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Centre for Metrological Traceability in Laboratory Medicine (CIRME)

Director: Prof. Mauro Panteghini

site: http://users.unimi.it/cirme

Importance of measurement uncertainty estimate in medical laboratories

Federica Braga

Research Centre for Metrological Traceability in Laboratory Medicine (CIRME)

Accurate Results for Patient Care Workshop 2019

A JCTLM Members' and Stakeholders' meeting

2-3 December 2019, BIPM

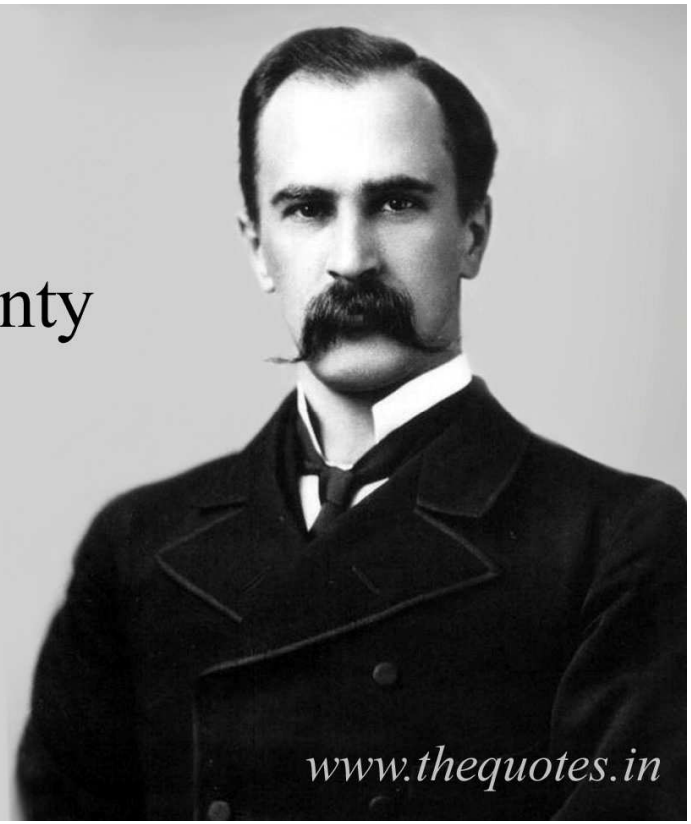
JCTLM

Accurate results for patient care

CERTAINTY IS AN ILLUSION

Medicine is a science of uncertainty
and an art of probability.

William Osler



MEASUREMENT UNCERTAINTY (MU) DEFINITION

Parameter characterizing the dispersion of the quantity values being attributed to a measurand

$$\text{Result} = x \pm u$$

quantity value — measurement uncertainty

The value of the measurand is assumed to lie within the interval $x - u$ to $x + u$ units, with a stated level of confidence.

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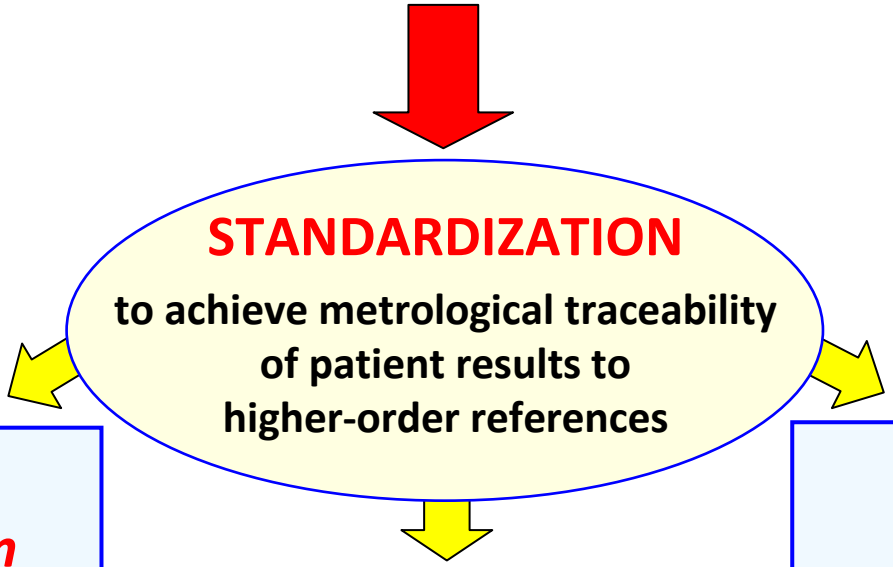
[International Vocabulary of Metrology Basic and general concepts and associated terms (VIM). 3rd ed. 2012]

"…In general use, the word uncertainty relates to the general concept of doubt… [however] uncertainty of measurement *does not imply doubt about the validity of a measurement*; on the contrary, knowledge of the uncertainty implies *increased confidence in the validity of a measurement result*… "

[Ellison SLR, Williams A, eds. (2012). Eurachem Guide: Quantifying Uncertainty in Analytical Measurement, Eurachem, 3rd ed.]

If I measure my uncertainty of measurement it is no longer an uncertainty. It is now the confidence limit within which the result will fall.

Laboratory users (i.e., doctors and patients) expect laboratory results to be equivalent and interpreted in a reliable and consistent manner



Unbroken traceability chain

Definition of higher order references to implement the appropriate trueness transfer process to commercial calibrators and patient results

Measurement uncertainty

With definition of allowable limits for clinical application of the measurements

Post-market surveillance

Survey the suitability of IVDs for clinical use and of laboratory performance in using them

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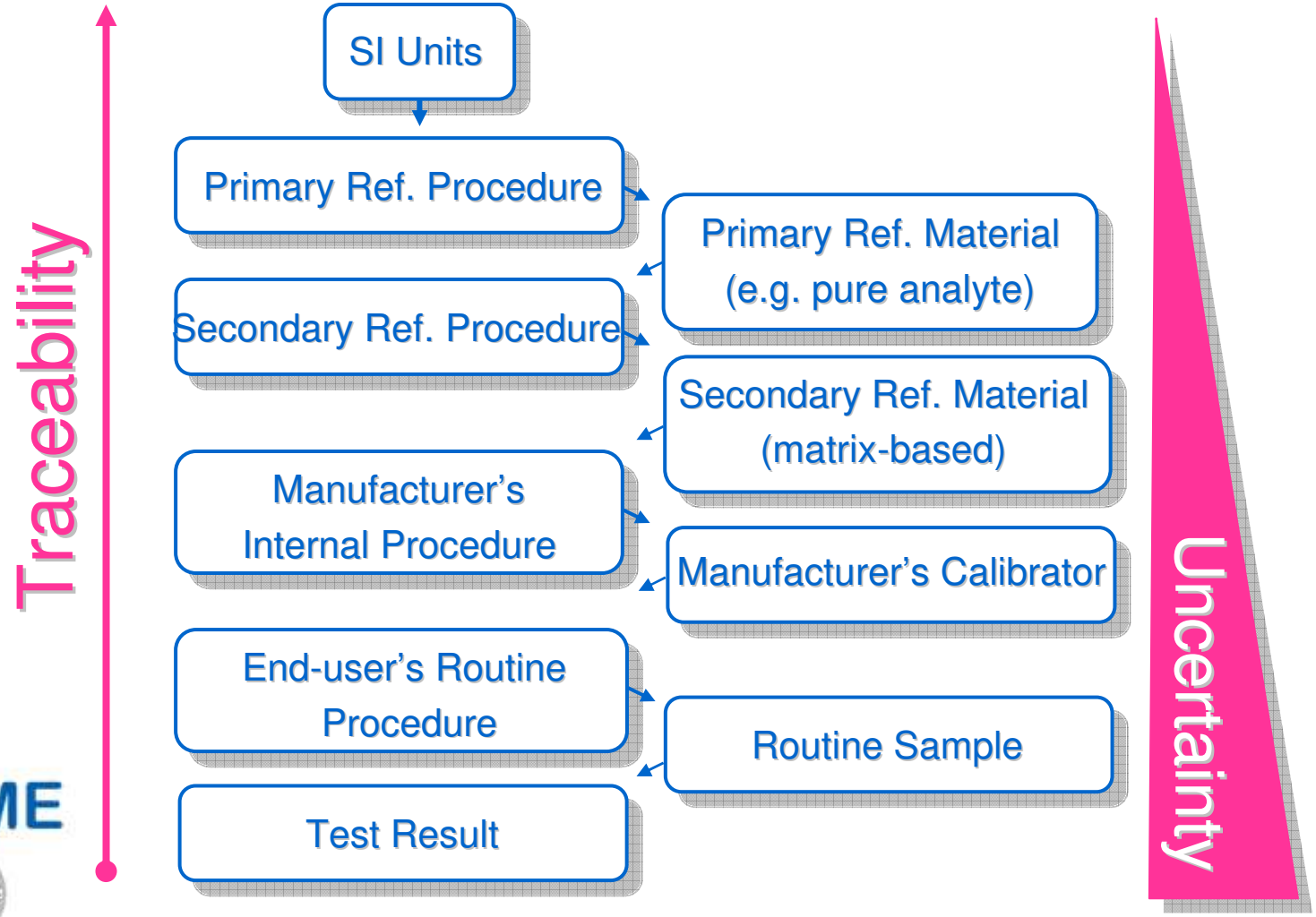
Infusino I, Panteghini M. Clin Biochem 2018;57:3

