

What to do when reference measurement procedures show different results?

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- Which procedures are of reference measurement procedures (RMP) of higher metrological order?
- If labs are using the same RMP, could results be different, too?
- When are results considered to be different?
- Are difference always errors of the performance?
- Who is right, who is wrong?
- Measurement uncertainty of individual result – Is the estimation appropriate?

Reference Measurement Procedures and Results

INTERNATIONAL
STANDARD

ISO
15193

Second edition
2009-05-01

***In vitro* diagnostic medical devices —
Measurement of quantities in samples of
biological origin — Requirements for
content and presentation of reference
measurement procedures**

*Dispositifs médicaux de diagnostic in vitro — Mesurage des grandeurs
dans des échantillons d'origine biologique — Exigences relatives au
contenu et à la présentation des procédures de mesure de référence*

Measurement principle and measurement method
Reagents and materials, apparatus, sampling and
sample ...

Calibration, design of analytical series, operation of
measuring system, data processing, calculation of
results ...

Method validation

- analytical influence quantities
- measurement trueness
- interlaboratory comparison
- **measurement uncertainty**
- ...



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2.9 (3.1)
measurement result
result of measurement

set of **quantity values** being attributed to a **measurand** together with any other available relevant information

NOTE 1 A measurement result generally contains “relevant information” about the set of quantity values, such that some may be more representative of the measurand than others. This may be expressed in the form of a probability density function (PDF).

NOTE 2 A measurement result is generally expressed as a single **measured quantity value and a measurement uncertainty**. If the measurement uncertainty is considered to be negligible for some purpose, the measurement result may be expressed as a single measured quantity value. In many fields, this is the common way of expressing a measurement result.

JCGM 200:2012



JCTLM Database



JCTLM Database: higher-order reference materials, methods and services

Search database ?

Please type a key word or select a criteria *

OTHER FILTERS

Type:

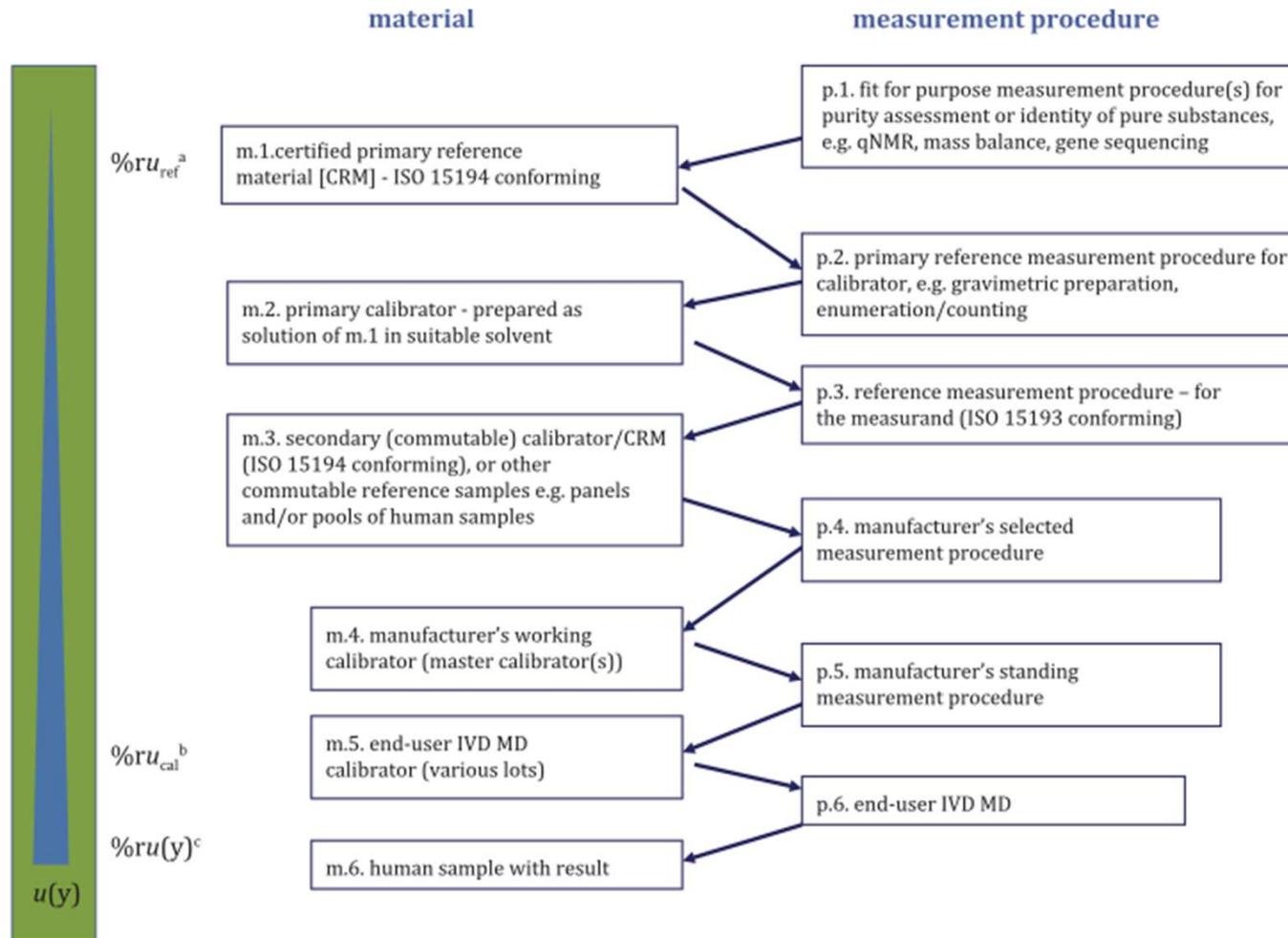
Analyte category:

Matrix category:

The JCTLM database content:

Reference Materials	265
Reference Measurement Procedures	215
Reference Measurement Services	224
of 20 calibration labs	

Traceability Hierarchy – ISO 17511



National Metrology Institutes

Calibration Laboratories

IVD Manufacturers

Clinical Laboratories

Services of Calibration Laboratories

The services of calibration laboratories can be applied to assign traceable values to

- calibrators,
- control materials,
- panels of patient samples.

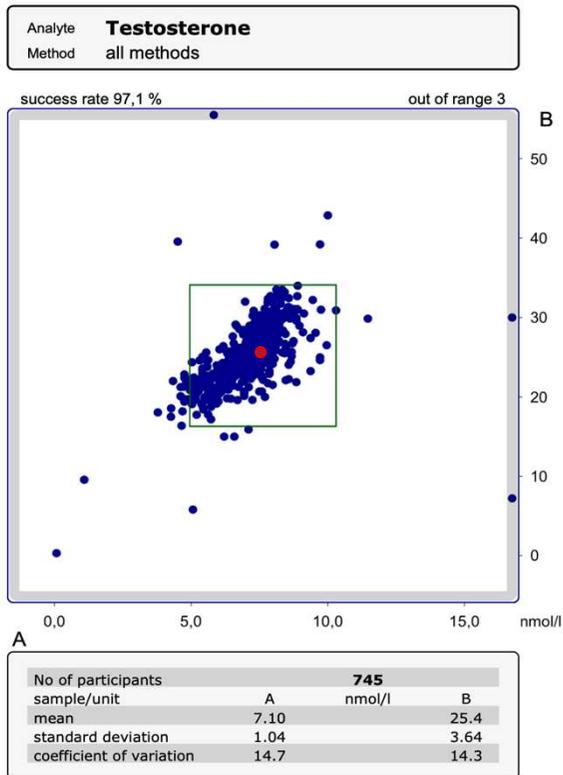
For metrological traceability, reference measurement procedure values with low measurement uncertainties are analyzed by appropriately calibration procedures.

