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Centre for Metrological Traceability in Laboratory Medicine (CIRME)

Director: Prof. Mauro Panteghini

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

13th International Scientific Meeting

THE INTERNAL QUALITY CONTROL IN THE TRACEABILITY ERA

MILANO, ITALY
November 28th, 2019

How to estimate measurement uncertainty in medical laboratories: the ISO Technical Specification 20914

Mauro Panteghini

University of Milan Medical School

Research Centre for Metrological Traceability in Laboratory Medicine (CIRME)

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.

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

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Why MU 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

ISO 15189:2012, 5.5.1.4, requires that "…(medical laboratories)… shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples."

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To estimate MU is not enough!

- MU is not a finding to be calculated only to fulfil accreditation parameters and then immediately forgotten
- Together with the MU, the laboratory must define the performance specifications (PS) to validate it
- All attempts must be made to improve on the MU value if PS are not achieved, including, as last option, the replacement of the measuring system
- MU must become a Key Quality Indicator in clinical laboratories because it can be used to describe both the performance of an IVD measuring system and the laboratory itself.

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

MU in medical labs is useful for a number of reasons

1. It gives objective information about quality of individual laboratory performance
2. It serves as management tool for clinical laboratories and IVD manufacturers, forcing them to investigate and eventually fix the identified problem
3. It helps those manufacturers that produce superior products and measuring systems to demonstrate the superiority of those products
4. It permits to identify analytes that need analytical improvement for their clinical use and ask IVD manufacturers to work for improving the quality of assay performance, when needed
5. It may oblige users (and consequently IVD industry) to abandon assays with demonstrated insufficient quality

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

How to calculate MU in laboratory

1. “Bottom-up” approach*

• 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*

CALCULATION OF COMBINED MU BY BOTTOM-UP APPROACH: 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:

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

How to calculate MU in laboratory

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

ISO/TS 20914:2019

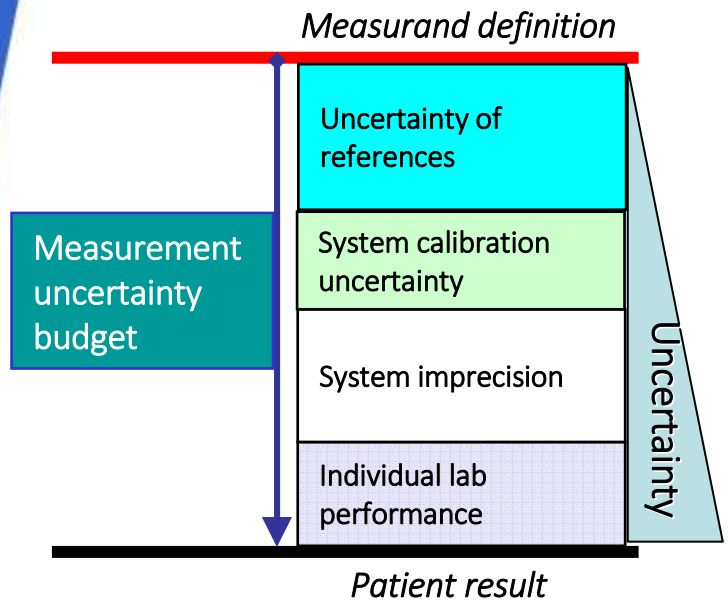
MEDICAL LABORATORIES -- PRACTICAL GUIDANCE FOR THE ESTIMATION OF MEASUREMENT UNCERTAINTY

Scope and main steps

- This document is concerned with practical approaches to estimation of MU, to be applied in medical laboratory settings for the purpose of estimating MU of values produced by measurement procedures intended to measure a broad range of biological measurands.
- New work item proposal to ISO: July 30, 2012; Dr Graham White (AU) – Project Leader.
- Draft #1: Jan 2013
- Toronto Draft: Sept 2014
- Draft #2: April 2015
- Geel Draft: Nov 2015
- London Draft: May 2016
- Kobe Draft: Oct 2016
- Minneapolis Draft: Jan 2017
- Brussels Draft: Nov 2017

8 drafts discussed and amended in 5.5 years

- Draft Technical Specification (DTS) Stage: July 2018
- Vote for publication by Sept 14, 2018 → 29 approval, 13 abstention, no disapproval
- First edition release: July 2019



THE INSPIRING CONCEPT: Estimate the combined uncertainty!

