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Sistema Socio Sanitario
Regione Lombardia
ASST Fatebenefratelli Sacco

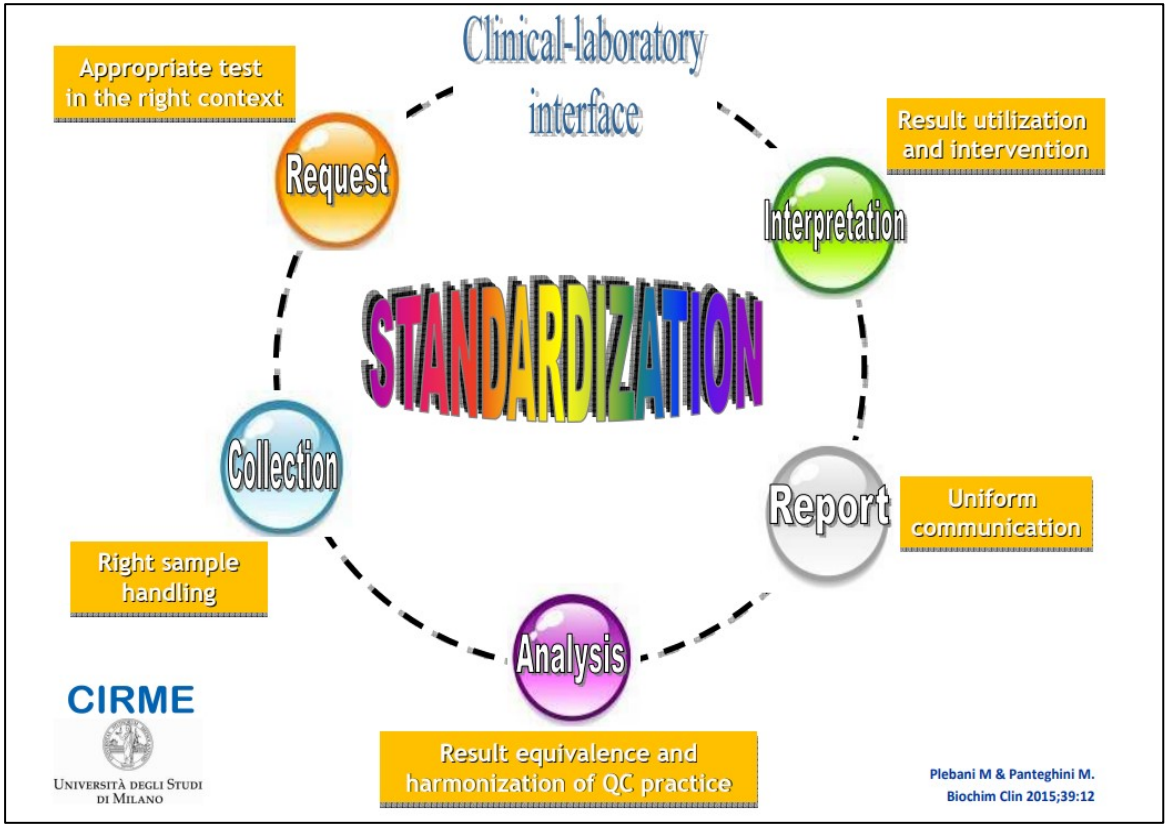
UNIVERSITÀ DEGLI STUDI DI MILANO

How to correctly validate the traceability of an IVD measuring system: practical examples

Dr. Elena Aloisio, M.D.
UOC Patologia Clinica
ASST Fatebenefratelli - Sacco

14th International Scientific Meeting
Implementation of metrological traceability in laboratory medicine: where we are and what is missing
Milan, November 30th 2022

STANDARDIZATION



"Standardization of laboratory tests has an ethical dimension as it aims to affect the way diagnostic tests are used in order to guarantee optimal care for patients in a global world."

Bossuyt X et al., Ann Rheum Dis 2008;67:1061

A matter of patient safety!!

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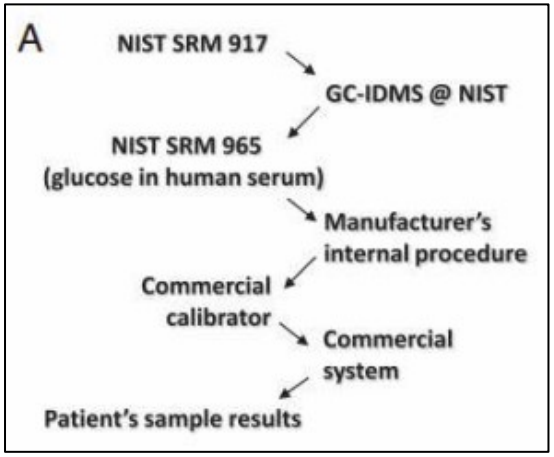
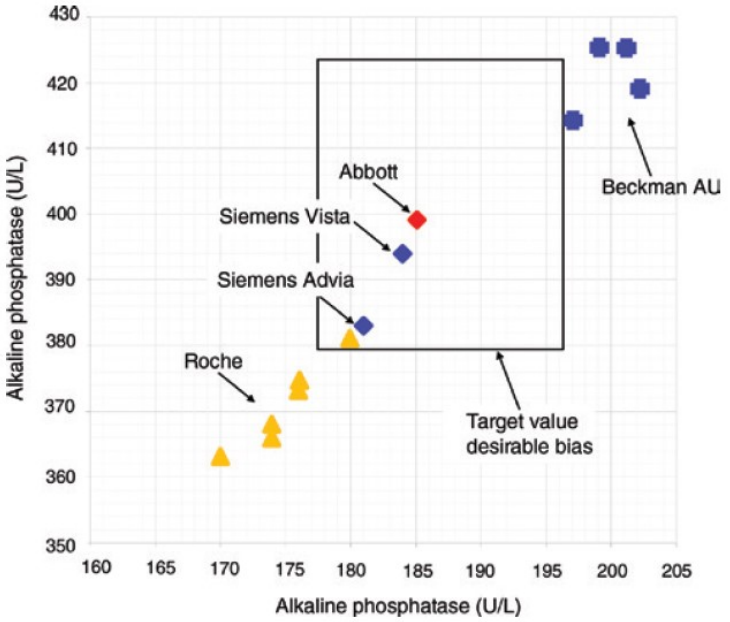
STANDARDIZATION