These values are used in

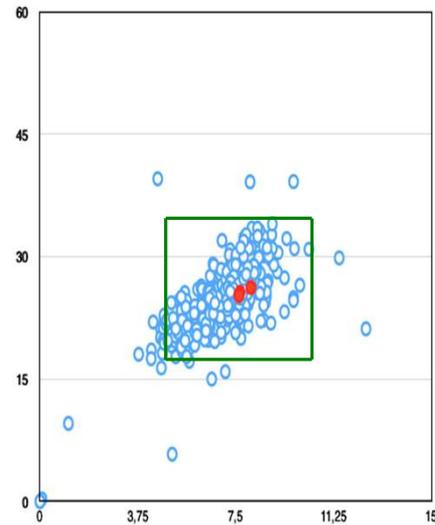
- the calibration of IVD procedures,
- the validation of IVD procedures,
- the quality assurance of clinical laboratories.



What does the end user see?



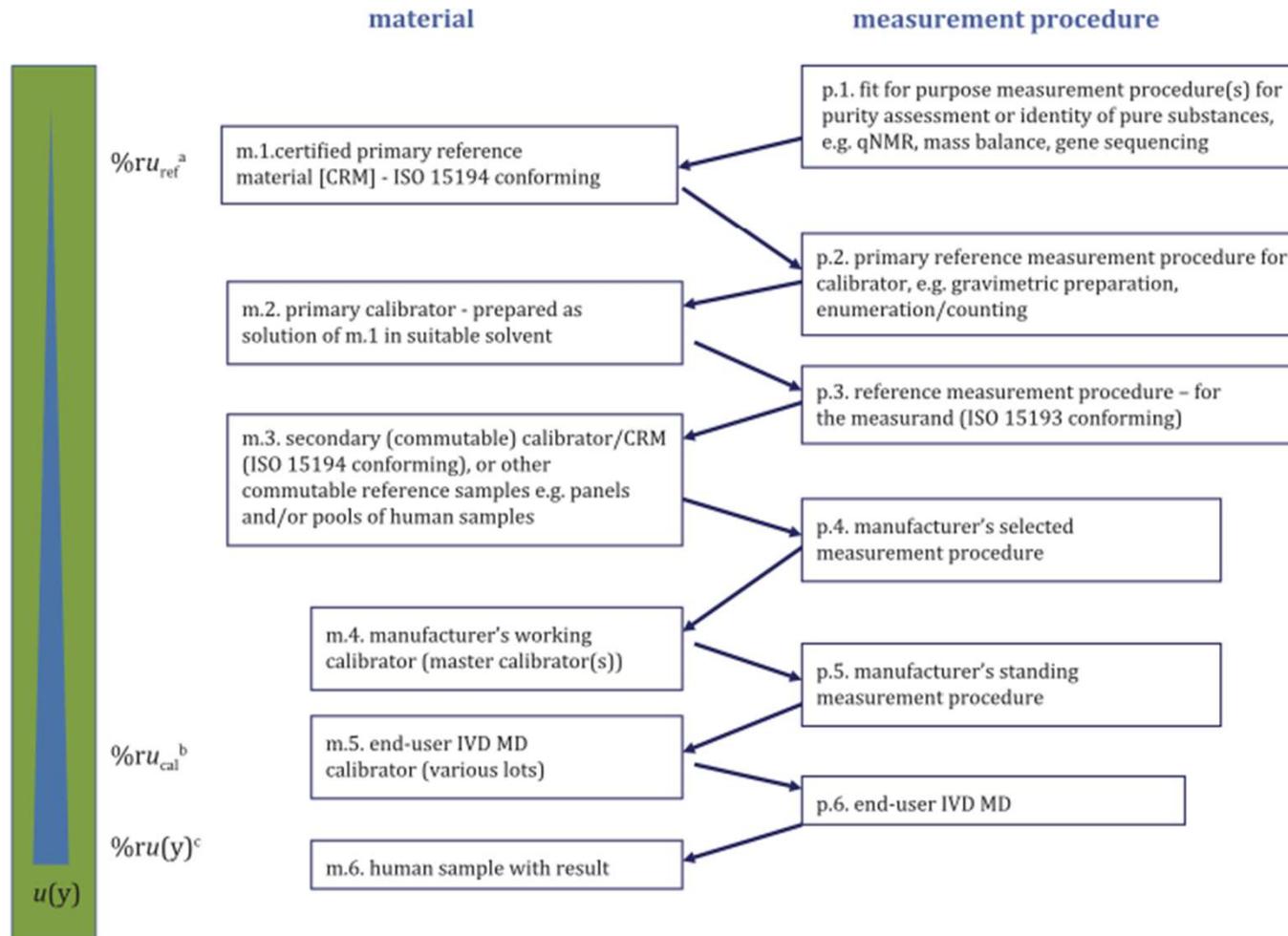
HM 1/16 vs. RELA2014



- 745 clinical labs
- 3 calibration labs
- Limits of Acceptance: 35%



Traceability Hierarchy – ISO 17511



National Metrology Institutes

CCQM comparisons

Calibration Laboratories

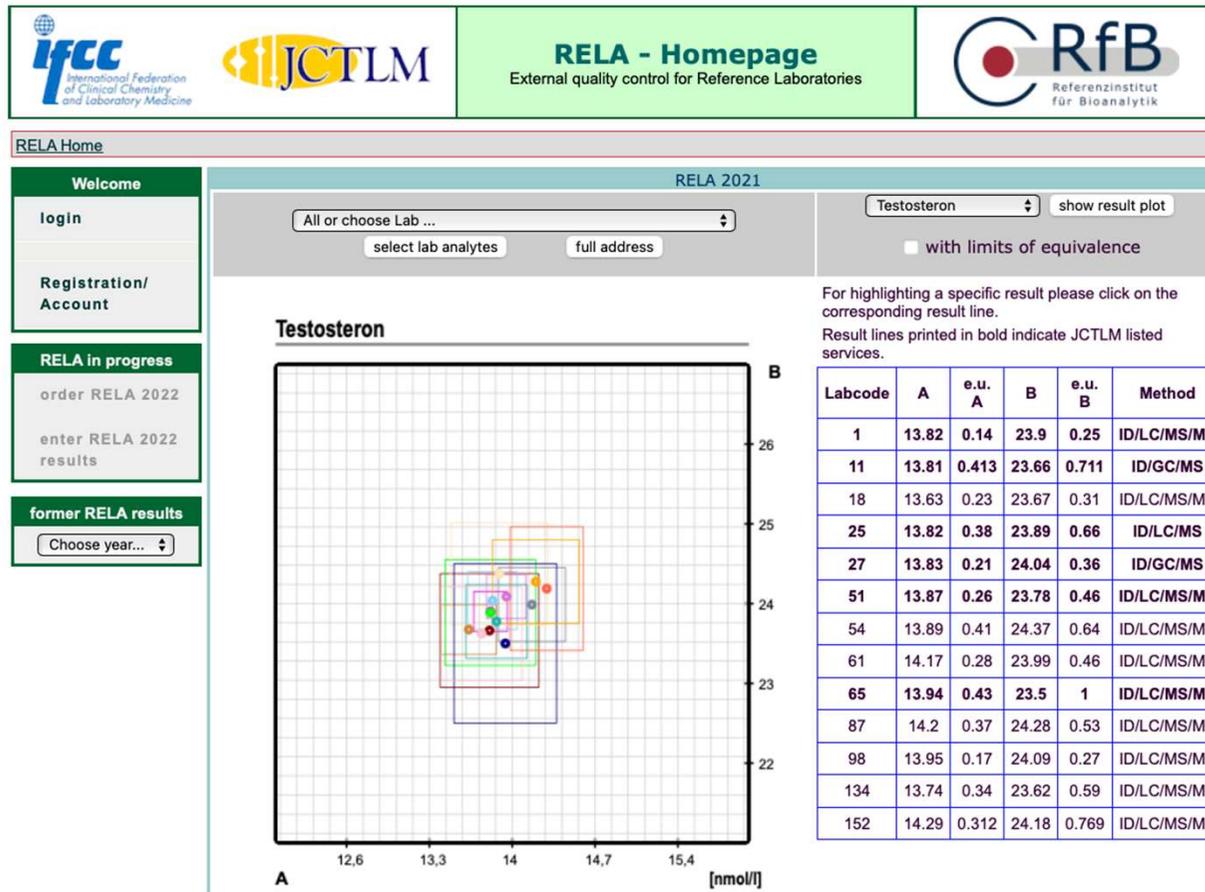
RELA surveys

IVD Manufacturers

Clinical Laboratories

EQA programs

EQA for Calibration Laboratories - RELA



Surveys for metabolites & substrates, electrolytes, enzymes, proteins, steroid hormones, drugs, ...

