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Centre for Metrological Traceability in Laboratory Medicine (CIRME)

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

Progress and impact of enzyme measurement standardization

Ilenia Infusino

Standardization in clinical enzymology: why?

- ✓ The determinations of most important enzymes are among the 20 most frequently ordered tests in clinical laboratories.
- ✓ These enzymatic determinations are important biomarkers for the diagnosis and monitoring of diseases of the liver, pancreas, skeletal muscle, bone, etc.

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Panteghini M, Bais R. Tietz Textbook of Clinical Chemistry & Molecular Diagnostics, 6th ed.

The lack of standardization may become an ethical issue

"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

Analytical improvements are matter of patient safety and key to future.

McLawhon RW. Clin Chem 2011;57:936

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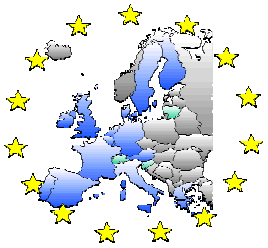

EU 98/79/EC-IVD Directive

→ To become *equivalent for long term*, results must be traceable to higher-order references.

Objective of traceability implementation:

to enable the results obtained by the calibrated routine procedure to be expressed in terms of the values obtained at the highest available level of the calibration hierarchy.

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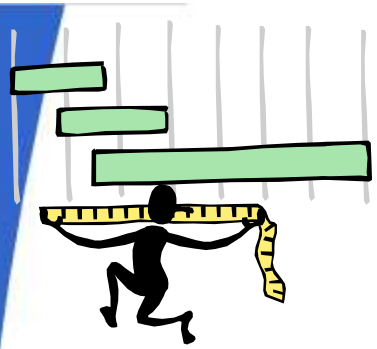

Basic requirements to establish traceability

- 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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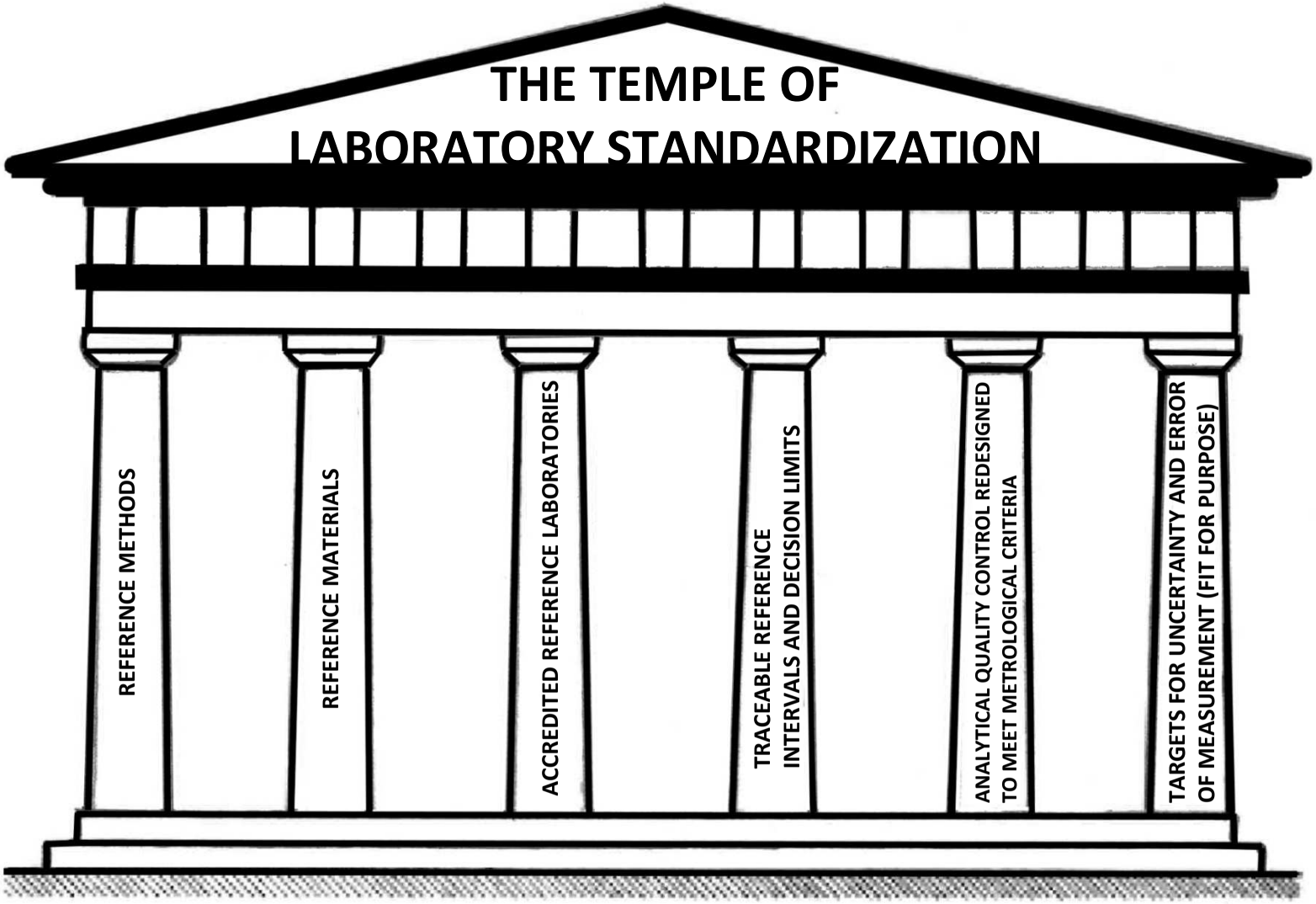
Fulfillment of the Requirements of the EU IVD Directive by Manufacturers

EU 98/79/EC-IVD Directive

THE TEMPLE OF LABORATORY STANDARDIZATION



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

Definition of Enzyme Catalytic Activity

An enzyme measurand cannot be described only by kind of quantity, name of enzyme and of system, but requires also the specified measurement procedure and especially the indicator component of the measured reaction.

Example:

Rate of conversion of NADH in the IFCC reference measurement procedure for lactate dehydrogenase (LDH)

Reaction:

$$\text{Lactate} + NAD^+ \xrightarrow[\text{LDH}]{} \text{Pyruvate} + NADH + H^+$$

ISO 18153:2003. In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials.

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Measurement of enzyme catalytic activity

→ The numerical results are method-dependent (i.e. depend entirely on the experimental conditions under which measurements are made)

Variables:

1. pH and nature of the buffer
2. substrate (nature and concentration)
3. activators and inhibitors
4. measurement temperature

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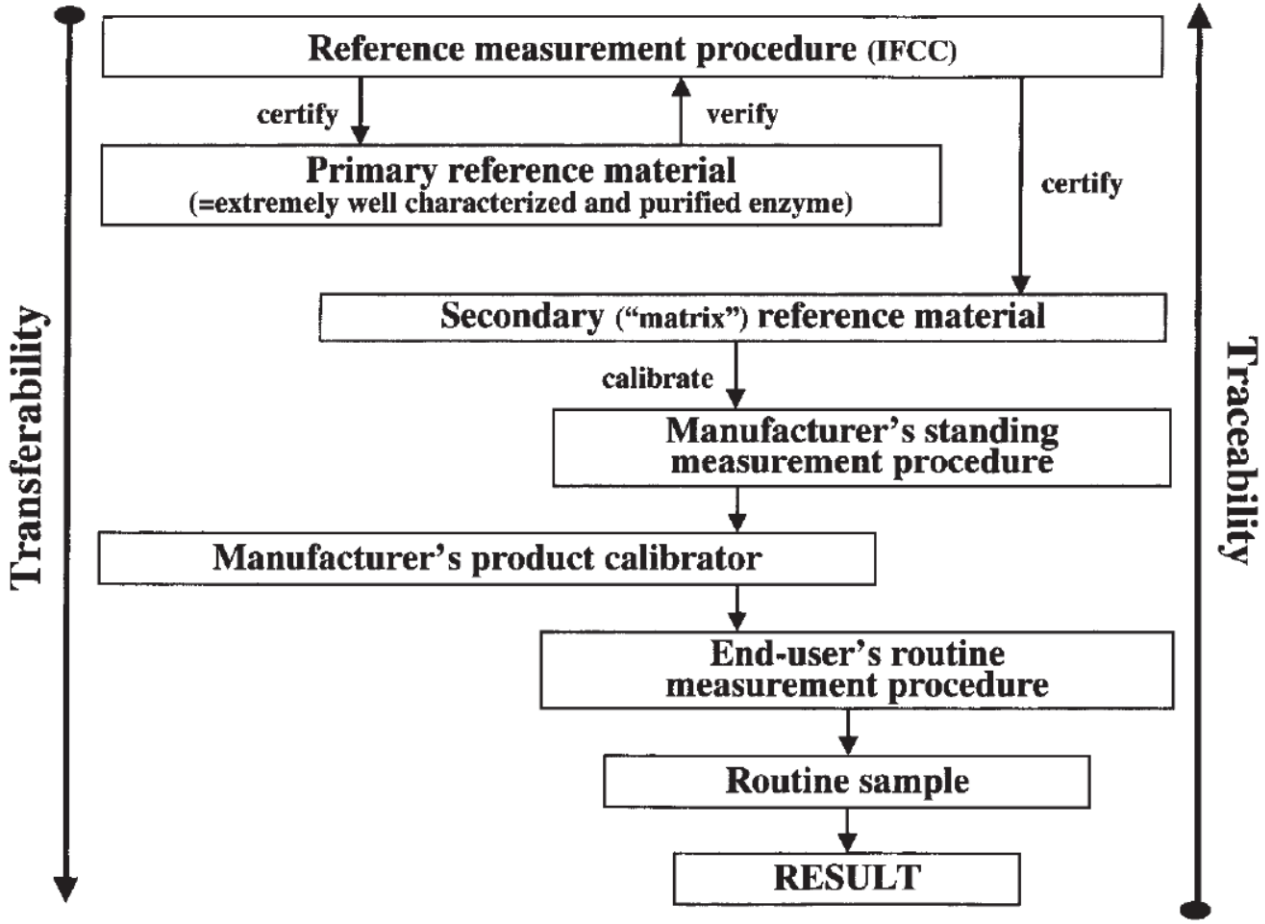
Clin Chem Lab Med 2001 39(9):795–800 © 2001 by Walter de Gruyter · Berlin · New York

