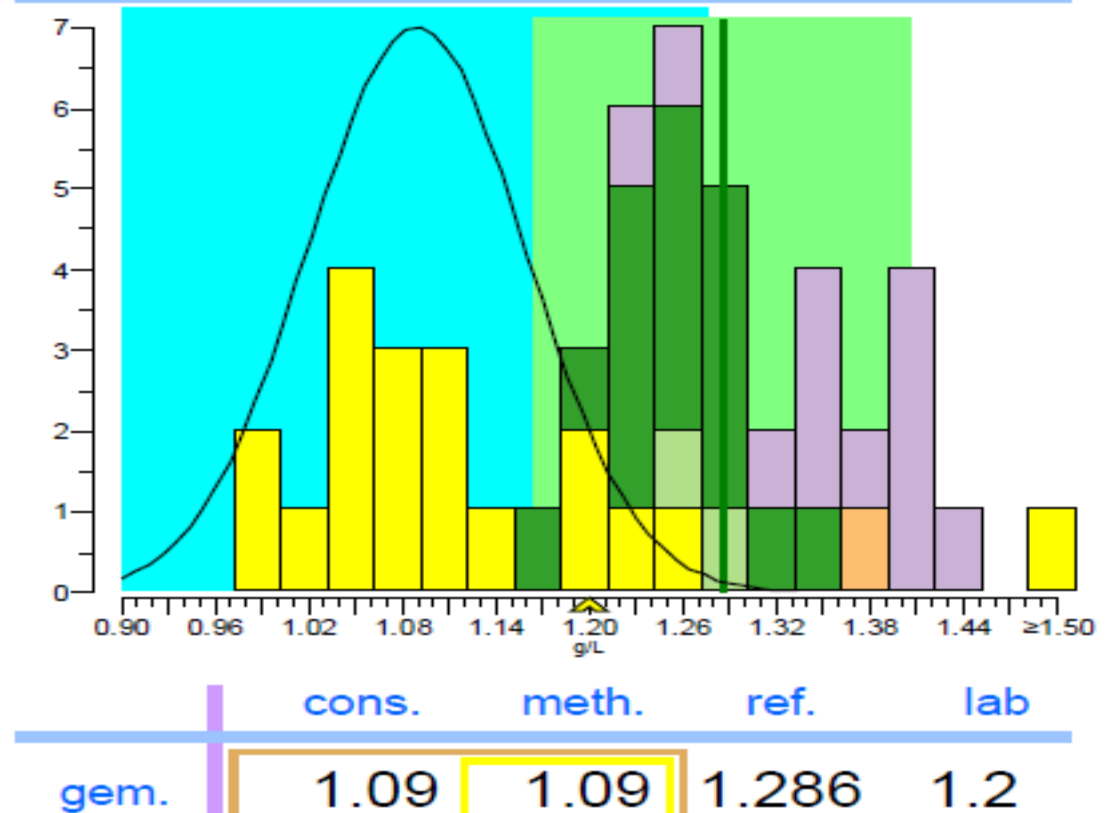
EQA February 2014

Mean Roche (n=14)  
Overall 1.07  
Suggests Factor 20%

Abbott (2):	1.29
Beckman (12):	1.36
Siemens (16):	1.26

Target: 1.286 g/L

2014.1 D



Legenda

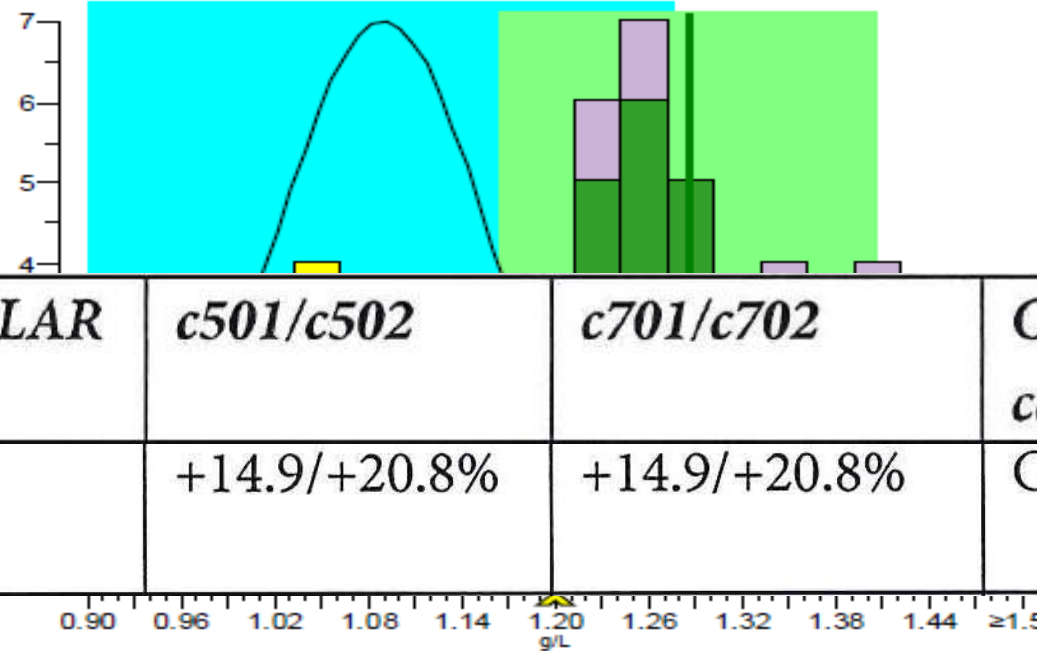
- Roche (turb)
- Beckman (turb/lum)
- Abbott (turb)
- Siemens (nefel)
- Siemens (turb/lum)
- Olympus (turb)
- Overig
- Ortho Diagnostics (turb)
- Beckman (nefel)

# A1AT

Target: 1.286 g/L

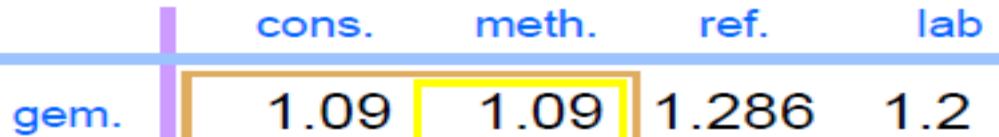
EQA February 2014

2014.1 D



Mean Roche (n=14)  
Overall 1.07  
Suggests Factor 20%

<i>Bepaling</i>	<i>INTEGRA</i>	<i>MODULAR</i> <P>	<i>c501/c502</i>	<i>c701/c702</i>	<i>C.f.a.s</i> <i>calibrator</i>
Tina-quant α-1 Antitrypsine	+21.1/+26.1%	None	+14.9/+20.8%	+14.9/+20.8%	C.f.a.s Protein

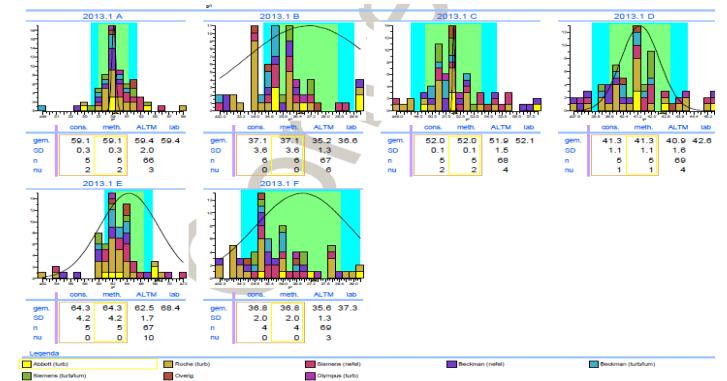


Abbott (2): 1.29  
Beckman (12): 1.36  
Siemens (16): 1.26

Legenda

- Roche (turb)
- Beckman (turb/lum)
- Abbott (turb)
- Siemens (nefel)
- Siemens (turb/lum)
- Olympus (turb)
- Overig
- Ortho Diagnostics (turb)
- Beckman (nefel)