The reported results of each lab consist of the **value and the estimated expanded uncertainty** for each sample.

Additional information:

- JCTLM listed RMP?
- JCTLM listed Service?

EQA for Calibration Laboratories - RELA

The screenshot shows the RELA 2021 homepage. At the top, there are logos for IFCC, JCTLM, RELA - Homepage (External quality control for Reference Laboratories), and RfB (Referenzinstitut für Bioanalytik). The main content area is titled 'RELA 2021' and features a search bar for 'Testosteron' with a 'show result plot' button. Below the search bar, there is a checkbox for 'with limits of equivalence'. A list of laboratories is displayed on the left, with a checkmark next to 'All or choose Lab ...'. On the right, a table shows results for 'Testosteron' with columns for Labcode, A, e.u. A, B, e.u. B, and Method. The table includes a note: 'For highlighting a specific result please click on the corresponding result line. Result lines printed in bold indicate JCTLM listed services.'

Labcode	A	e.u. A	B	e.u. B	Method
1	13.82	0.14	23.9	0.25	ID/LC/MS/MS
11	13.81	0.413	23.66	0.711	ID/GC/MS
18	13.63	0.23	23.67	0.31	ID/LC/MS/MS
25	13.82	0.38	23.89	0.66	ID/LC/MS
27	13.83	0.21	24.04	0.36	ID/GC/MS
51	13.87	0.26	23.78	0.46	ID/LC/MS/MS
54	13.89	0.41	24.37	0.64	ID/LC/MS/MS
61	14.17	0.28	23.99	0.46	ID/LC/MS/MS
65	13.94	0.43	23.5	1	ID/LC/MS/MS
87	14.2	0.37	24.28	0.53	ID/LC/MS/MS
98	13.95	0.17	24.09	0.27	ID/LC/MS/MS
134	13.74	0.34	23.62	0.59	ID/LC/MS/MS
152	14.29	0.312	24.18	0.769	ID/LC/MS/MS

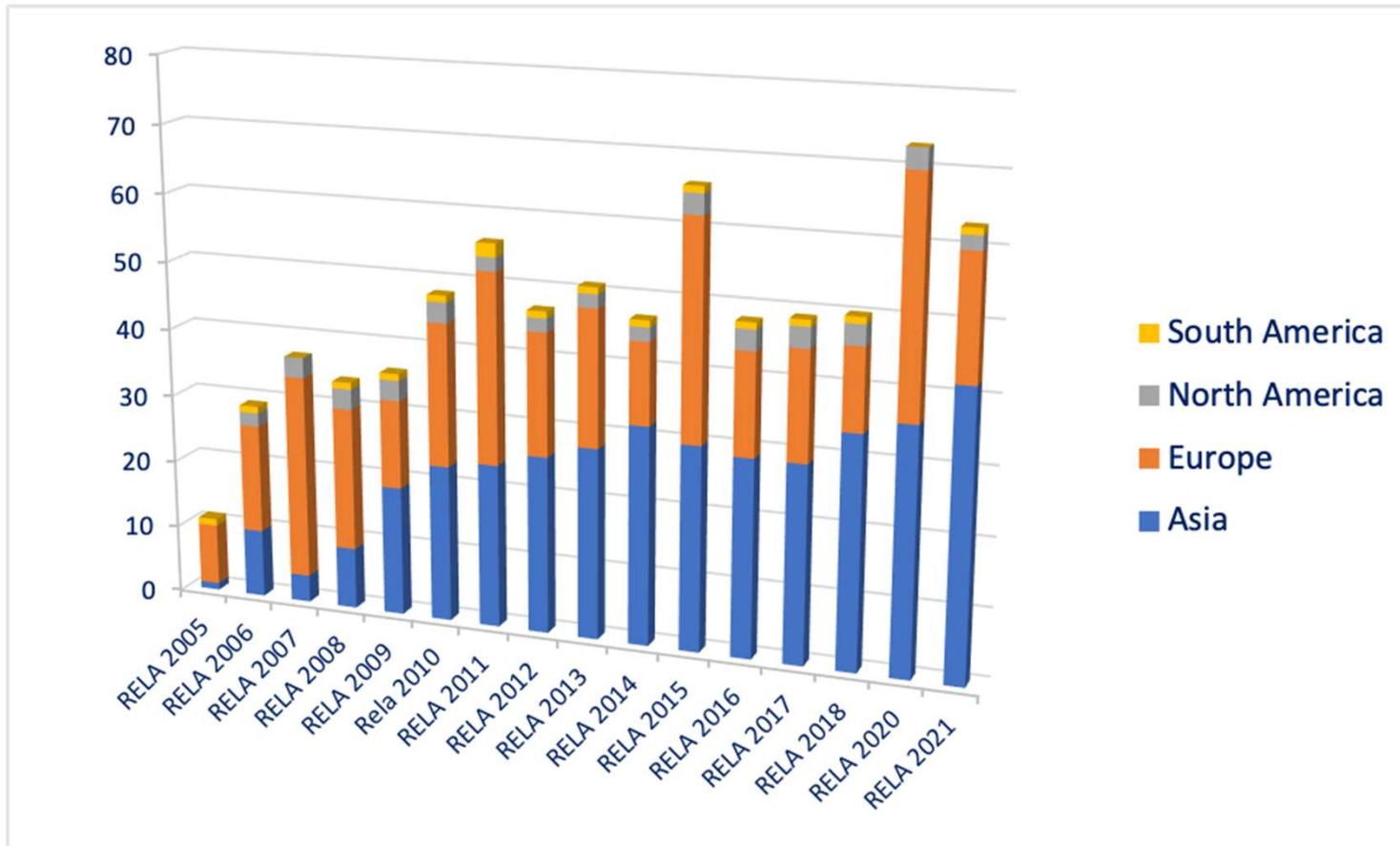
Surveys for metabolites & substrates, electrolytes, enzymes, proteins, steroid hormones, drugs, ...

The reported results of each lab consist of the **value and the estimated expanded uncertainty** for each sample.

Additional information:

- JCTLM listed RMP?
- JCTLM listed Service?
- Identity and address of the lab

RELA Participants 2005 to 2021



Number of laboratories:

2005 11 Labs

2021 63 Labs

18 participants are accredited according to ISO 17025 AND 15195.

Number of results:

2003 111

2021 658

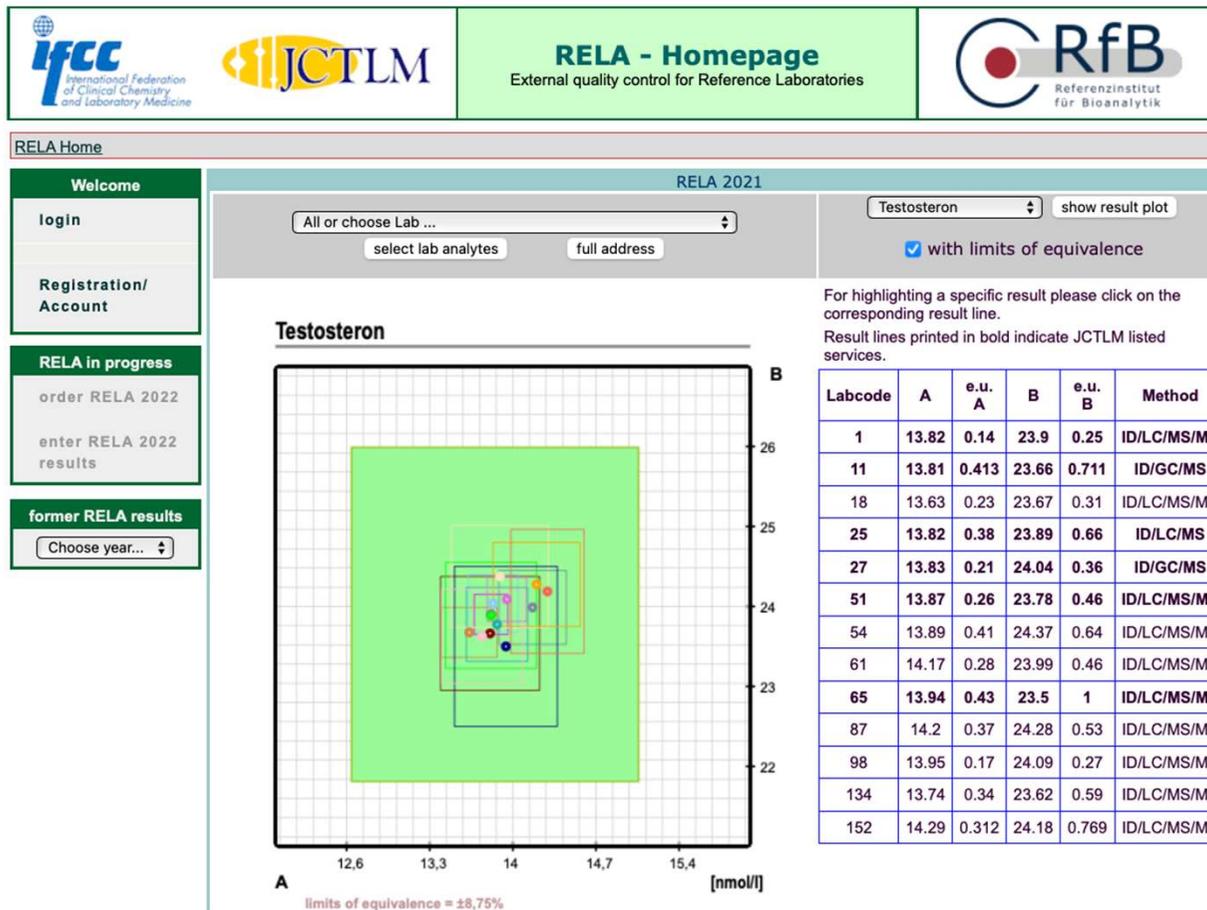
Objective of Interlaboratory Comparisons

The **purpose of measurement intercomparisons** between participants is to test, **whether measurements** performed by the laboratories **are consistent taking into account the uncertainties** assigned to the measurements. If an **inconsistency is detected**, the participating laboratories should take the **corrective actions needed to obtain consistency**. Otherwise, measurement results exchanged cannot be considered equivalent (without be in disharmony with the concept of the SI system of units).

L.Nieslen, Report DFM-99-R39, 3208 LN, Danish Institute of Fundamental Metrology, Lyngby, Denmark (2000)



Limits of Equivalence



Limits of Equivalence (LoE) are

- prescribed in the RELA procedure manual
- applied, if at least 5 labs use a JCTLM listed RMP

Are the values and their expanded MU within the LoE?

Analysis and Action

Each calibration lab is responsible for the interpretation of its EQA results. If it observes any considerable differences, it has to organise further investigations by itself.

C-TLM as the advisory board could recommend additional experiments and support the participants.

The organiser of RELA can provide additional materials to the calibration labs.

Examples for comparisons between

- few participating laboratories (Digitoxin)
- laboratories using RMP with certified reference material (Calcium)
- laboratories using a conventional RMP (HbA1c)

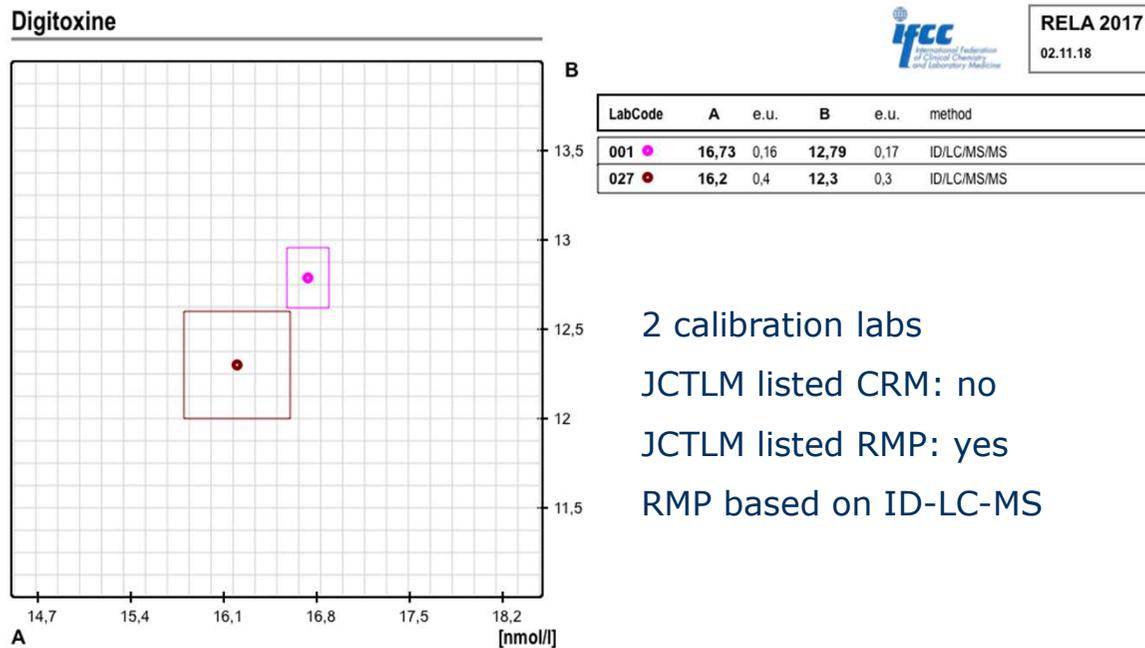


Differences at Comparison with n<5 Laboratories

RELA 2017

No. of participants: 2

Digitoxine



2 calibration labs
JCTLM listed CRM: no
JCTLM listed RMP: yes
RMP based on ID-LC-MS

Accreditation body states the difference of the results and threatens to withdraw the accreditation status

Differences at Comparison with n<5 Laboratories

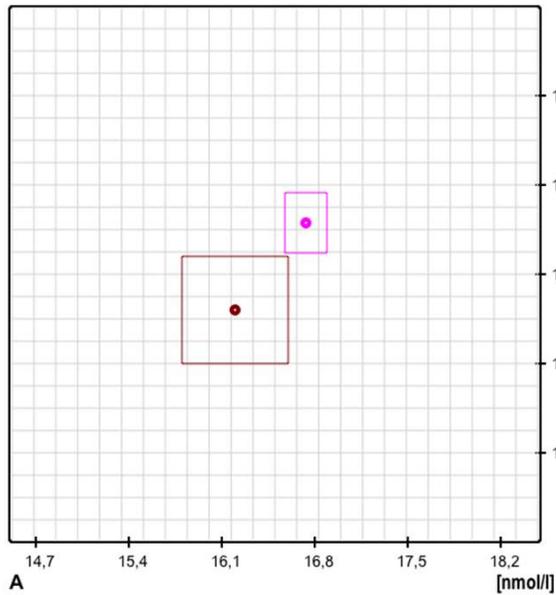
RELA 2017

No. of participants: 2

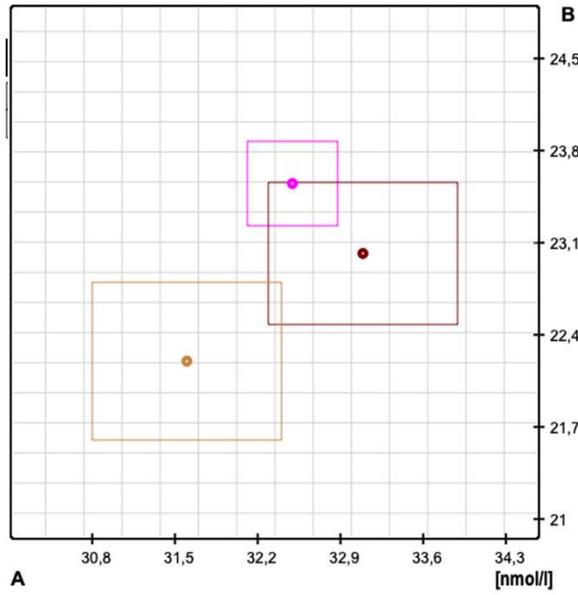
RELA 2018

No. of participants: 3

Digitoxine



Digitoxine



RELA 2018
12.11.20

LabCode	A	e.u.	B	e.u.	method
001	32,49	0,38	23,55	0,32	ID/LC/MS
008	33,09	0,8	23,02	0,54	ID/LC/MS
027	31,6	0,8	22,2	0,6	ID/LC/MS/MS