Opinion Paper

Establishing a Reference System in Clinical Enzymology

Mauro Panteghini[1], Ferruccio Ceriotti[2], Gerhard Schumann[3] and Lothar Siekmann[4]



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Aspects to be controlled in performing reference measurement procedures for enzymes

- ✓ Gravimetry controlled by calibrated test weights
- ✓ Volumetry controlled by gravimetry
- ✓ Temperature controlled by calibrated thermometer
- ✓ pH controlled by calibrated equipment
- ✓ Photometric wavelength controlled by certified filters or solutions of holmium
- ✓ Photometric absorbance checked by certified test solutions

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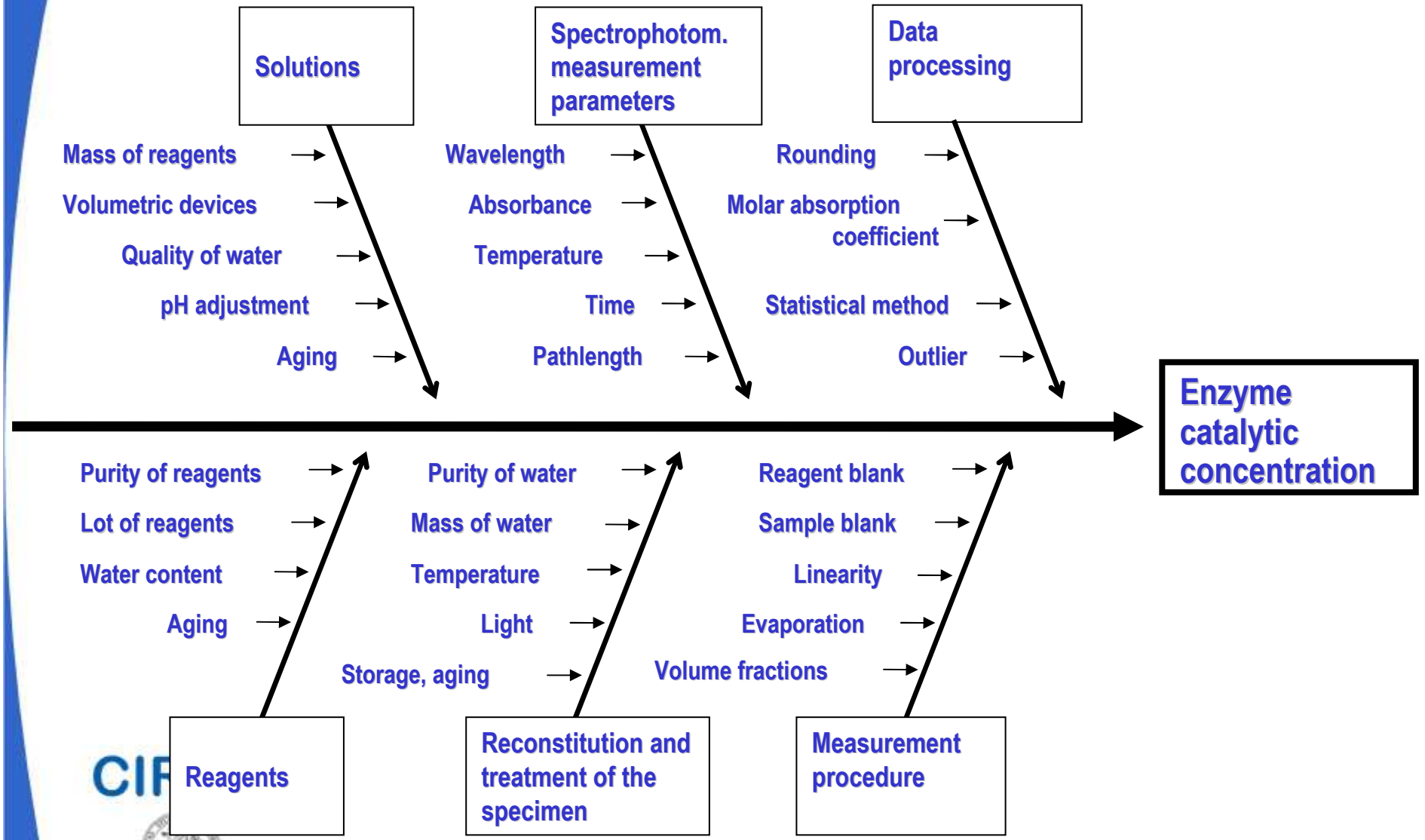
Infusino I, Schumann G, Ceriotti F, Panteghini M. CCLM 2010;48:301

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



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

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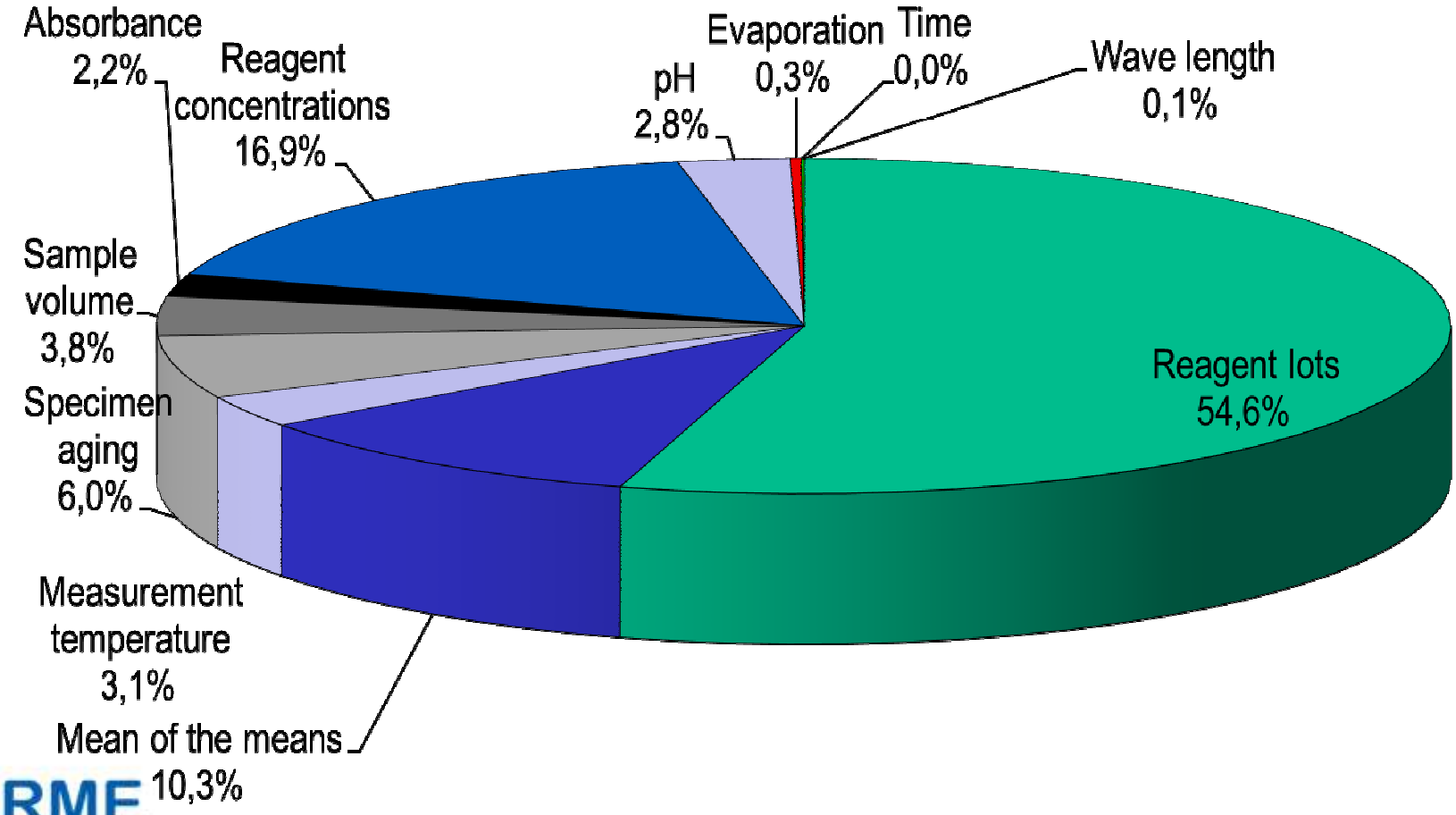
Example of uncertainty budget for ALT reference measurement procedure