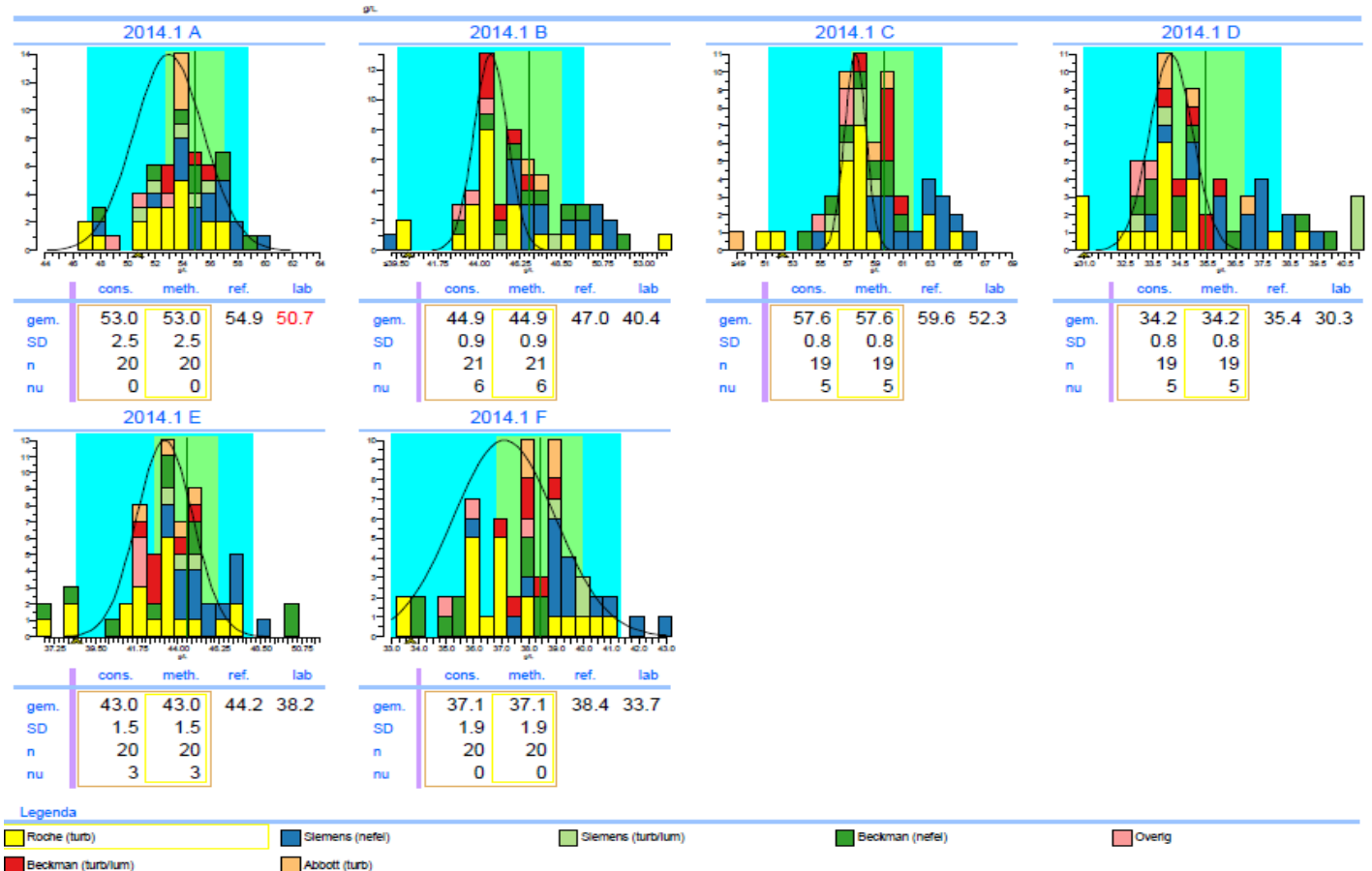
- **Gebruik van referentie materiaal ondersteund met een doorloper**
- **Herstandaardisatie door firma**



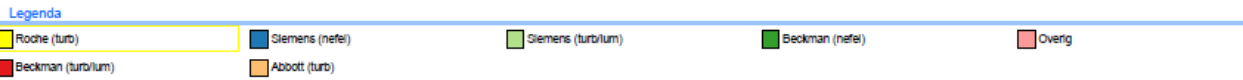
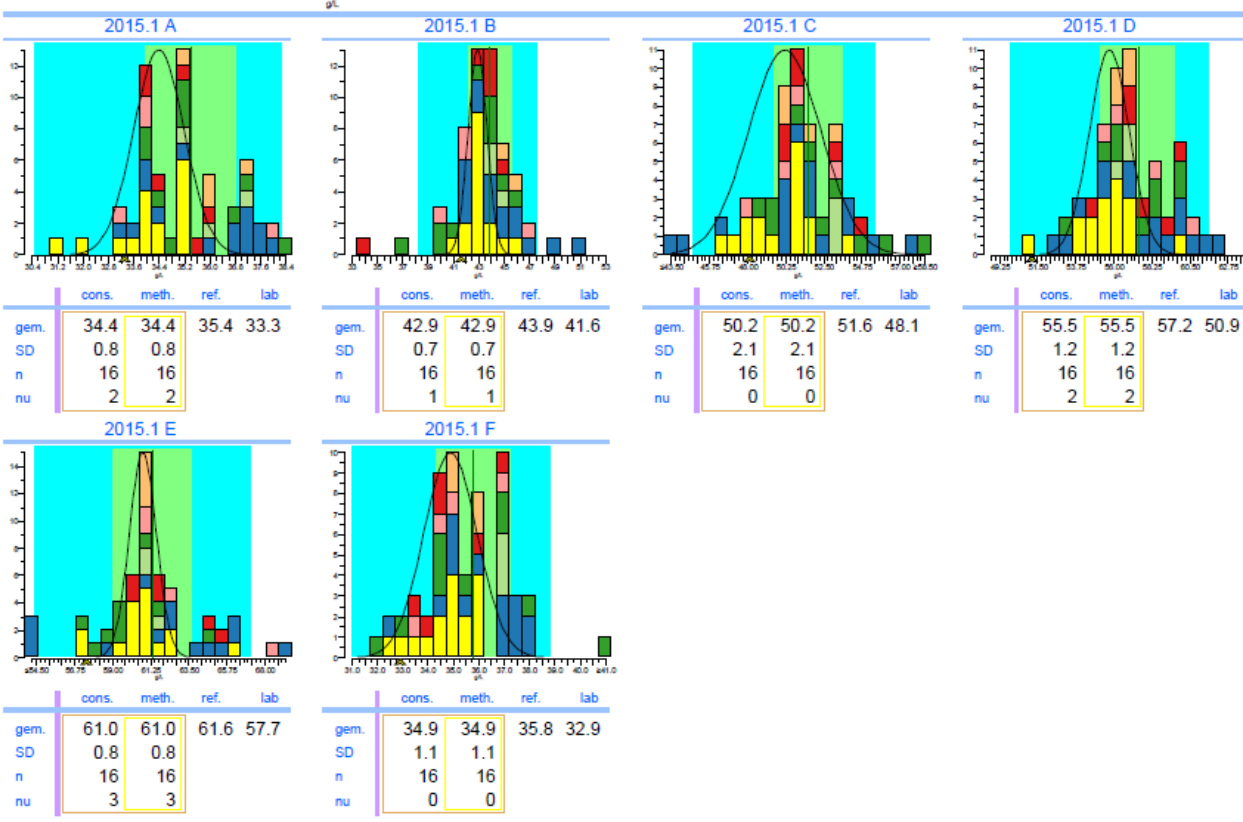
Jan 2014

Bij parameters zoals C3, IgG, IgA, IgM, a1-antitrypsine, CRP en ferritine zijn duidelijk gebruikers van verschillende firma's te onderscheiden: er ontstaan 'groepen'. Indien men eigen referentie intervallen heeft vastgesteld is dit te verklaren. Enige jaren gelden is de discussie voor standaardisatie en harmonisatie opgestart welke van groot belang zijn. Voor de standaardisatie van eiwitten kunt u gebruik maken van het SKML Trueness Verificator monster (albumine, prealbumine, IgG, IgA, IgM, haptoglobine, AAT, transferine, C3, C4, te bestellen via de SKML) en/of het ERM-DA470k/IFCC materiaal (a2 macroglobuline, a1 acid glycoprotein, a1 anti-trypsine, albumine, C3, C4, haptoglobine, IgA, IgG, IgM, transferrine, transthyretine). Het ERM is te bestellen via de IRMM-site maar ook andere firma's verkopen dit materiaal.