2 calibration labs

1 National Metrology Institute

JCTLM listed CRM: no

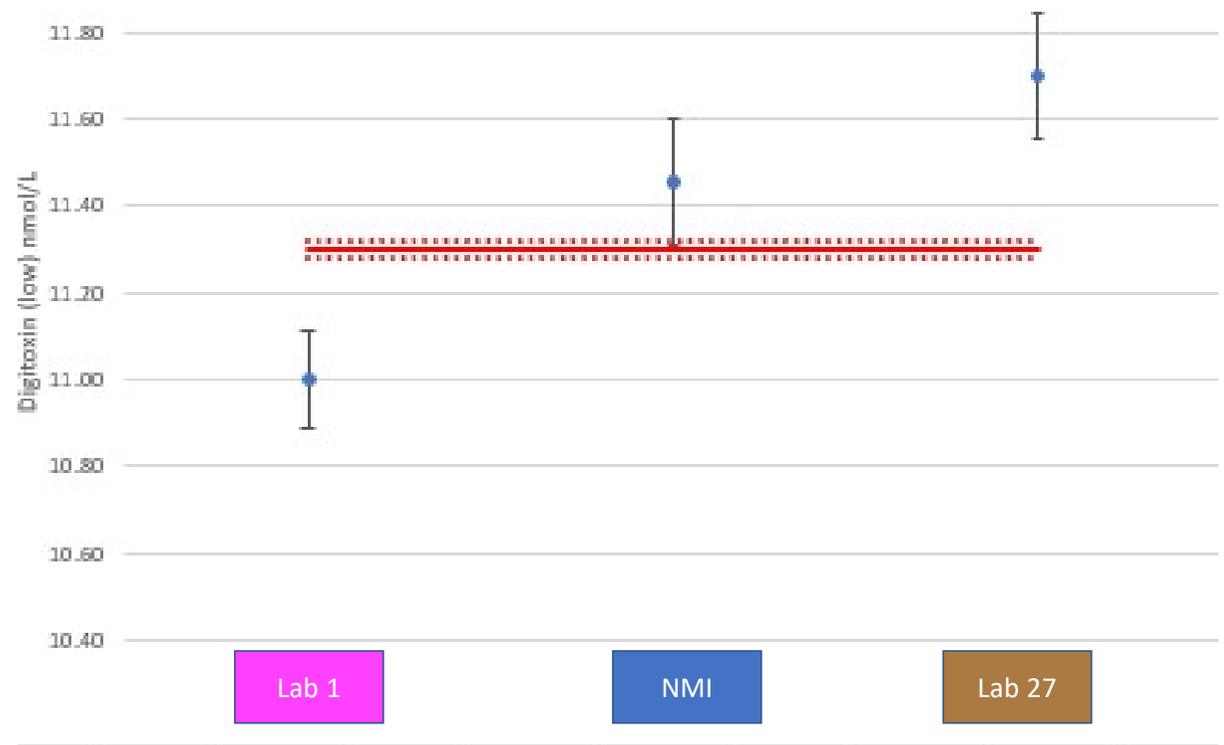
Additional Study with Spiked Material - Sample A

Calibration labs and NMI exchanged and used the same calibration material

Determined amount concentration of Digitoxin in spiked serum

— gravimetric reference value

..... standard uncertainty



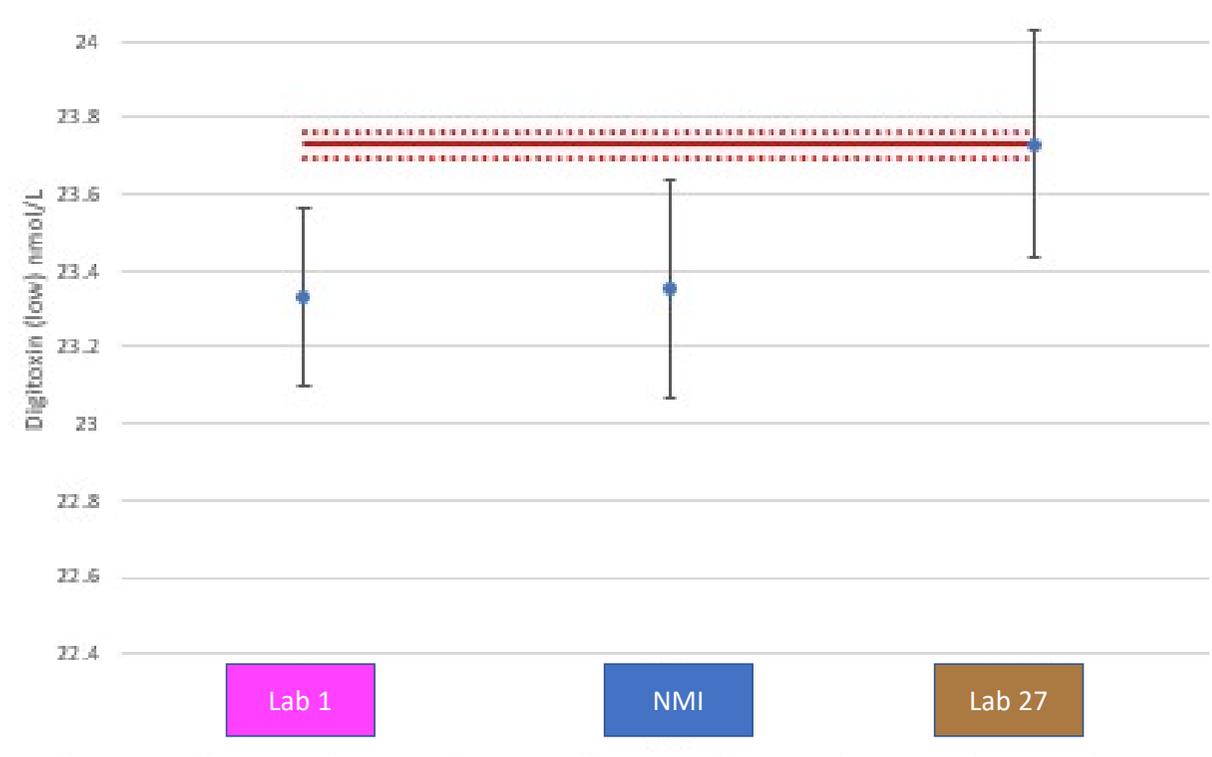
Additional Study with Spiked Material - Sample B

Calibration labs and NMI exchanged and used the same calibration material

Determined amount concentration of Digitoxin in spiked serum

— gravimetric reference value

..... standard uncertainty



Differences at Comparison with n<5 Laboratories

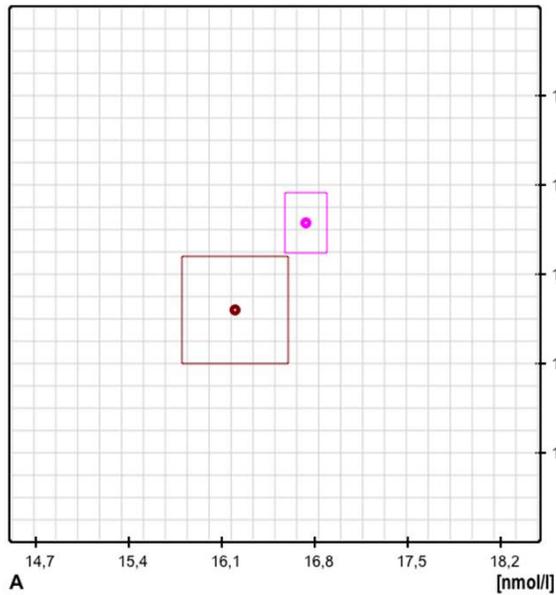
RELA 2017

No. of participants: 2

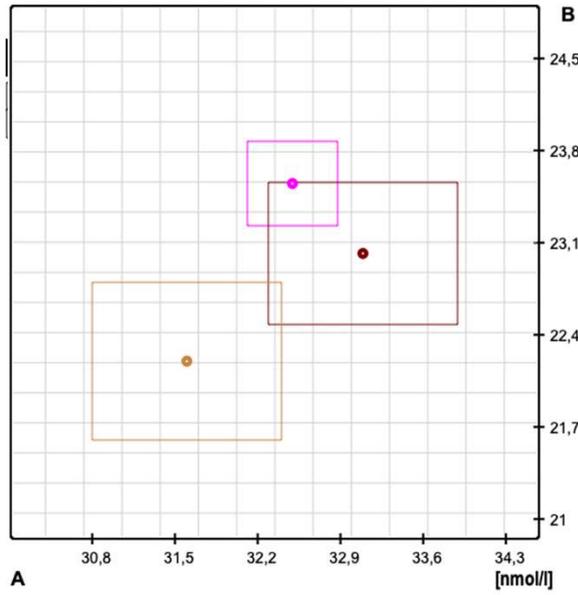
RELA 2018

No. of participants: 3

Digitoxine



Digitoxine



RELA 2018
12.11.20

LabCode	A	e.u.	B	e.u.	method
001	32,49	0,38	23,55	0,32	ID/LC/MS
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027	31,6	0,8	22,2	0,6	ID/LC/MS/MS