To become *equivalent*, results must be traceable to higher-order references



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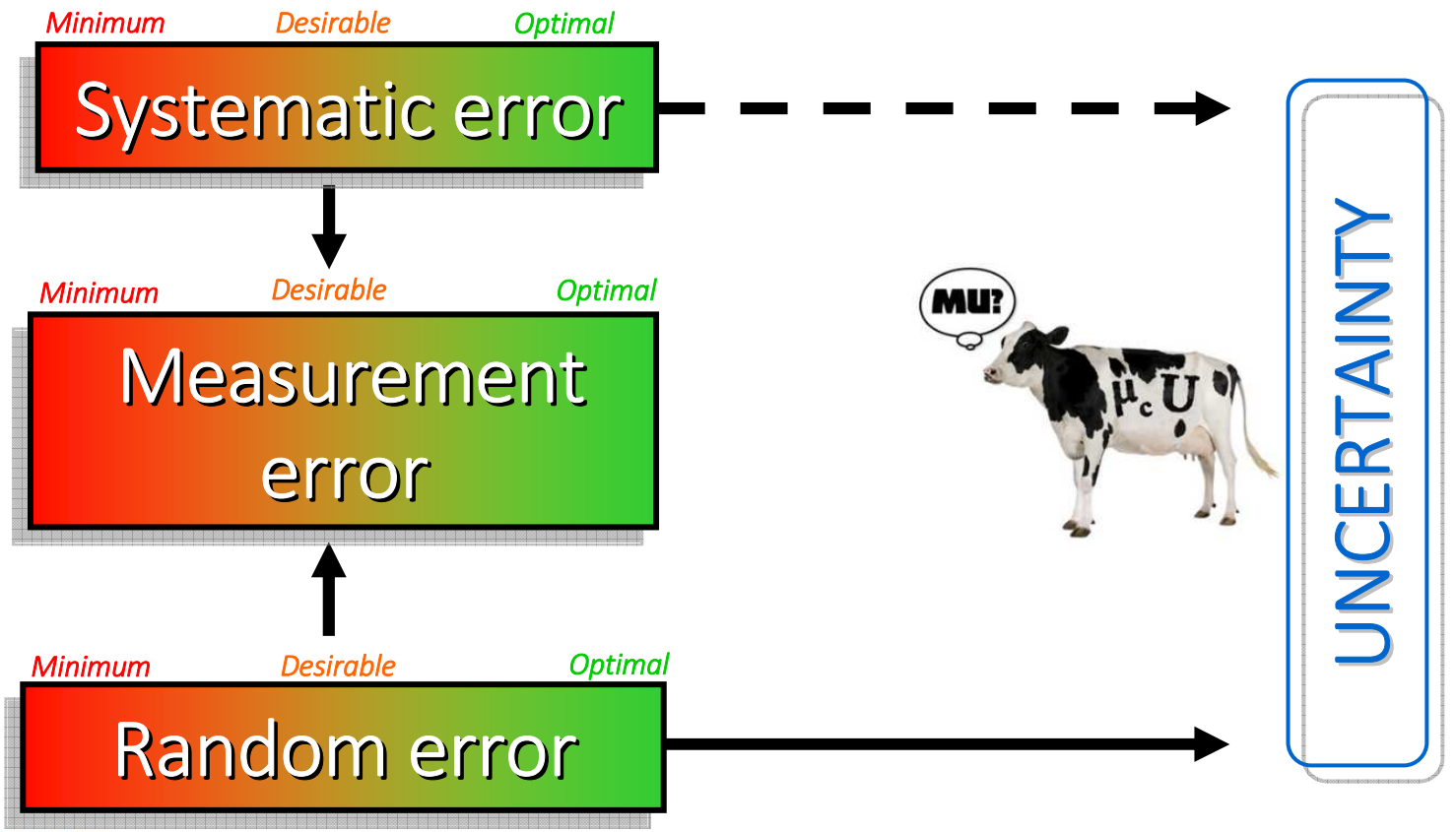
Assumption behind the *uncertainty concept*: the bias should be appropriately eliminated



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WHY MEASUREMENT UNCERTAINTY IS NEEDED

ISO 15189:2012 AND MEDICAL LABORATORIES ACCREDITATION

ISO 15189:2012 introduced the estimation of measurement uncertainty as a specific requirement for the accreditation of medical laboratories

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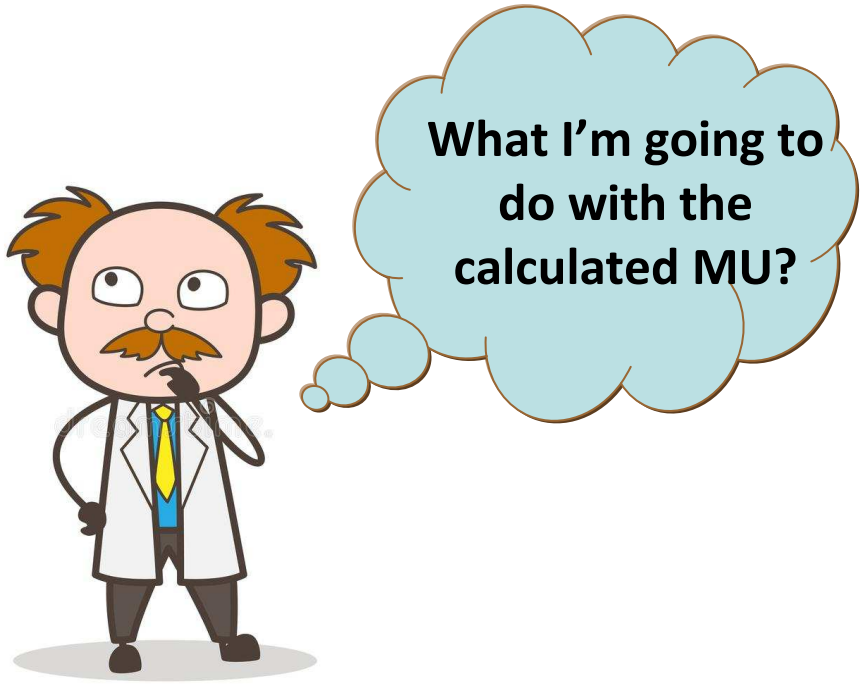
Certainty of MU

- You ***must*** calculate MU, and many labs do
- Most labs do nothing with MU after that



www.westgard.com/mu-global-survey.htm

Westgard QC

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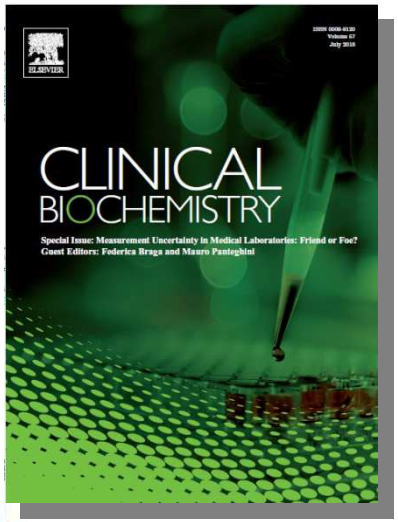
11th International Scientific Meeting

MEASUREMENT UNCERTAINTY IN MEDICAL LABORATORIES: FRIEND OR FOE?

MILANO, ITALY
November 30th, 2017

AULA MAGNA - LITA SEGRATE
Via Fratelli Cervi, 93 - Segrate, Milano



Special Issue: Measurement Uncertainty in Medical Laboratories: Friend or Foe?
Guest Editors: Federica Braga and Mauro Panteghini

CLINICAL BIOCHEMISTRY

Volume 57, July 2018

How to calculate MU in laboratory

1. "Bottom-up" approach

- **Originally proposed by JCGM in GUM***
- **Based on a comprehensive dissection of the measurement, in which each potential source of uncertainty is identified, quantified and combined to generate a combined uncertainty of the result using statistical propagation rules.**

2. "Top-down" approach

- **It estimates MU of laboratory results by using internal quality control data to derive the random components of uncertainty and commercial calibrator information.**

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**Evaluation of measurement data – Guide to the expression of uncertainty in measurement (GUM). JCGM 100:2008*