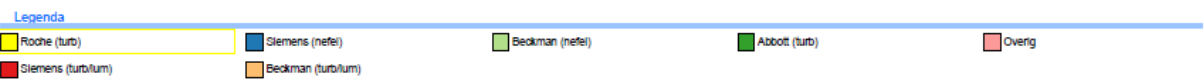
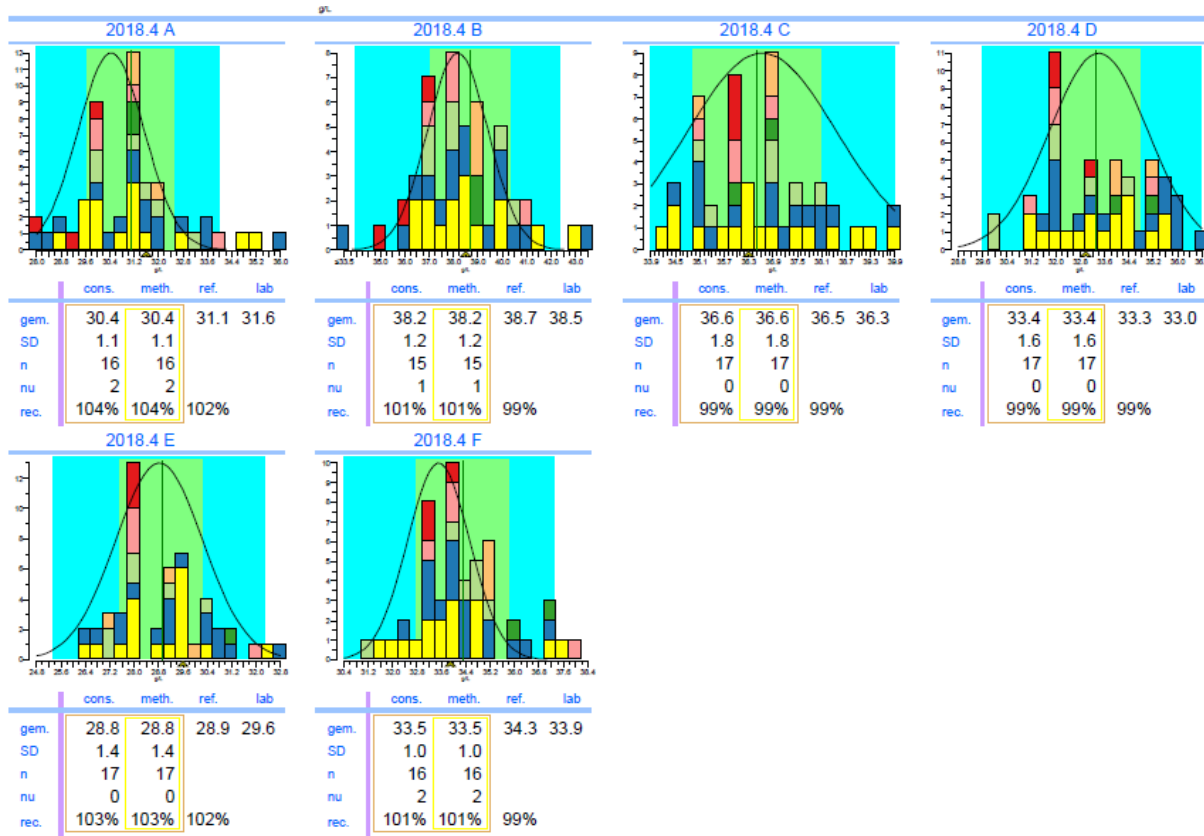
Alb –TQ  
Na invoeren ERM-DA470k



Alb TQ  
2015



Alb TQ  
2018

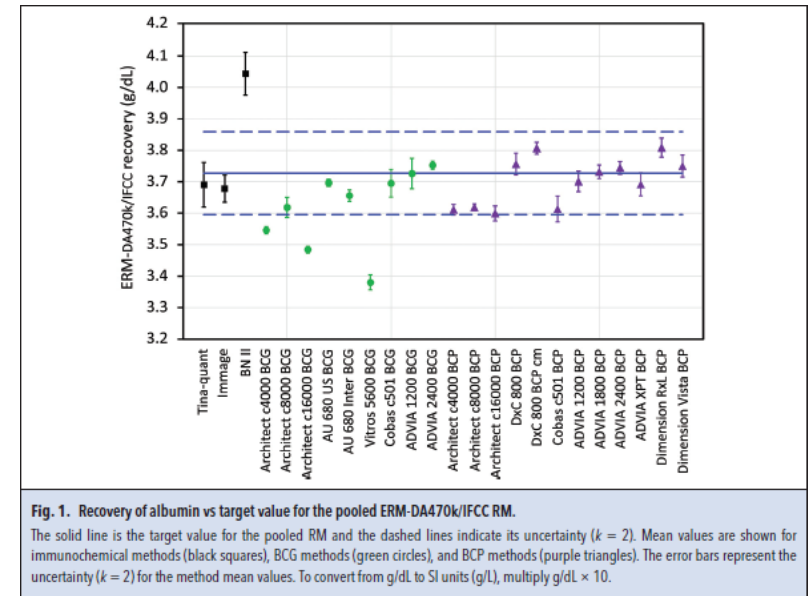


# Factor ingevoerd



# State of Harmonization of 24 Serum Albumin Measurement Procedures and Implications for Medical Decisions

Lorin M. Bachmann,<sup>1\*</sup> Min Yu,<sup>2</sup> James C. Boyd,<sup>2</sup> David E. Bruns,<sup>2</sup> and W. Greg Miller<sup>1</sup>



www.kidney-international.org 2019

technical notes

## The bias between different albumin assays may affect clinical decision-making

Anne-Els van de Logt<sup>1,3</sup>, Sanna R. Rijpma<sup>2,3</sup>, Coralien H. Vink<sup>1</sup>, Elma Prudon-Rosmulder<sup>2</sup>, Jack F. Wetzels<sup>1</sup> and Miranda van Berkel<sup>2</sup>

<sup>1</sup>Radboud University Medical Center, Radboud Institute for Health Sciences, Department of Nephrology, Nijmegen, the Netherlands; and

<sup>2</sup>Radboud University Medical Center Department of Laboratory Medicine, Nijmegen, the Netherlands

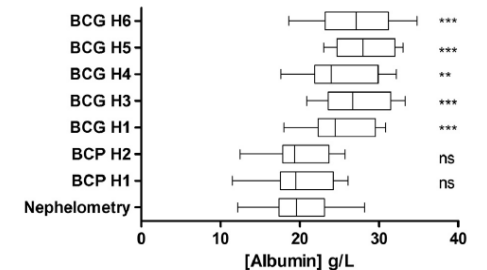


Figure 1 | Albumin concentrations in patients with membranous nephropathy measured in different hospital (H) laboratories. Box and whisker plots are shown of albumin

## Advies van Firma:

### Aanbeveling

De Albumine BCG test is al vele decennia in gebruik. Het criterium van deze colorimetrische test voor albumine in humane serum- en plasmamonsters op de **cobas c** en de COBAS INTEGRA® 400 plus analyzers is een recovery binnen  $\pm 10\%$  van de initiële waarden bij een albumine-concentratie van 35 g/l (532  $\mu\text{mol/l}$ ). Voor diagnostische doeleinden moeten de testresultaten altijd worden beoordeeld samen met de medische geschiedenis van de patiënt, klinisch onderzoek en andere bevindingen.

