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Centre for Metrological Traceability in Laboratory Medicine (CIRME)

Director: Prof. Mauro Panteghini

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

Who, what and when to do in validation/verification of methods

Mauro Panteghini

Term definitions

- ***VALIDATION***
  - → **Validation of a laboratory method relates to the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled.**
- ***VERIFICATION***
  - → **Verification checks that the available evidence is sufficient to determine that a given assay fulfils specified requirements.**

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Analytical validation and verification: what to do?

- Calibration characterization & traceability
- Assay selectivity
- Indicators of trueness and precision
- Performance characteristic limits (LoD, LoQ)
- Indicators of measurement range
- Interferences & pre-analytical factors
- Reference interval

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Analytical validation and verification of assays: whose responsibility is it?

VALIDATION **VERIFICATION**

edma
DIAGNOSTICS FOR HEALTH

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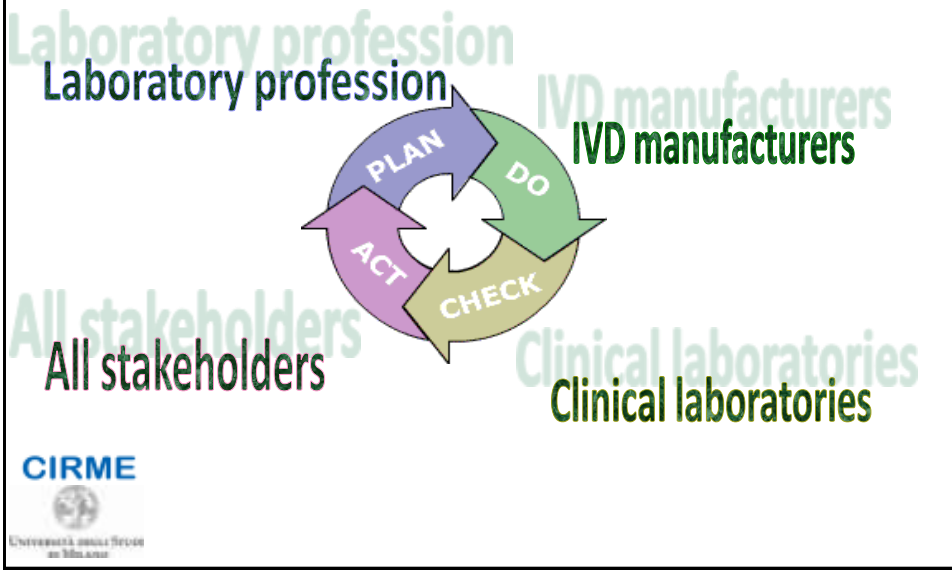

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Profession (e.g., JCTLM, EFLM):	**Define analytical objectives: reference measurement systems (traceability chain) and associated clinically acceptable uncertainty (fitness for purpose)**
	⬇
Diagnostic manufacturers:	**Implement suitable analytical systems (platform, reagents, calibrators, controls) fulfilling the above established goals**
	⬇
End users (clinical laboratories):	**Survey assay and laboratory performance through IQC and EQA redesigned to meet metrological criteria**

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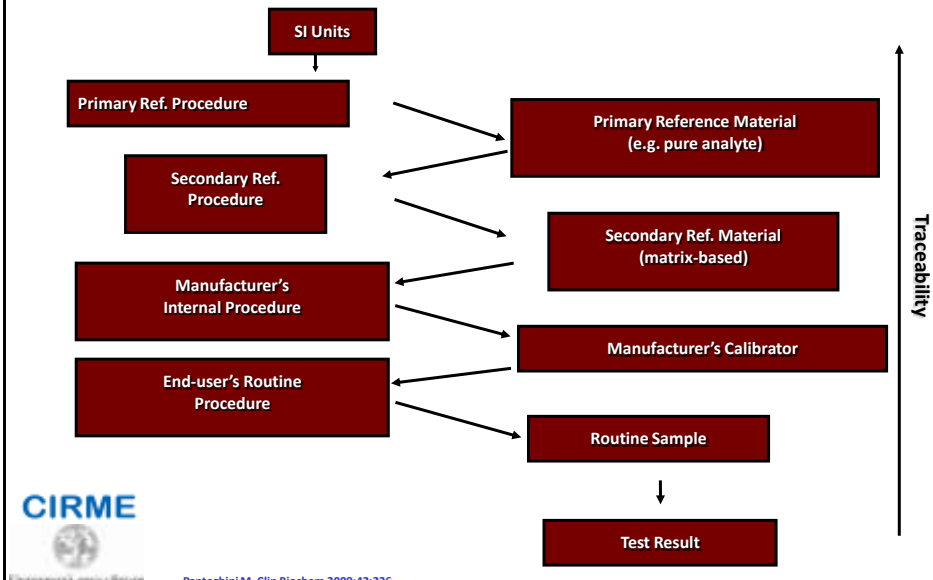
Adapted from Panteghini M, Clin Chem Lab Med 2010;48:7