$$u_{result} = (u^2_{ref} + u^2_{cal} + u^2_{imp})^{½}$$

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

FAKE NEWS

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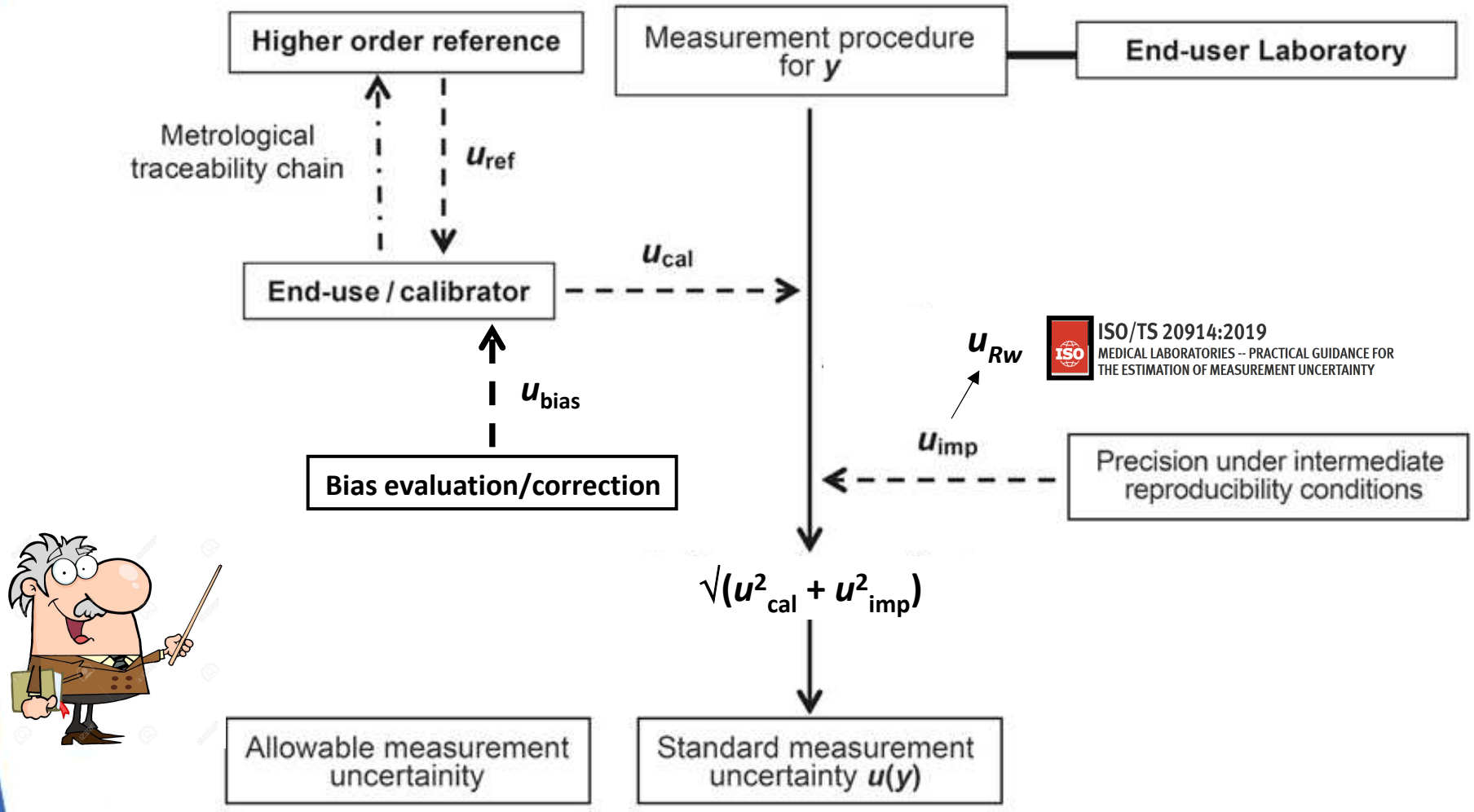
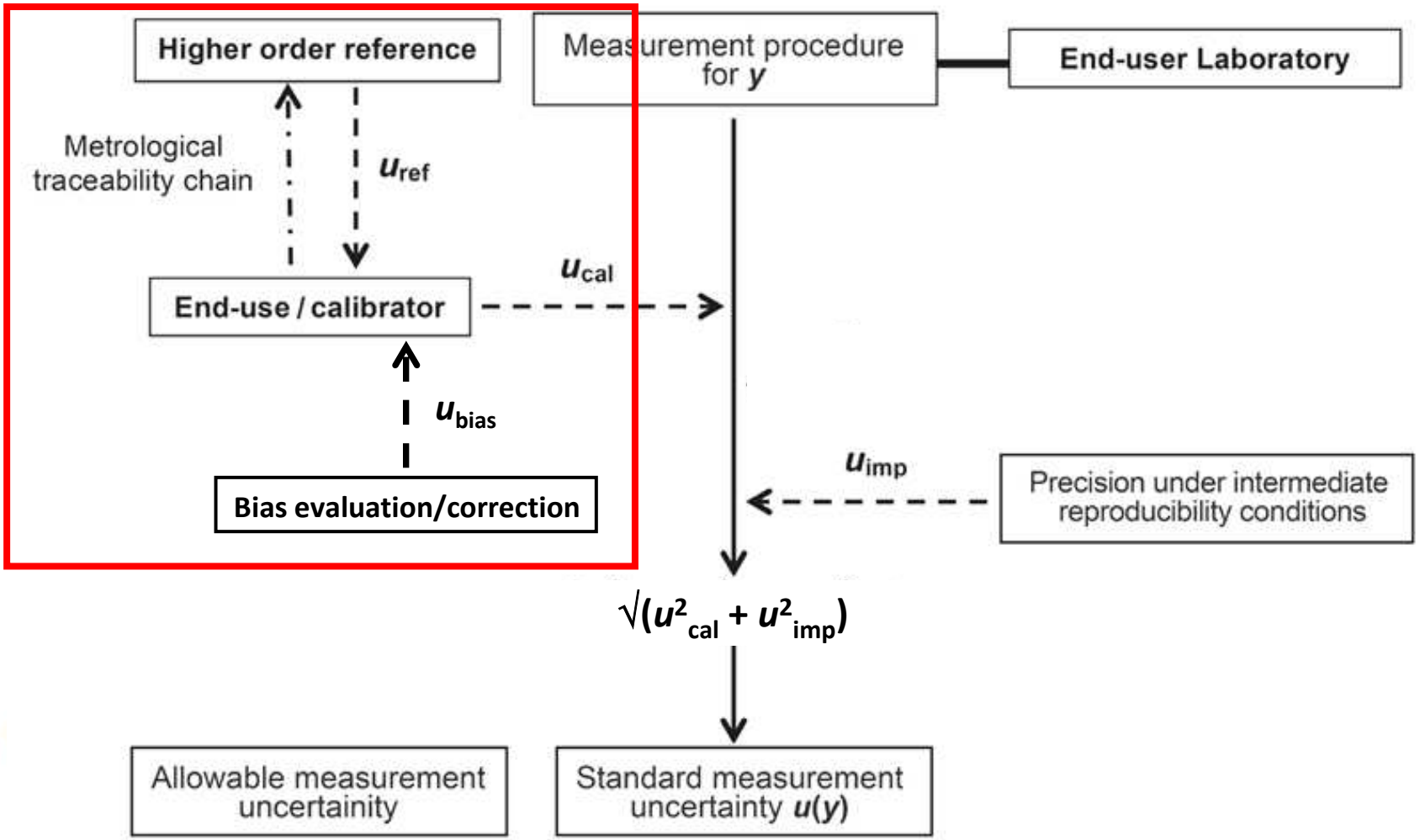
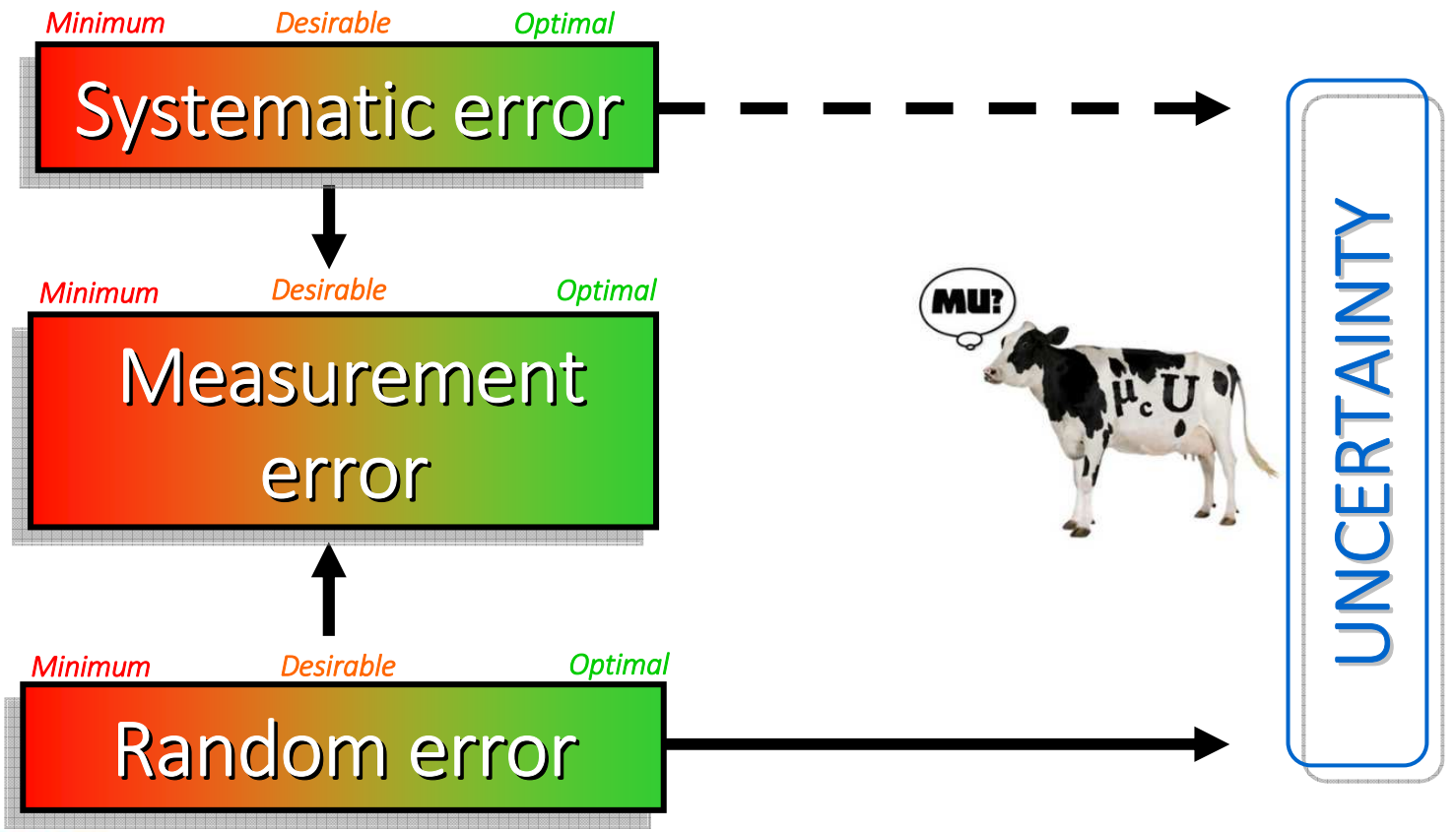
Sources of MU with the 'top-down' approach



Sources of MU with the 'top-down' approach



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



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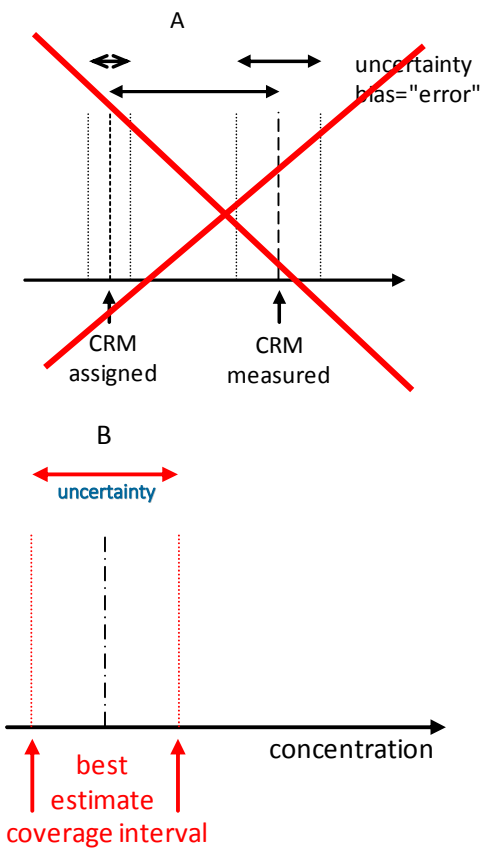
Role of IVD manufacturers



1) Elimination of measurement bias relative to the higher-order reference selected

CRM = certified reference material

2) Estimation of combined MU @ the calibrator level

Clinical laboratories have to rely on the manufacturers who must ensure traceability of their analytical systems to the highest available level. Therefore, *estimation of a bias by the end-user laboratory should be rarely required*.