To become equivalent for being interpreted in a consistent manner, results must be traceable to higher order references

EU 98/79/EC-IVD Directive

Traceability is not always correctly implemented by manufacturers



Clin Chem Lab Med 2017; 55(3): e47–e50

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TRACEABILITY IMPLEMENTATION

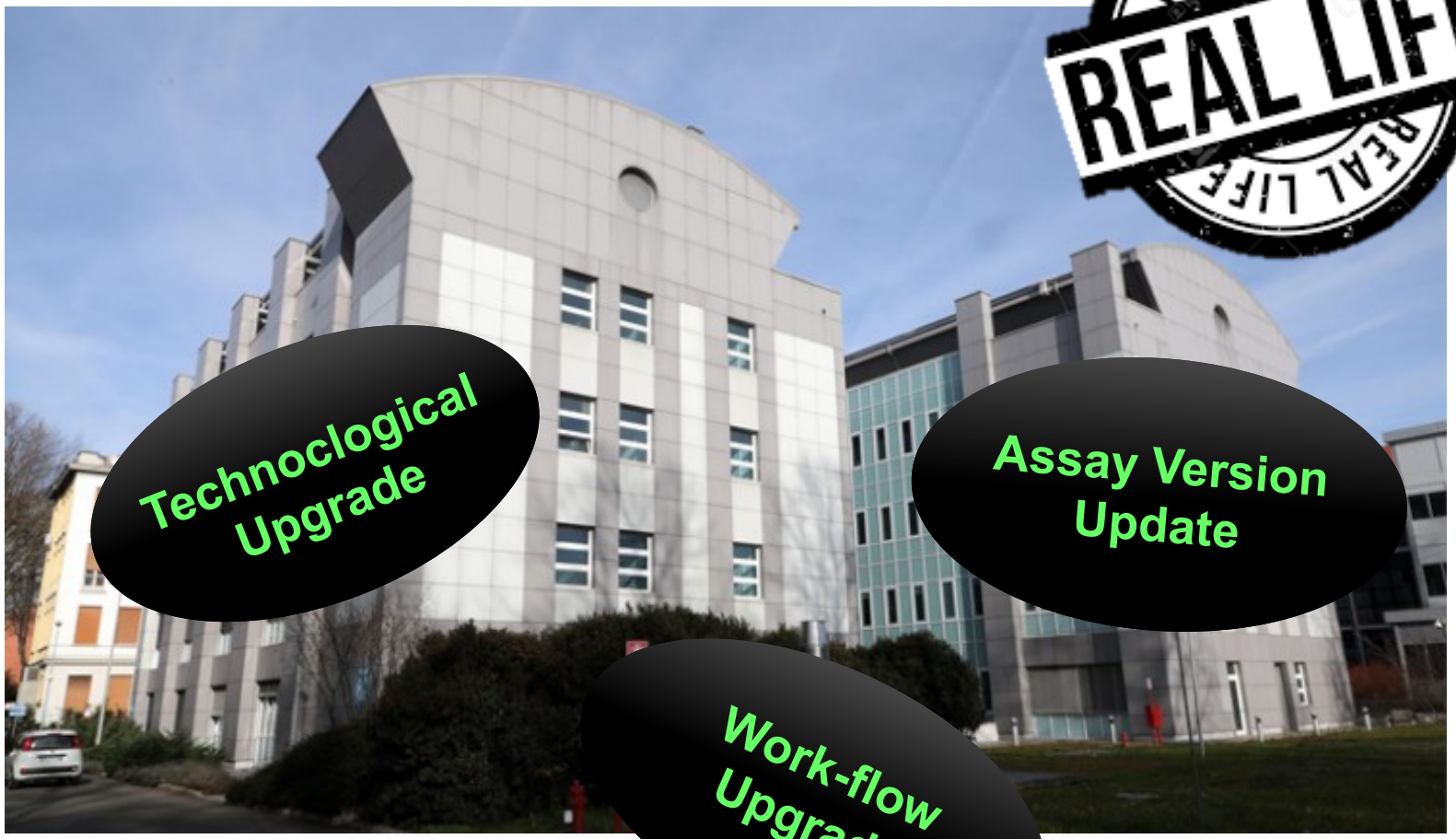
Profession
(e.g., IFCC, JCTLM)

Diagnostic
manufacturers

End users
(clinical laboratories)

Laboratory professionals should independently verify the correct implementation of metrological traceability of commercial measuring systems and determine if their performance is fit for purpose.