Reference Measurement System



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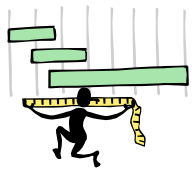
Panteghini M, Clin Biochem 2009;42:236



Basic requirements to establish traceability

- Establishment of a calibration hierarchy
- Establishment of the metrological traceability for the measurement results (understand the measurements)
- Elimination of measurement bias
- Adequate estimation of measurement uncertainties

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Joint Committee for Traceability in Laboratory Medicine (JCTLM)

The World's only quality-assured database of:

a) Higher Order Reference Materials
b) Higher Order Reference Measurement Procedures
c) Accredited Laboratory Reference Measurement Services

For use by (primarily):

a) IVD industry (to assist them in following the EU Directive on compliance and traceability of commercial systems)
b) Regulators (to verify that results produced by IVDs are traceable to)

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Panteghini M. Clin Biochem 2009;42:236

DE GRUYTER

Sverre Sandberg*, Callum G. Fraser, Andrea Rita Horvath, Rob Jansen, Graham Jones, Wytze Oosterhuis, Per Hyltoft Petersen, Heinz Schimmel, Ken Sikaris and Mauro Panteghini

Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine

Model 1: Based on the effect of analytical performance on clinical outcomes

a. Done by direct outcome studies – investigating the impact of analytical performance of the test on clinical outcomes;

b. Done by indirect outcome studies – investigating the impact of analytical performance of the test on clinical classifications or decisions and thereby on the probability of patient outcomes, e.g., by simulation or decision analysis.

Model 2: Based on components of biological variation of the measurand.

Model 3: Based on state of the art of the measurement (i.e., the highest level of analytical performance technically achievable).

Possible criteria for allocation of laboratory tests to different models for performance specifications

1. The measurand has a central role in diagnosis and monitoring of a specific disease ⇒ outcome model
2. The measurand has a high homeostatic control ⇒ BV model
3. Neither central diagnostic role nor sufficient homeostatic control ⇒ state-of-the-art model

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Role of IVD manufacturers

IVD manufacturers should define a calibration hierarchy to assign traceable values to their system calibrators and to fulfil during this process uncertainty limits, which represent a proportion of the uncertainty budget allowed for clinical laboratory results.