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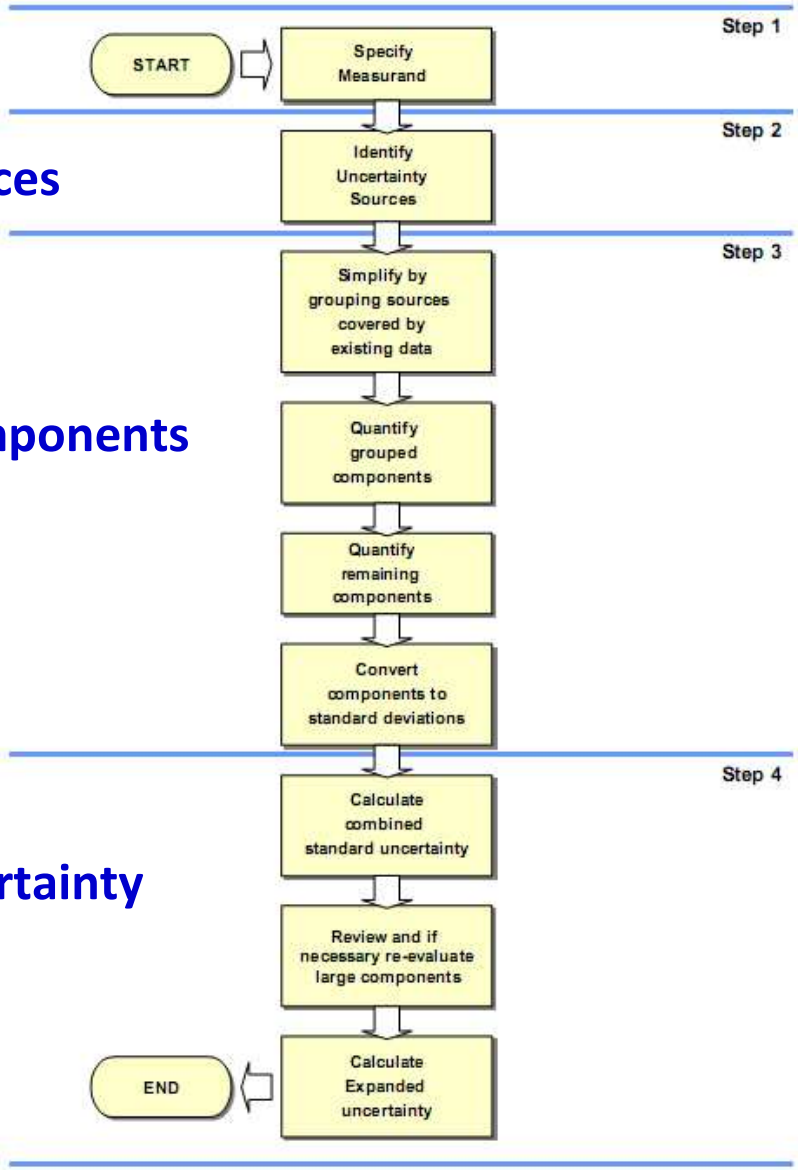
"BOTTOM-UP" APPROACH

Step 1: Specify the measurand

Step 2: Identify uncertainty sources

Step 3: Quantify uncertainty components

Step 4: Calculate combined uncertainty



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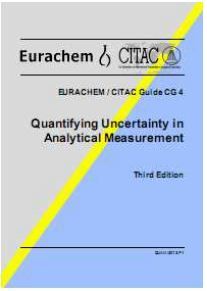
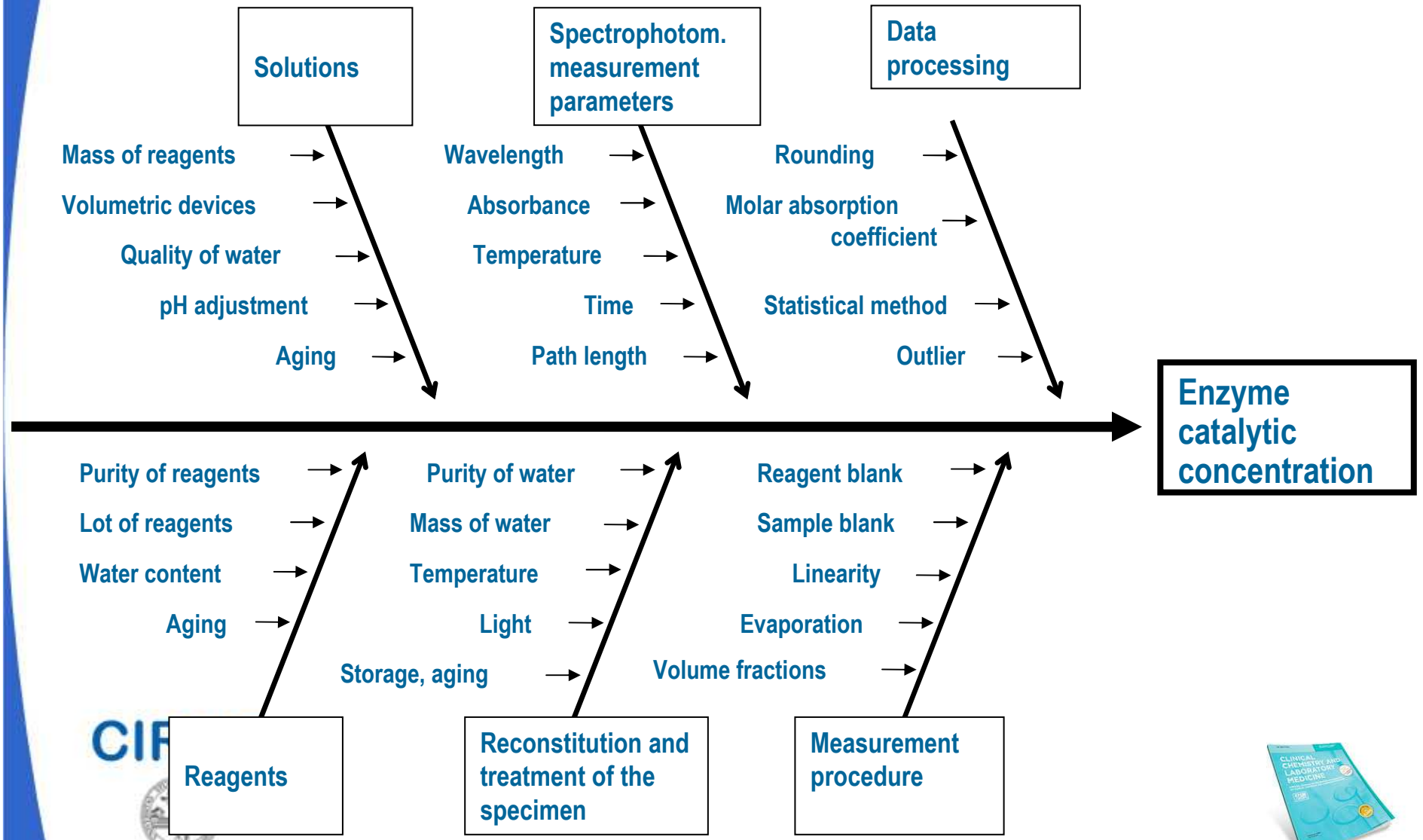
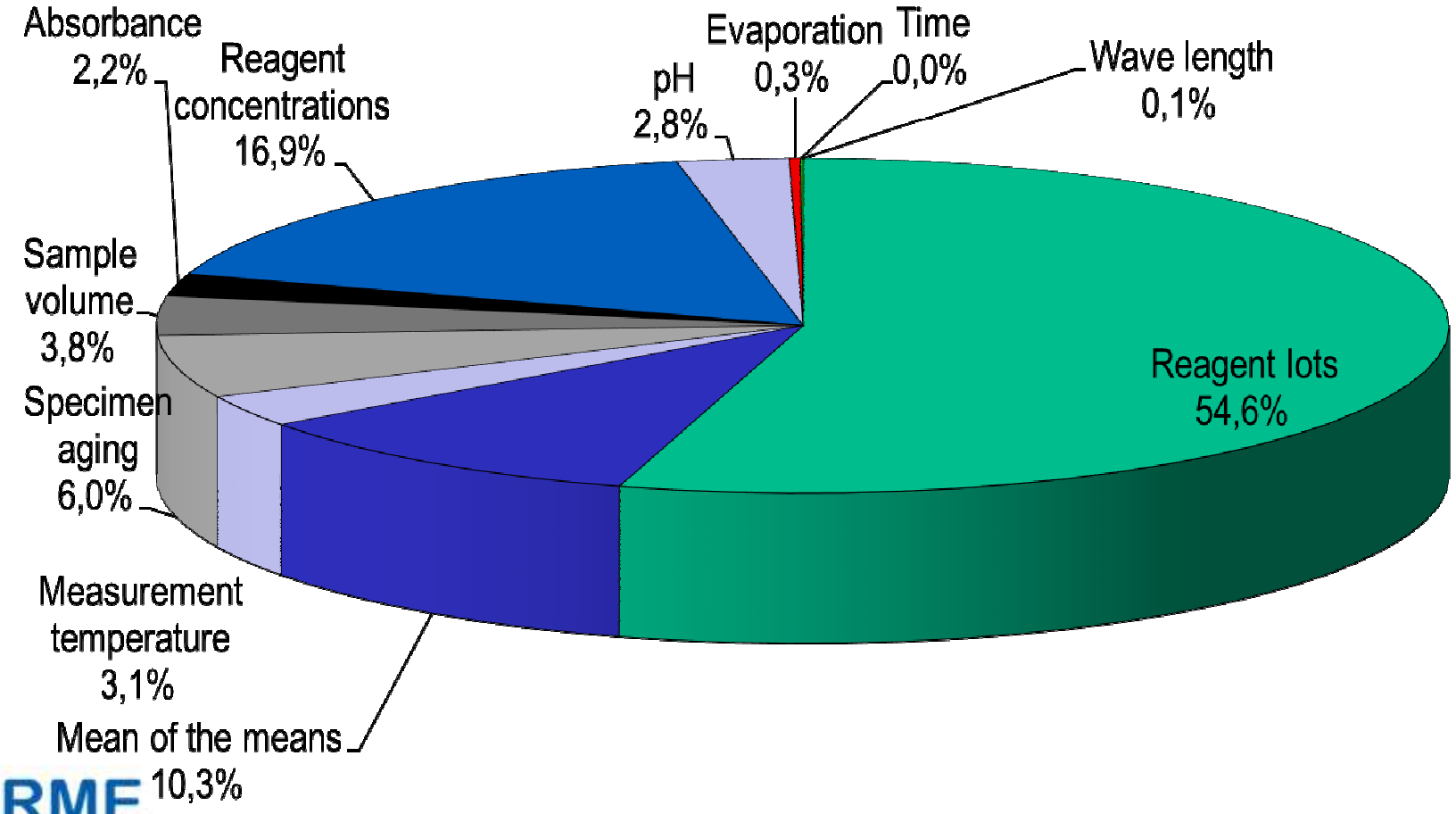