Colorimetrische methoden die worden gebruikt voor het bepalen van albumine kunnen leiden tot vals verhoogde testresultaten bij patiënten die lijden aan nierfalen of insufficiëntie als gevolg van interferentie met andere eiwitten. Immunoturbidimetrische methoden worden minder beïnvloed.

Voorheen bestond er geen referentiemethode voor de albumine test, maar inmiddels wees de JCTLM (Joint Committee for Traceability in Laboratory Medicine) de immuno-assay aan als referentiemethode van keuze<sup>1</sup>.

In Nederland hebben vrijwel alle gebruikers van de **cobas c** en/of COBAS INTEGRA® 400 plus analyzers de 'Tina-quant Albumine Gen.2' immunoturbidimetrische test al in gebruik voor het bepalen van (micro)albumine in urine en liquor, terwijl voor het testen op albumine in serum of plasma nog gebruik wordt gemaakt van de oudere colometrische testen Albumine BCG (broomcresol green) en/of Albumine BCP (broomcresol purple).

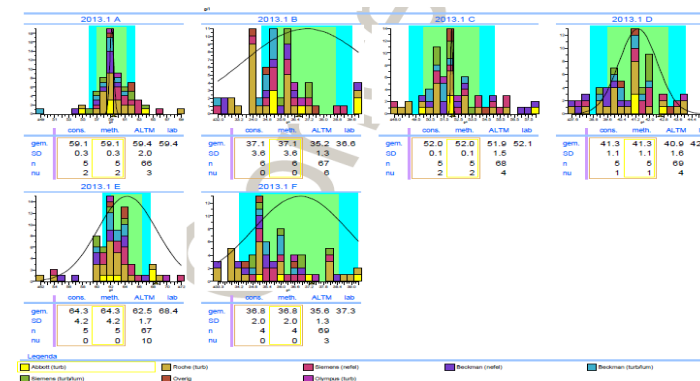
Nu het JCTLM de immuno-assay als referentiemethode heeft aangewezen adviseert Roche vanuit het oogpunt van kwaliteitsverbetering en standaardisatie om ook voor uw serum of plasma albumine testen over te stappen op de Tina-quant Albumin Gen.2 test. Deze immunoturbidimetrische in-vitro test is ontwikkeld voor de kwantitatieve bepaling van albumine in menselijke urine, serum, plasma en liquor.

# ACTIE?

## Ja/Nee?

## Hoe dan?

- Gebruik van referentie materiaal ondersteund met een doorloper
- Herstandaardisatie door firma
- Factor door laboratorium ingevoerd
- Aanbeveling door firma



# Inter-laboratory discordance of beta-2 microglobulin results: impact on the validity of the international staging system for multiple myeloma

© 2014 John Wiley & Sons Ltd  
*British Journal of Haematology*, 2014, **166**, 946–957

Patient	Lab 1	Lab 2	Lab 3	Lab 4
3	1	1	1	1
5	1	1	1	1
1	2	2	2	2
2	2	2	2	2
4	2	1	1	2
6	2	1	1	2
8	2	2	2	2
9	2	2	2	2
10	2	1	1	1
12	2	1	2	2
13	2	2	2	2
14	2	2	2	2
15	2	1	2	2
19	2	2	2	2
21	2	2	2	2
7	3	3	3	3
11	3	2	3	3
16	3	1	2	2
17	3	3	3	3
18	3	2	2	2
20	3	2	3	3

Fig 2. Discordance of International Staging System scores for individual patients derived by different laboratories. Green: Stage 1 ( $\beta 2M < 3.5 \text{ mg/l}$  plus albumin  $\geq 35 \text{ g/l}$ ). Yellow: Stage 2 (neither stage I nor III). Red: Stage III ( $\beta 2M \geq 5.5 \text{ mg/l}$ ).