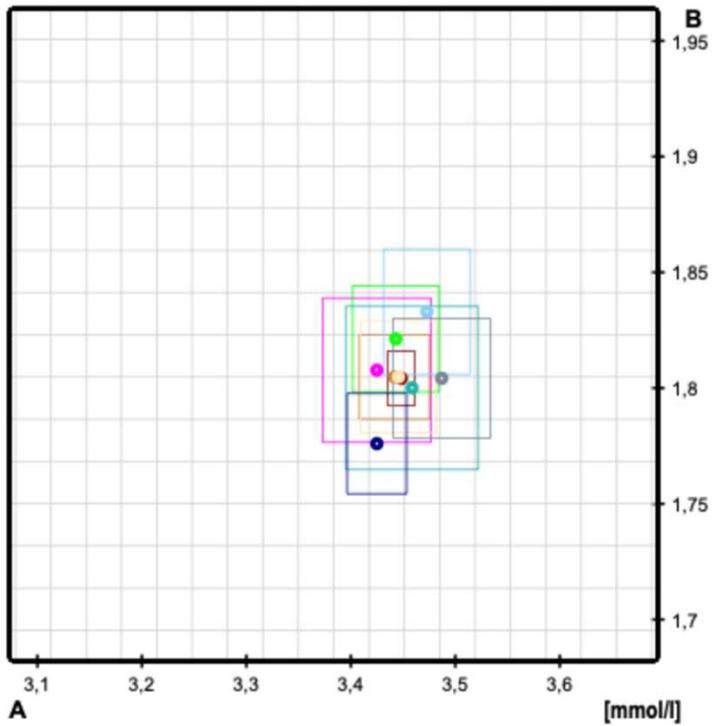


RMP with CRM

RELA 2021

No. of participants: 9

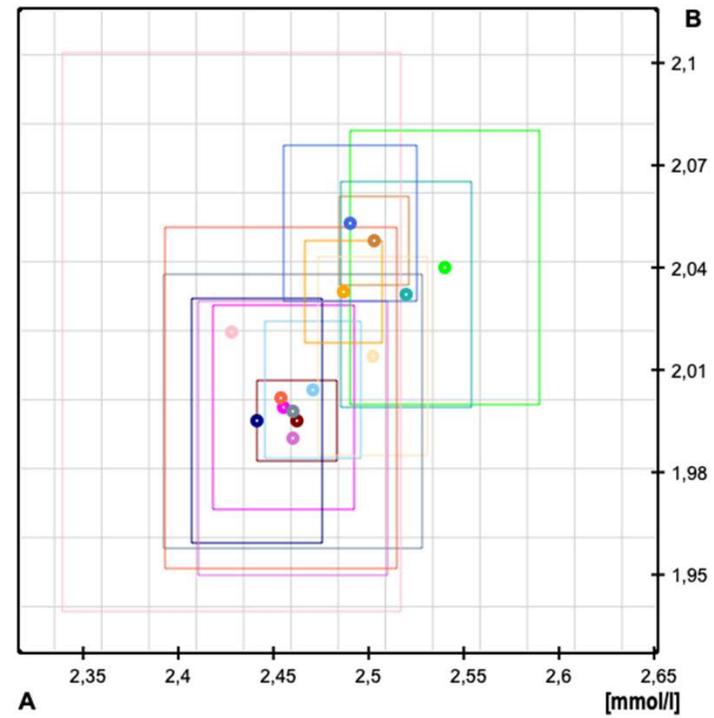
Calcium



RELA 2022 (preliminary)

No. of participants: 14

Calcium

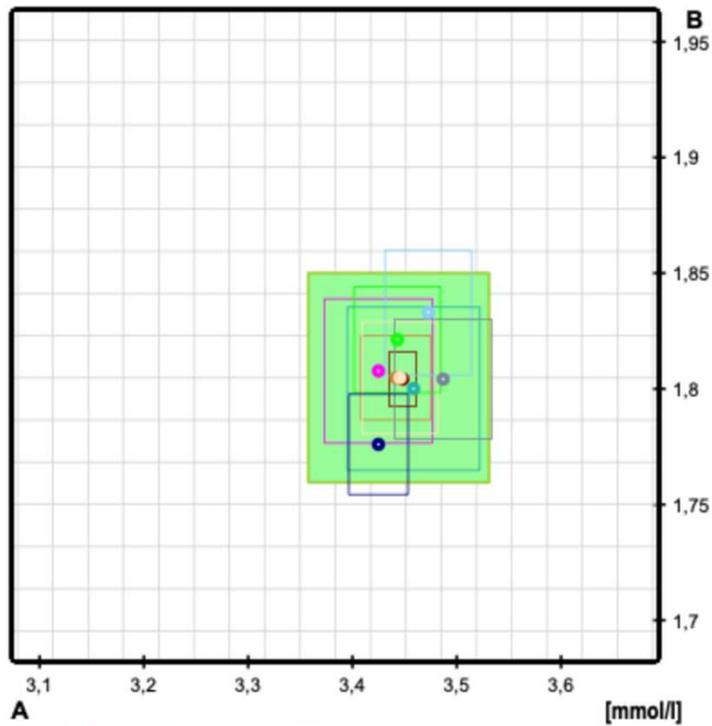


RMP with CRM

RELA 2021

No. of participants: 9

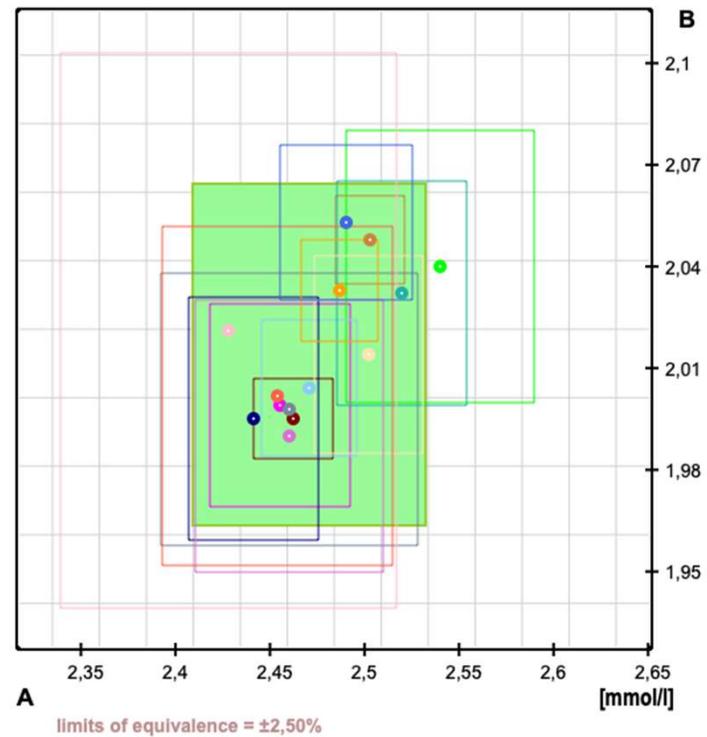
Calcium



RELA 2022 (preliminary)

No. of participants: 14

Calcium





National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 915b

Calcium Carbonate

This Standard Reference Material (SRM) is intended for use as an analytical standard of known purity. It is intended primarily for use in the calibration and standardization of procedures for calcium (Ca) determinations employed in clinical analysis and for routine critical evaluation of the daily working standards used in these procedures. This lot of calcium carbonate (CaCO₃) was prepared to ensure a material of high purity and homogeneity and has been assayed after heating at 200 °C to 210 °C. A unit of SRM 915b consists of a single glass bottle containing 20 g of the material.