Our experience



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1st Example: Technological upgrade

SPRING/SUMMER 2019

ARCHITECT C16000

ALINITY c

Abbott

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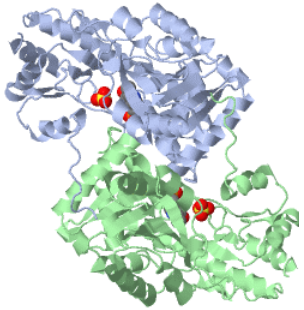
1st Example: Technological upgrade

Clin Chem Lab Med 2020; 58(8): 1250–1256 DE GRUYTER

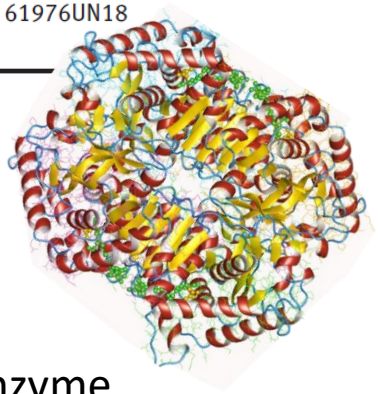
Elena Aloisio*, Erika Frusciante, Sara Pasqualetti, Ilenia Infusino, Magdalena Krintus, Grazyna Sypniewska and Mauro Panteghini

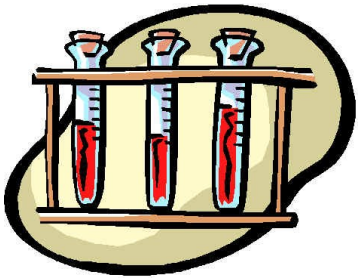
Traceability validation of six enzyme measurements on the Abbott Alinity c analytical system

Traceability validation experiment



Enzyme	Code no.	Method principle	Calibration principle	Declared traceability	Employed reagent lot
ALT	08P1820	Kinetic spectrophotometric (NADH with pyridoxal-5′-phosphate)	Calibration factor (7658)	IFCC RMP	75228UN18
ALP	08P2020	Kinetic spectrophotometric (para-nitrophenyl phosphate)	Calibration factor (2290)[a]	IFCC RMP	86495UN18
AST	08P2320	Kinetic spectrophotometry (NADH with pyridoxal-5′-phosphate)	Calibration factor (6835)	IFCC RMP	61147UN18
CK	08P4220	Kinetic spectrophotometric (N-acetyl-L-cysteine)	Calibration factor (9081)	Not declared[b]	00675UN19
γGT	07P7320	Kinetic spectrophotometric (L-gamma-glutamyl-3-carboxy-4-nitroanilide)	Calibration factor (8372)[a]	IFCC RMP	68895UN18
LDH	07P7420	Kinetic spectrophotometric (lactate to pyruvate)	Calibration factor (11180)	IFCC RMP	61976UN18





PREPARATION FOR EXPERIMENT:

- Three fresh-frozen human serum pools per enzyme
- Clinical Laboratory and Standards Institute (CLSI) C37-A
- Catalytic concentrations of pools distributed across reportable range for each enzyme (L, M, H)

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Traceability validation experiment

CIRME
Università degli Studi di Milano

Centre for Metrological Traceability in Laboratory Medicine (CIRME)

Enzyme **target values and corresponding uncertainties** were assigned to each pool in the CIRME reference laboratory (University of Milan), by using the corresponding IFCC reference measurement procedure

Enzyme	Pool ID	RMP	
		Target[a], U/L	U[b], %
ALT	L	38.9	5.12
	M	78.1	3.43
	H	201.3	2.64
ALP	L	95.9	3.00
	M	310.1	2.57
	H	504.9	2.64
AST	L	30.0	2.53
	M	70.4	2.52
	H	172.2	2.60
CK	L	108.6	3.98
	M	243.0	3.21
	H	461.6	2.55
γGT	L	43.0	3.47
	M	84.3	2.68
	H	211.3	2.72
LDH	L	220.6	2.70
	M	361.3	2.57
	H	442.7	2.66

IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37°C. Part 1. The Concept of Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes

L. Siekmann, R. Bonora, C.A. Burtis, F. Ceriotti, P. Clerc-Renaud, G. Férard, C.A. Ferrero, J.-C. Forest, P.F.H. Franck, F.-J. Gella, W. Hoelzel, P.J. Jørgensen, T. Kanno, A. Kessner, R. Klauke, N. Kristiansen, J.-M. Lessinger and T.P.J. Linsinger

Clin Chem Lab Med. 2002 Jun;40(6):631-4. doi: 10.1515/CCLM.2002.109.

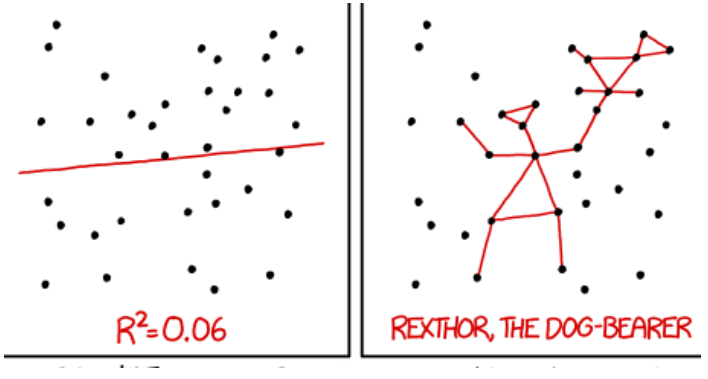
Traceability validation experiment

Experiment:

- **System alignment** to manufacturer's specifications was checked by using IQC component I, befor and after analytical run;

MULTICHEM S PLUS (Technopath)

- Measurements of each pool were performed **in triplicate;**
- Correlations were performed by **Passing-Bablok regression**;
- **Bias** (Alinity vs RMP) was estimated.