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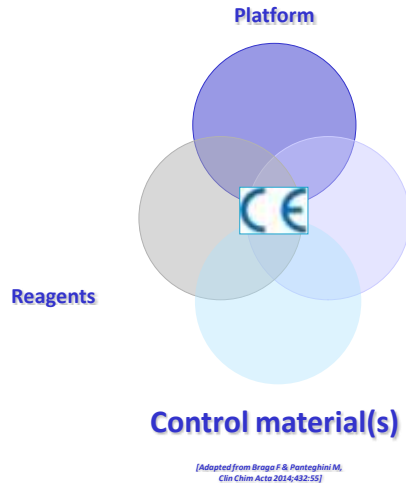
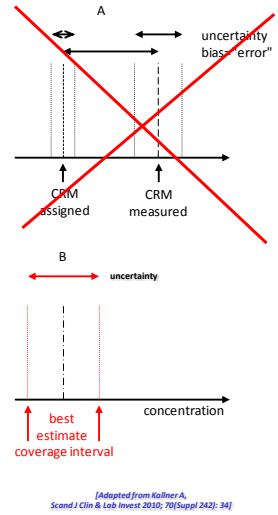
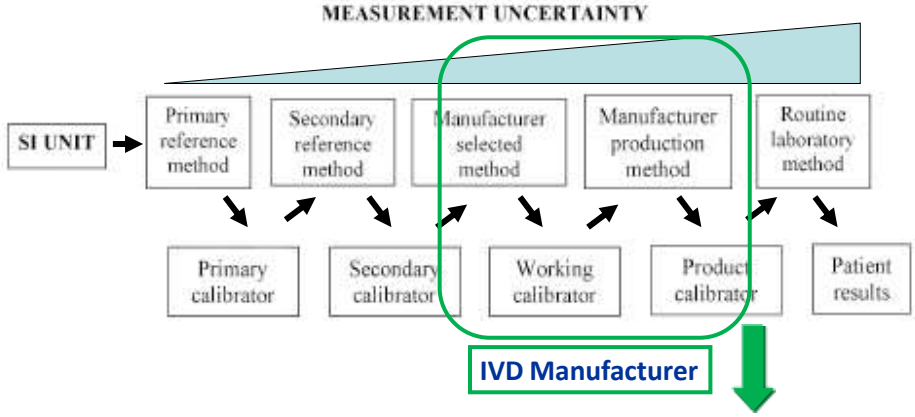
Braga F & Panteghini M, Clin Chim Acta 2014;432:55



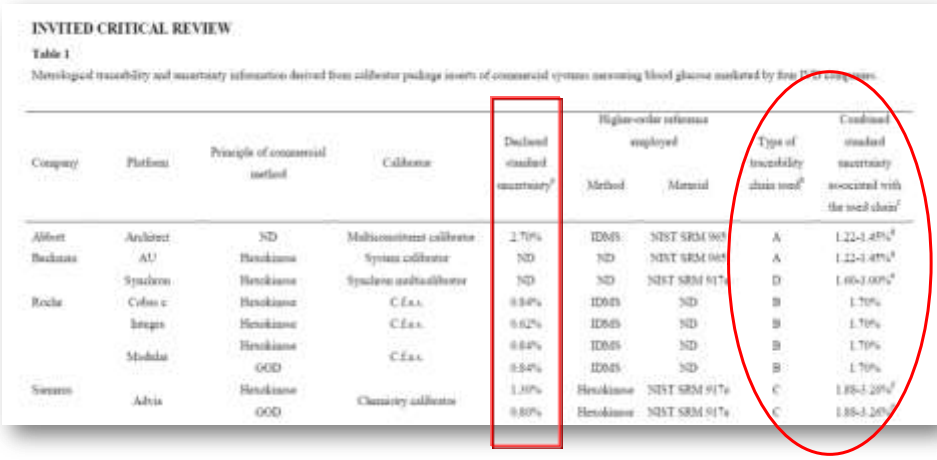


Thus, clinical laboratories need to rely on the manufacturers who must ensure traceability of their analytical system to the highest available level

Measurement uncertainty budget



The manufacturer must indicate the *combined* uncertainty associated with calibrators when used in conjunction with other components of the analytical system (platform and reagents). Such uncertainty estimates provided by the manufacturer should include the uncertainty associated with higher levels of the metrological traceability chain.



INVITED CRITICAL REVIEW

Table 1

Metrological traceability and uncertainty information derived from calibrator package inserts of commercial systems measuring blood glucose marketed by four IVD companies.