Overview of potentially relevant uncertainty components of the enzyme measurements using reference procedures



Infusino I, Schumann G, Ceriotti F, Panteghini M. CCLM 2010;48:301

Example of uncertainty budget for ALT reference measurement procedure



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Frusciante E, Infusino I, Panteghini M. Biochim Clin 2011;35:20

EXAMPLE: CALCULATION OF COMBINED MU FOR ALT MEASUREMENT WITH IFCC REFERENCE PROCEDURE

ALT

Parameter	Declared uncertainty		Reference	Distribution of uncertainty	Type of uncertainty	Standard uncertainty	Coefficient of sensitivity	Pro		Relative standard uncertainty
wavelenght	0,1	nm	manufacturer's specification	rectangular	B	0,06	0,14	1	nm	0,01
absorbance	0,3	%	manufacturer's specification	rectangular	B	0,17	1	1	%	0,17
pH	0,05	pH	IFCC-document	rectangular	B	0,03	0,14	0,05	pH	0,08
temperature	0,1	°C	IFCC-document	rectangular	B	0,06	4,14	1	°C	0,24
reagent concentration	1,5	%	IFCC-document	rectangular	B	0,87	0,26	1	%	0,23
lot of reagent	1,5	%	IFCC-document	rectangular	B	0,87	1	1	%	0,87
volume fraction of sample	0,4	%	data basis	rectangular	B	0,22	1	1	%	0,22
time	0,03	%	experiment	rectangular	B	0,02	1	1	%	0,02
evaporation	0,1	%	experiment	rectangular	B	0,06	1	1	%	0,06
aging of specimen	0,5	%	IFCC-document	rectangular	B	0,29	1	1	%	0,29
linearity	0,6	%	experiment	normal	B	0,30	1	1	%	0,30
mean of the means	0,8	U/L	result of the RMV investigation	normal	A	0,40	1	1	U/L	0,40

Combined standard uncertainty = square root of the sum of the variances (calculated from the standard uncertainty components)

Coverage probability *p*	Coverage factor *k*
90%	1.64
95%	1.96
95.45%	2.00
99%	2.58
99.73%	3.00

$[u_c]^2 = u(wl)^2 + u(abs)^2 + u(pH)^2 + u(temp)^2 + u(reag)^2 + u(lot)^2 + u(vol)^2 + u(time)^2 + u(evap)^2 + u(aging)^2 + u(lin)^2 + u(mean)^2 = 1.3$

$[u_c] = 1.14\ \%$

The appropriate coverage factor should be applied to give an expanded uncertainty (U): U = *k* x u_c. The choice of the factor *k* is based on the desired level of confidence:

$U\ (k=1.96) = \pm 2.23\%$

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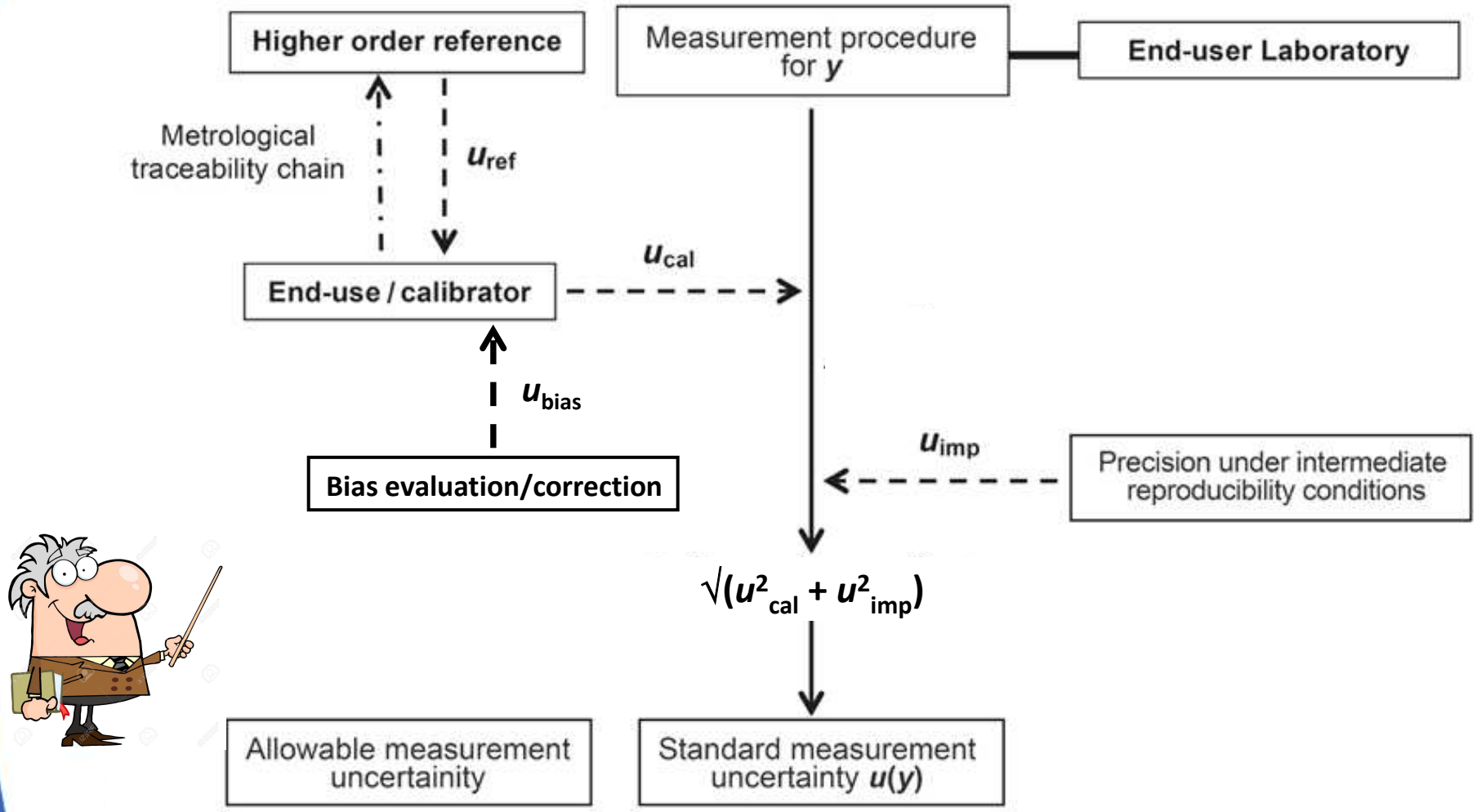
SOURCES OF MU WITH THE 'TOP-DOWN' APPROACH



MU must be defined across the entire traceability chain,

→ starting with the provider of reference materials,

→ extending through the IVD manufacturers and their processes for assignment of calibrator values, and

→ ultimately to the final result reported to clinicians by end users (i.e. clinical laboratories).