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

JCTLM database

Enzymes reference measurement procedures

JCTLM
Accurate results for patient care

Enzyme	Reference
ALP	*Clin Chem Lab Med* **2011**;49:1439-46
ALT	*Clin Chem Lab Med* **2002**;40:718-24
Amylase	*Clin Chem Lab Med* **2006**;44:1146-55
AST	*Clin Chem Lab Med* **2002**;40:725-33
CK	*Clin Chem Lab Med* **2002**;40:635-42
GGT	*Clin Chem Lab Med* **2002**;40:734-38
LDH	*Clin Chem Lab Med* **2002**;40:743-48

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JCTLM database

Enzymes reference materials

JCTLM
Accurate results for patient care

	IRMM
AST	ERM-AD457 (IFCC)
ALT	-
γGT	ERM-AD452 (IFCC)
LDH	-
CK	ERM-AD455 (IFCC)
AMY	IRMM/IFCC 456
ALP	-

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Reproduction of ERMs for ALT, CK and LDH

- **New ERMs produced**
- **Characterisation completed**
- **Stability & homogeneity data available by 09/2016**

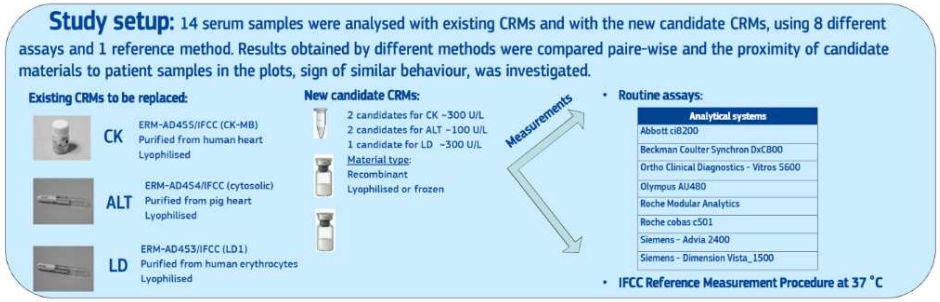
Poster Abstracts – IFCC WorldLab Istanbul 2014 – Istanbul, 22-26 June 2014 • DOI 10.1515/cclm-2014-4057
Clin Chem Lab Med 2014; 52, Special Suppl, pp S1 – S1760, June 2014 • Copyright © by Walter de Gruyter • Berlin • Boston S1657

Standardisation, accreditation and harmonisation

Cod: 1516

COMMUTABILITY STUDY ON CANDIDATE MATERIALS FOR THREE NEW ENZYME CERTIFIED REFERENCE MATERIALS

B. Toussaint[4], F. Ceriotti[8], H. Schimmel[4], R. Rej[10], M. Besozzi[6], F.J. Gella[2], G. Giana[7], J. Lessinger[5], M. McCusker[1], M. Orth[9], M. Panteghini[3]



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SEP
05
2016

The JRC released three new Certified Reference Materials (CRMs) applicable for in-vitro diagnostics to monitor the function and state of heart, liver and soft tissues.

European Commission

ERM
European Reference Materials

CERTIFICATE OF ANALYSIS

ERM®-AD454k/IFCC

ENZYME IN BUFFER		
	Catalytic activity concentration [1]	
	Certified value [2]	Uncertainty [3]
Alanine aminotransferase (ALT)	103.8 U/L 1.73 µkat/L	2.6 U/L 0.05 µkat/L

1) Catalytic activity concentration of alanine aminotransferase (ALT) in the reconstituted material, as obtained by the IFCC primary reference measurement procedure for the measurement of catalytic activity concentration of alanine aminotransferase at 37 °C.

2) Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of units (SI). Values were converted from U/L into µkat/L by multiplication with the factor f= 0.01667.

3) The uncertainty is the expanded uncertainty of the certified value with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

CERTIFICATE OF ANALYSIS

ERM®-AD455k/IFCC

ENZYME IN BUFFER		
	Catalytic activity concentration [1]	
	Certified value [2]	Uncertainty [3]
Creatine kinase isoenzyme MM (CK-MM)	314 U/L 5.23 µkat/L	6 U/L 0.10 µkat/L

1) Catalytic activity concentration of creatine kinase isoenzyme MM (CK-MM) in the reconstituted material, as obtained by the IFCC primary reference measurement procedure for the measurement of the catalytic activity concentration of creatine kinase at 37 °C.

2) Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI). Values were converted from U/L into µkat/L by multiplication with the factor f= 0.01667.

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CERTIFICATE OF ANALYSIS

ERM®-AD453k/IFCC

ENZYME IN BUFFER		
	Catalytic activity concentration [1]	
	Certified value [2]	Uncertainty [3]
Lactate dehydrogenase isoenzyme 1 (LD1)	330 U/L 5.50 µkat/L	7 U/L 0.12 µkat/L

1) Catalytic activity concentration of lactate dehydrogenase isoenzyme 1 (LD1) in the reconstituted material, as obtained by the IFCC primary reference measurement procedure for the measurement of catalytic activity concentration of lactate dehydrogenase at 37 °C.

2) Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI). Values were converted from U/L into µkat/L by multiplication with the factor f= 0.01667.

3) The uncertainty is the expanded uncertainty of the certified value with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

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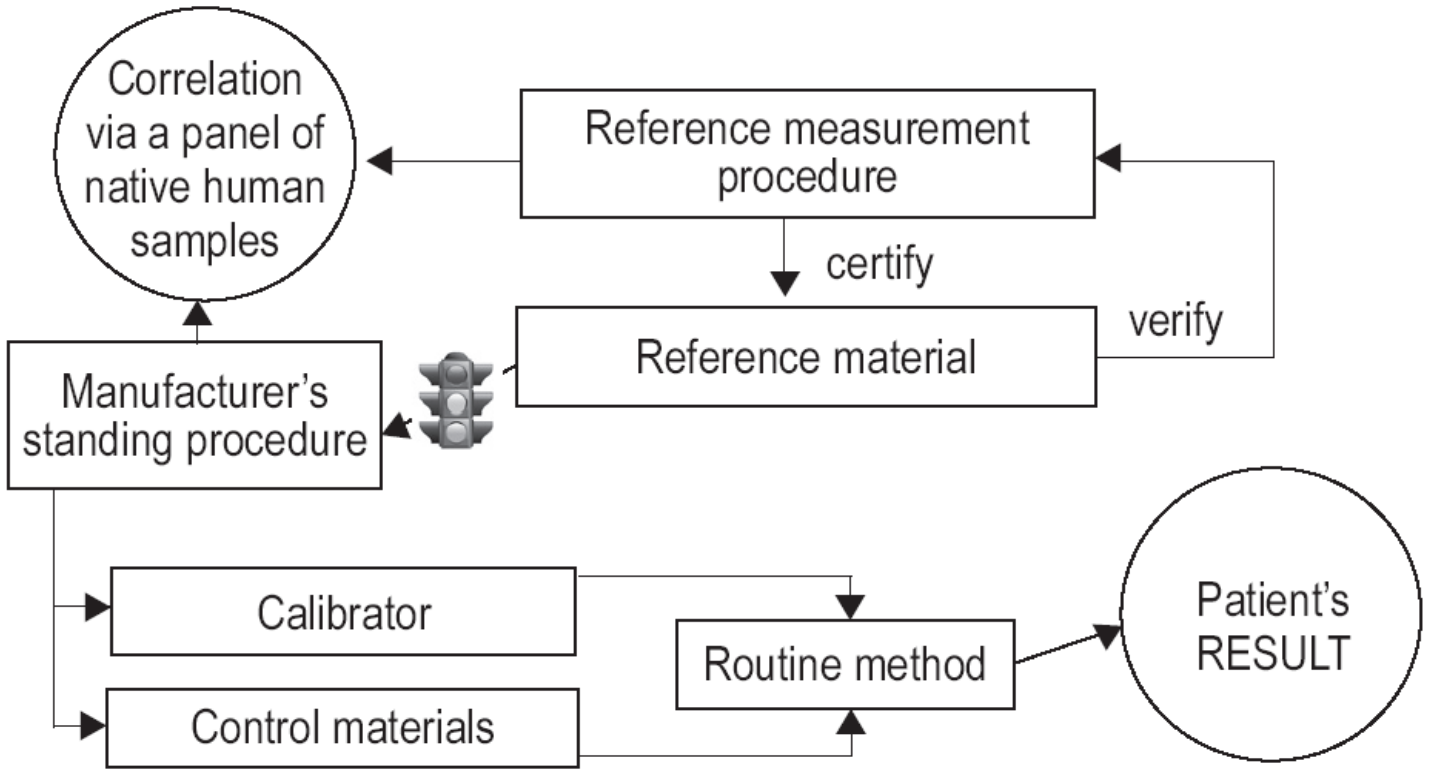