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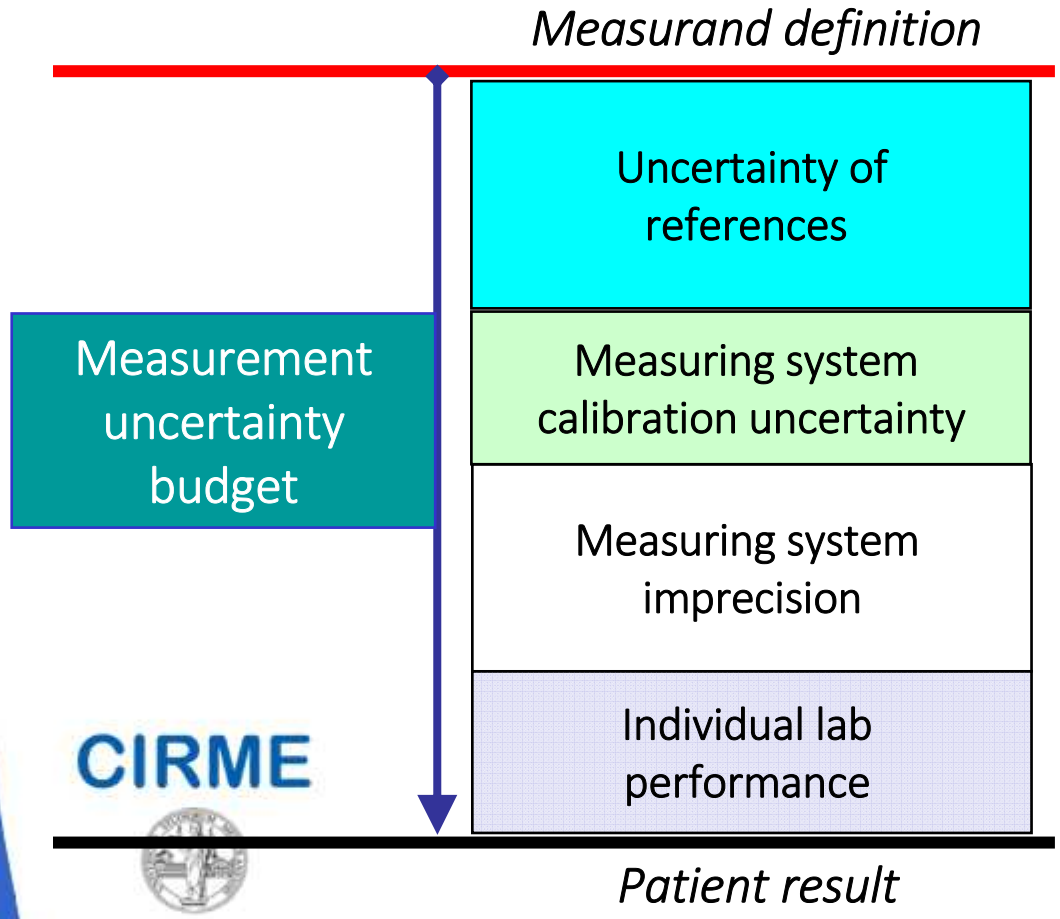
Commercial calibrator MU [u_{cal}]



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u_{cal} must be a combination of all uncertainties introduced by the selected calibration hierarchy for the measurand beginning with the highest available reference down to the assigned value of the calibrator for the end-user IVD medical device.

Manufacturers should estimate the combined uncertainty!

$$u_{cal} = (u^2_{ref} + u^2_{value\ ass})^{½}$$

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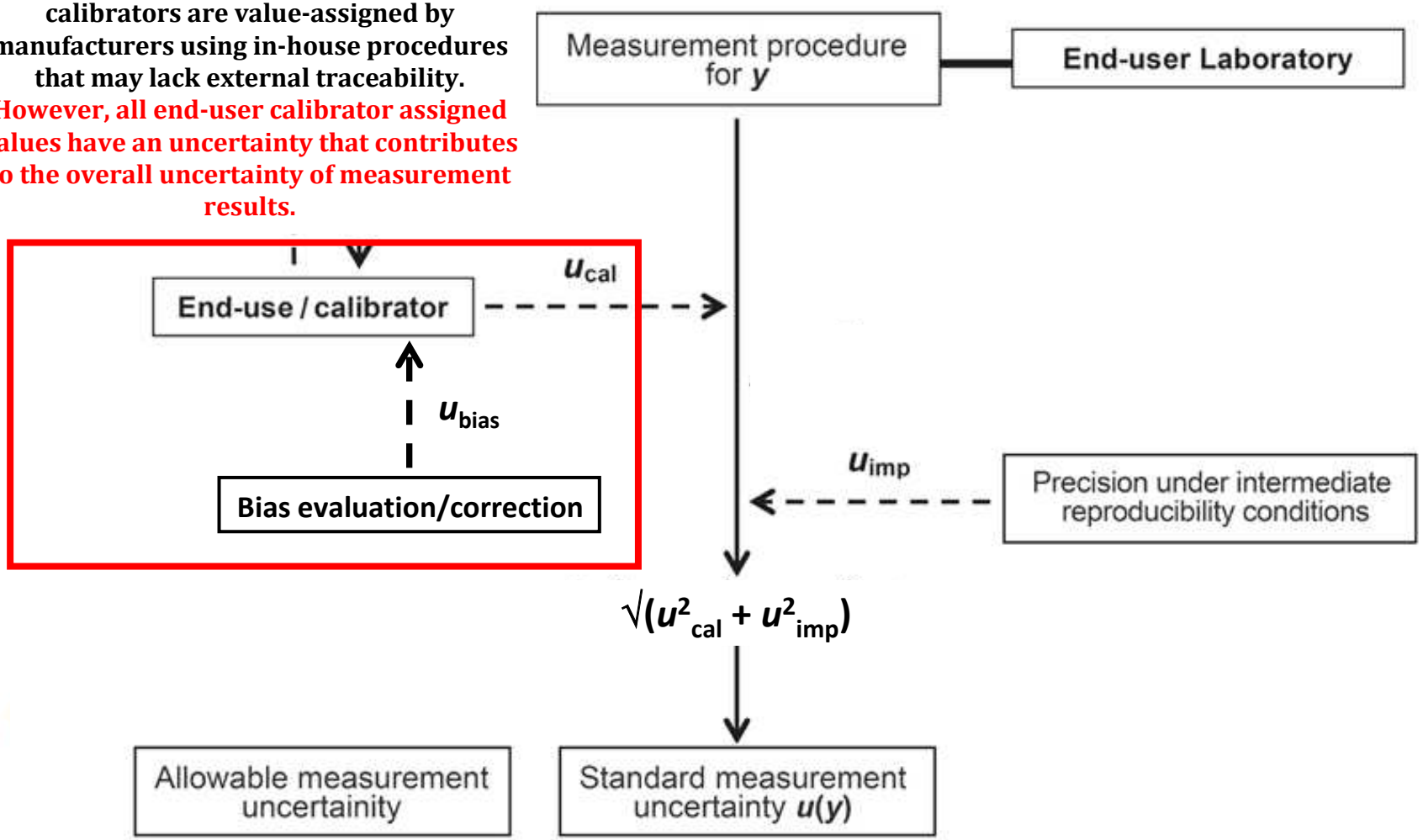
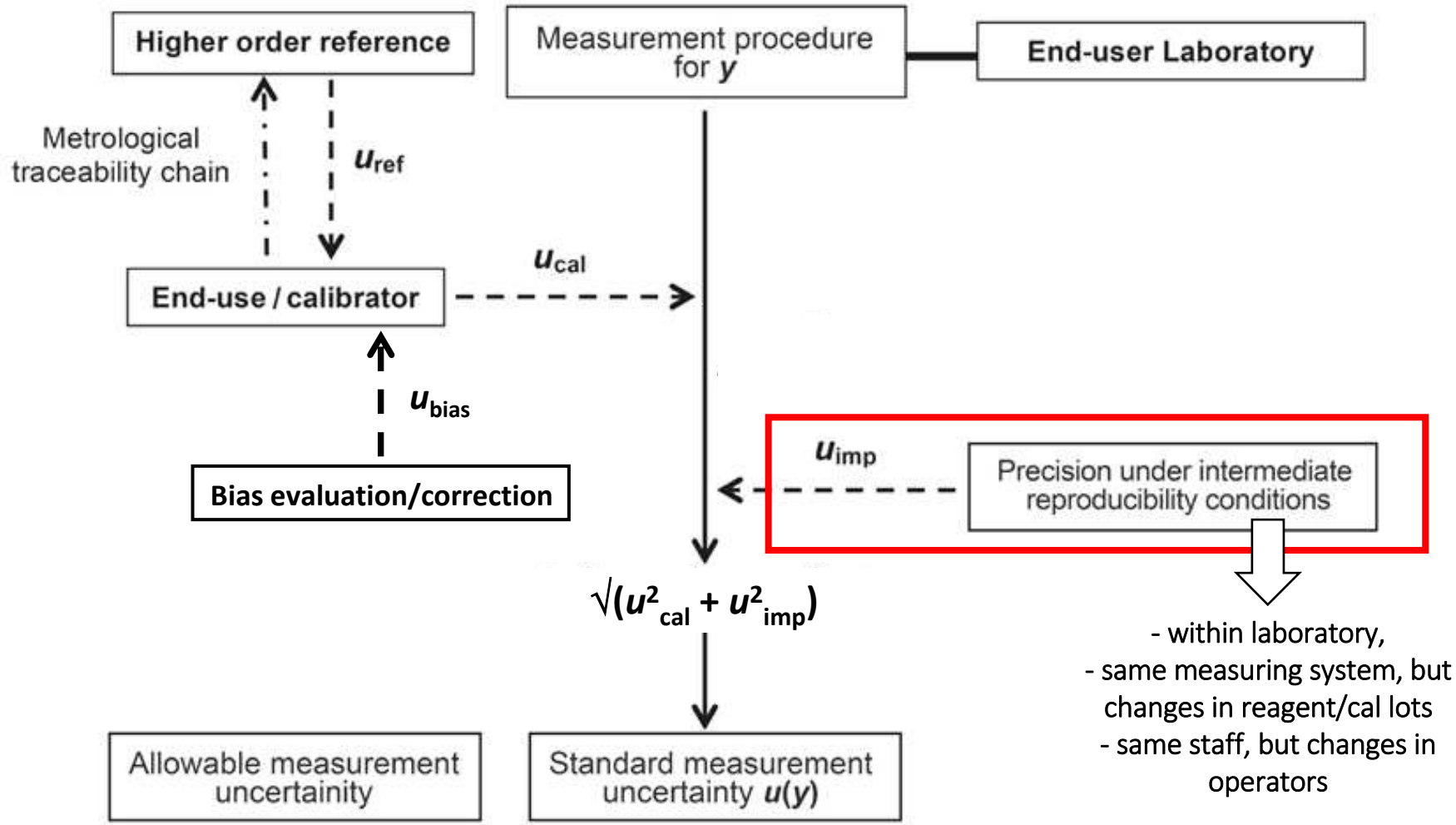
Sources of MU with the 'top-down' approach