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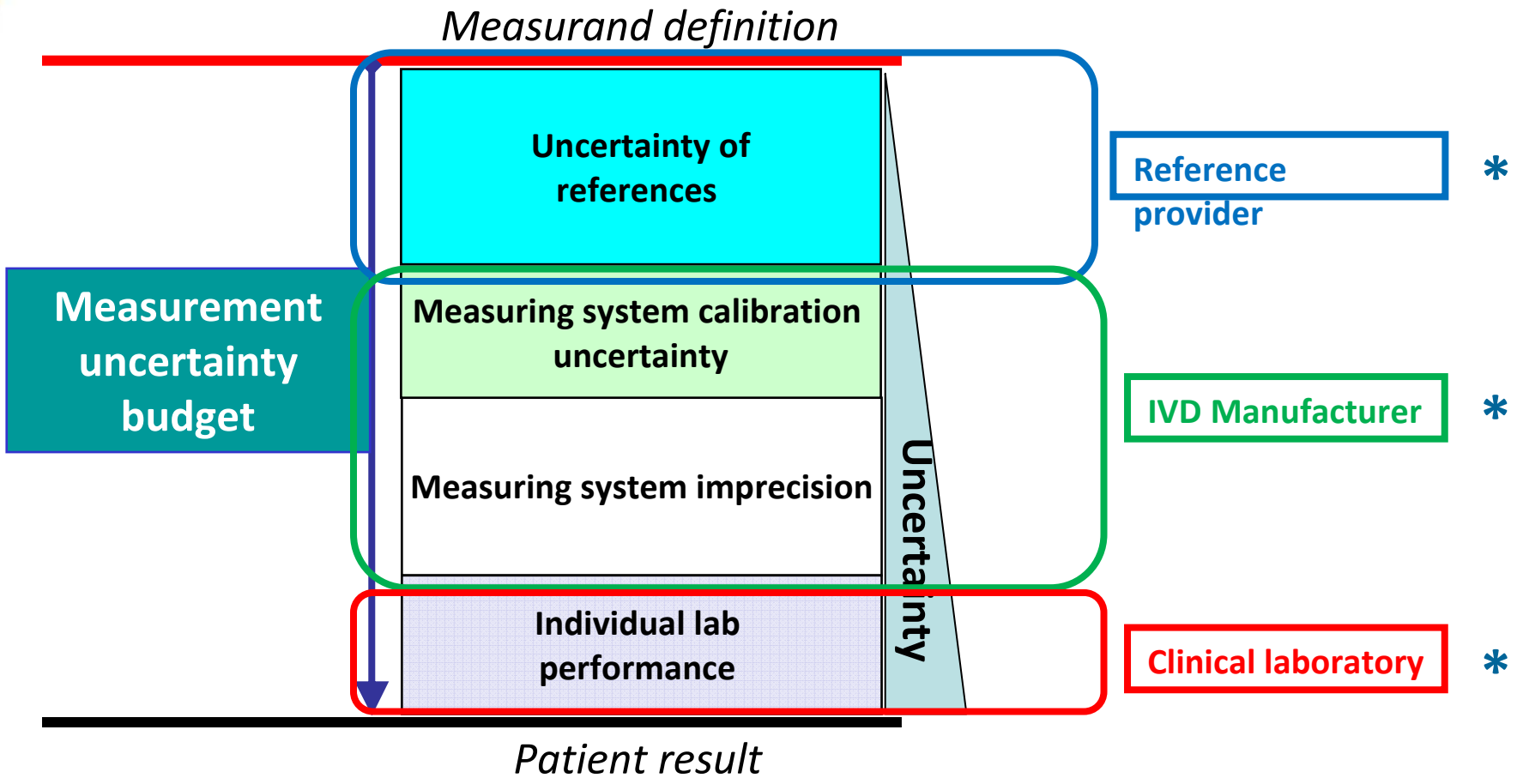
[Panteghini M, Clin Chem Lab Med 2012;50:1237]



*** Although independent in the tasks, their performances contribute together to the total MU budget**

[Braga F, Panteghini M. Clin Biochem 2018;57:7]

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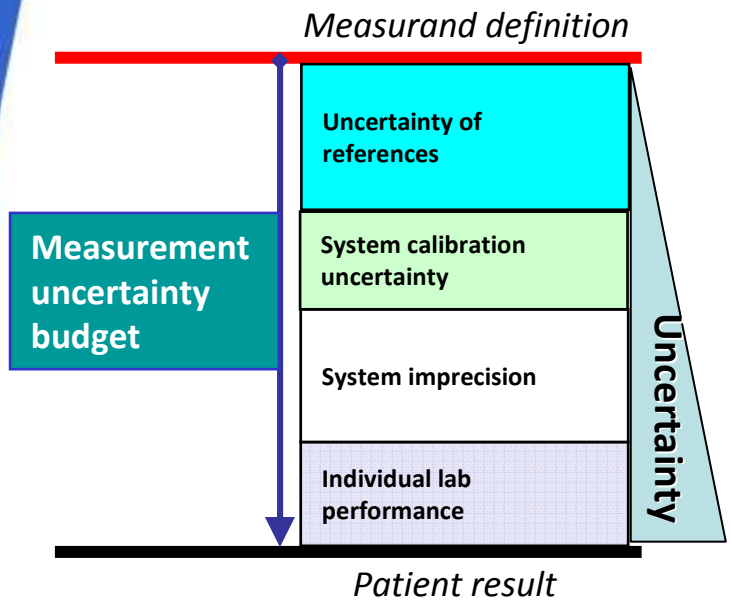

Estimate the combined uncertainty!

$$U_{result} = (U^2_{ref} + U^2_{cal} + U^2_{imp})^{1/2}$$

Avoid the common misconception that the reproducibility of a measurement result equals its overall MU FAKE NEWS

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WHY MEASUREMENT UNCERTAINTY MATTERS

- Uncertainty of higher-order references → to define their suitability
- Uncertainty of IVD calibrators → to verify quality of IVD products
- Uncertainty of clinical results → to provide evidence of clinically unsuitable results and stimulate work for improving the quality of assay performance

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Adapted from Infusino I, Panteghini M. Clin Biochem 2018;57:3

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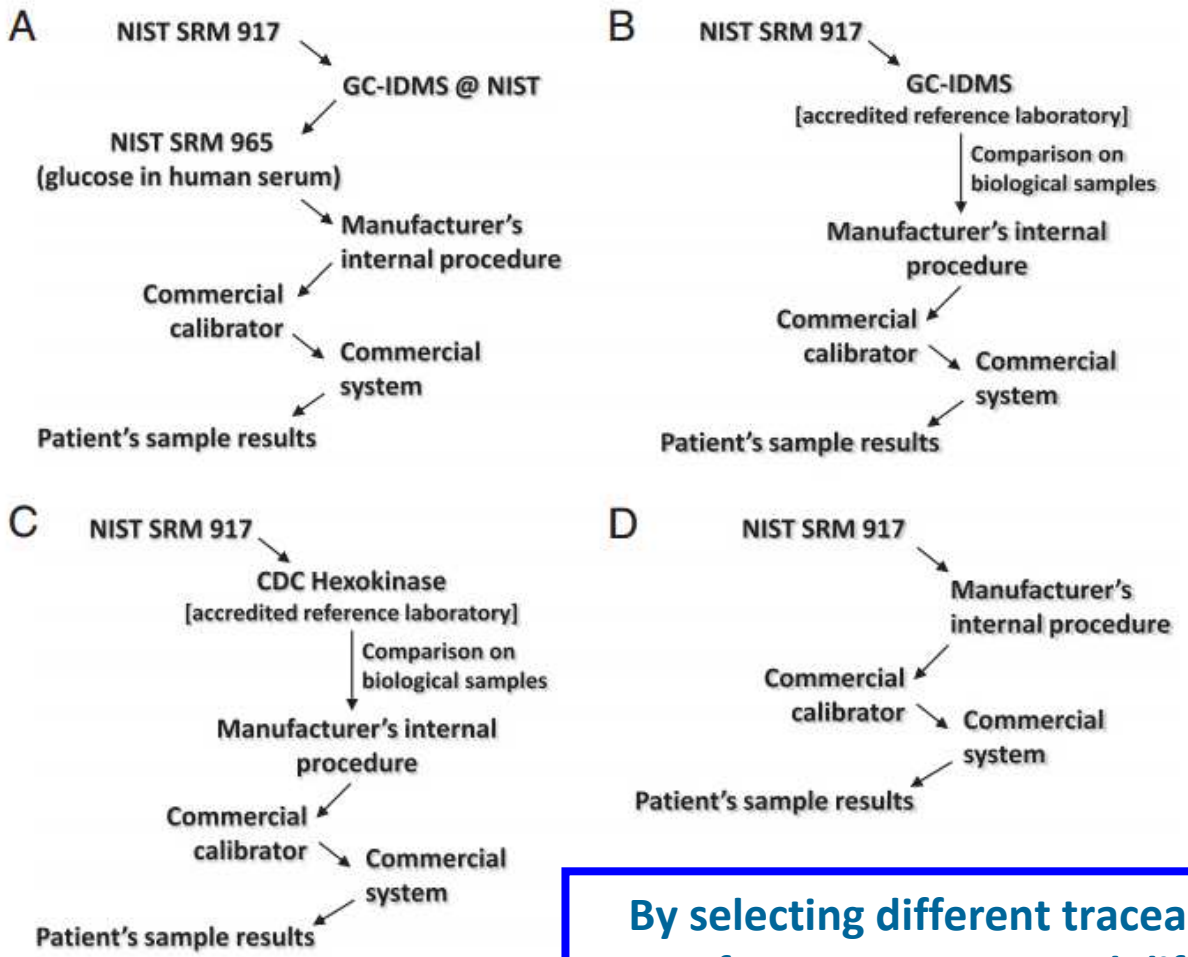
TRACEABILITY CHAINS AVAILABLE FOR IVD MANUFACTURERS FOR PLASMA GLUCOSE



By selecting different traceability chains, IVD manufacturers may spend different amounts of the total MU budget in implementing traceability of their measuring systems

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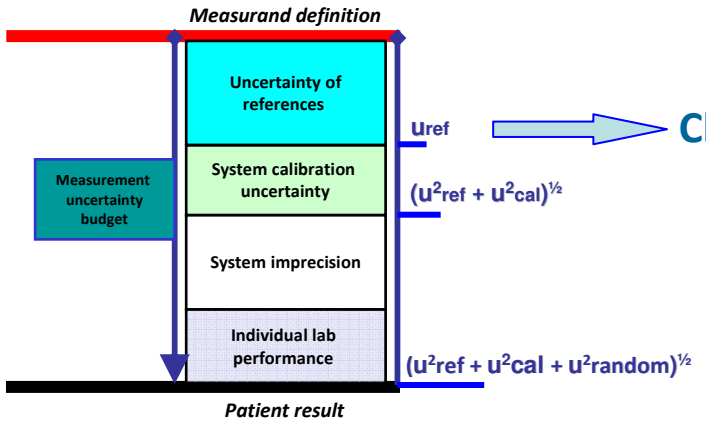
Braga F & Panteghini M, Clin Chim Acta 2014;432:55
Pasqualetti S, Braga F, Panteghini M, Clin Biochem 2017; 50:587.