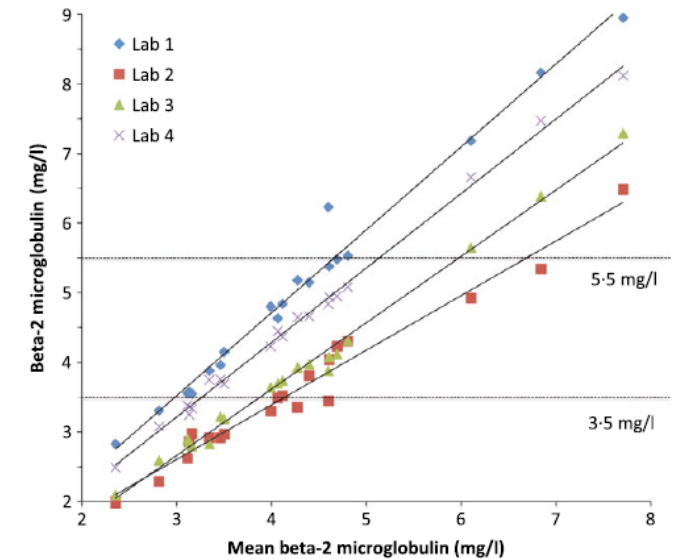
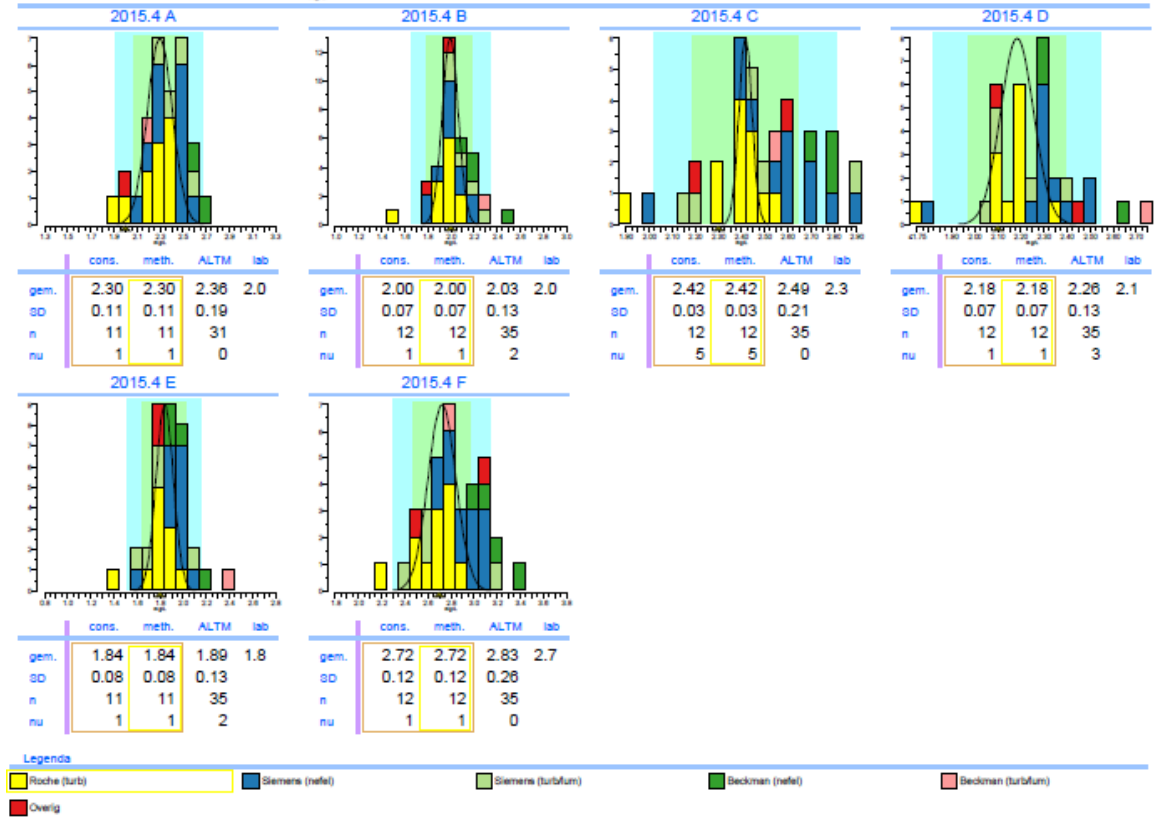
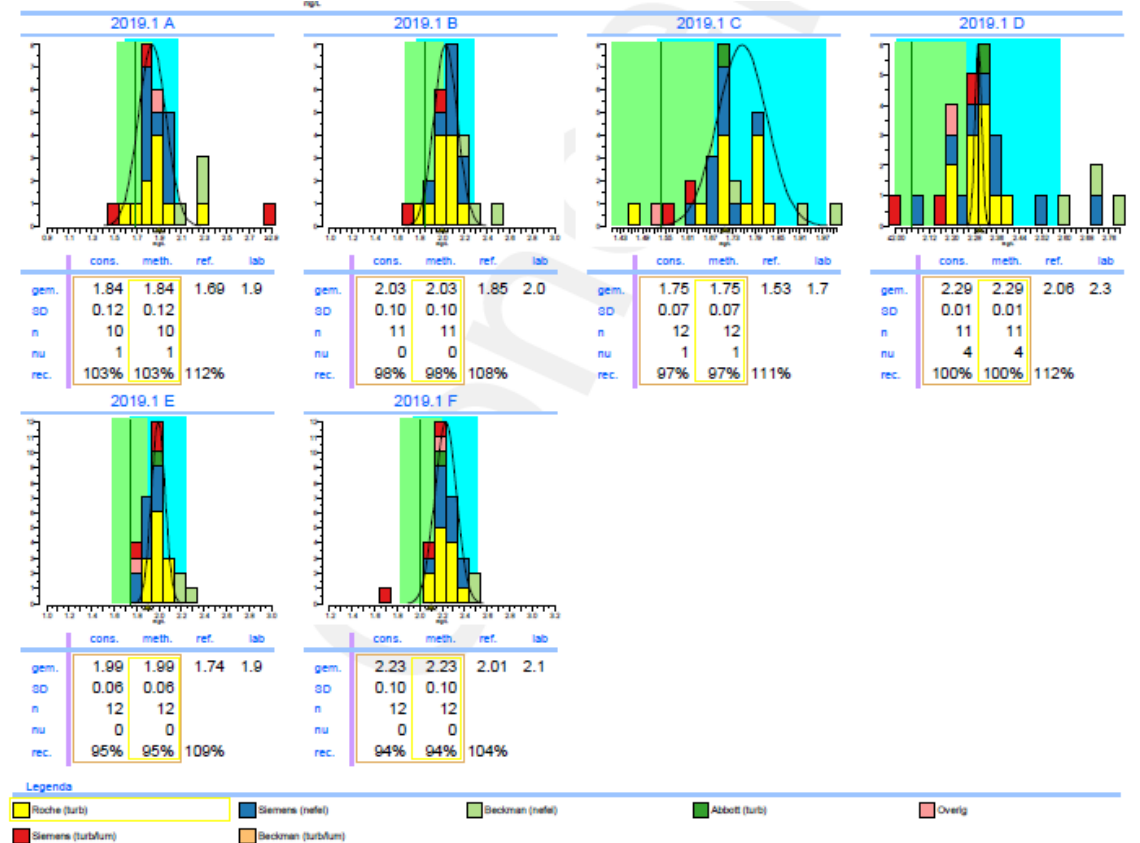
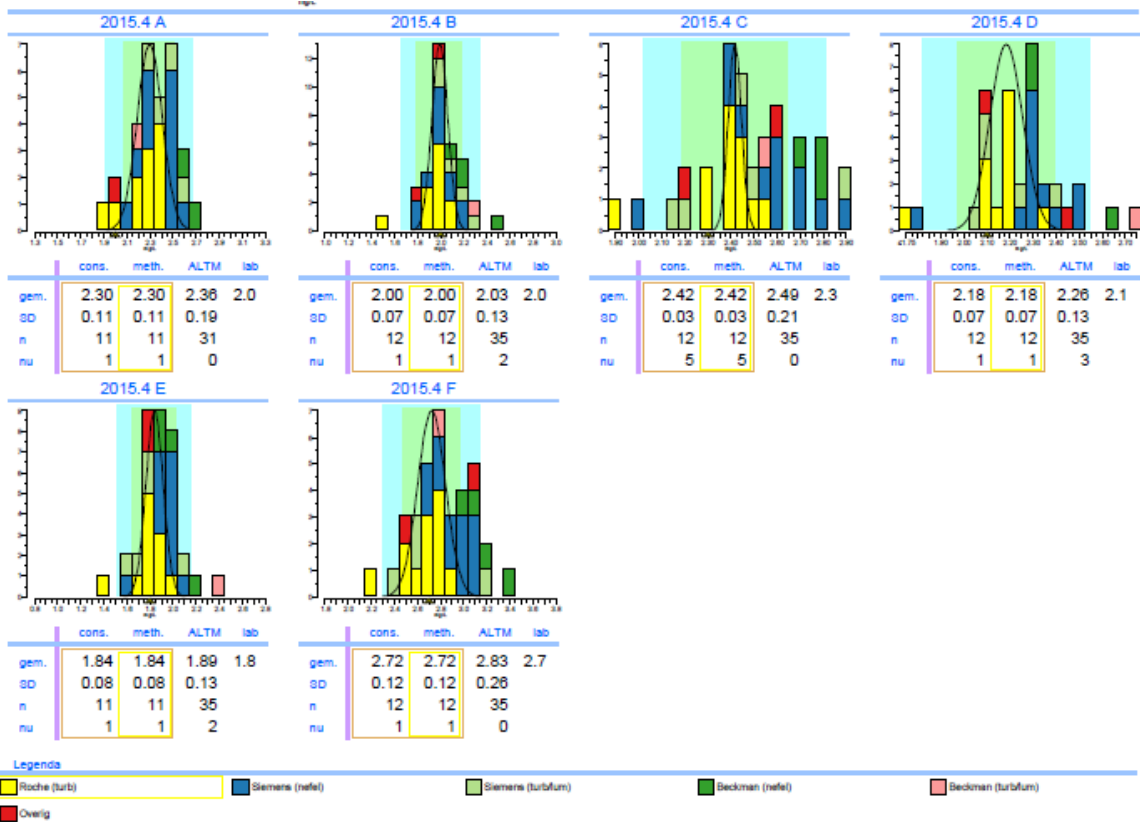


Fig 1. Comparison of individual laboratory beta-2 microglobulin results versus the mean of all four laboratories. Lab 1: nephelometric (Immagine 800; Beckman Coulter); Lab 2: chemiluminescence (Immulate 2000; Siemens); Lab 3: turbidimetric (Cobas c502; Roche) and Lab 4: turbidimetric (Architect c16200; Abbott).