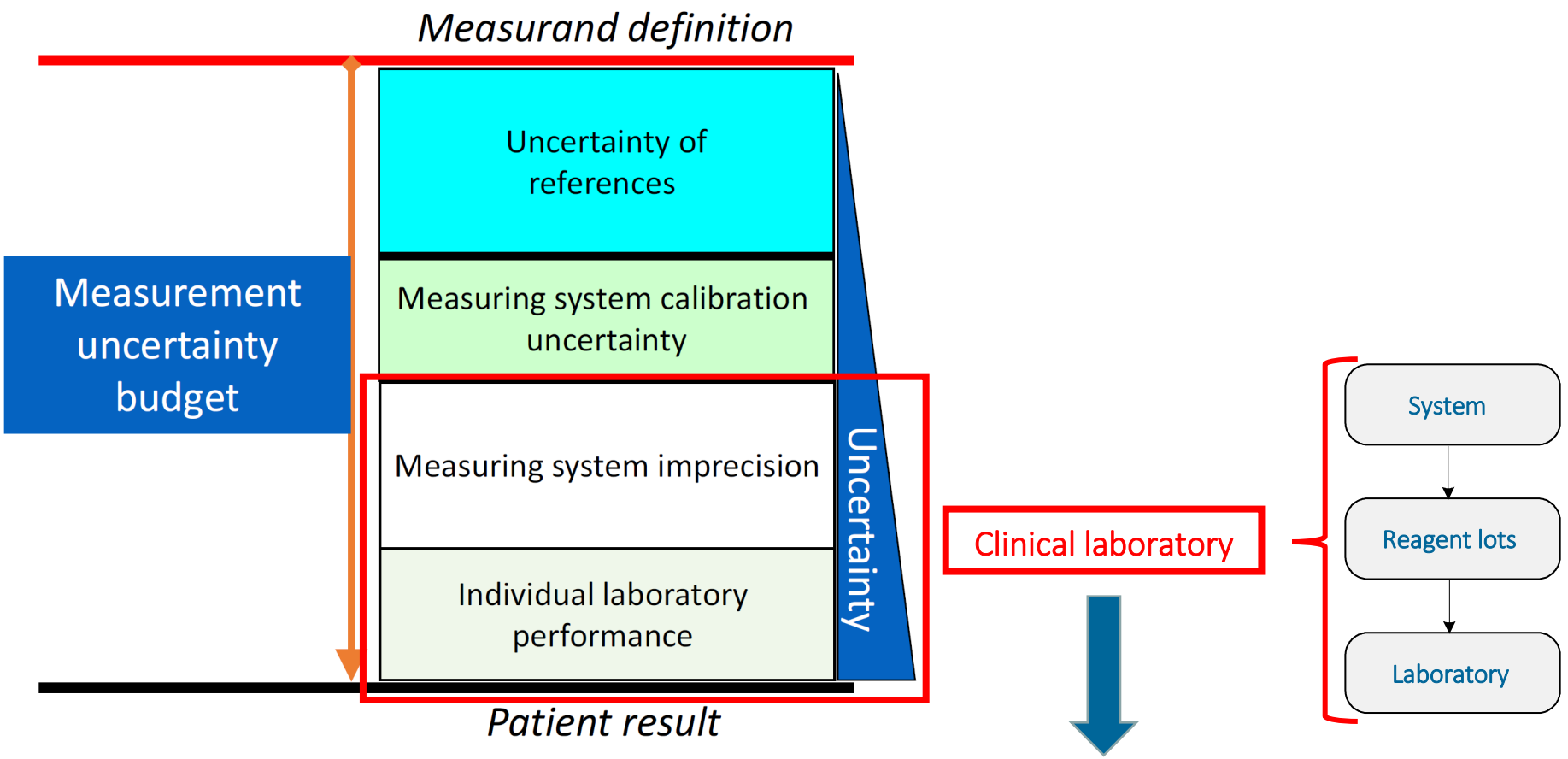
Higher-order references do not currently exist for some measurands, in which case calibrators are value-assigned by manufacturers using in-house procedures that may lack external traceability.
However, all end-user calibrator assigned values have an uncertainty that contributes to the overall uncertainty of measurement results.



Sources of MU with the 'top-down' approach



Uncertainty margins for clinical laboratories



The individual laboratory should monitor the variability of the measuring system used locally through the Internal Quality Control

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Testing MU due to the random effects [u_{Rw}]: characteristics of control material

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Additional attributes to be considered in selection of suitable IQC materials for estimating u_{Rw} include but are not limited to:

— material provided preferably by a third-party (i.e. different from that used to check the alignment of the measuring system);

— material that closely resembles authentic clinical samples (ideally a commutable material);

— material(s) with an amount of substance (measurand concentration) appropriate to the intended medical application of the analyte[26].

— Braga et al.: Performance criteria for combined uncertainty budget

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Clin Chem Lab Med 2015;53:905

Table 1: Main characteristics for a control material to be used in the internal quality control component II program in order to derive the uncertainty of the analytical system due to the random effects.

Characteristic	Remarks
Matrixed material from a third-party independent source should be used (e.g., fresh-frozen pool)	Material must be different from the system control material used for checking its alignment
Material should closely resemble to authentic patient samples (fulfil commutability)	Commercial non-commutable controls may provide a different impression of imprecision performance
Material concentrations should be appropriate to the clinical application of the analyte	When clinical decision cut-points are employed for a given analyte, samples around these concentrations should preferentially be selected

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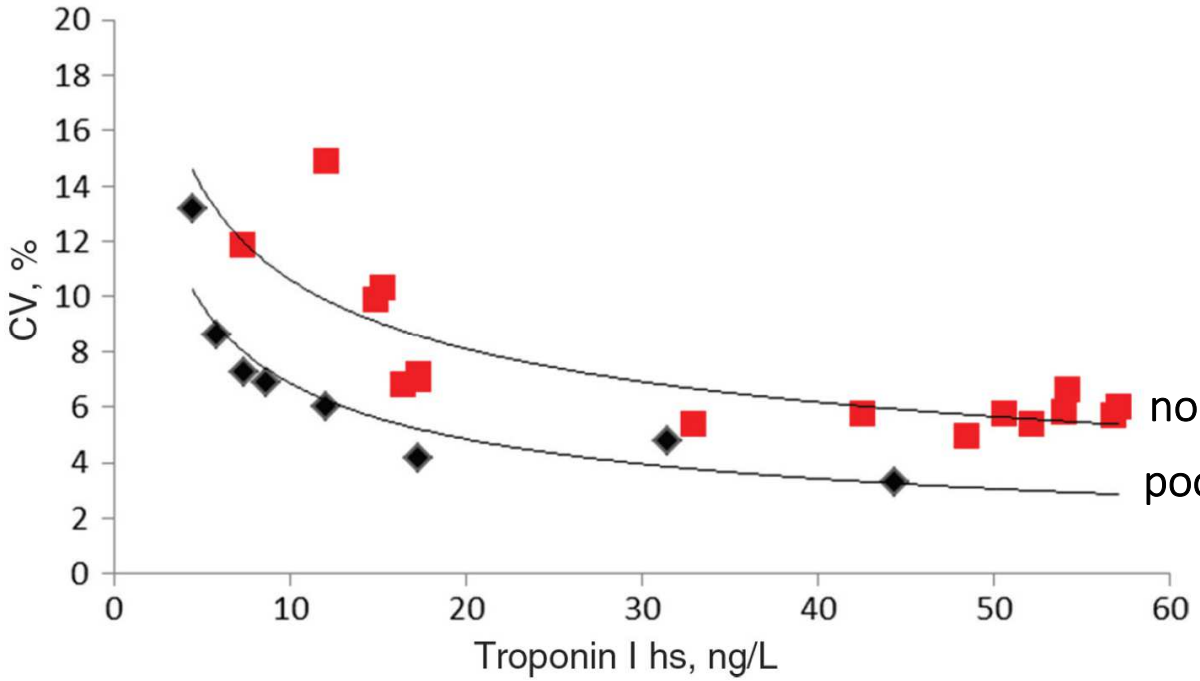
It is generally assumed that for a given measurement procedure the magnitude of imprecision for both IQC and typical human samples is similar, so that a standard uncertainty calculated for an IQC material is considered applicable to human samples with similar measurand values. This assumption should be validated by a performing a precision study of representative human samples and relevant IQC material(s) and their variances compared



Hage-Sleiman et al. Clin Chem Lab Med 2019;57:e49

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Testing MU due to the random effects [u_{Rw}]

Within-laboratory imprecision for a period sufficient to include most changes to measuring conditions... This uncertainty will be a suitable estimate of the uncertainty expected during daily or regular use of a measuring system.