Traceability validation experiment

The traceability of Alinity assays to the RMP was considered rightly implemented if the mean percentage bias on the three pools was less than the bias specifications

(derived from biological variability data, https://biologicalvariation.eu)

Enzyme	Allowable bias[a]		Allowable measurement uncertainty[b]	
	Desirable	Optimum	Desirable	Optimum
ALT	±7.7%	±3.9%	±10.1%	±5.1%
ALP	±5.5%	±2.8%	±6.0%	±3.0%
AST	±5.7%	±2.8%	±9.8%	±4.9%
CK	±8.8%	±4.4%	±15.4%	±7.7%
γGT	±10.5%	±5.3%	±8.6%	±4.3%
LDH	±3.4%	±1.7%	±5.2%	±2.6%

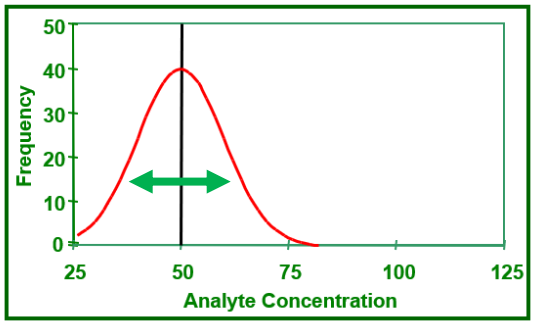
Enzyme	Pool ID	RMP		Mean of three Alinity replicates, U/L	Bias, U/L	Bias, %	Mean bias, %	Regressi parameters
		Target[a], U/L	U[b], %					
ALT	L	38.9	5.12	40.0	1.1	2.83	0.44	y = 8x + 1.9 U/L,
	M	78.1	3.43	77.7	−0.4	−0.51		R 1.0000
	H	201.3	2.64	199.3	−2.0	−0.99		
ALP	L	95.9	3.00	97.0	1.1	1.15	1.78	y = 1.05x − 3.5 U/L,
	M	310.1	2.57	310.3	0.2	0.06		R^2 = 0.9991
	H	504.9	2.64	525.7	20.8	4.12		
AST	L	30.0	2.53	32.3	2.3	7.67	4.44	y = 1.01x + 2.0 U/L,
	M	70.4	2.52	72.7	2.3	3.27		R^2 = 1.0000
	H	172.2	2.60	176.3	4.1	2.38		
CK	L	108.6	3.98	110.7	2.1	1.93	0.61	y = 1.02x + 0.1 U/L,
	M	243.0	3.21	238.3	−4.7	−1.93		R^2 = 0.9992
	H	461.6	2.55	470.0	8.4	1.82		
γGT	L	43.0	3.47	41.3	−1.7	−3.95	−1.83	y = 1.01x − 2.1 U/L,
	M	84.3	2.68	83.0	−1.3	−1.54		R^2 = 1.0000
	H	211.3	2.72	211.3	0.0	0.0		
LDH	L	220.6	2.70	224.0	3.4	1.54	0.83	y = 0.99x + 5.2 U/L,
	M	361.3	2.57	363.5	2.2	0.60		R^2 = 1.0000
	H	442.7	2.66	444.3	1.6	0.36		

Measurement uncertainty

$$u_{result} = \sqrt{(u^2_{cal} + u^2_{rw} + u^2_{bias})}$$

Long term intermediate reproducibility:

- Third party commutable control material
- Measured daily for 5 months
- Estimation of mean, SD and CV



$$\sqrt{[bias^2 + (SD_{bias}/\sqrt{n})^2 + u_{ref}^2]}$$

- the average difference between the pool mean value and corresponding RMP target value (**bias**)
- the **bias variability**
- the relative standard **uncertainty of target value** assigned to the pool by the RMP (u_{ref})

Measurement uncertainty

Enzyme	Catalytic activity concentration (order of magnitude compared to the upper reference limit [URL] in adult white males[a])	u_{bias}	u_{Rw}	u_{result}	U^b
ALT	88 U/L (1.5×URL)	1.9%	1.1%	2.2%	4.4%
ALP	345 U/L (3.0×URL)	1.3%	1.3%	1.9%	3.8%
AST	204 U/L (6.0×URL)	2.7%	0.7%	2.8%	5.6%
CK	513 U/L (3.0×URL)	2.2%	0.6%	2.3%	4.6%
γGT	184 U/L (2.7×URL)	1.4%	1.4%	2.0%	4.0%
LDH	374 U/L (1.7×URL)	1.6%	1.4%	2.1%	4.2%

Enzyme	Allowable bias[a]		Allowable measurement uncertainty[b]	
	Desirable	Optimum	Desirable	Optimum
ALT	±7.7%	±3.9%	±10.1%	±5.1%
ALP	±5.5%	±2.8%	±6.0%	±3.0%
AST	±5.7%	±2.8%	±9.8%	±4.9%
CK	±8.8%	±4.4%	±15.4%	±7.7%
γGT	±10.5%	±5.3%	±8.6%	±4.3%
LDH	±3.4%	±1.7%	±5.2%	±2.6%

Inter-laboratory verification

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UNIWERSYTET MIKOŁAJA KOPERNIKA W TORUNIU
Collegium Medicum
im. Ludwika Rydygiera w Bydgoszczy

Experiment:

- **System alignment** to manufacturer's specifications was checked by using IQC component I, befor and after analytical run;
- **Measurements** of each pool were performend in triplicate;
- **Bias** (Milan vs Bydgoszcz) was estimated.