Braga F, Panteghini M. Clin Chim Acta 2014;432:55-61

Company	Platform	Principle of commercial method	Calibrator	Declared standard uncertainty[a]	Higher-order reference employed		Type of traceability chain used[b]	Combined standard uncertainty associated with the used chain[c]
					Method	Material		
Abbott	Architect	ND	Multiconstituent calibrator	2.70%	IDMS	NIST SRM 965	A	1.22–1.45%[d]
Beckman	AU	Hexokinase	System calibrator	ND	ND	NIST SRM 965	A	1.22–1.45%[d]
	Synchron	Hexokinase	Synchron multicalibrator	ND	ND	NIST SRM 917a	D	1.60–3.00%[e]
Roche	Cobas c	Hexokinase	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
	Integra	Hexokinase	C.f.a.s.	0.62%	IDMS	ND	B	1.70%
	Modular	Hexokinase	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
		GOD	C.f.a.s.	0.84%	IDMS	ND	B	1.70%
Siemens	Advia	Hexokinase	Chemistry calibrator	1.30%	Hexokinase	NIST SRM 917a	C	1.88–3.26%[f]
		GOD	Chemistry calibrator	0.80%	Hexokinase	NIST SRM 917a	C	1.88–3.26%[f]



Chain A = 0.73% vs. Chain C = 1.63%

The quality of glucose measurement may be dependent on the type of traceability chain selected for trueness transferring, sometimes making difficult (e.g., chain C) to achieve the suitable limits for MU on clinical samples

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Braga F & Panteghini M, Clin Chim Acta 2014;432:55
Pasqualetti S, Braga F, Panteghini M, Clin Biochem 2017; 50:587.

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Clinical Chemistry 65:3
473-483 (2019)

Trueness Evaluation and Verification of Interassay Agreement of 11 Serum IgA Measuring Systems: Implications for Medical Decisions

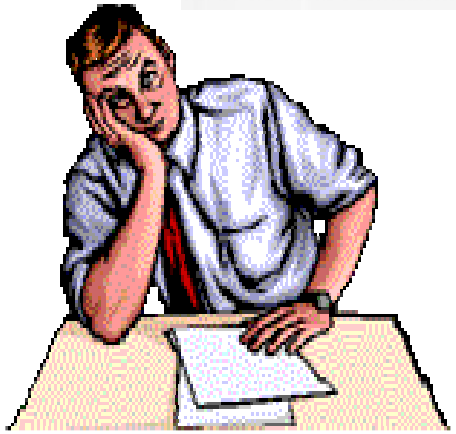
Federica Braga,[1*] Ilenia Infusino,[1] Erika Frusciante,[1] Ferruccio Ceriotti,[2] and Mauro Panteghini[1]

Table 3. Metrological traceability and mean relative combined measurement uncertainty (expanded by a coverage factor of 2) on patient pools for each evaluated IgA measuring system.[a]

Measuring system	Calibrator type	Stated traceability	Standard uncertainty associated with reference material, %	Calibrator standard uncertainty, %[b]	Bias vs ERM-DA470k/ IFCC target value (1.8 g/L), %[c]	Method CV, %[d]	Mean combined uncertainty on patient pools, %[e]
Architect c4000	Specific proteins multiconstituent calibrator	CRM 470	1.02	0.75 (the same for 5 levels from 1.05 to 7.00 g/L)	Not significant	1.24	3.54
AU 480	Serum protein multicalibrator 1	CRM 470	1.02	1.21-3.06[f] (5 levels, from 0.49 to 6.22 g/L)	4.17	1.81	**9.57[g]**
Immage 800	Serum protein multicalibrator 1	CRM 470	1.02	1.25-2.22[f] (5 levels, from 0.45 to 6.21 g/L)	7.50	2.99	**16.34[h]**
Optilite	IgA calibrator	ERM-DA470k/IFCC	1.39	3.00 (1 level, 0.64 g/L)	Not significant	2.07	7.29
Cobas c702	C.f.a.s. proteins	CRM 470	1.02	0.55 (1 level, 4.02 g/L)	Not significant	1.53	3.84
Mindray BS480	Specific proteins calibrator	ERM-DA470k/IFCC	1.39	3.57-3.91[f] (5 levels, from 0.42 to 6.30 g/L)	5.56	1.08	**13.67[i]**
ADVIA XPT	Liquid specific protein calibrators	CRM 470	1.02	0.60-0.81[f] (2 levels, from 0.62 to 2.49 g/L)	4.78	0.85	**9.99[j]**
Dimension Vista	PROT1 CAL	CRM 470	1.02	1.77 (1 level, 2.03 g/L)	10.3	3.59	**22.10**
Taurus	Proteins calibrators	ERM-DA470k/IFCC	1.39	0.41-5.75[f] (5 levels, from 0.69 to 6.94 g/L)	Not significant	1.54	5.36[k]
ILab 650	Proteins calibrators	ERM-DA470k/IFCC	1.39	0.41-5.75[f] (5 levels, from 0.69 to 6.94 g/L)	Not significant	1.46	5.28[k]

$$2 * \sqrt{(u^2_{cal} + u^2_{bias} + u^2_{imp})} = U$$

Houston
we have a problem.



The manufacturer's internal quality specifications to validate the calibrator traceability to higher-order references are not established on the basis of suitable APS

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EXAMPLE

Abbott - Creatinine Enzymatic Assay -

Abbott Diagnostics in a document released on August 2014 informed customers that the internal release specification for CAL was ±5% from the target value of NIST SRM 967a Level 1

			Insert Range	Lot 30410Y600 (Mean)	Lot 40043Y600 (Mean)	Lot 40150Y600 (Mean)	Lot 40252Y600 (Mean)
NIST SRM 967A			Target: 0.85*	0.82	0.88	0.88	0.83

*Manufacturer's release specification is +/- 5% from the target.

–3.53%	+3.53%	+3.53%	–2.44%

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But APS for MU of creatinine measurement on clinical samples are:

Biological variation model

$CV_I = 4.4\%$

$\leq 0.75 \times CV_I$ (minimum) = 3.3%

$\leq 0.50 \times CV_I$ (desirable) = 2.2%

$\leq 0.25 \times CV_I$ (optimum) = 1.1%

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	 SRM 967a level 1	SRM 967a level 2
Multigent Clin Chem Calibrator lot no. 40043Y600		
Imprecision (u_{Rw})	0.47%	0.40%
Bias (u_{bias})	3.57%	7.05%
Relative combined standard uncertainty [$u_c = (u_{bias}^2 + u_{Rw}^2)^{0.5}$]	3.60%	7.06%
Expanded uncertainty ($U = k \times u_c$)	7.20%	14.12%

From MILAN APS MODEL 2

3.3% minimum

2.2% desirable

1.1% optimum

Our study shows that this validation criterion for traceability of different CAL lots adopted by the manufacturer is however too large to comply with the U goal for creatinine measurements in biological samples with an acceptable confidence.

Letter to the Editor

The calibrator value assignment protocol of the Abbott enzymatic creatinine assay is inadequate for ensuring suitable quality of serum measurements

Pasqualetti S et al. CCA 2015;450:125

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EXAMPLE

BECKMAN COULTER - Serum folate -

Beckman Coulter in their technical bulletin released on 2011 informed customers that the internal release specification for CAL was ±10% from the target value of WHO IS 03/178.

Accuracy to WHO International Standard 03/178

Fifty-four (54) measurements were made using multiple reagent pack and calibrator lots on the Access 2 Immunoassay system. The mean Folate assay result was accurate within ±10% of the WHO International Standard 03/178 assigned value of 5.33 ng/mL.