The intermediate reproducibility should be estimated from consecutive 6-month data in order to capture systematic sources of uncertainty, such as those caused by different lots of reagents, different calibrations, different environmental conditions such as room temperature and humidity.

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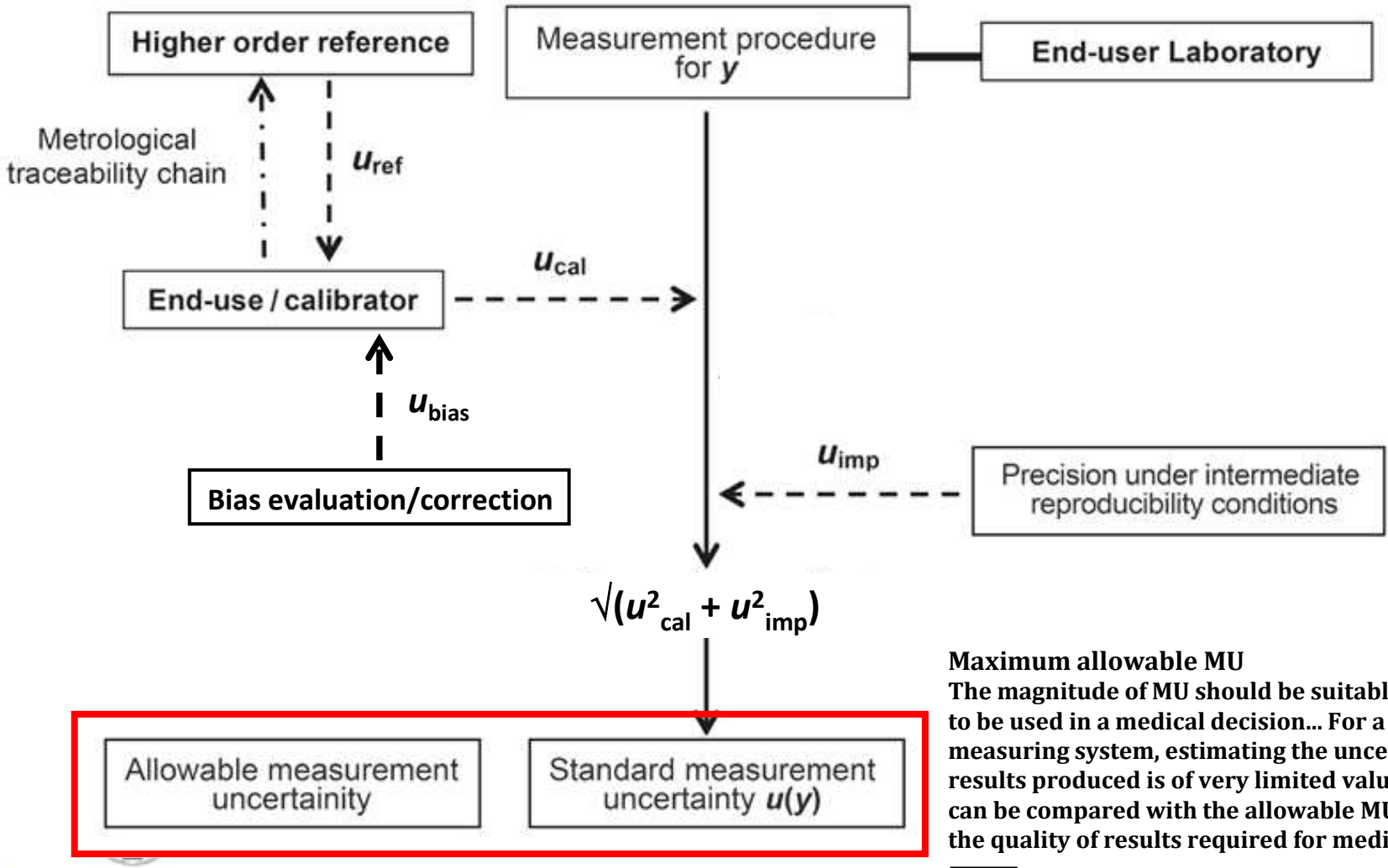
Sources of MU with the 'top-down' approach



Maximum allowable MU

The magnitude of MU should be suitable for a result to be used in a medical decision... For a given measuring system, estimating the uncertainty of the results produced is of very limited value unless it can be compared with the allowable MU based on the quality of results required for medical use.

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DE GRUYTER

Clin Chem Lab Med 2017; 55(2): 189–194

Opinion Paper

Ferruccio Ceriotti*, Pilar Fernandez-Calle, George G. Klee, Gunnar Nordin, Sverre Sandberg, Thomas Streichert, Joan-Lluis Vives-Corrons and Mauro Panteghini, on behalf of the EFLM Task and Finish Group on Allocation of laboratory tests to different models for performance specifications (TFG-DM)

Criteria for assigning laboratory measurands to models for analytical performance specifications defined in the 1st EFLM Strategic Conference

APS model 1: outcome-based	APS model 2: biological variation	APS model 3: state-of-the-art
P-Cholesterol+ester	P-Sodium ion	U-Sodium ion
P-Cholesterol+ester in LDL	P-Potassium ion	U-Potassium ion
P-Cholesterol+ester in HDL	P-Chloride	U-Chloride
P-Triglycerides	P-Bicarbonate	U-Calcium ion
P-Glucose	P-Calcium ion	U-Magnesium ion
B-Hemoglobin A_{1c}	P-Magnesium ion	U-Phosphate (inorganic)
P-Albumin	P-Phosphate (inorganic)	U-Creatinine
P-Troponin T and P-troponin I	P-Creatinine	U-Urate
P-Thyrotropin	P-Cystatin C	
B-Hemoglobin	P-Urate	
B-Platelets	P-Proteins	
B-Neutrophil leukocytes	B-Erythrocytes	
	B-Erythrocyte volume fraction	
	B-Erythrocyte volume	
	P-Prothrombin time	
	P-activated partial thromboplastin time	

The measurand has a central role in diagnosis and monitoring of a specific disease

The measurand has a high homeostatic control

Neither central diagnostic role nor sufficient homeostatic control

EXAMPLE

Creatinine in serum has a strict metabolic control

Apply
MILAN APS MODEL 2

Clinical Chemistry 63:9
1527–1536 (2017)

Other Areas of Clinical Chemistry

The EuBIVAS Project: Within- and Between-Subject Biological Variation Data for Serum Creatinine Using Enzymatic and Alkaline Picrate Methods and Implications for Monitoring

Anna Carobene,[1,11*] Irene Marino,[1] Abdurrahman Coşkun,[2,11] Mustafa Serteser,[2] Ibrahim Unsal,[2] Elena Guerra,[1] William A. Bartlett,[3,11] Sverre Sandberg,[4,5,11] Aasne Karine Aarsand,[4,11] Marit Sverresdotter Sylte,[4] Thomas Røraas,[5,11] Una Ørvim Sølvik,[6] Pilar Fernandez-Calle,[7,11] Jorge Díaz-Garzón,[7] Francesca Tosato,[8] Mario Plebani,[8] Niels Jonker,[9,11] Gerhard Barla,[9] and Ferruccio Ceriotti[10] on behalf of the European Biological Variation Study of the EFLM Working Group on Biological Variation

Mean intra-individual biological variation (CV_I)
4.4%

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Setting APS for MU from Biological Variation (BV): Concept

If the intra-individual BV is high, the analytical requirements are relatively low.
If, on the other hand, the intra-individual BV is low, it increases the necessity to reduce the analytical part of the total variation.