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	1.22-1.48%[d]
Beckman	AU	Hexokinase	System calibrator	ND	ND	NIST SRM 965	A	1.22-1.48%[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		0.84%	IDMS	ND	B	1.70%
Siemens	Advia	Hexokinase	Chemistry calibrator	1.10%	Hexokinase	NIST SRM 917a	C	1.88-3.20%[f]
		GOD		0.80%	Hexokinase	NIST SRM 917a	C	1.88-3.20%[f]

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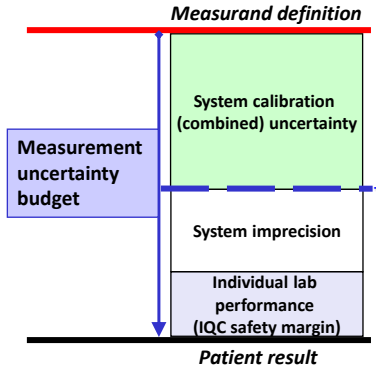
Braga F & Panteghini M, Clin Chim Acta 2014;432:55

Need to define criteria for manufacturers that can be achieved for their calibrators leaving enough uncertainty budget for the laboratories to produce clinically acceptable results.



→ **Allowable limit for the combined uncertainty of manufacturer's commercial calibrators @ 50% of the goals**

[note that these are goals for random variability, as at the calibrator level the systematic error (bias), in agreement with the metrological traceability theory, must be corrected if present in a non negligible amount]

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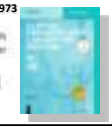
Opinion Paper

Clin Chem Lab Med 2013; 51:973



Defining acceptable limits for the metrological traceability of specific measurands

Pasqualetti S, Infusino I, Carnevale A, Szőke D, Panteghini M.

Clin Chim Acta. 2015 Aug 11. pii: S0009-8981(15)00378-2. doi: 10.1016/j.cca.2015.08.007. [Epub ahead of print]

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

Letter to the editor

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

Note: For serum creatinine measurements on patient samples, the acceptable limits for expanded uncertainty derived from its CV_I are 6.0% (desiderable) and 9.0% (minimum quality level), respectively.

Table 1
Uncertainties for each contributing factor in determination of serum creatinine with Abbott enzymatic assay on Architect c16000 platform after calibration with two different lot of system calibrator. Data obtained by measurements of NIST SRM 967a reference material (certified value ± expanded uncertainty: L1, 0.847 mg/dL ± 0.018 mg/dL and L2, 3.877 mg/dL ± 0.082 mg/dL).

	SRM 967a level 1	SRM 967a level 2
Multigent Clin Chem Calibrator lot no. 40043V600		
Imprecision (u_{Rw})	0.47%	0.40%
Bias (u_{bias})	3.57%	7.05%
Relative combined standard uncertainty [$u_c = (u_{bias}^2 + u_{Rw}^2)^{1/2}$]	3.60%	7.06%
Expanded uncertainty ($U = k \times u_c$)	7.20%	14.12%
Multigent Clin Chem Calibrator lot no. 40495V600		
Imprecision (u_{Rw})	0.53%	0.42%
Bias (u_{bias})	4.02%	1.71%
Relative combined standard uncertainty [$u_c = (u_{bias}^2 + u_{Rw}^2)^{1/2}$]	4.05%	1.76%
Expanded uncertainty ($U = k \times u_c$)	8.10%	3.52%

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Availability and quality of information about IVD metrological traceability and uncertainty

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Currently, the full information about calibration is usually not available

Manufacturers only provide the name of higher order reference material or procedure to which the assay calibration is traceable, without any description of implementation steps and their corresponding uncertainty.

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DE GRUYTER Clin Chem Lab Med 2025; 63(6): 905–912

Opinion Paper

Federica Braga*, Ilenia Infusino and Mauro Panteghini

Performance criteria for combined uncertainty budget in the implementation of metrological traceability

Table 2: The information that in vitro diagnostics manufacturers should provide to laboratory users about the implementation of metrological traceability of their commercial systems. Adapted from [7].

a) An indication of higher order references (materials and/or procedures) used to assign traceable values to calibrators;
b) Which internal calibration hierarchy has been applied by the manufacturer, and
c) A detailed description of each step;
d) The (expanded) combined uncertainty value of commercial calibrators, and
e) Which, if any, acceptable limits for uncertainty of calibrators were applied in the validation of the analytical system.