Mean		Target		Difference		Two-Sided 95% CL[a]		% Difference
(ng/mL)	(nmol/L)	(ng/mL)	(nmol/L)	(ng/mL)	(nmol/L)	(ng/mL)	(nmol/L)	
5.05	11.44	5.33	12.08	-0.28	-0.063	4.44 – 5.65	10.06 – 12.08	-5.3%

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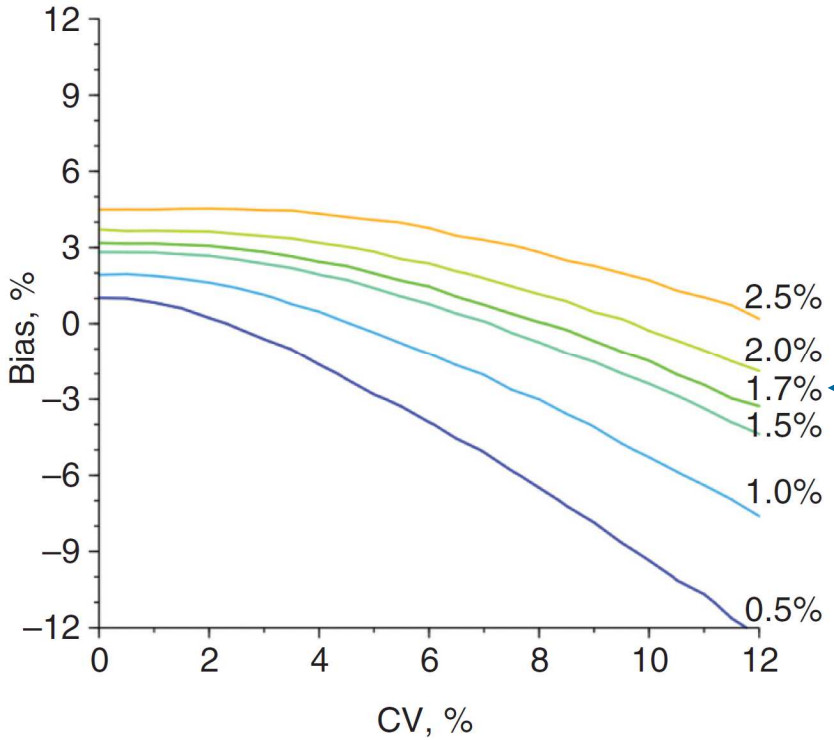
DE GRUYTER Clin Chem Lab Med 2019; aop

Letter to the Editor

Simona Ferraro*, Andrew W. Lyon, Federica Braga and Mauro Panteghini

Definition of analytical quality specifications for serum total folate measurements using a simulation outcome-based model



Clinically acceptable misclassification

Plots of the fraction of population misclassification rate [in terms of false negatives] as a function of assay bias and imprecision at mean folate of 4.0 µg/L

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Table 3. Mean relative combined measurement uncertainty (expanded by a coverage factor of 2) on patient pools for each evaluated total folate measuring system.

Measuring system	Calibrator type	Calibrator standard uncertainty, %[a]	Bias vs WHO IS 03/178 target value (5.33 µg/L), %[b]	Method CV, %[c]	Mean combined uncertainty on patient pools, %[d]
Alinity i	Folate calibrators	0.33-0.23[e] (5 levels, from 1.5 to 20.0 µg/L)	5.07	3.31	12.12[f]
Dxl Access	Access Folate calibrators	2.02-1.69[e] (5 levels, from 1.24 to 24.8 µg/L)	9.01	1.60	18.61[g]
Cobas e801	CalSet Folate	6.31-1.45 (2 levels, 1.75 and 15.8 µg/L)	–8.26	3.58	18.24[h]
Advia Centaur	FOL calibrators	5.25-2.75 (2 levels, 2.7 and 16.5 µg/L)	8.26	2.05	17.89[i]

[a] Except for Advia Centaur, not combined with uncertainty of corresponding higher-order reference material.
[b] Data from this study.
[c] The mean imprecision of measuring systems was obtained from duplicate measurements of 6 clinical sample pools with serum total te concentrations between 3.0 and 7.0 µg/L.
[d] Expanded by multiplying the standard uncertainty by a coverage factor of 2 (95.45% level of confidence). For suitable clinical application of serum total folate measurements, the expanded measurement uncertainty at the patient sample level should remain within ±5.0%.

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[Braga F et al., submitted]

WHY MEASUREMENT UNCERTAINTY MATTERS

- Uncertainty of higher-order references → to define their suitability
- Uncertainty of IVD calibrators → to verify quality of IVD products
- Uncertainty of clinical results → to provide evidence of clinically unsuitable results and stimulate work for improving the quality of assay performance

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Infusino I, Braga F, Mozzi R, Valente C, Panteghini M
Clinica Chimica Acta 412 (2011) 791–792

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

Letter to the Editor

Is the accuracy of serum albumin measurements suitable for clinical application of the test?

Probably not

Table 1
Relative standard uncertainties for each contributing factor in determination of serum albumin with Roche Tina-quant immunoturbidimetric assay on Cobas c 501 platform. Data obtained by measurements of ERM-DA 470k/IFCC Human Serum Proteins reference material (certified value ± expanded uncertainty, 37.2 g/L ± 1.2 g/L).

Factor	Result
Imprecision (u_{Rw})	1.88%
Bias (u_{bias})	6.42%
Relative combined standard uncertainty [$u_c = (u_{bias}^2 + u_{Rw}^2)^{0.5}$]	6.69%

2.4% minimum
1.6% desirable
0.8% optimum

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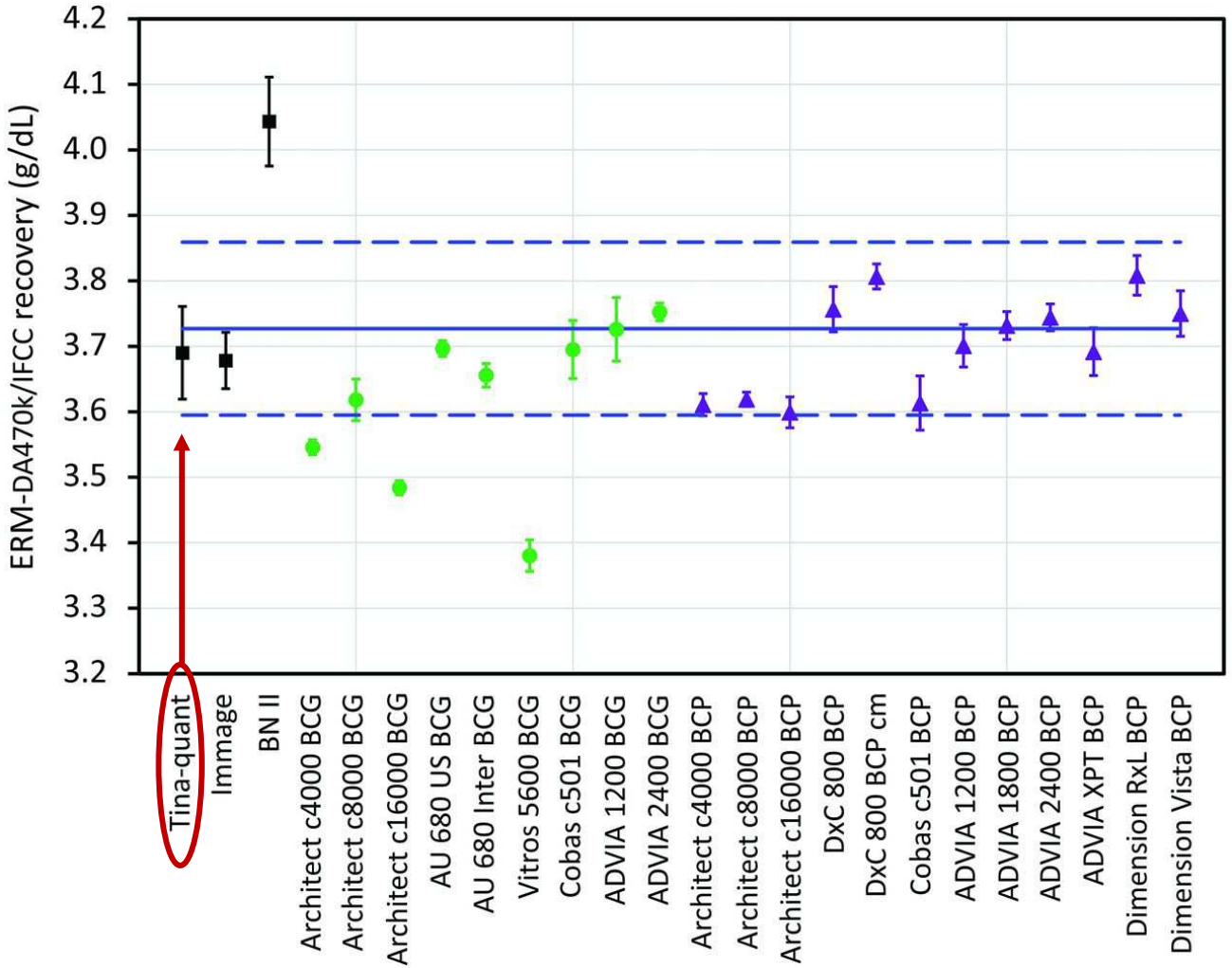
2017 State of Harmonization of Serum Albumin Measurements



[Bachmann LR et al. Clin Chem 2017;63:770]