$$V_{TOT} = (MU^2 + CV_I^2)^{1/2}$$

Measurement uncertainty

Intra-individual biological variability

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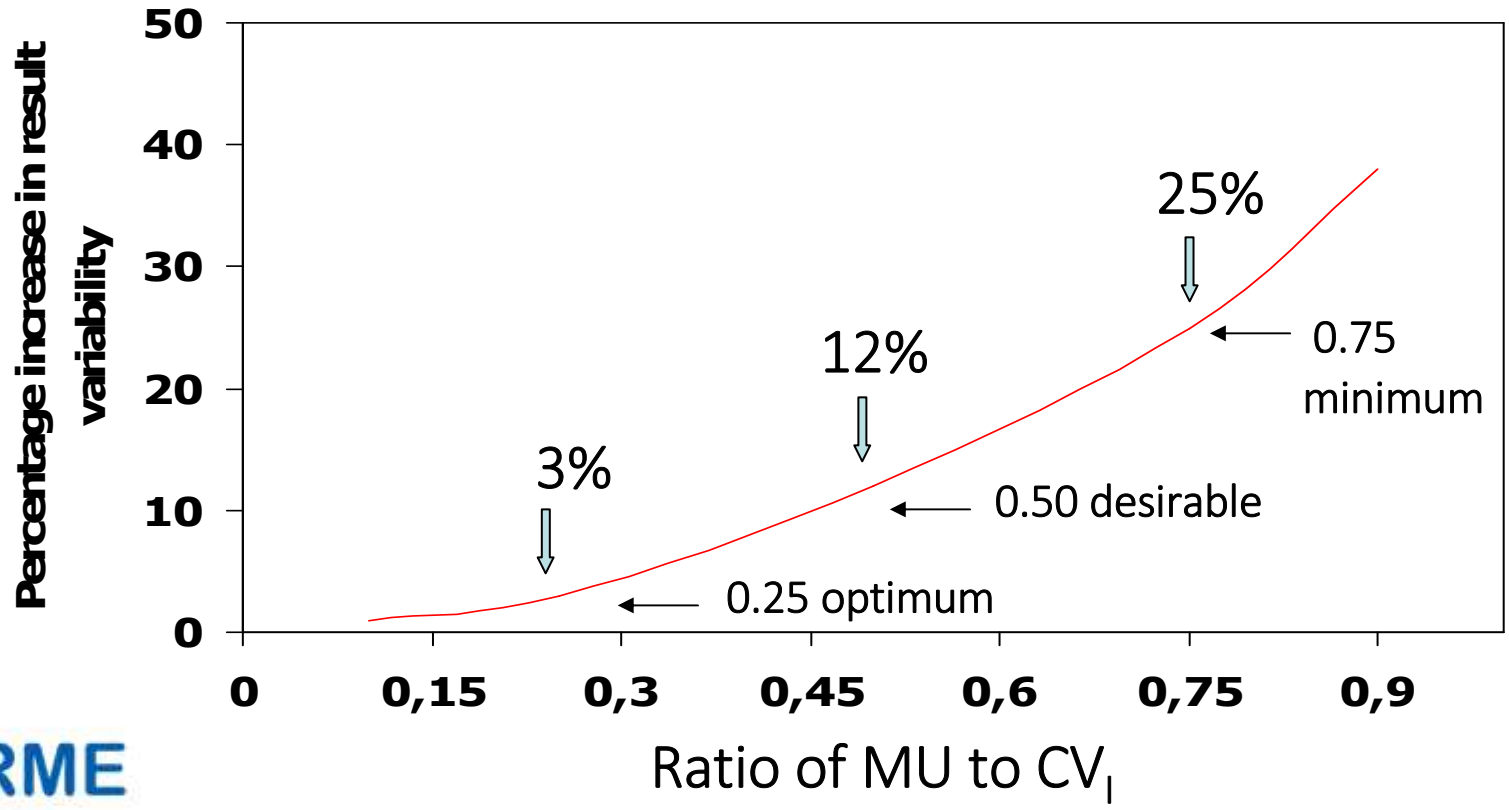
Impact of MU on total variability



[Adapted from Fraser CG et al. Ann Clin Biochem 1997;34:8]

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APS for MU of creatinine measurement on clinical samples

Biological variation model

Average CV_I = 4.4%

≤0.75 x CV_I (minimum) = 3.3%

≤0.50 x CV_I (desirable) = 2.2%

≤0.25 x CV_I (optimum) = 1.1%

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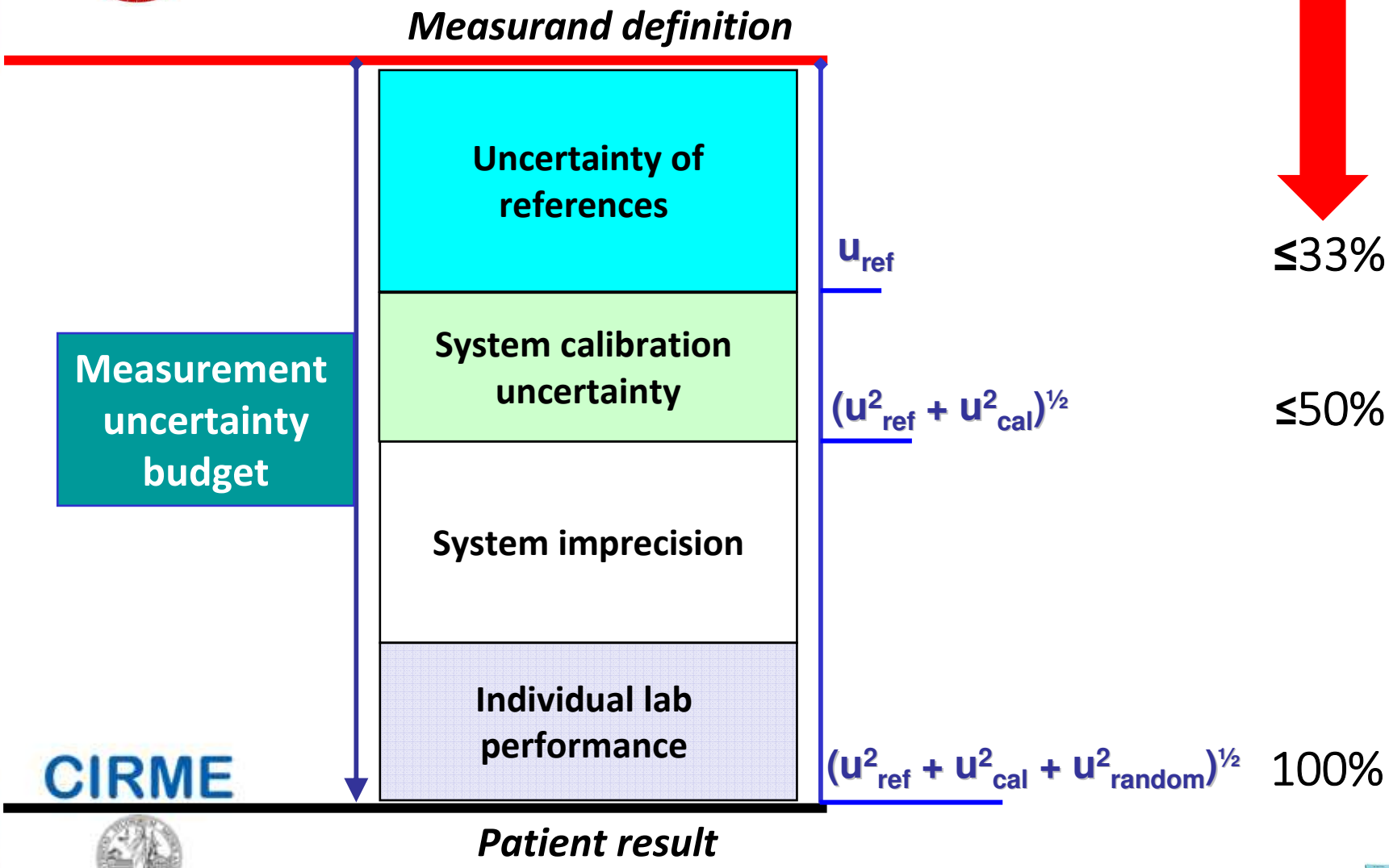
Recommended limits for combined MU budget (expressed as percentage of total budget goal)



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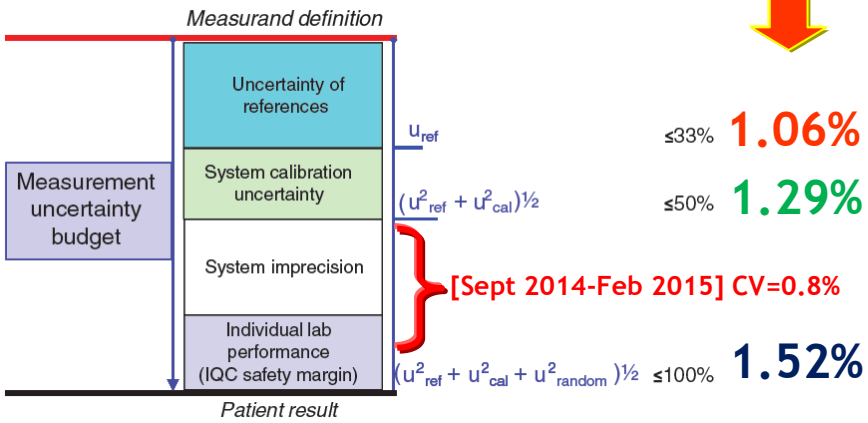
Braga F, Infusino I, Panteghini M. Clin Chem Lab Med 2015;53:905

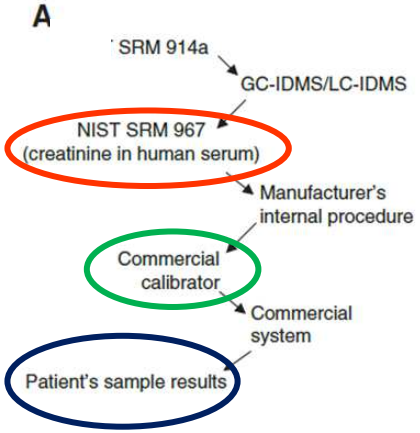
EXAMPLE

Performance in terms of MU of the Abbott Architect enzymatic creatinine assay

Abbott

Creatinine enzymatic assay (cod. 8L24)
Clin Chem Calibrator (LN 6K30)





From MILAN APS MODEL 2

3.3% minimum

2.2% desirable

1.1% optimum

Allowable limits for the standard MU of serum creatinine measured on clinical samples

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Clinical Biochemistry 57 (2018) 7–11

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Defining permissible limits for the combined uncertainty budget in the implementation of metrological traceability

Federica Braga*, Mauro Panteghini

Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy

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Time to move to practice

Now that the theory has been consolidated, it is necessary to widespread apply it in the laboratory medicine practice. Particularly, it becomes mandatory to verify for each analyte measured in the clinical laboratory if the status of the uncertainty budget of its measurement associated with the proposed metrological traceability chain is suitable for clinical application of the test.