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Infusino I, Bonora R, Panteghini M. Clin Biochem Rev 2007;28:155

JCTLM database

Enzyme reference measurement service providers

JCTLM
Accurate results for patient care

CIRME (Centro Interdipartimentale per la Riferibilita' Metrologica in Medicina di Laboratorio - Universita' di Milano), Italy – Contact person: Prof. M Panteghini
DGKL (Reference Institute of the German Society of Clinical Chemistry and Laboratory Medicine), Germany – Contact person: Prof. G Schumann
Instand e.V., Germany – Contact person: Dr. P Kaiser
NCCL (National Center for Clinical Laboratories), China – Contact person: Prof. Wenxiang Chen
Beijing Aerospace General Hospital Reference Laboratory, China – Contact person: Dr. Baorong Chen
LREC (Clinical Enzymology Reference Laboratory - Universitat Autònoma de Barcelona), Spain – Contact person: Dr. F Canalias
SCCL (Shanghai Center for Clinical Laboratory), China – Contact person: Dr. Yuan Lu
CLNU (Center of Laboratory Medicine, Affiliated Hospital of Nantong University), China – Contact person: Dr. Huimin Wang
MakerBio-RSP, China – Contact person: Dr. Lei Lv
GPHCM (Guangdong Provincial Hospital of Chinese Medicine), China – Contact person: Jianbing Wang, Xianzhang Huang

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For use by (primarily): a) IVD industry (to ensure that results produced by IVDs are traceable to)
b) Regulators (to verify that results produced by IVDs are traceable to)
c) EQAS providers (to assign true values to EQAS materials)

Lack of proper reference intervals may hamper the implementation of standardization in enzymology

- The implementation of standardization can modify enzyme results
- Without adequate R.I. this situation can impair the interpretation of the results and, paradoxically, worsen the patient's outcome
- The absence of reliable R.I. for the newly standardized commercial methods may hamper their adoption

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

Clin Chem Lab Med 2010;48(11):1593–1601 © 2010 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2010.315

Research Article

Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in serum: results from an IFCC multicenter study

Ferruccio Ceriotti[1,*], Joseph Henny[2], Josep Queraltó[3], Shen Ziyu[4], Yeşim Özarda[5], Baorong Chen[6], James C. Boyd[7] and Mauro Panteghini[8] on behalf of the IFCC Committee on Reference Intervals and Decision Limits (C-RIDL) and Committee on Reference Systems for Enzymes (C-RSE)

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Clin Chem Lab Med 2003; 41(7):970–971 © 2003 by Walter de Gruyter · Berlin · New York

Letter to the Editor

Reference Interval for Lactate Dehydrogenase Catalytic Activity in Serum Measured According to the New IFCC Recommendations

Franca Pagani, Roberto Bonora and Mauro Panteghini*

ELSEVIER

Clinica Chimica Acta 327 (2003) 69–79

www.elsevier.com/locate/clinchim

New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects

Gerhard Schumann*, Rainer Klauke

Klinische Chemie, Medizinische Hochschule Hannover, D-30623 Hannover, Germany

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Traceable pediatric reference intervals for ALP

Tabella 2

Limiti di riferimento standardizzati per l'attività della fosfatasi alcalina del siero ottenuti nel presente studio

Femmine			Maschi		
Età (anni)	Limite inferiore (U/L)	Limite superiore (U/L)	Età (anni)	Limite inferiore (U/L)	Limite superiore (U/L)
<1	140	500	<1	140	500
1-<2	140	400	1-<2	140	400
2-<5	140	365	2-<5	140	365
5-<9	140	400	5-<12	140	400
9-<11	140	430	12-<13	140	465
11-<12	140	400	13-<14	110	465
12-<13	100	350	14-<15	90	400
13-<14	75	300	15-<16	65	275
14-<15	55	200	16-<17	50	200
15-<16	40	150	17-<18	50	150
16-<18	40	110	18-<19	43	135
			19-<20	43	120
≥18[a]	33	98	≥20[a]	43	115

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Ceriotti F, Panteghini M, Guerra E et al. Biochim Clin 2017;41:166-74.

Are Asian results different?

DE GRUYTER DOI 10.1515/cclm-2012-0421 — Clin Chem Lab Med 2013; 51(7): 1429–1442

Kiyoshi Ichihara*, Ferruccio Ceriotti, Tran Huu Tam, Shigeo Sueyoshi, Priscilla M.K. Poon, Mee Ling Thong, Yasushi Higashiuesato, Xuejing Wang, Hiromi Kataoka, Akemi Matsubara, Shu-Chu Shiesh, Dewi Muliaty, Jeong-Ho Kim, Masakazu Watanabe, Christopher W.K. Lam, Lothar Siekmann, Joseph B. Lopez, Mauro Panteghini and on behalf of the Committee on Reference Intervals and Decision Limits, International Federation for Clinical Chemistry and Laboratory Medicine, and the Science Committee for the Asia-Pacific Federation of Clinical Biochemistry

The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes

DE GRUYTER Clin Chem Lab Med 2016; 54(4): 659–665

Liqiao Han, Jianbing Wang, Qiaoxuan Zhang, Peifeng Ke, Xiaobin Wu, Zemin Wan, Haibiao Lin, Ruili Zeng, Xianzhang Huang* and Junhua Zhuang*

Development of reference intervals for serum alkaline phosphatase among adults in Southern China traced to the new IFCC reference measurement procedure

Common reference intervals for enzymes in adults

Premise

Use of commercial assays that provide traceable results permits to derive and employ traceable reference intervals.

Enzyme	European		Asian	
	Females	Males	Females	Males
AST	11–34		14–32	
ALT	8–41	9–59	11–31	14–54
GGT	6–40	12–68	15–43	15–68
LDH	125–220		138–235	
CK	34–145	46–171	40–152	58–261
AMY	31–107		47–136	
ALP	33–98	43–115	40–106	48–131

Infusino I et al. Clin Chem Lab Med 2017; 55:334-40.

Expected consequences

1. Experts defines reference measurement systems
2. Industry implements traceability to them
3. Users (and industry) abandon non-specific methods
4. EQAS provide commutable materials and accuracy-based grading
5. Professionals establish clinically allowable errors
6. Individual laboratories monitor their performance by participating to EQAS and applying allowable limits

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

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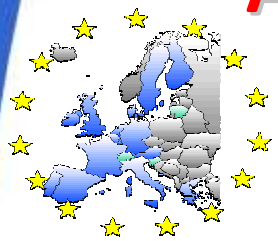
Adapted from Infusino I, Schumann G, Ceriotti F, Panteghini M. CCLM 2010;48:301