# B2-microglobuline in 2015





# B2-microglobuline in 2015 en 2019

## Waar wordt tegen gestandaardiseerd?

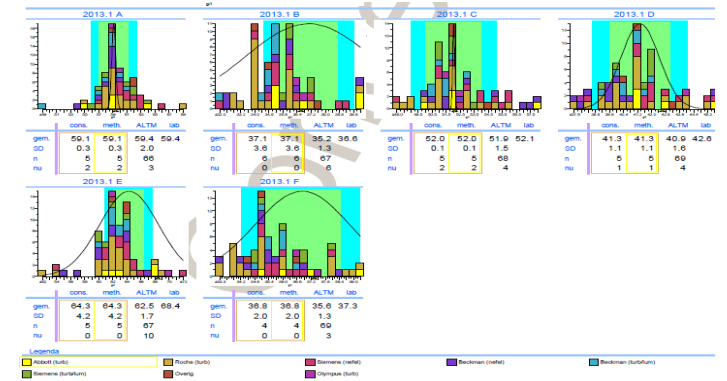
### ERM470k (SKML) of WHO 1987 (firma)

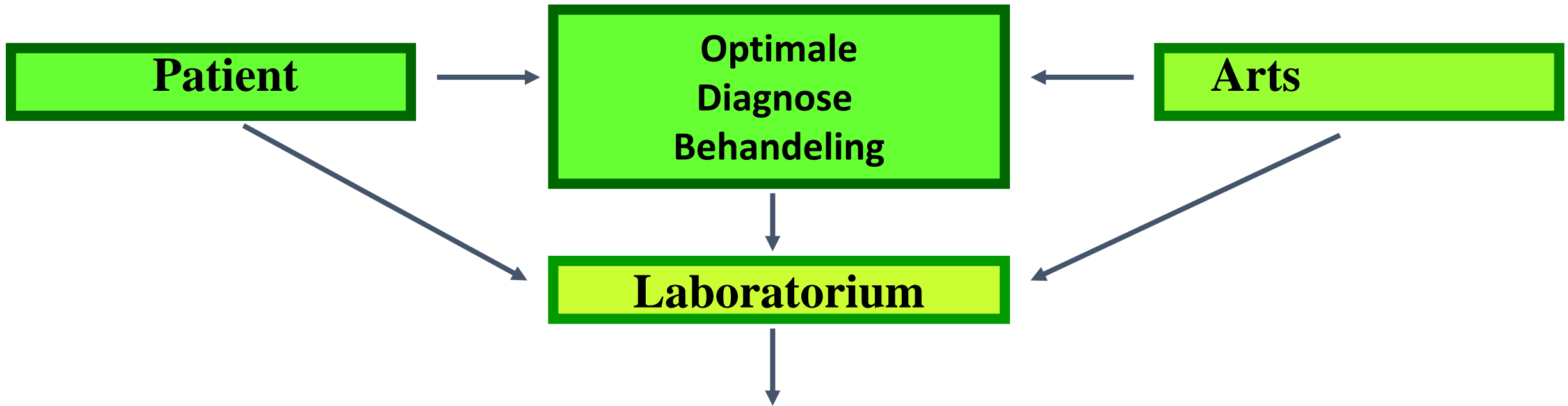
# ACTIE?

## Ja/Nee?

## Hoe dan?

- Gebruik van referentie materiaal ondersteund met een doorloper
- Herstandaardisatie door firma
- Factor door laboratorium ingevoerd
- Aanbeveling door firma
- Gebruik van dezelfde standaard? Invloed op ref waarden?

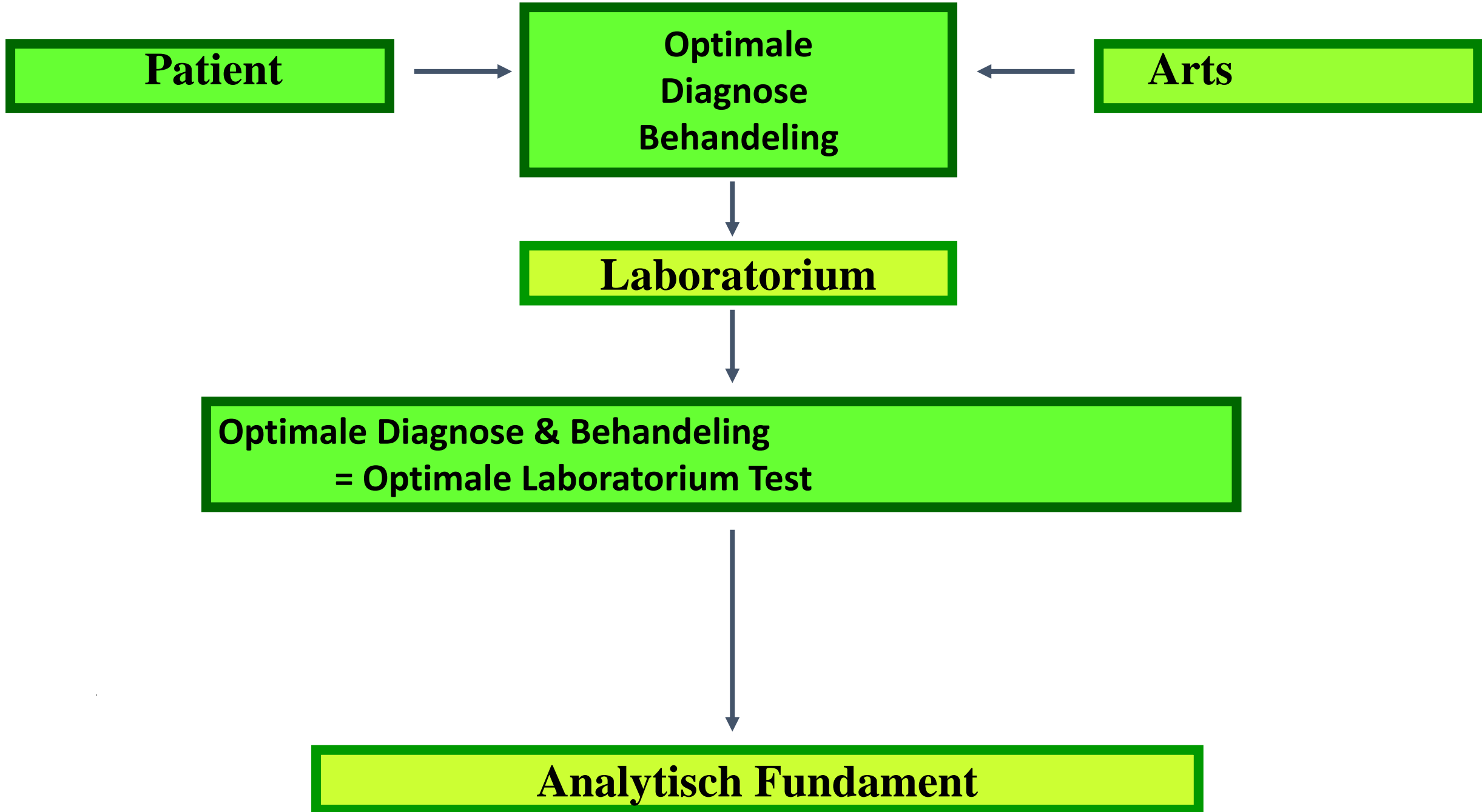




**Optimale Diagnose & Behandeling  
= Optimale Laboratorium Test:**

- Zelfde Uitkomst
- Zelfde Referentiewaarden
- Zelfde Beslisgrenzen
- Zelfde Literatuur





**Patient**

**Optimale  
Diagnose  
Behandeling**

**Arts**

**Laboratorium**

**Optimale Diagnose & Behandlung  
= Optimale Laboratorium Test**

**Analytisch Fundament**

# Optimale Immuno Chemie

Patient

Arts

Uniform  
= Optimaal

## Analytisch Fundament

- Referentie Methode? Arb. (IRMM)
- Referentie Materiaal? Ja (CRM 470)
- Wereldwijde Erkenning? Ja
- Wereldwijd Gebruik
  - door Rondzenders? SKML 2014
  - door Fabrikanten? .....

Sandra Secchiero\*, Laura Sciacovelli and Mario Plebani

# Harmonization of units and reference intervals of plasma proteins: state of the art from an External Quality Assessment Scheme

The main step toward achieving the standardization of plasma protein measurements has been the production, by the Committee on Plasma Proteins of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), of the Certified Reference Materials, CRM470 for 15 human serum proteins

Units

Reference intervals

Inter-laboratory variability

**Table 1:** Units of measure (%) utilized by participants to CRB External Quality Assessment program for 23 plasma proteins.