Example 1: Glucose (Plasma)

		Desirable MU limit
Reference material	(NIST SRM 965b) **0.61-0.73%** (depends on the concentration level)	0.9% — 33% TB_U
XY manufacturer's calibrator	C1: 120 ± 2.4 mg/dL C2: 497 ± 10.0 mg/dL **≤1.25%**	1.35% — 50% TB_U
Clinical samples	***The end user has a margin until a CV of 2.4%***	 2.7% — TB_U

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The uncertainty of this measuring system has a *high probability* to fulfil the desirable APS for the total uncertainty budget (TB_u)

Example 2: Creatinine (Serum)

		Desirable MU limit
Reference material	(NIST SRM 967a) L1: 0.847 ± 0.018 mg/dL L2: 3.877 ± 0.082 mg/dL **1.06%**	0.75% — 33% TB_U
XY manufacturer's calibrator	4.0 ± 0.12 mg/dL **1.50%**	1.1% — 50% TB_U
Clinical samples	***The end user has a margin until a CV of 2.0%***	 2.2% — TB_U

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The uncertainty of this measuring system has a *medium probability* to fulfil the desirable APS for the total uncertainty budget (TB_u)

Example 3: Sodium (Serum)

		Desirable MU limit
Reference material	(NIST SRM 956d) 120 ± 0.7 mg/dL **0.29%**	0.17% — 33% TB_U
XY manufacturer's calibrator	C1: 120 ± 1.5 mmol/L **0.63%** C2: 160 ± 1.5 mmol/L **0.47%**	0.25% — 50% TB_U
Clinical samples	***The end user has no margin to fulfil specifications***	0.50% — TB_U

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The uncertainty of this measuring system has *no possibility* to fulfil the desirable APS for the total uncertainty budget (TB_u)

The importance of grading different quality levels for APS

To move, in case, from desirable to minimum quality goals and, in the meantime, ask reference providers/IVD manufacturers to work for improving the quality of assay performance

Panteghini et al.: Definition of performance specifications: 3 years from the Milan Conference Clin Chem Lab Med 2017

Example 3: Sodium (Serum)

		Minimum MU limit	
Reference material	(NIST SRM 956d) 120 ± 0.7 mg/dL **0.29%**	0.25%	33% TB_U
XY manufacturer's calibrator	C1: 120 ± 1.5 mmol/L **0.63%** C2: 160 ± 1.5 mmol/L **0.47%**	0.38%	50% TB_U
Clinical samples	***The end user has a margin until a CV of 0.6%***	0.75%	TB_U

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The uncertainty of this measuring system has a *realistic possibility* to fulfil the minimum APS for the total uncertainty budget (Tb_u)

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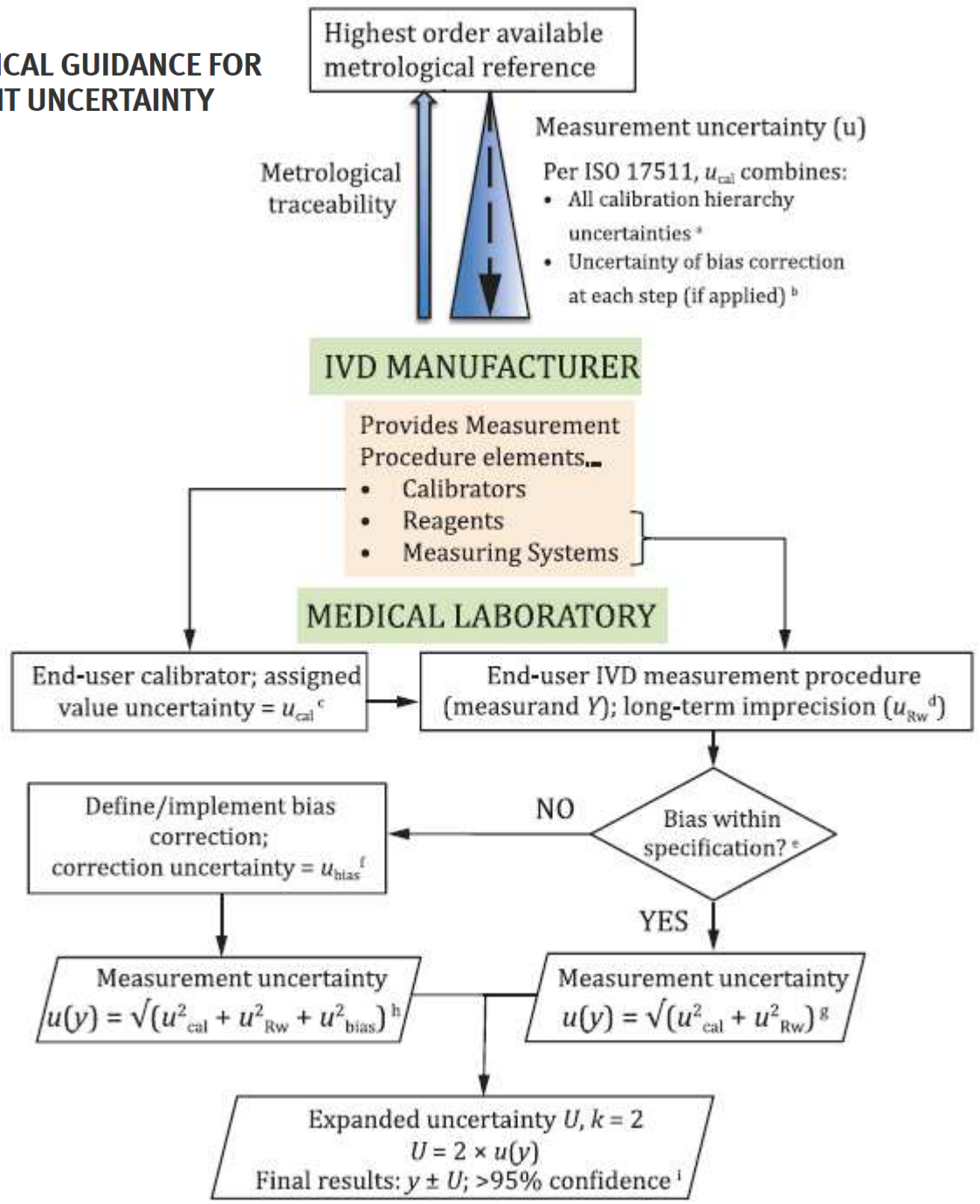

***Bias correction*: Appearance of a medically unacceptable measurement bias can be detected by EQA surveillance, but caution needs to be exercised. In addition to accounting for the commutability of the EQA material, the EQA target value can itself be biased, depending on how the value is assigned to the material.**

If unresolved by the manufacturer, the laboratory can introduce a correction factor. If so, the uncertainty of the correction factor, u_{bias}, needs to be estimated and included in the calculation of $u(y)$. Use of bias correction factors are not permitted by some national regulations.

How to deal with potential bias on clinical measurements

1. As the IVD measuring system is CE-marked and correct alignment to higher-order references is expected, just consider the uncertainty of the value assigned to the calibrator (that should include the uncertainty of the bias correction)
2. If a medically significant bias is shown in ongoing EQA surveillance (providing that they are organized as category IA/IIA), the bias against a reference (material or procedure) should be estimated and its values included in the estimate of MU of clinical samples
3. If this uncertainty is not fulfilling the predefined performance specification, it is the responsibility of the manufacturer to take an immediate investigation and eventually fix the problem with a corrective action (e.g. by improving the calibrator value-assignment protocol)

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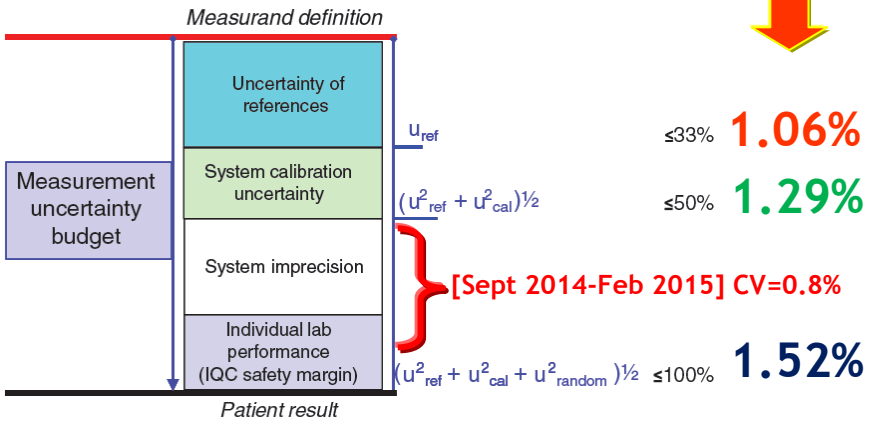
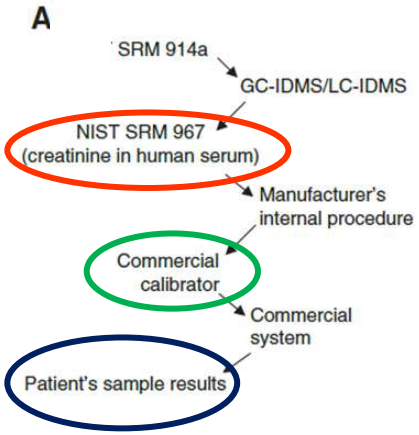
EXAMPLE