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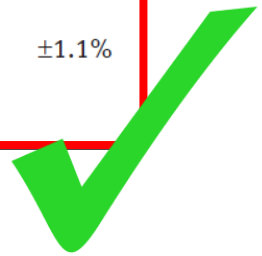
Inter-laboratory verification

Enzyme	Pool ID	Milan mean of triplicates, U/L	Bydgoszcz mean of triplicates, U/L	Bias, U/L	Bias, %	Mean bias, %	Goal[a]
ALT	1	39.7	40.3	0.6	1.5	1.23	±2.6%
	2	79.0	80.0	1.0	1.3		
	3	230.7	232.7	2.0	0.9		
ALP	1	113.3	113.3	0	0	−0.30	±1.8%
	2	515.7	512.7	−3.0	−0.6		
	3	811.3	809.0	−2.3	−0.3		
AST	1	38.0	36.7	−1.3	−3.4	−1.83	±1.9%
	2	82.3	81.3	−1.0	−1.2		
	3	193.7	192.0	−1.7	−0.9		
CK	1	95.0	96.0	1.0	1.1	0.97	±2.9%
	2	213.0	215.7	2.7	1.3		
	3	491.3	493.7	2.4	0.5		
γGT	1	37.0	37.0	0	0	−0.53	±3.5%
	2	72.0	70.3	−1.7	−2.4		
	3	226.0	227.7	1.7	0.8		
LDH	1	225.0	226.7	1.7	0.8	0.13	±1.1%
	2	370.7	368.0	−2.7	−0.7		
	3	878.7	881.3	2.6	0.3		

[a]1/3 of allowable desirable bias reported in Table 2.



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2nd Example: Assay update to new generation

Alinity c

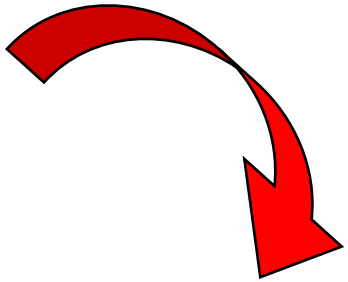
GGT
07P73
G71324R03
B7P730

Gamma-Glutamyl Transferase Reagent Kit

SUMMER 2022

Abbott

- No calibrator (fixed factor: 8372)
- Traceability to IFCC RMP verified in previous experiment



Gamma-Glutamyl Transferase2

GGT2
04T96
G93226R02
B4T960

FOR USE WITH
Alinity c

- Multiparametric calibration material (Consolidated Chemistry Calibrator)
- Declared traceability to IFCC RMP through ERM-AD452/IFCC

2nd Example: Assay update to new generation

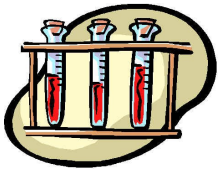
Clin Chem Lab Med 2022; 60(10): e228–e231 DE GRUYTER

Letter to the Editor

Giorgia Bianchi*, Giulia Colombo, Sara Pasqualetti and Mauro Panteghini

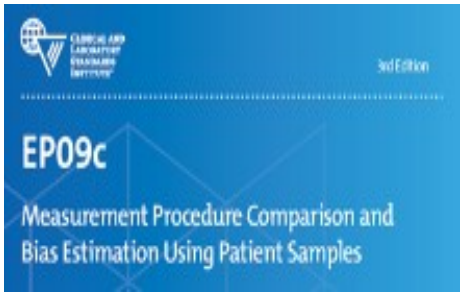
Alignment of the new generation of Abbott Alinity γ-glutamyltransferase assay to the IFCC reference measurement system should be improved

Correlation experiment



Protocol:

- Clinical Laboratory and Standards Institute (CLSI) EP09
- Thirty-six leftover native serum samples
- Catalytic concentrations ranging from 9 to 777 U/L