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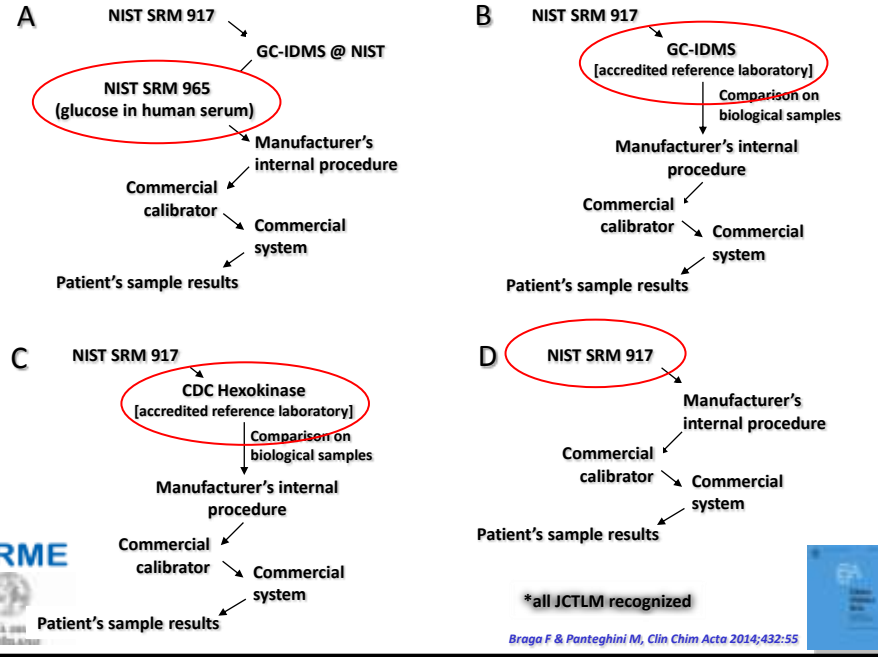
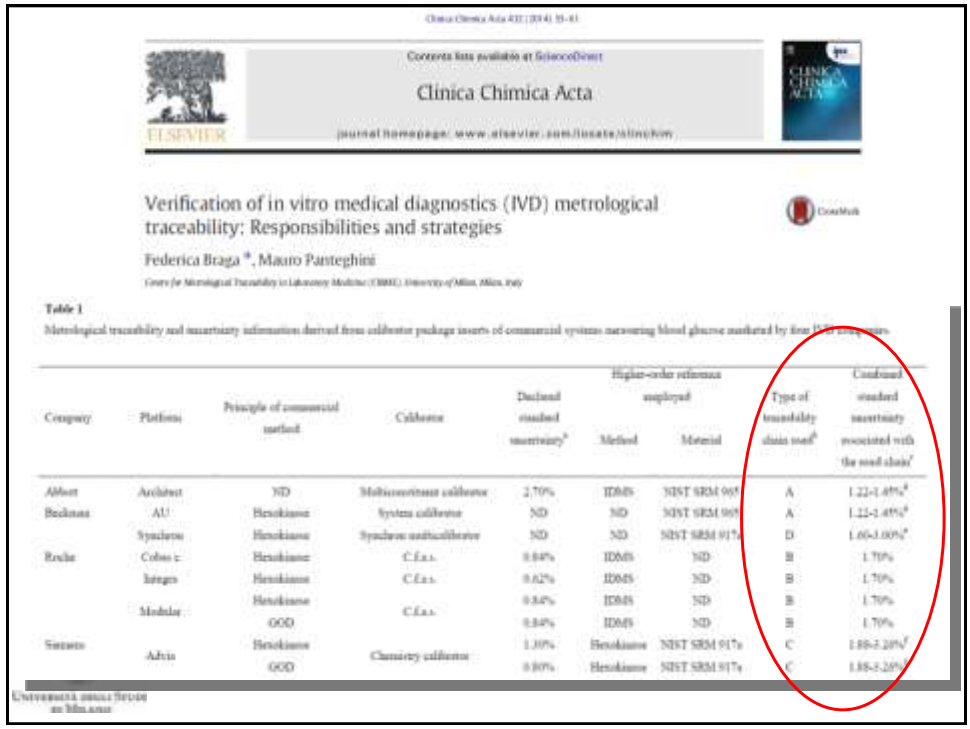
Types of metrological chains that can be used to implement the traceability of blood glucose results*