Assessment of enzyme measurements in 70 European laboratories



R. Jansen et al. / Clinica Chimica Acta 368 (2006) 160–167

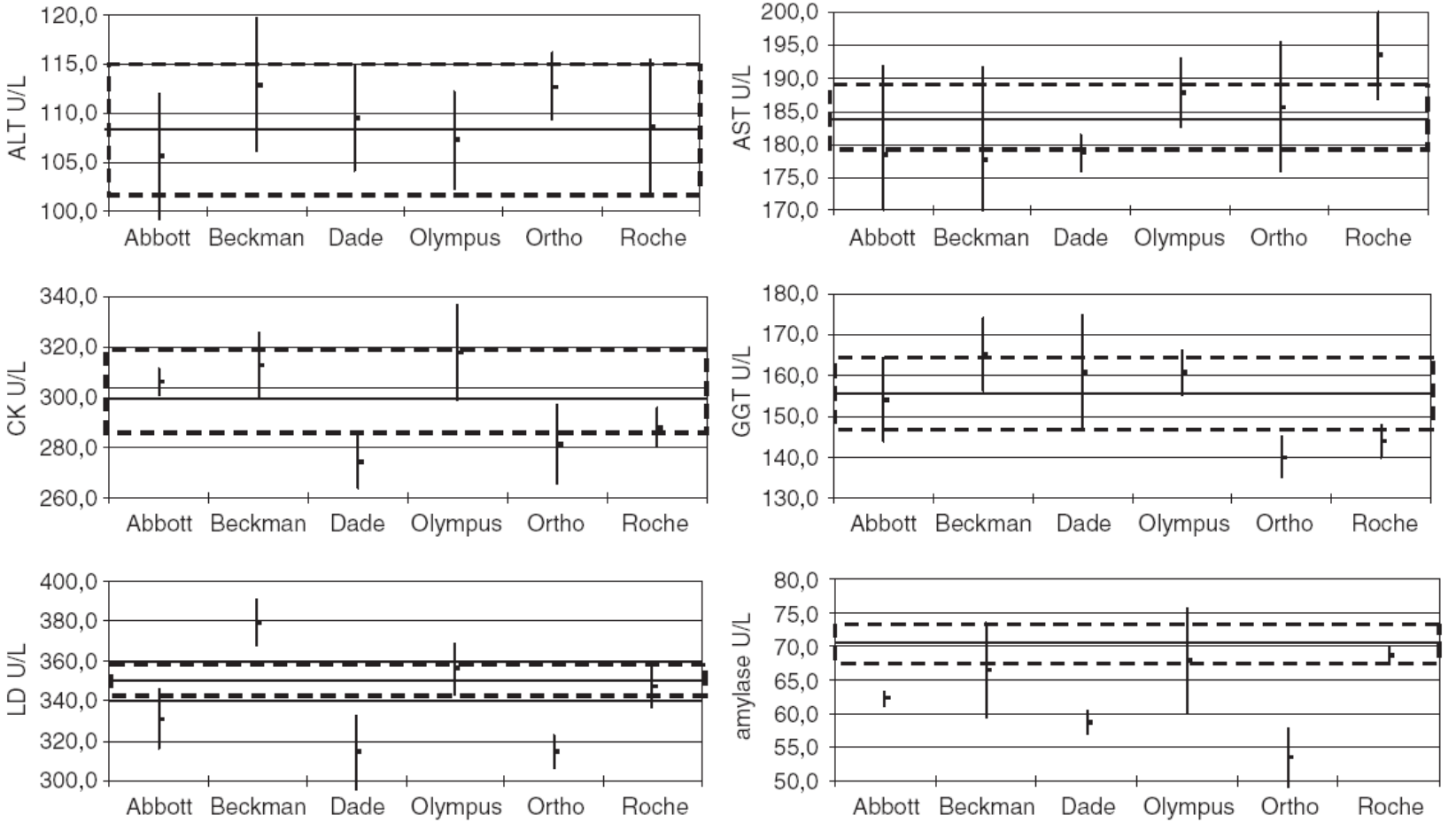


Fig. 1. Target value (fat line), means ± SD_{bl} (U/L) for each company system, and the area (dashed) of maximum allowable SD_{bl} in absence of significant bias.

Assessment of enzyme measurements in 4 European countries

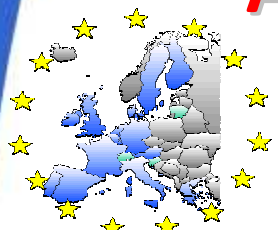


Table 1
TE_A, average TE scores, and %TE scores ≥95%.

Analyte	TE_A %	NL Av TE score (%)	NL % TE sc >95%	PT Av TE score (%)	PT % TE sc >95%	ES Av TE score (%)	ES % TE sc >95%	UK Av TE score (%)	UK % TE sc >95%
ALT	14.6	93	84	80	63	83	45	87	40
Amylase	26.3	85	77	53	43	59	40	90	90
AST	15.2	94	82	76	38	88	64	79	30
CK	30.3	99	96	83	63	98	91	100	100
Gamma-GT	22.2	97	93	83	75	90	91	89	80
LDH	11.4	84	76	24	13	63	55	9	0

CK is nicely standardized and a substantial improvement in analytical performance of marketed GGT assays was demonstrated. Conversely, aminotransferases, LDH and AMY still showed major disagreement suggesting the need for improvement in implementing traceability to higher order references.

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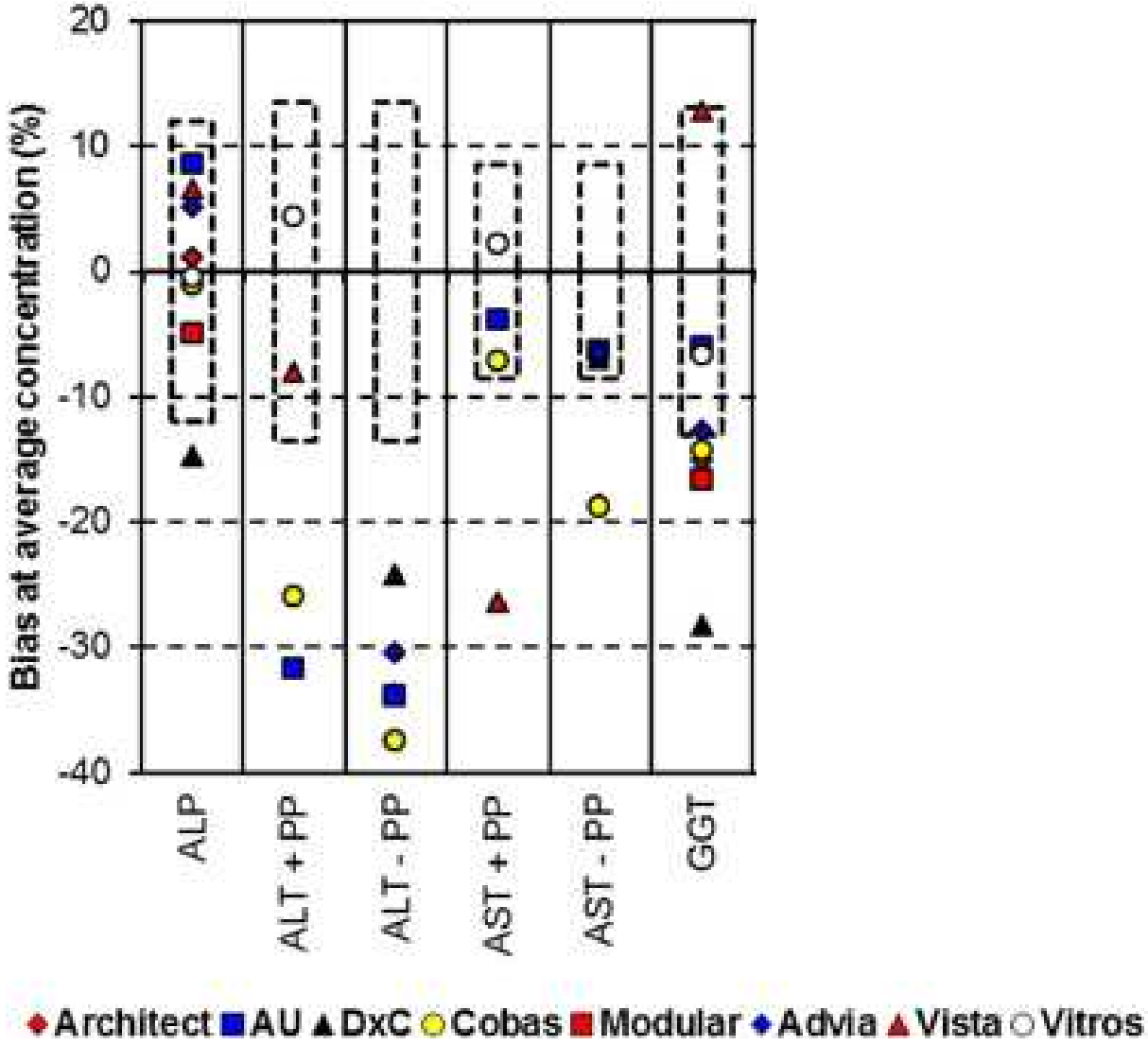
Analytical systems measuring serum ALT marketed by four IVD companies

Company	Platform	Principle of method	Calibrator	Declared uncertainty	Higher-order reference employed
Abbott	Architect	with P-5-P	Calibration factor	NA	IFCC Reference Method
		without P-5-P	Calibration factor	NA	NADH molar extinction factor
Beckman	AU	with P-5-P	System calibrator	6%	IFCC Reference Method
		without P-5-P	System calibrator	NA	Beckman Coulter Master Calibrator
	Synchron	with P-5-P	Enzyme Validator Level 1	14.48%	IFCC Reference Method
			Enzyme Validator Level 2	7.53%	IFCC Reference Method
Roche	Cobas c	with P-5-P	C.f.a.s.	0.66%	IFCC Reference Method
		without P-5-P	C.f.a.s.	0.66%	IFCC Reference Method modified
	Integra	with P-5-P	C.f.a.s	1.50%	IFCC Reference Method
		without P-5-P	C.f.a.s	1.50%	IFCC Reference Method modified
	Modular	with P-5-P	C.f.a.s	1.09%	IFCC Reference Method
		without P-5-P	C.f.a.s	1.09%	IFCC Reference Method modified
		without P-5-P HiCo	C.f.a.s	1.09%	IFCC Reference Method modified
Siemens	Dimension Vista	with P-5-P	Enzyme II Calibrator Level 2	5.21%	IFCC Reference Method
			Enzyme II Calibrator Level 3	5.24%	IFCC Reference Method
	Advia	with P-5-P	Chemistry calibrator control 1	2.71%	IFCC Reference Method
			Chemistry calibrator control 2	2.40%	IFCC Reference Method
		without P-5-P	Chemistry calibrator control 1	2.50%	IFCC Reference Method
			Chemistry calibrator control 2	1.30%	IFCC Reference Method