INSTRUCTIONS FOR USE

Drying Instructions: Dry the material at 200 °C to 210 °C for 4 h. After the SRM has been dried, store it in a desiccator over anhydrous magnesium perchlorate.

Product Search

Browse NIST products by name.

My Cart

Calcium Carbonate



Calcium Carbonate

SKU: 915b

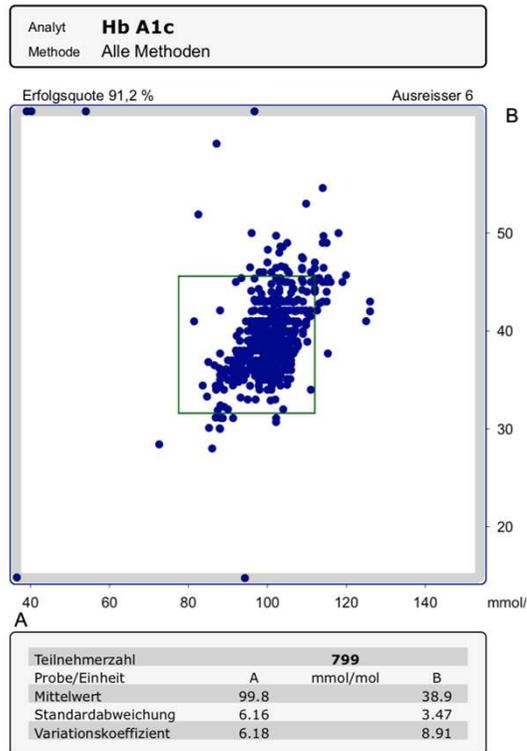
Availability: Out of Stock - Product is not available at this time.

Please contact srminfo@nist.gov to be added to the Waitlist for this material

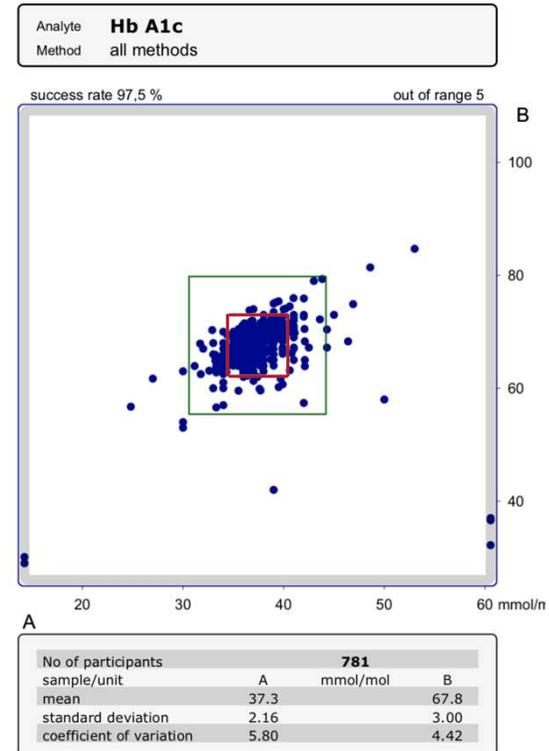
Certified reference materials are an important anchor to avoid different results. If no CRM is available, calibration labs should use the same preparation and lot of a reference material.