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Verification of in vitro medical diagnostics (IVD) metrological traceability: Responsibilities and strategies

Federica Braga *, Mauro Panteghini

Table 1

Company	Platform	Principle of commercial method	Calibrator	Declared standard uncertainty[a]	Higher-order reference employed: Method	Higher-order reference employed: Material	Type of traceability chain used[b]	Combined standard uncertainty associated with the used chain[c]
Abbott	Architect	ND	Multiconstituent calibrator	2.70%	IDMS	NIST SRM 965	A	1.22-1.48%[d]
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Siemens	Advia	Hexokinase	Chemistry calibrator	1.10%	Hexokinase	NIST SRM 917a	C	1.88-3.20%[d]
		GOD		0.80%	Hexokinase	NIST SRM 917a	C	1.88-3.20%[d]

The role of the end users: “check”

1. Availability and quality of information about IVD metrological traceability and uncertainty

2. Post-marketing surveillance of IVD system traceability

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Braga F & Panteghini M, Clin Chim Acta 2014;432:55

Profession (e.g., JCTLM, EFLM): Define analytical objectives: reference measurement systems (traceability chain) and associated clinically acceptable uncertainty (fitness for purpose)

Diagnostic manufacturers: Implement suitable analytical systems (platform, reagents, calibrators, controls) fulfilling the above established goals

Post-marketing surveillance of IVD metrological traceability

End users (clinical laboratories): Survey assay and laboratory performance through IQC and EQA redesigned to meet metrological criteria

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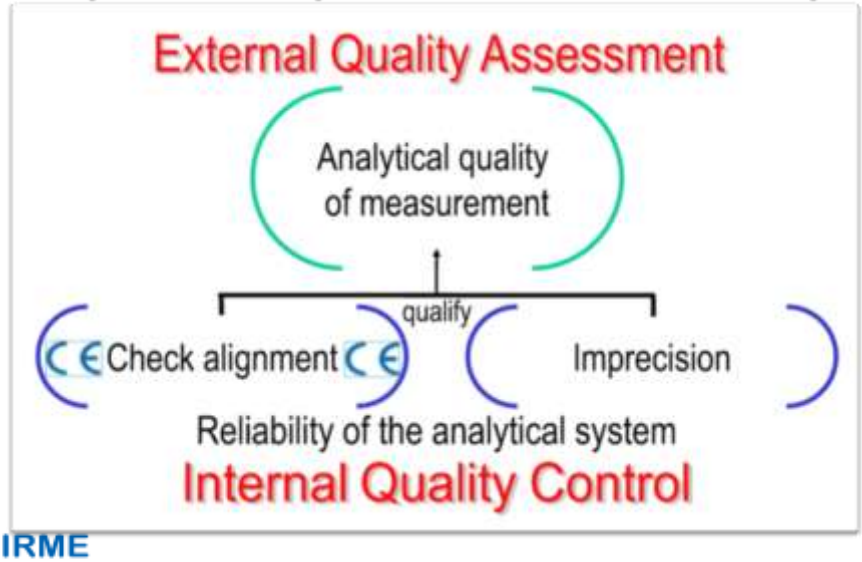
Adapted from Panteghini M, Clin Chem Lab Med 2010;48:7

Analytical Quality Control in the Traceability Era



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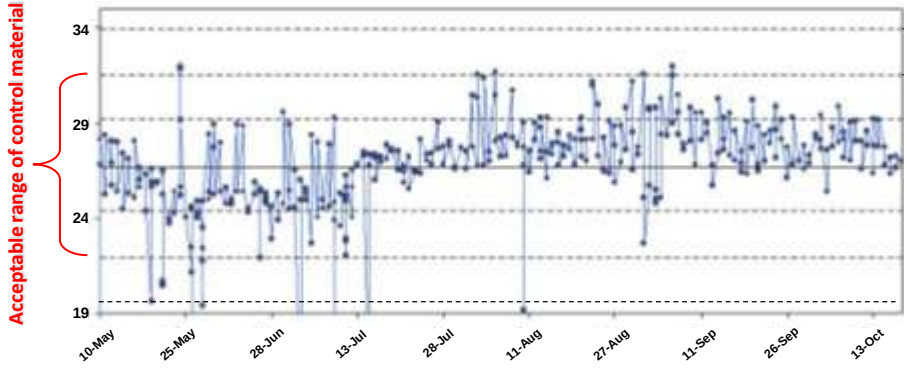
Panteghini M, Clin Chem Lab Med 2010;48:7
Braga F & Panteghini M, Clin Chim Acta 2014;432:55