Proteins Units, %

g/dl  
Ig A-G-M  
C3-C4  
AAT  
TTR  
ALB 58.4  
AAG  
A2M  
B2M  
HPT  
TRF  
CRP  
CER  
Ig E  
RF  
ASO  
TLC ( $\kappa - \lambda$ )  
FLC ( $\kappa - \lambda$ )  
RBP

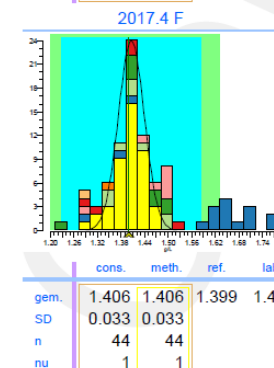
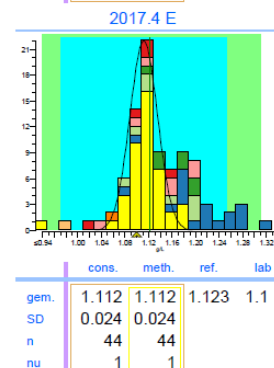
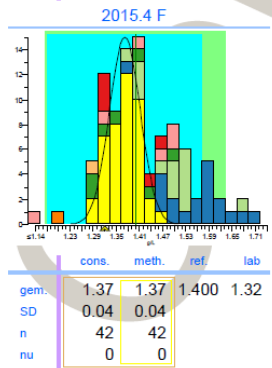
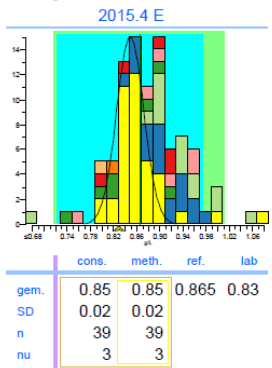
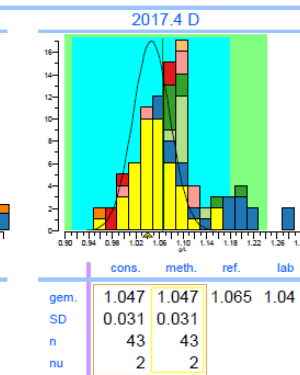
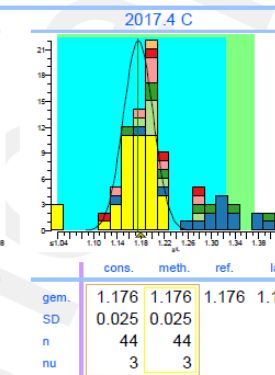
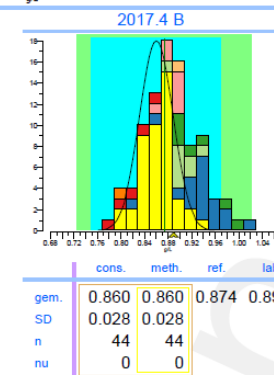
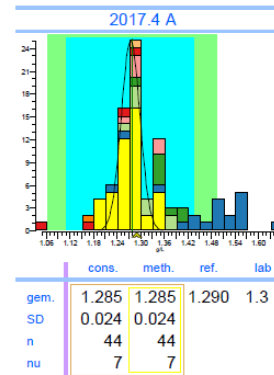
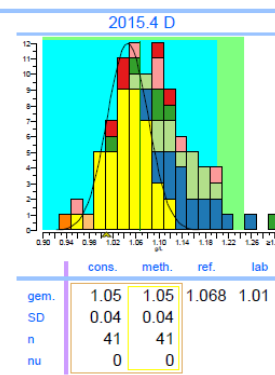
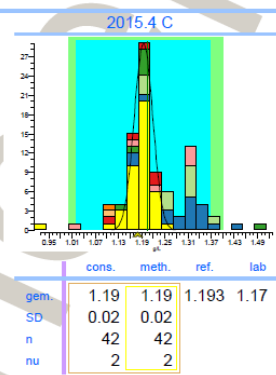
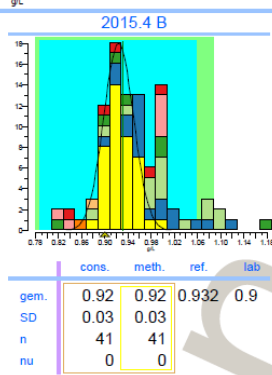
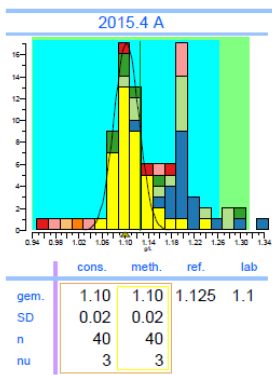
DE GRUYTER

Clin Chem Lab Med 2018; aop

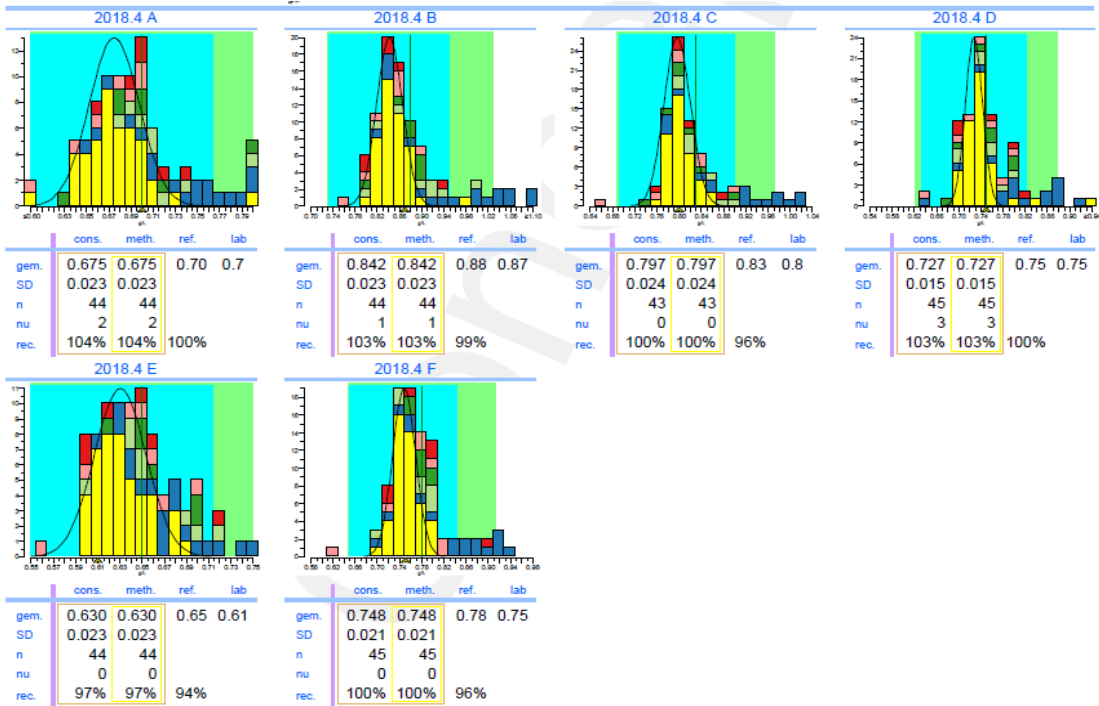
Wendy P.J. den Elzen<sup>a,\*</sup>, Nannette Brouwer<sup>a</sup>, Marc H. Thelen, Saskia Le Cessie, Inez-Anne Haagen and Christa M. Cobbaert