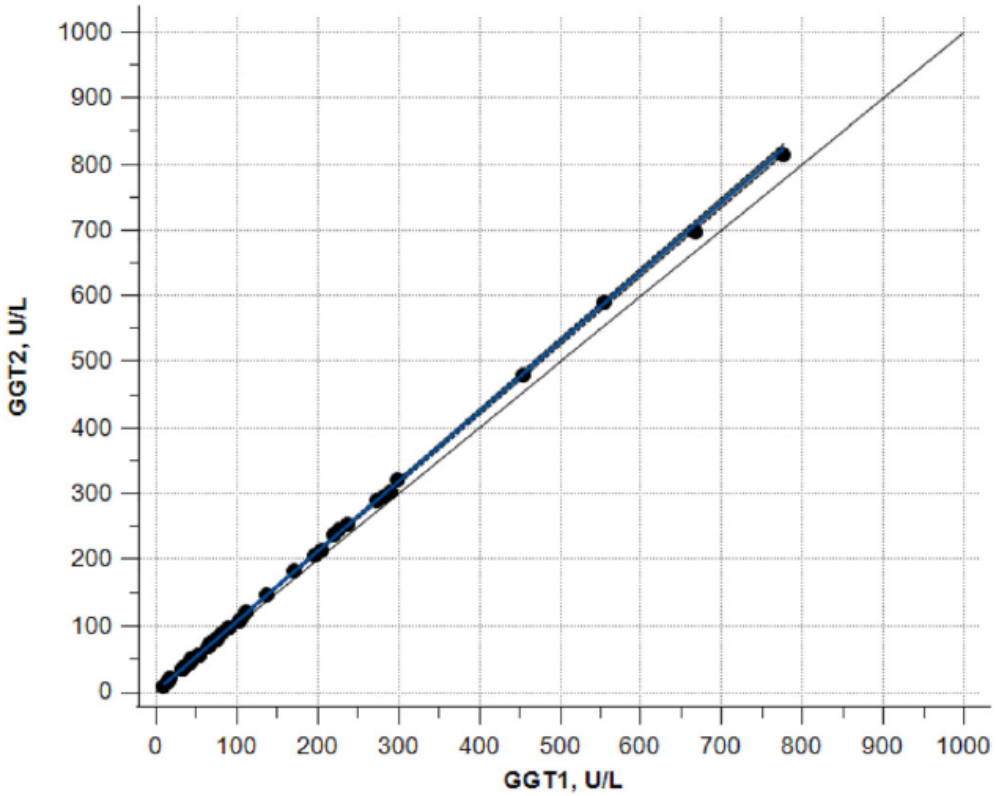


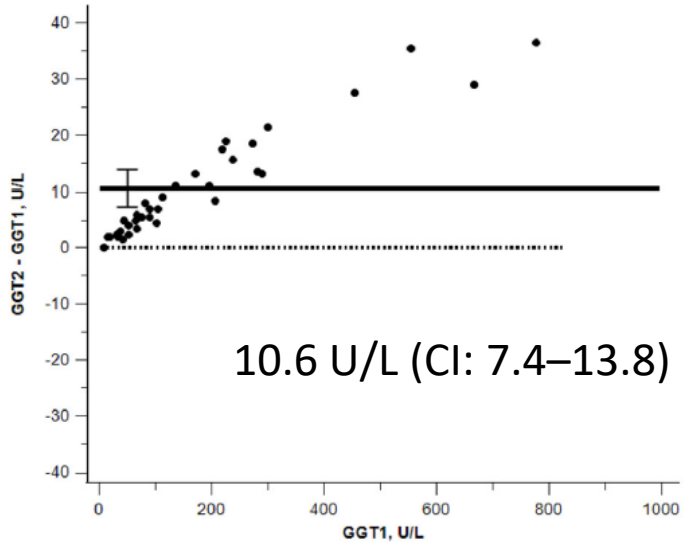
Experiment:

- **System alignment** to manufacturer's specifications was checked by using IQC component I, befor and after analytical run;
- **Measurements** of each sample were performend in duplicate;
- Correlation was performed by **Passing-Bablok regression**;
- **Bias** (GGT2 vs GGT) was estimated by bias plots.

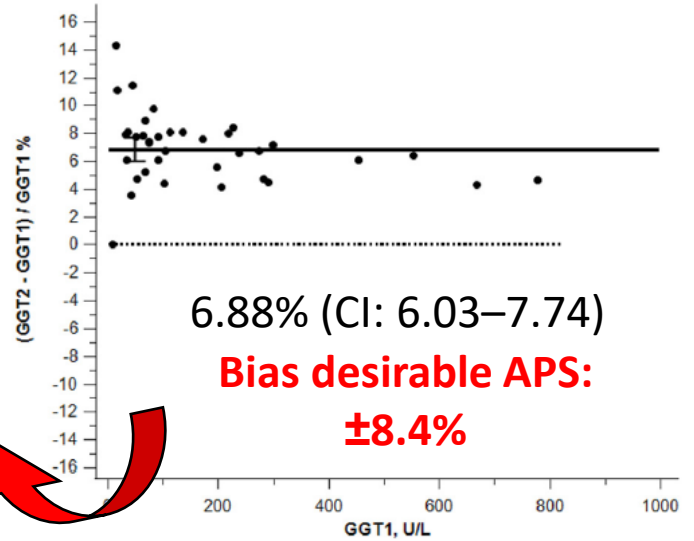
Correlation experiment

GGT2=**1.061** (95%CI: 1.050–1.069) GGT1 + **0.9** (95%CI: 0.1–1.6) U/L]





10.6 U/L (CI: 7.4–13.8)



6.88% (CI: 6.03–7.74)

Bias desirable APS: ±8.4%

What effect on MU?

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Bias & MU evaluation

CIRME
Università degli Studi di Milano
Centre for Metrological Traceability in Laboratory Medicine (CIRME)

VS

Gamma-Glutamyl Transferase2

Pool ID	RMP		Mean of three Alinity GGT2 replicates, U/L	Bias, U/L	$2*u_{bias}$, U/L[a]	Bias, %	Regression parameters
	Target, U/L	u_{ref}, U/L					
L	43.0	0.75	46.0	3.0	1.5	6.98	y=1.089x – 0.8 U/L,
M	84.3	1.13	89.7	5.4	2.4	6.41	R^2=1.0000
H	211.3	2.87	230.0	18.7	6.2	8.85	

(same pools as 1° experiment)

Mean bias:
7.41%

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Bias & MU evaluation

TECHNICAL SPECIFICATION

ISO/TS 20914

First edition
2019-07

Medical laboratories — Practical guidance for the estimation of measurement uncertainty

Laboratoires médicaux — Lignes directrices pratiques pour l'estimation de l'incertitude de mesure

An estimate of measurement bias is considered statistically significant if:

Bias > 2 x u_{bias}

$$\sqrt{(SD_{mean}^2 + u_{ref}^2)}$$

$$u_{result} = \sqrt{(u_{bias}^2 + u_{cal}^2 + u_{Rw}^2)}$$

Pool ID	Bias, U/L	2*u_{bias}, U/L[a]	Bias, %
L	3.0	1.5	6.[illegible]
M	5.4	2.4	[illegible]
H	18.7	6.2	[illegible]85

$$u_{result} = \sqrt{(bias^2 + u_{bias}^2 + u_{cal}^2 + u_{Rw}^2)} = 9.14\%$$

Minimum MU APS: 6.68%

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Bias & MU evaluation

- The presence of a positive proportional bias when GGT2 is employed **may denote some problems in the calibrator value-assignment protocol** used for transferring trueness from higher-order references to the multiparametric calibrator.