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Kenneth Goossens, Katleen Van Uytfanghe, Linda M. Thienpont. Clinica Chimica Acta, Volume 442, 2015, 44–45

Expected consequences

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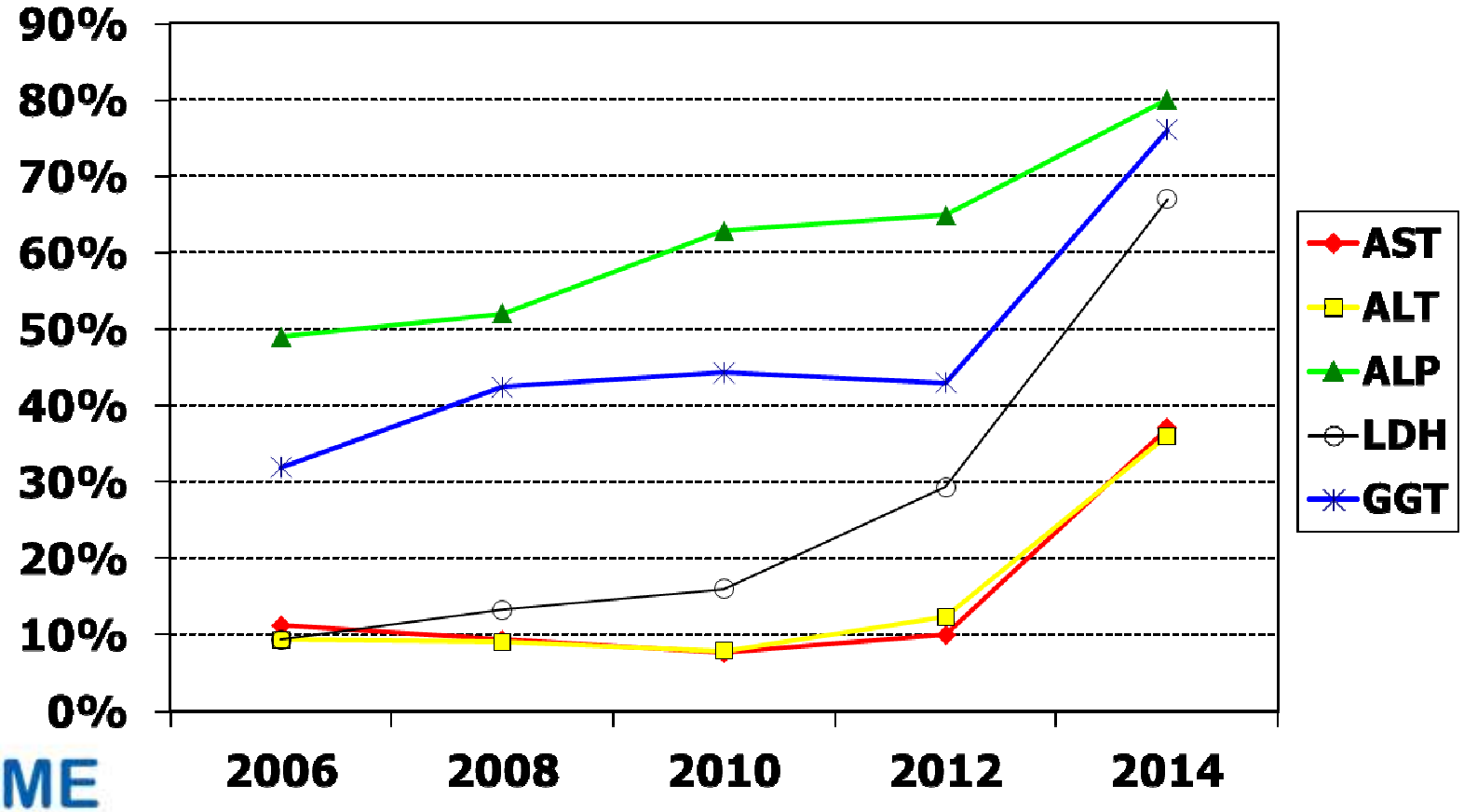
Adapted from Infusino I, Schumann G, Ceriotti F, Panteghini M. CCLM 2010;48:301

Analytical systems measuring serum LDH marketed by four IVD companies

Company	Platform	Principle of method	Calibrator	Declared uncertainty	Higher-order reference employed
Abbott	Architect	Lactate to pyruvate	Calibration factor	NA	IFCC Reference Method
Beckman	AU	SCE 1982 (37 °C)	System calibrator	7.67%	Beckman Coulter Master Calibrator
		LDH-L (AMP buffer)	System calibrator	NA	Beckman Coulter Master Calibrator
		IFCC (37 °C)	System calibrator	5.33%	IFCC Reference Method
	Synchron	Lactate to pyruvate	Enzyme Validator Level 1	3.03%	IFCC Reference Method
			Enzyme Validator Level 2	1.24%	IFCC Reference Method
Roche	Cobas c	IFCC liquid ver. 2	C.f.a.s.	0.66%	IFCC Reference Method
		DGKC	C.f.a.s.	0.75%	Roche reagent, manual measurement
	Integra	IFCC liquid ver. 2	C.f.a.s	0.60%	IFCC Reference Method
		DGKC	C.f.a.s	2.50%	Roche reagent, manual measurement
	Modular	IFCC liquid	C.f.a.s	0.66%	IFCC Reference Method
		DGKC	C.f.a.s	0.75%	Roche reagent, manual measurement
Siemens	Dimension Vista	IFCC method	Enzyme I Calibrator Level 2	2.17%	IFCC Reference Method
		IFCC method	Enzyme I Calibrator Level 3	2.65%	IFCC Reference Method
	Advia	Lactate to pyruvate	Chemistry calibrator control 1	0.90%	IFCC Reference Method
			Chemistry calibrator control 2	0.60%	IFCC Reference Method
		Pyruvate to lactate	Chemistry calibrator control 1	1.00%	Molar extinction coefficient of reaction product
			Chemistry calibrator control 2	0.40%	Molar extinction coefficient of reaction product

Percentage of Italian laboratories declaring to use methods employing the IFCC analytical principles



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But, those who said to report enzyme results traceable to the IFCC RMPs, did they accurately recover the targets set by the reference laboratory?

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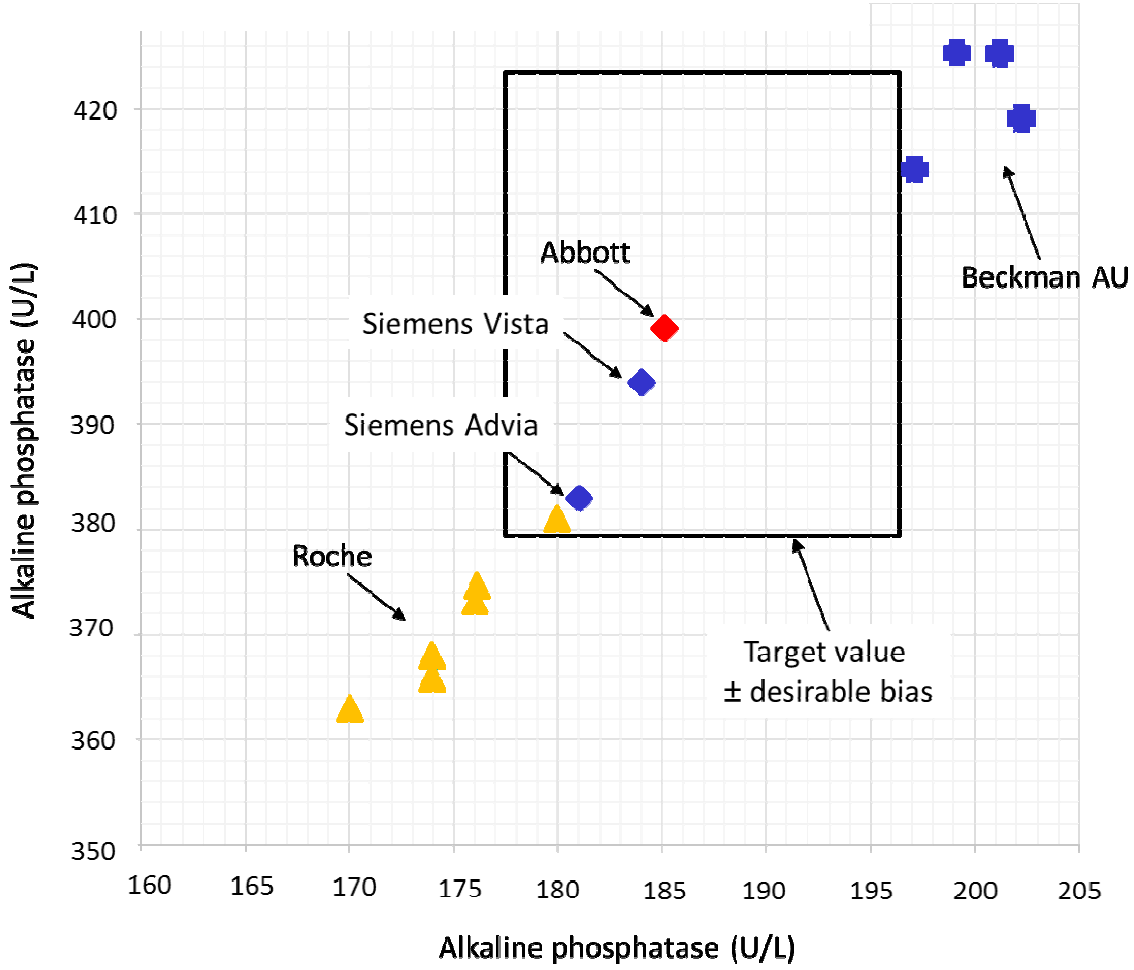
DE GRUYTER Clin Chem Lab Med 2017; 55(3): e47–e50