## NUMBER: standardized reference intervals in the Netherlands using a ‘big data’ approach

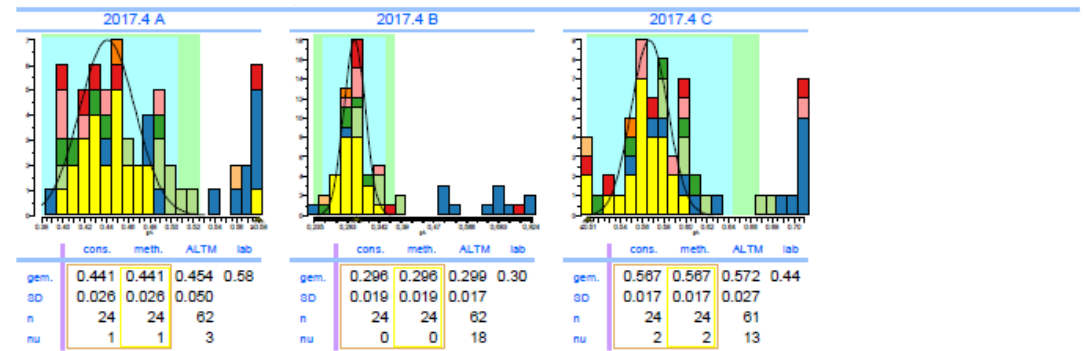
Combi immunochemie  
Sectie humorale immunologie



## IgM in 2015 en 2017



IgM in 2018



IgM in de M-prot SKML rondzending 2017.4

# IgM, Wat is er gedaan:

## In samenwerking met Firma:

Probleem: voor **gevriesdroogde monsters** is er een matrix effect voor Siemens IgM (BNII en ProSpec) in de SKML rondzending.

Probleem wordt niet gezien in de Instand (D'land)rondzending met plasma en/of serum, lyophilised.

### Acties SKML:

- a. **ingevroren serum** geprobeerd: is beter maar nog steeds matrix effect
- b. **gefiltreerd serum** en serum uit andere bron dat ingevroren is geprobeerd: probleem blijft

Opmerkelijk is dat de ProSpec een lab geen matrix probleem heeft als met "eigen protocol" gewerkt wordt. Dit wekt de suggestie dat er iets subtiels is met de meting/reagens van Siemens. Bij patiënten ook grote kans op problemen?

### c. **gedelipideerd serum**, waarneming met IgM ontvet:

1. *geeft verhoogde waarden op Roche-TQ, turbidimetrie.*
2. Atellica Solution-turbidimetrie: ingevroren poolserum en gedelipideerd gemeten waarden overeenkomend met ref materiaal (delipideren heeft geen effect, gemeten waarden waren goed en blijven goed.)
3. gemeten op BNII, Atellica-nefelometrie (afh van protocol, zie eigen protocol) waarden overeenkomend met ref materiaal

### Voorstel:

Scoren op methodegroep

### Conclusie:

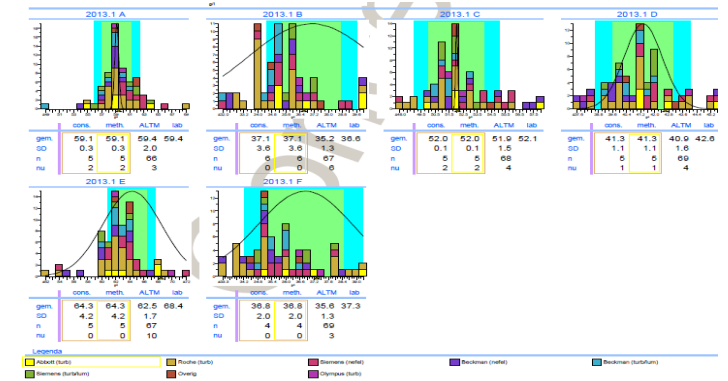
**Wat is er aan de hand?**

# ACTIE?

## Ja/Nee?

## Hoe dan?

- Gebruik van referentie materiaal ondersteund met een doorloper
- Herstandaardisatie door firma
- Factor door laboratorium ingevoerd
- Aanbeveling door firma
- Gebruik van dezelfde standaard?
- Blijf communiceren



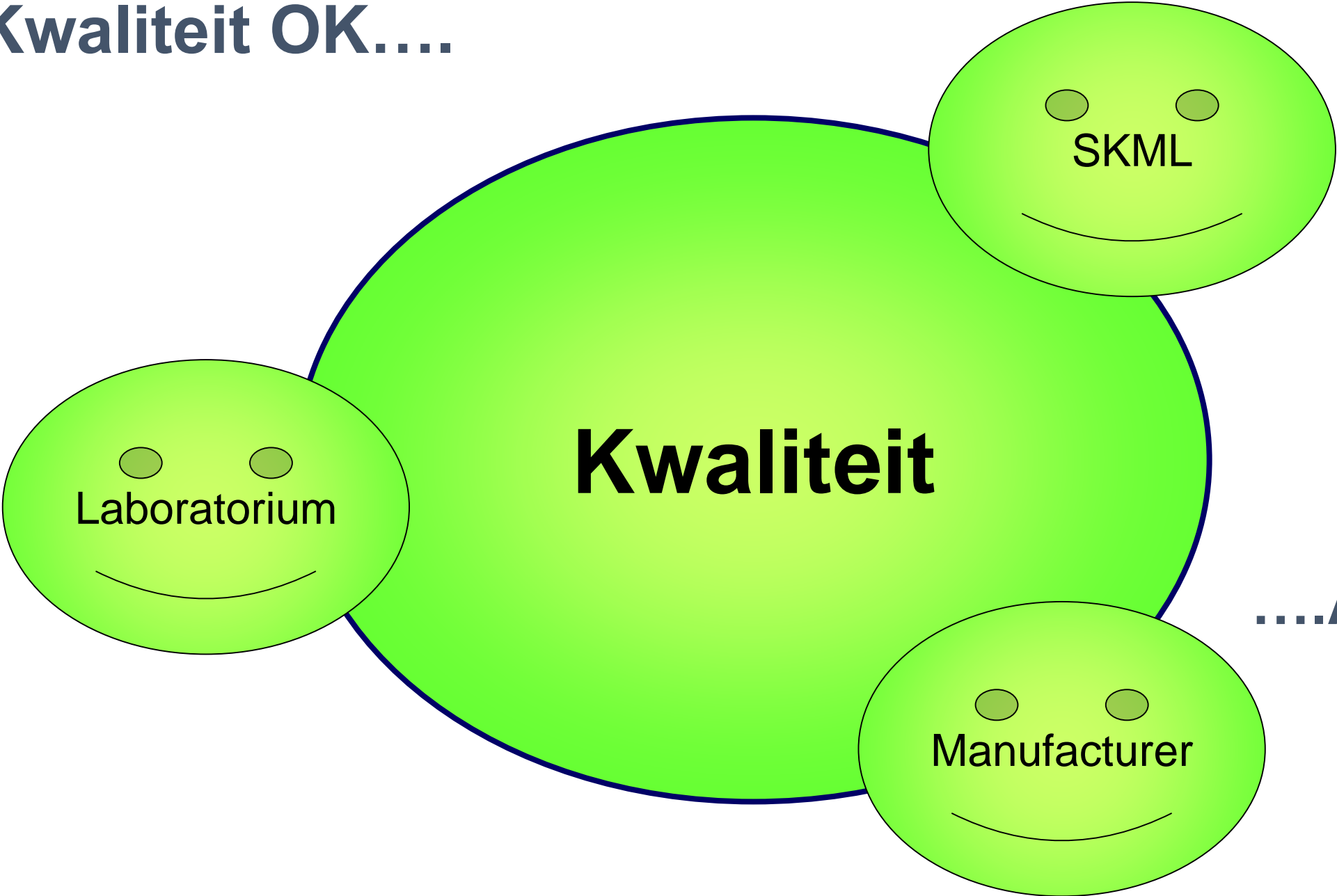
# Analytisch Fundament

**Standardize if possible**

**Harmonize if standardization is not possible**



# Kwaliteit OK....



....**All  
Smile**