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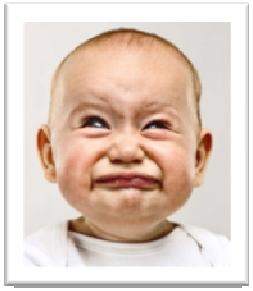
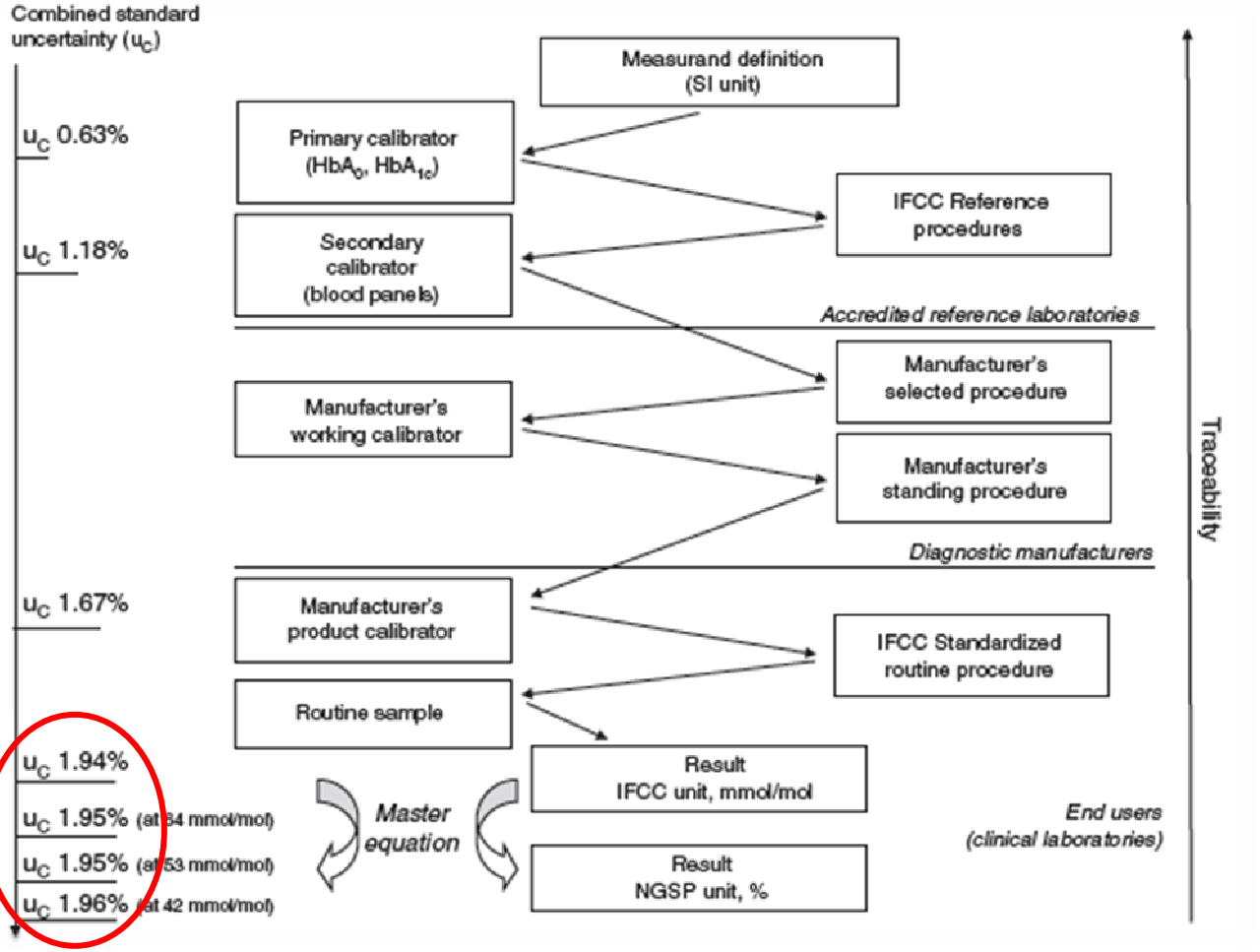
HbA1c reference system and associated combined standard uncertainty



Analytical goals for HbA_{1c} measurement

Quality level	U_C
Optimal	≤0.6
Desirable	≤1.3
Minimal	≤1.9



[Braga F & Panteghini M, Clin Chem Lab Med 2013;51:1719]

Federica Braga* and Mauro Panteghini

Standardization and analytical goals for glycated hemoglobin measurement

Clin Chem Lab Med 2013;51:1719–26

Further advances are needed to:

1. reduce uncertainty associated with higher-order metrological references (reference materials and procedures)
2. decrease the imprecision (i.e. random uncertainty) of commercial HbA1c assays

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Letter to the Editor

Dominika Szőke*, Assunta Carnevale, Sara Pasqualetti, Federica Braga, Renata Paleari and Mauro Panteghini

More on the accuracy of the Architect enzymatic assay for hemoglobin A_{1c} and its traceability to the IFCC reference system

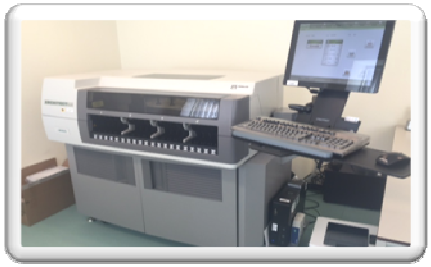

Table 3
Analytical goals for HbA_{1c} measurement

Quality level	U_C
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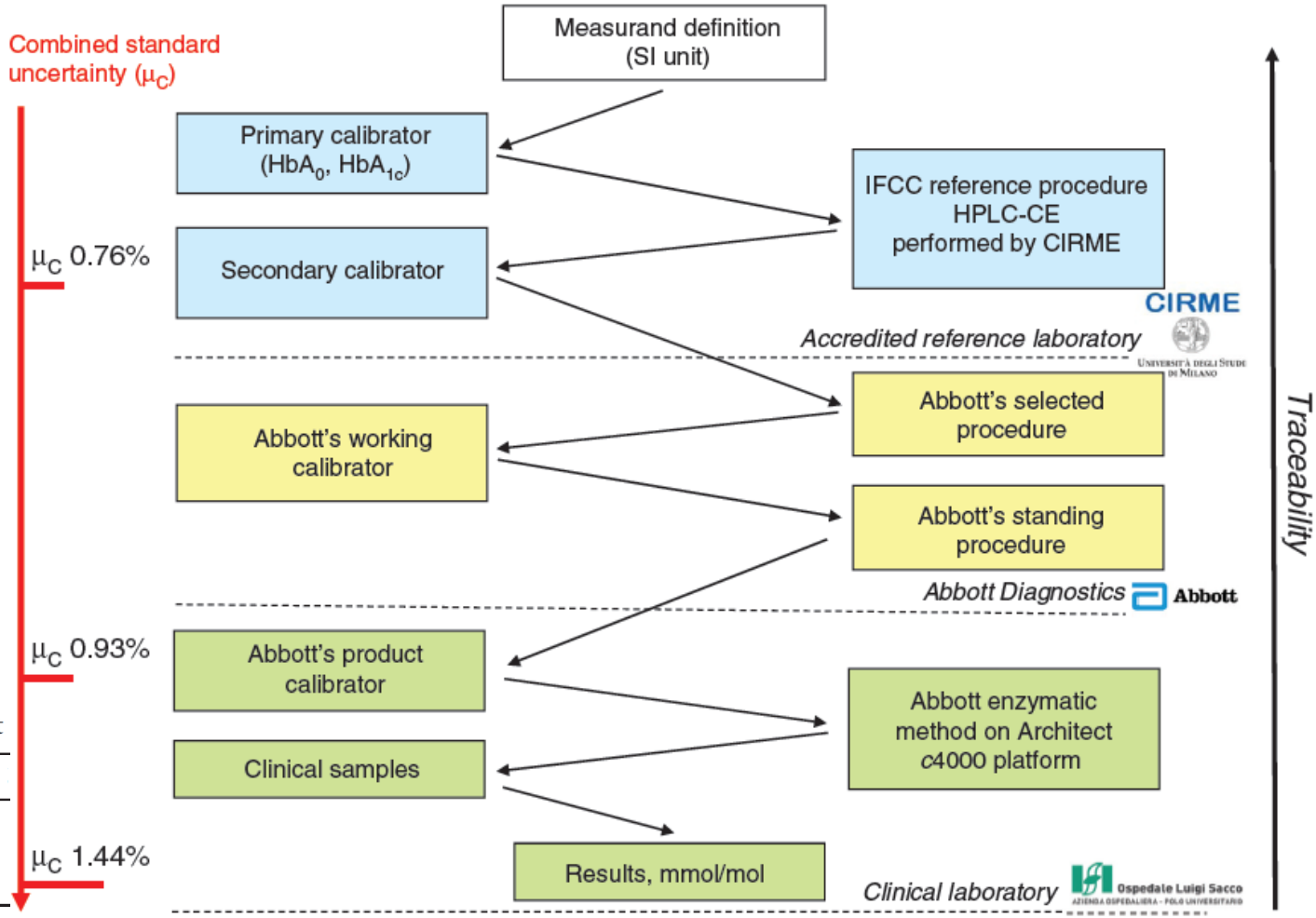

MU is useful for a number of reasons

- Giving objective information about quality of individual laboratory performance
- Serving as management tool for the clinical laboratory and IVD manufacturers, forcing them to investigate and eventually fix the identified problem
- Helping those manufacturers that produce superior products and measuring systems to demonstrate the superiority of those products
- Identifying analytes that need analytical improvement for their clinical use and ask IVD manufacturers to work for improving the quality of assay performance
- Abandonment by users (and consequently by industry) of assays with demonstrated insufficient quality

Thank you for your kind attention!

F. Braga

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https://sites.unimi.it/cirme/

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