- **Commutability of reference materials** is also fundamental to guarantee the correct transfer of trueness.
  No verification of commutability for ERM-AD452/IFCC material → possible bias introduction during trueness transferring process.

End-user laboratory could introduce a **correction factor for the detected bias.**
However this may **alter the status of the measuring system**, neglecting responsibilities from the manufacturer and interrupting the system originally provided through CE marking

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3rd Example: Work-flow upgrade

AUTUMN 2020

ARCHITECT c4000
STAND ALONE

ALINITY c
TOTAL LABORATORY AUTOMATION

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Abbott

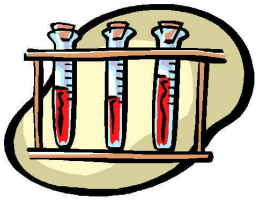
3rd Example: Work-flow upgrade

› Clin Chem Lab Med. 2022 Jan 18;60(3):441-450. doi: 10.1515/cclm-2021-1249. Print 2022 Feb 23.

A step towards optimal efficiency of HbA_{1c} measurement as a first-line laboratory test: the TOP-HOLE (Towards OPtimal glycoHemOgLobin tEsting) project

Sara Pasqualetti [1 2], Assunta Carnevale [1], Alberto Dolci [1 3], Mauro Panteghini [1 2 3]

Traceability validation experiment

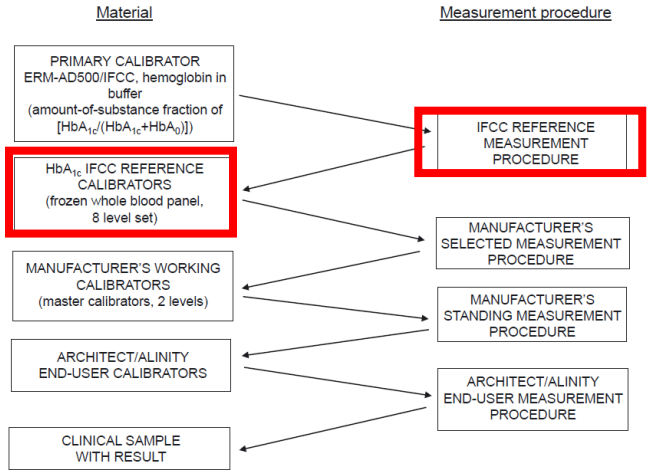


REFERENCE MATERIALS:

- 8 frozen human K_2EDTA whole-blood samples prepared by European Reference Laboratory for Glycohemoglobin
- Values assigned by the IFCC network laboratories

Experiment:

- **System alignment** to manufacturer's specifications was checked by using IQC component I, befor and after analytical run;
- **Measurements** of each sample were performend in triplicate;
- **Bias** (Alinity c vs target value) was estimated.



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Traceability validation experiment

Bias was considered statistically significant if >2 × u_{bias}

TCM no.	Target value, mmol/mol	Standard uncertainty associated with TCM, %	Mean of three Architect replicates, mmol/mol	Bias vs. TCM target, %	Mean of three Alinity replicates, mmol/mol	Bias vs. TCM target, %
1	31.9	0.78	30.9	−3.13[c]	30.9	−3.1[c]
2	37.6	0.66	37.3	−0.80	37.2	−1.06
3	48.7	0.72	48.1	−1.23	48.5	−0.41
4	58.2	0.77	58.2	0	58.5	0.52
5	67.3	0.67	67.9	0.89	67.8	0.74
6	78.6	0.70	78.2	−0.51	79.3	0.89
7	87.2	0.63	89.5	2.64[c]	91.2	4.59[c]
8	99.5	0.65	100.2	0.70	102.7	3.22[c]

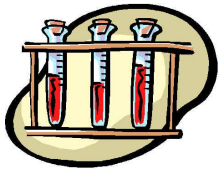
[a]Reagent and calibrator lot no. 55712UQ11 and 56194UQ11, respectively. [b]Reagent and calibrator lot no. 56350UQ01 and 56853UQ03, respectively. [c]Statistically significant bias.

ISO/TS 20914:

"Even if a measurement bias is statistically significant, the final assessment of significance of any correction for the bias should be based on its impact on medical interpretation of measurement results."

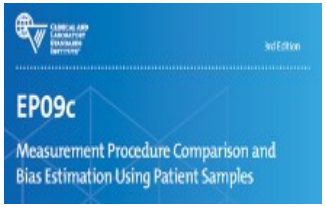
For HbA_{1c} concentrations covering the cut-offs adopted in the clinical setting (42-64 mmol/mol) the bias was negligible, with no risk of patient misclassification

Correlation experiment Alinity c vs Architect c4000



Protocol:

- Clinical Laboratory and Standards Institute (CLSI) EP09
- Forty leftover WB samples
- Concentrations ranging from 27.7 to 105.0 mmol/mol