Letter to the Editor

Federica Braga*, Erika Frusciante, Ilenia Infusino, Elena Aloisio, Elena Guerra, Ferruccio Ceriotti and Mauro Panteghini

Evaluation of the trueness of serum alkaline phosphatase measurement in a group of Italian laboratories



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Analytical systems measuring serum ALP marketed by four IVD companies

Company	Platform	Principle of method	Calibrator	Declared uncertainty	Higher-order reference employed
Abbott	Architect	p-nitrophenyl-phosphate	Calibration factor	NA	IFCC Reference Method (2011)
		p-nitrophenyl-phosphate	Calibration factor	NA	p-nitrophenol molar extinction factor
Beckman	AU	IFCC method	System calibrator	6%	Beckman Coulter Master Calibrator
		DEA	System calibrator	NA	Beckman Coulter Master Calibrator
	Synchron	AMP	Enzyme Validator Level 1	6.22%	IFCC Reference Method (2011)
			Enzyme Validator Level 2	1.86%	
		AMP	Enzyme Validator Level 1	3.64%	DGKC Standard Method
			Enzyme Validator Level 2	1.27%	
Roche	Cobas c	IFCC Gen.2	C.f.a.s.	0.59%	IFCC Reference Method (1983)
	Integra	IFCC Gen.2	C.f.a.s	1.22%	IFCC Reference Method (1983)
	Modular	IFCC liquid	C.f.a.s	0.65%	IFCC Reference Method (1983)
		DGKC	C.f.a.s	0.91%	Roche reagent, manual measurement
Siemens	Dimension Vista	AMP	ALPI calibrator	4.51%	IFCC Reference Method (2011)
	Advia	AMP	Chemistry calibrator control 1	3.70%	IFCC Reference Method (2011)
			Chemistry calibrator control 2	1.00%	IFCC Reference Method (2011)
		DEA	Chemistry calibrator control 1	1.40%	Molar extinction coefficient of reaction product
			Chemistry calibrator control 2	1.30%	Molar extinction coefficient of reaction product

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Expected consequences

1. Experts defines reference measurement systems
2. Industry implements traceability to them
3. Users (and industry) abandon non-specific methods
4. EQAS provide commutable materials and accuracy-based grading
5. Professionals establish clinically allowable errors
6. Individual laboratories monitor their performance by participating to EQAS and applying allowable limits

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

Need of post-market vigilance of IVD systems

True value assignment to EQAS materials allows objective evaluation of the performance of enzyme measurements through an accuracy-based (instead of inferior consensus-based) grading of the competency of participating clinical laboratories.

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

Clinica Chimica Acta 414 (2012) 234–240

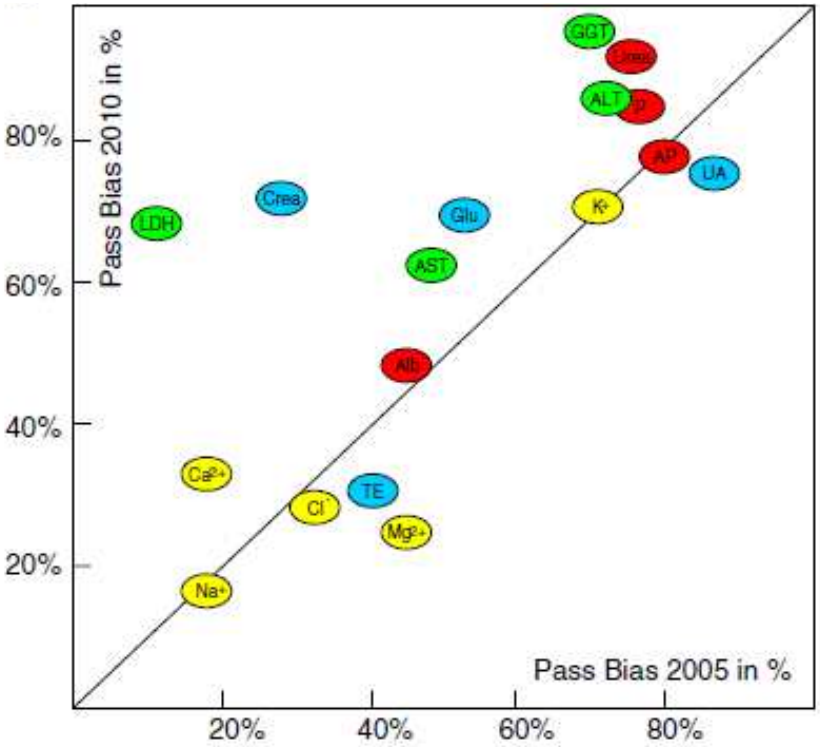
Contents lists available at SciVerse ScienceDirect

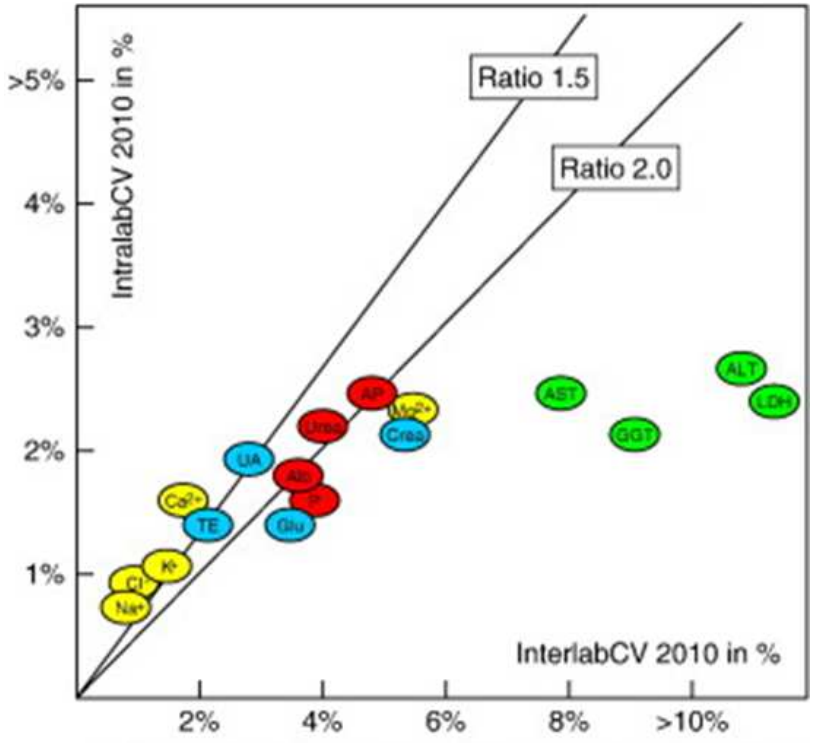
Clinica Chimica Acta

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

Systematic monitoring of standardization and harmonization status with commutable EQA-samples—Five year experience from the Netherlands

Christa Cobbaert [a,*], Cas Weykamp [b], Paul Franck [c], Robert de Jonge [d], Aldy Kuypers [e], Herman Steigstra [f], Jacqueline Klein Gunnewiek [g], Douwe van Loon [h], Rob Jansen [f]





Expected consequences

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

DE GRUYTER Clin Chem Lab Med 2015; 53(6): 833–835

Consensus Statement

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).