Abbott

Creatinine enzymatic assay (cod. 8L24)
Clin Chem Calibrator (LN 6K30)





From MILAN APS MODEL 2

3.3% minimum

2.2% desirable

1.1% optimum

Allowable limits for the standard MU of serum creatinine measured on clinical samples

CIRME

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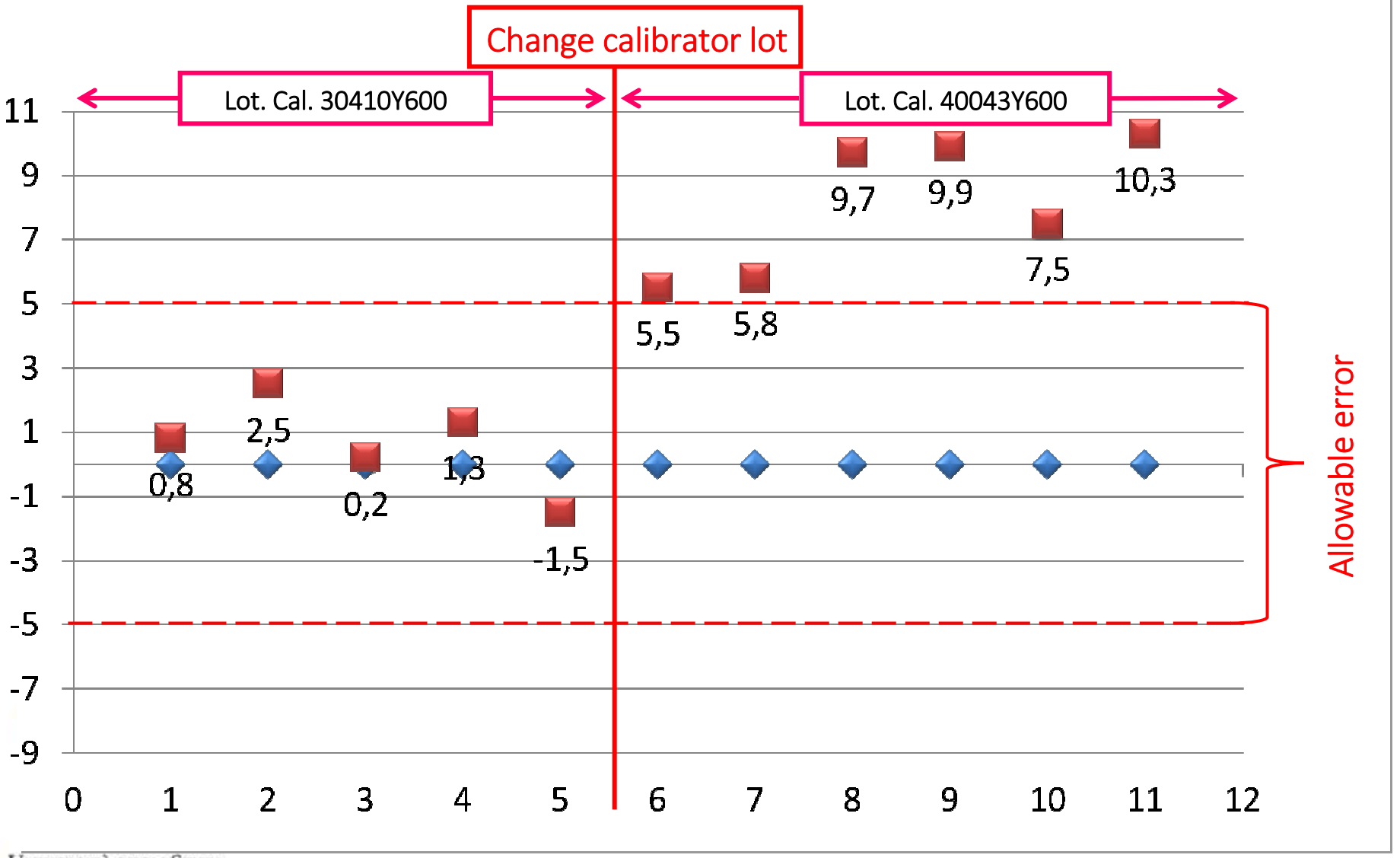
Case study: Creatinine



Pasqualetti S et al. CCA 2015;450:125

		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

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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%

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.

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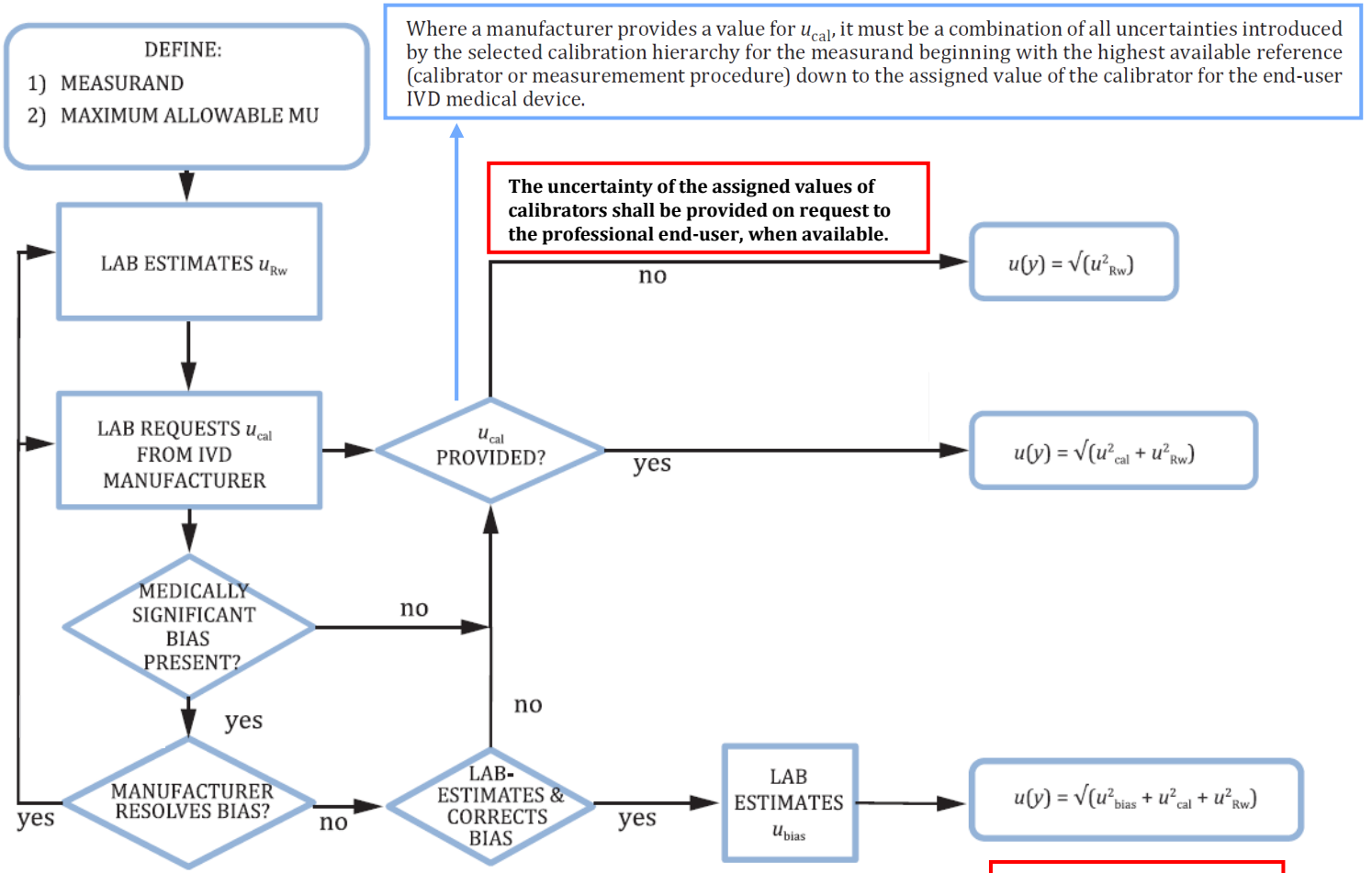
ISO/TS 20914:2019

MEDICAL LABORATORIES -- PRACTICAL GUIDANCE FOR THE ESTIMATION OF MEASUREMENT UNCERTAINTY



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Clinical Chemistry 63:9 (2017) 1551

the Clinical Chemist — *Unveiling the Right Side*

An Ode to "Measurement Uncertainty"

Usha Anand*

Once we learn how to calculate "measurement uncertainty" half the battle is won.
If we then ascertain if it affects the interpretation of our results, our job is almost done.