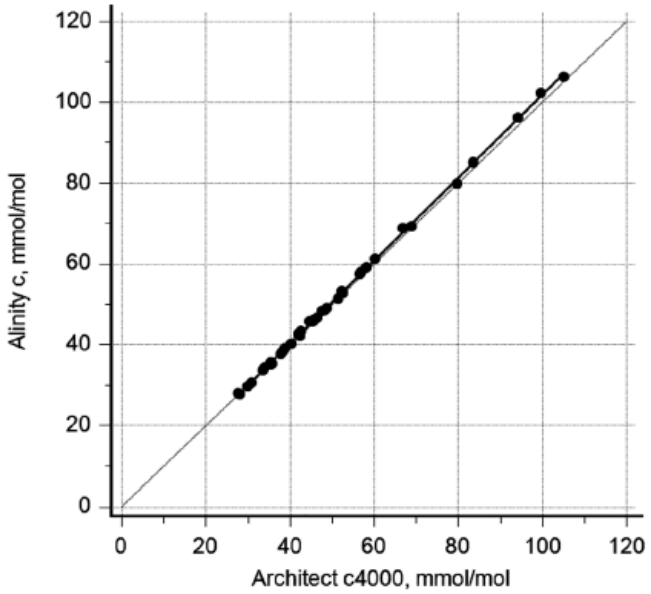
Experiment:

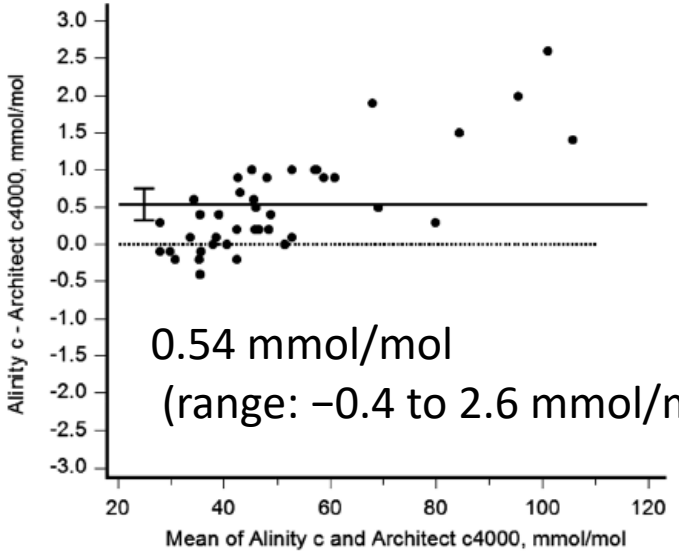
- **System alignment** to manufacturer's specifications was checked by using IQC component I, befor and after analytical run;
- **Measurements** of each sample were performend in duplicate;
- Correlation was performed by **Deming regression**;
- **Bias** (Alinity vs Architect) was estimated by bias plots.

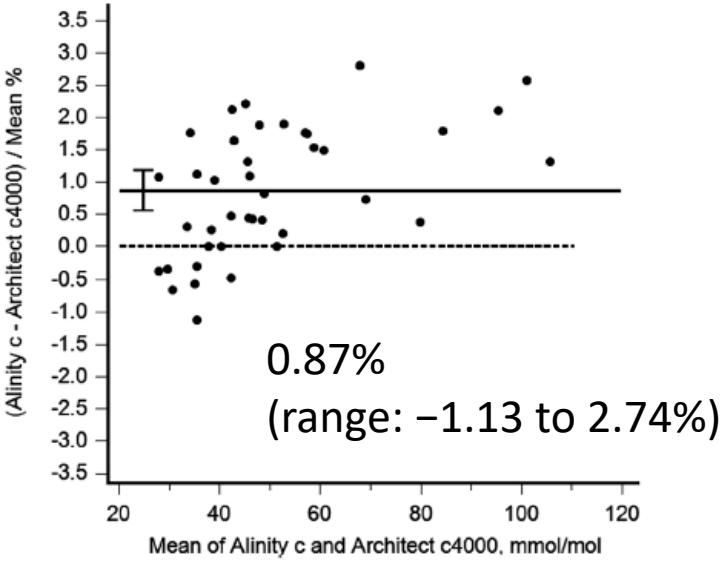
Correlation experiment

Alinity= **1.027** (CI: 1.016–1.034) Architect − **0.8** (CI: −1.3 to −0.3) mmol/mol

correlation coefficient:
0.9998 (95% CI: 0.9996–0.9999)







A bias lower than 1 mmol/mol has very little impact on the misclassification rate if MU is maintained <3.0%

Nielsen AA et al. Clin Chem Lab Med 2014;52:1069–77

MU evaluation

Estimated bias non-significant

$$u_{result} = \sqrt{(u_{bias}^2 + u_{cal}^2 + u_{Rw}^2)}$$

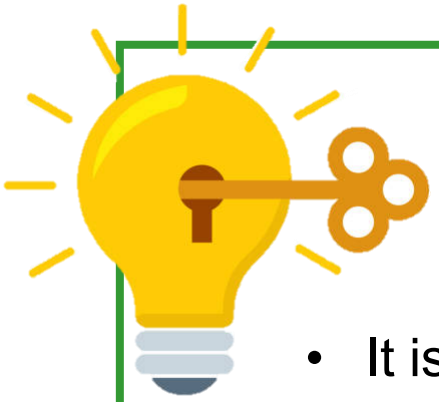
$$u_{result} = 1.58\%$$

Desirable MU APS: 3.0%

("outcome-based APS")

Nielsen AA et al. Clin Chem Lab Med 2014;52:1069–77

Key messages



- Flawed traceability implementation may have a **negative impact on patient care**
- It is a **duty of end-users (clinical laboratories) to verify traceabilty claims** made by manufacturers, in order to determine if performances of analytical systems are fit for purpose
- Design of verification experiments and interpretation of their results should always be drived by the **biological nature of the analyte and its clinical application**

Sistema Socio Sanitario

Regione Lombardia

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