Demostrating Traceability - HbA1c

GH 3/17: lyophilized hemolysate

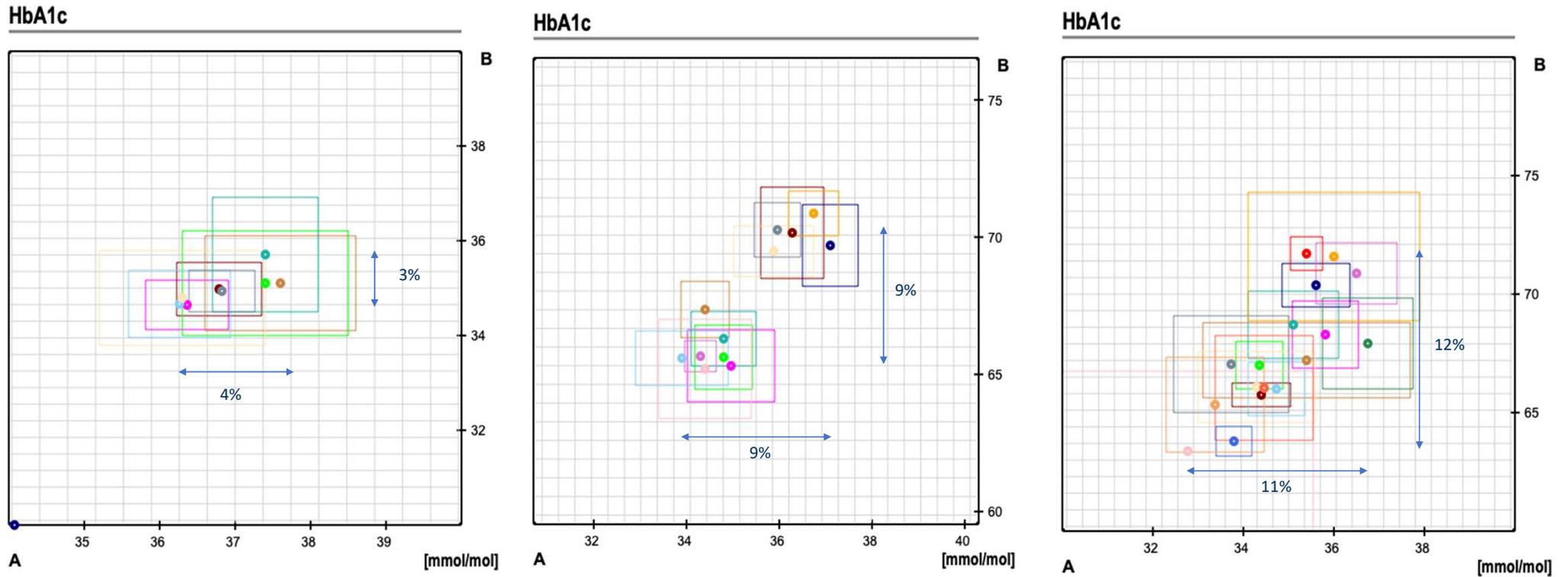


GH 1/18: fresh whole blood



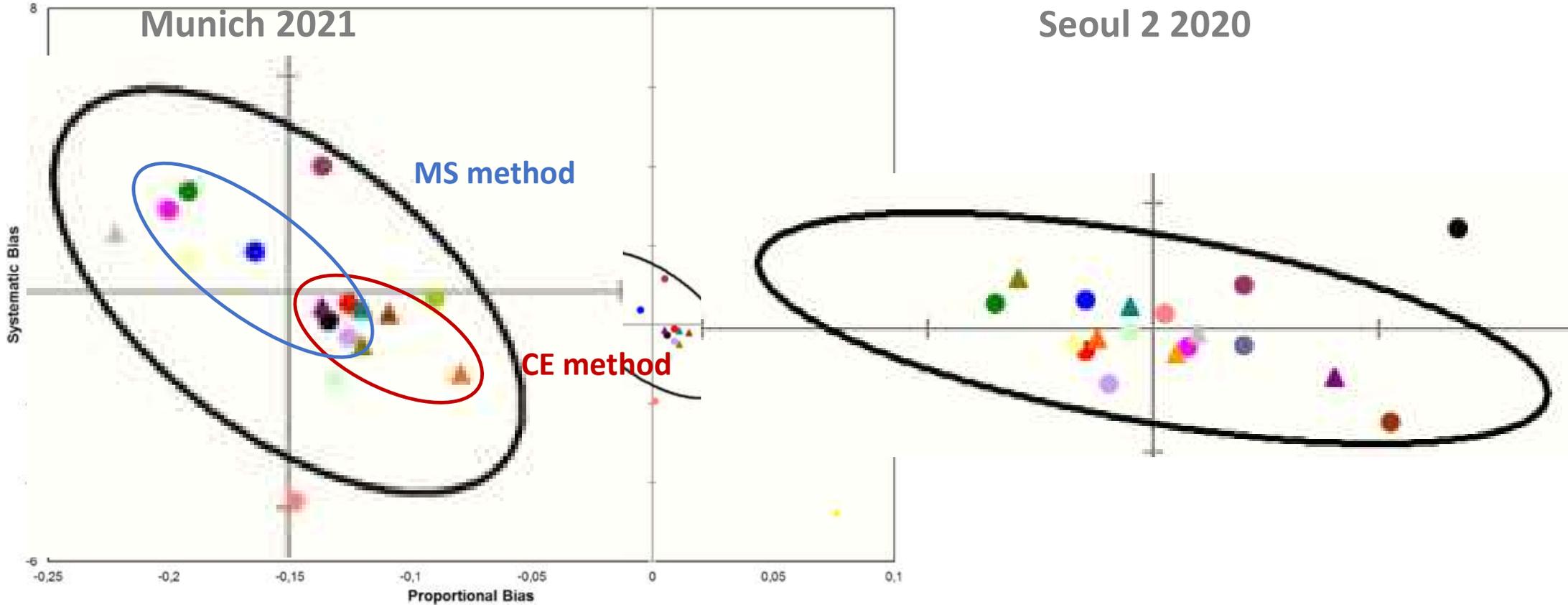
New Limits of Acceptance:
+/- 8%

HbA1c – RELA 2018-2021



HbA1c – IFCC Network Comparison

Approval Lab Elliptic (Study 2021 Munich)



IFCC RMP HbA1c – Critical steps?

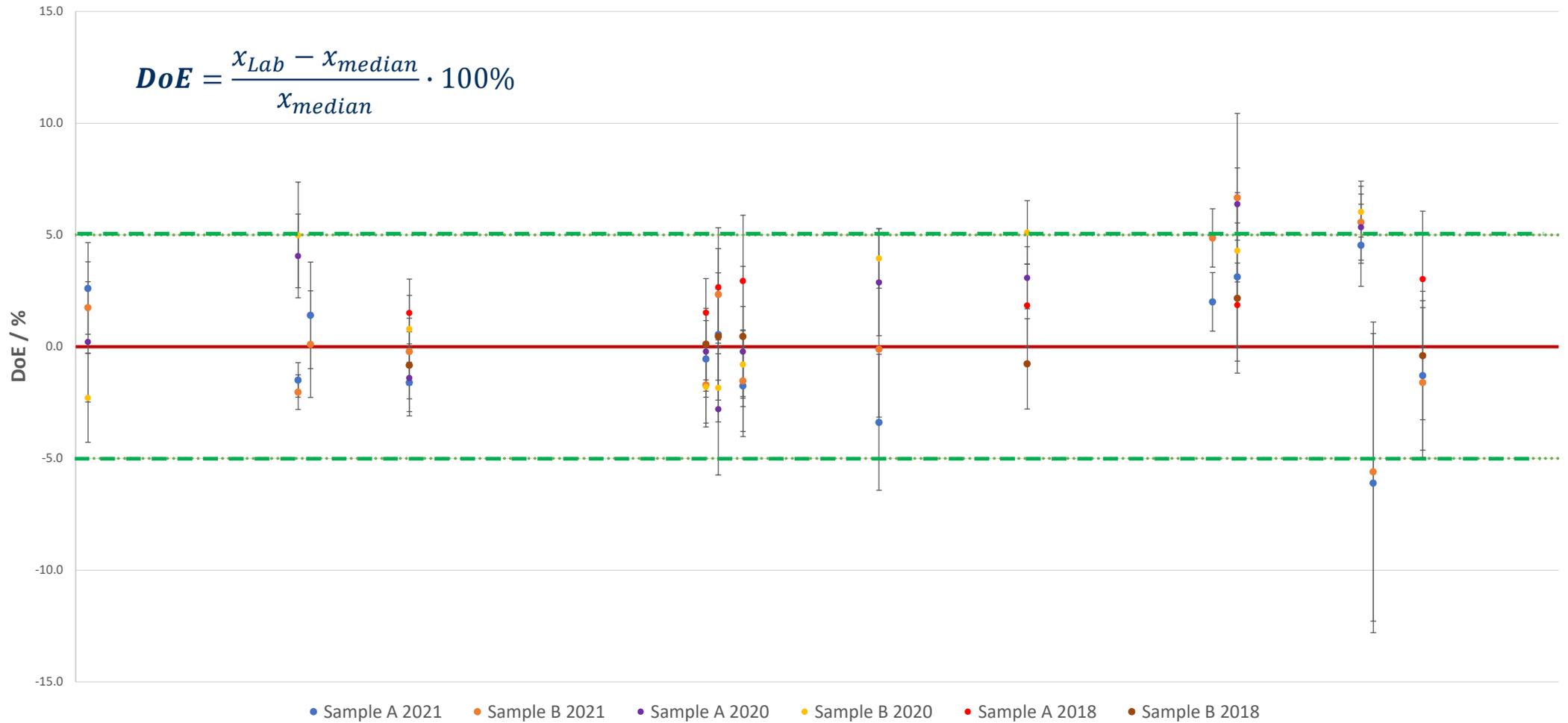
Sample Preparation		sample types		
		fresh whole blood	lyophilized	prepared whole blood
		<i>EurA1c study</i>	<i>RELA</i>	<i>IFCC network</i>
whole blood washing	Mixed blood (1.5 mL) is washed twice with 10 mL saline (NaCl, 0.15 mol/L) centrifugation for 10 min at 3000 g at 8°C	√	-	(√)
elimination of pre-HbA1c	incubation of sedimented cells in 10 mL saline at 37° C for 4 hour centrifugation for 10 min at 3000 g at 8°C	√	-	(√)
hemolysis	addition of 1 mL dest. water	√	-	(√)
Hb concentration	determination of total hemoglobin concentration dilution of hemolysates to 50 mg/mL total hemoglobin by mixing with buffer, MES 50 mmol/L, EDTA 1 mmol/L and potassium cyanide 10 mmol/L, pH 6.2, adjusted by sodium hydroxide 4 mol/L	√	√	(√)
remove of cell debris	centrifugation for 20 min at 3000 g determination of total hemoglobin concentration, storage at -70°C for maximum of three years	√	√	(√)

to be done by:

√ participant
 (√) network coordinator
 - not relevant

Calculation of Degree of Equivalence

DoE RELA 2018-2021



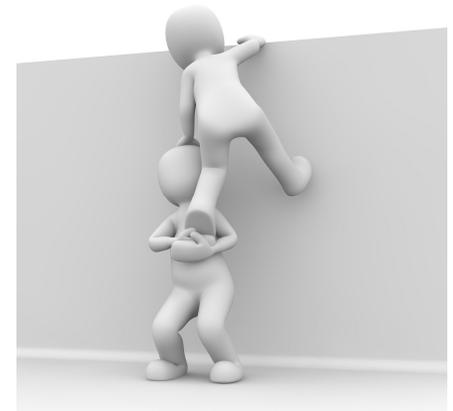
Suggestion for further actions ...

Individual network lab: Review RMP and data
→ Critical steps? Challenges?



Special network meeting:

- Combine & discuss results of review
- Plan experiments / measures



Conclusions

What to do when reference measurement procedures show different results?



Five steps in the ISO 17025 laboratory risk management process

Conclusions



Even if a RMP is well described according to ISO 15193, many factors influence the results. A reference measurement procedure is a tool. It needs to be maintained for a lifetime.

Well trained technicians and practical knowledge as well as continuous monitoring are essential to minimize the risk of different results.

Calibration labs are responsible to look for any considerable differences. If inconsistencies are detected, further investigations are needed by the individual lab and in cooperation with colleagues.



Thank you for your attention!

rela@spmd-rfb.de