Monitoring the reliability of the analytical system through IQC: Component I. Check alignment ("system traceability")

This program checks whether in the course of an analytical run the performance of an analytical system complies with the set goals, represented by the acceptable ranges of control materials.



Clinical laboratories must verify the consistency of declared performance during routine operations performed in accordance with the manufacturer's instructions, by checking that values of control materials provided by the manufacturer as component of the analytical system are in the established control range, with no clinically significant changes in the assumed traceable results.

Internal Quality Control (Component I)

Acceptance/rejection of the analytical run in "real time"

Testing alignment ["system traceability"]

Any "out of control" signal must be made available with sufficient time to allow immediate corrective actions to bring again the situation under control (virtually "unbiased") and before reports related to the samples analyzed in the affected analytical run are issued.

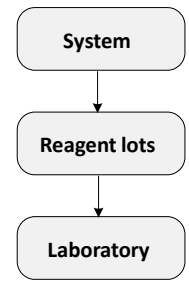
Braga F et al. J Med Biochem 2015;34:282-7

Internal Quality Control (Component II)

System stability at medium/long term

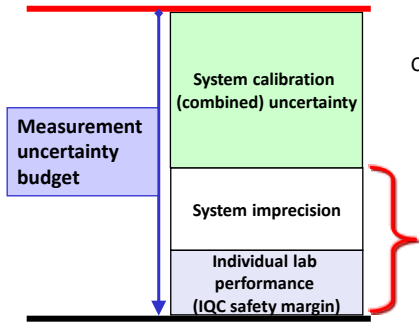
Estimating the measurement uncertainty due to random effects ("imprecision")

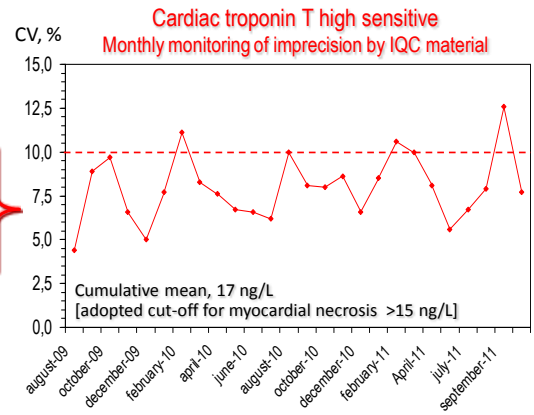
This program provides, through mechanisms of retrospective evaluation, data useful to the knowledge of variability of the analytical system and of its use by the individual laboratory.



Braga F et al. J Med Biochem 2015;34:282-7

Monitoring the reliability of the analytical system through IQC: Component II. Evaluate the system + individual lab imprecision





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Characteristics of a material to be used for the IQC component II programme

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

CI

Braga F, Infusino I, Panteghini M. J Med Biochem 2015;34:234-41
Braga F, Infusino I, Panteghini M. Clin Chem Lab Med 2015;53:905–12

Requirements for the applicability of EQAS results in the evaluation of the performance of participating laboratories in terms of traceability of their measurements

Feature	Aim
EQAS materials value-assigned with reference procedures by an accredited ref. laboratory	To check traceability of commercial system to reference systems
Proved commutability of EQAS materials	To allow transferability of participating laboratory performance to the measurement of patient samples
Definition and use of the clinically allowable measurement error	To verify the suitability of laboratory measurements in clinical setting

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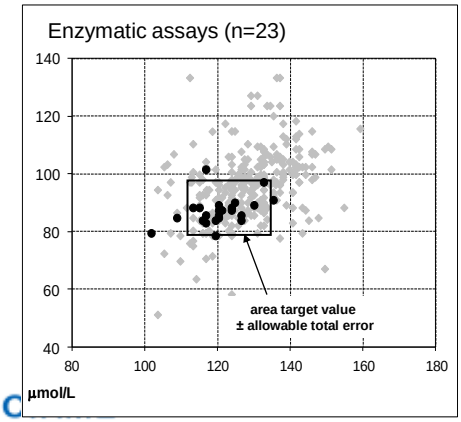
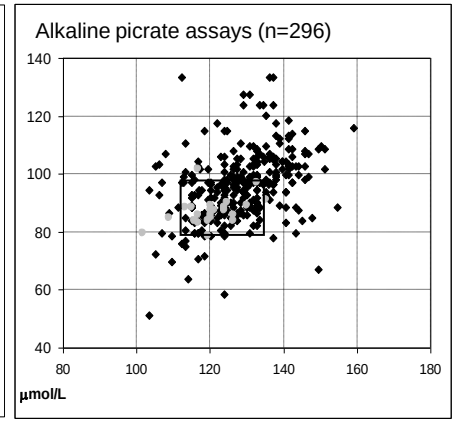
Panteghini M, CCLM 2010;48:7
Infusino I et al., CCLM 2010;48:301
Braga F & Panteghini M. CCLM 2013;51:1719
Braga F & Panteghini M, Clin Chim Acta 2014;432:55

EQAS materials with physiologic (88.4 μmol/L) and borderline (123.8 μmol/L) creatinine concentrations vs. the desirable goal for TE (±8.9%).
The vast majority (87%) of laboratories using systems employing enzymatic assays were able to fulfill the desirable performance, while only one third of laboratories using picrate-based systems were able to meet the target.





Carobene A et al., Clin Chim Acta 2014;427:100.

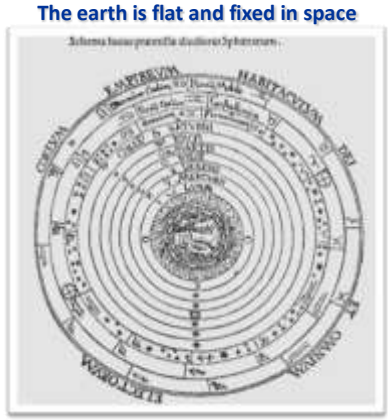
Unique benefits of EQAS that meet metrological criteria

- Giving objective information about quality of individual laboratory performance
- Creating evidence about intrinsic standardization status/ equivalence of the examined assays
- Serving as management tool for the laboratory and IVD manufacturers, forcing them to investigate and eventually fix the identified problem
- Helping manufacturers that produce superior products and systems to demonstrate the superiority of those products
- Identifying analytes that need improved harmonization and stimulating and sustaining standardization initiatives that are needed to support clinical practice guidelines
- Abandonment by users (and consequently by industry) of nonspecific methods and/or of assays with demonstrated insufficient quality

Braga F et al. J Med Biochem 2015;34:282-7
Ferraro S et al, Clin Chem Lab Med 2015; in press

What COPERNICUS did was take the existing 'a priori' concept of the world and pose an alternative 'a priori' concept

The earth is flat and fixed in space



The earth is spherical and moves around the sun

Equivalency-based grading

Trueness-based grading

What TRACEABILITY does is take the existing 'a priori' concept of the Quality Control and pose an alternative 'a priori' concept

Take a home massage

Who, what and when to do in validation/ verification of methods

- The major role of IVD manufacturers is to implement suitable analytical systems fulfilling the requirements for a specific intended use
- The uncertainty of the IVD manufacturer's calibrators should include the uncertanty associated with higher levels of metrological traceability chain
- The main scope of the IQC component I to check the alignment of the analytical system and verify the consistency of declared traceability
- The requirements for the applicability of EQA results in the performance evaluation of participating laboratories in terms of standardization and traceability of measurements need: a) use of commutable control materials, b) assignment of values (and uncertainty) to control materials with reference procedures performed by an accredited laboratory, c) apply a clinically acceptable allowable error limit

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