Analytical performance specification (APS) derivation should be added to the Miller's EQAS categorization

[Miller WG et al. Clin Chem 2011;57:1670]

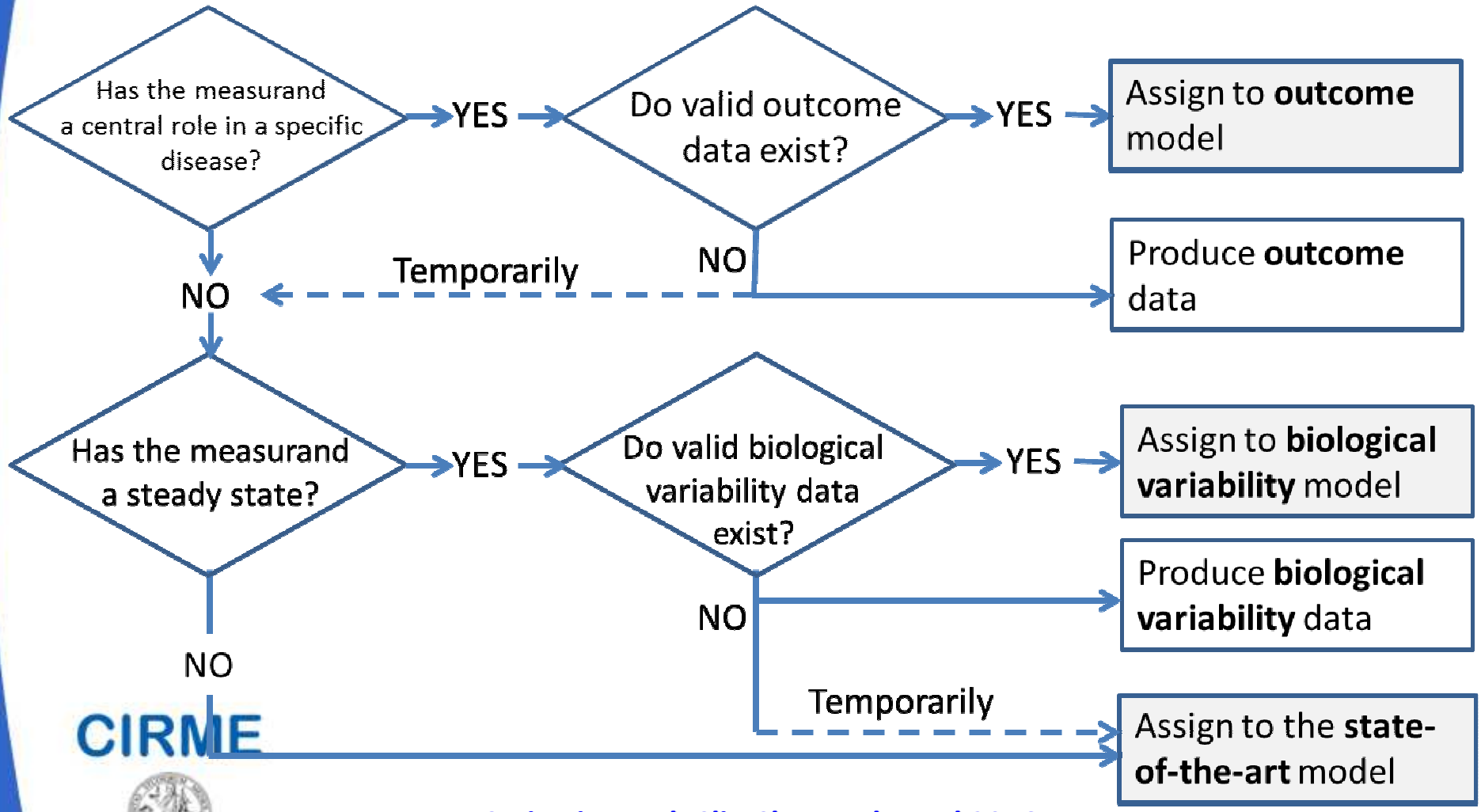
				Evaluation capability				
				Accuracy				
				Individual laboratory				
	Sample characteristics				Relative to participant results		Reproducibility	
Category	Commutable	Value assigned with RMP[a] or CRM	Replicate samples in survey	Absolute vs RMP or CRM	Overall	Peer group	Individual laboratory intralab CV	Measurement procedure interlab CV
1	Yes	Yes	Yes	X	X	X	X	X
2	Yes	Yes	No	X	X	X		X

Category 1/2A → Milan model 1 or 2 as basis for APS

Category 1/2B → Other models

Infusino I et al. Clin Chem Lab Med 2016;in press.

Workflow for allocation of laboratory measurands to different models for performance specifications



Ceriotti F et al, Clin Chem Lab Med 2016

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Quantifying Biological Variation

How do you do the experiment?

✓ Subjects	How many?
✓ Collect specimens	Number? Frequency?
✓ Analyse specimens	Minimise analytical variation?
✓ Analyse data	Outliers? Statistics?

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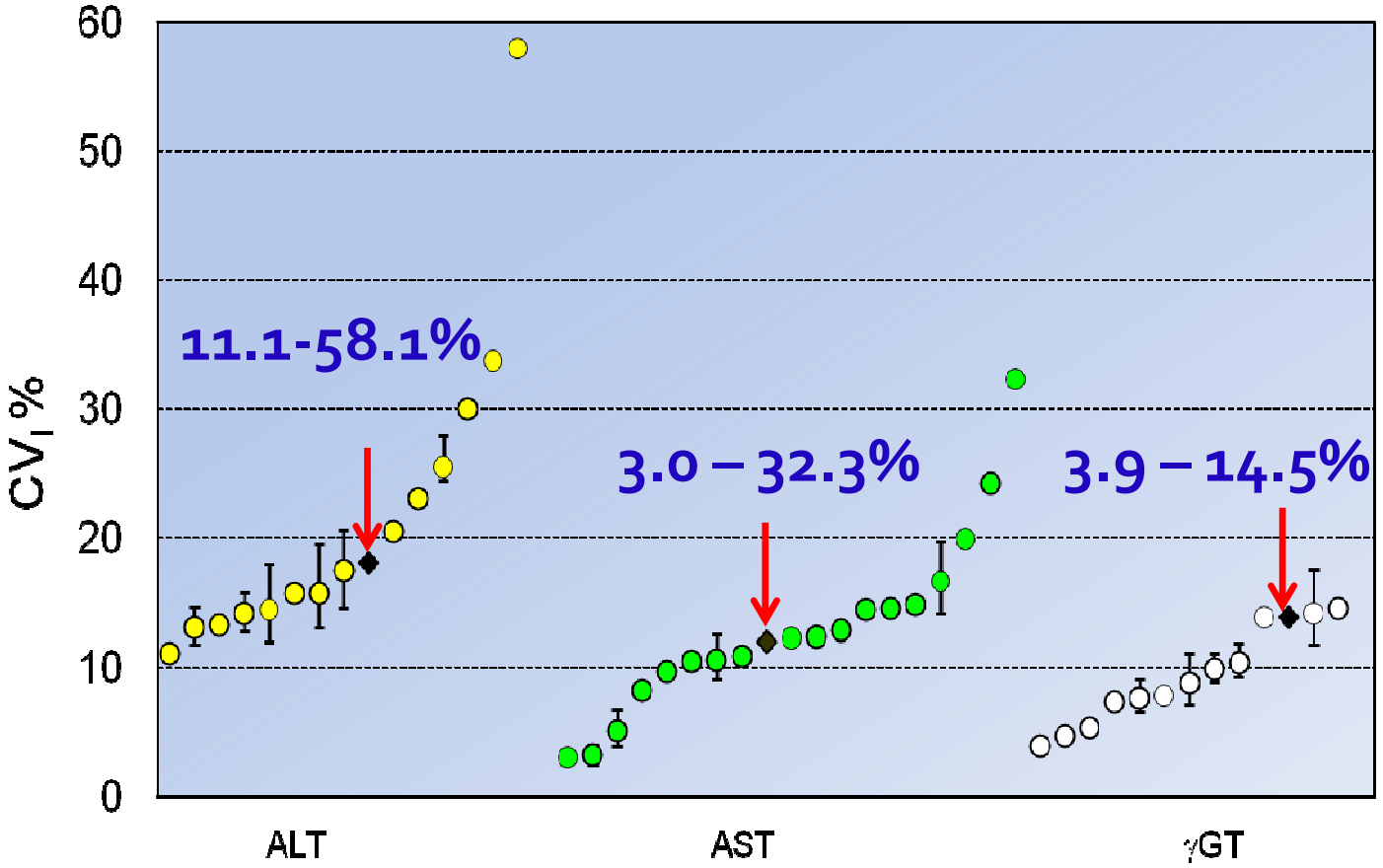
Braga F, Panteghini M. Crit Rev Clin Lab Sci. 2016 Mar 14:1-13

ALT, AST and γGT

Within-subject biological variation (CV_I)



The arrows show the values currently present in the Ricos' database

Carobene A et al., Clin Chem Lab Med 2013;51:1997

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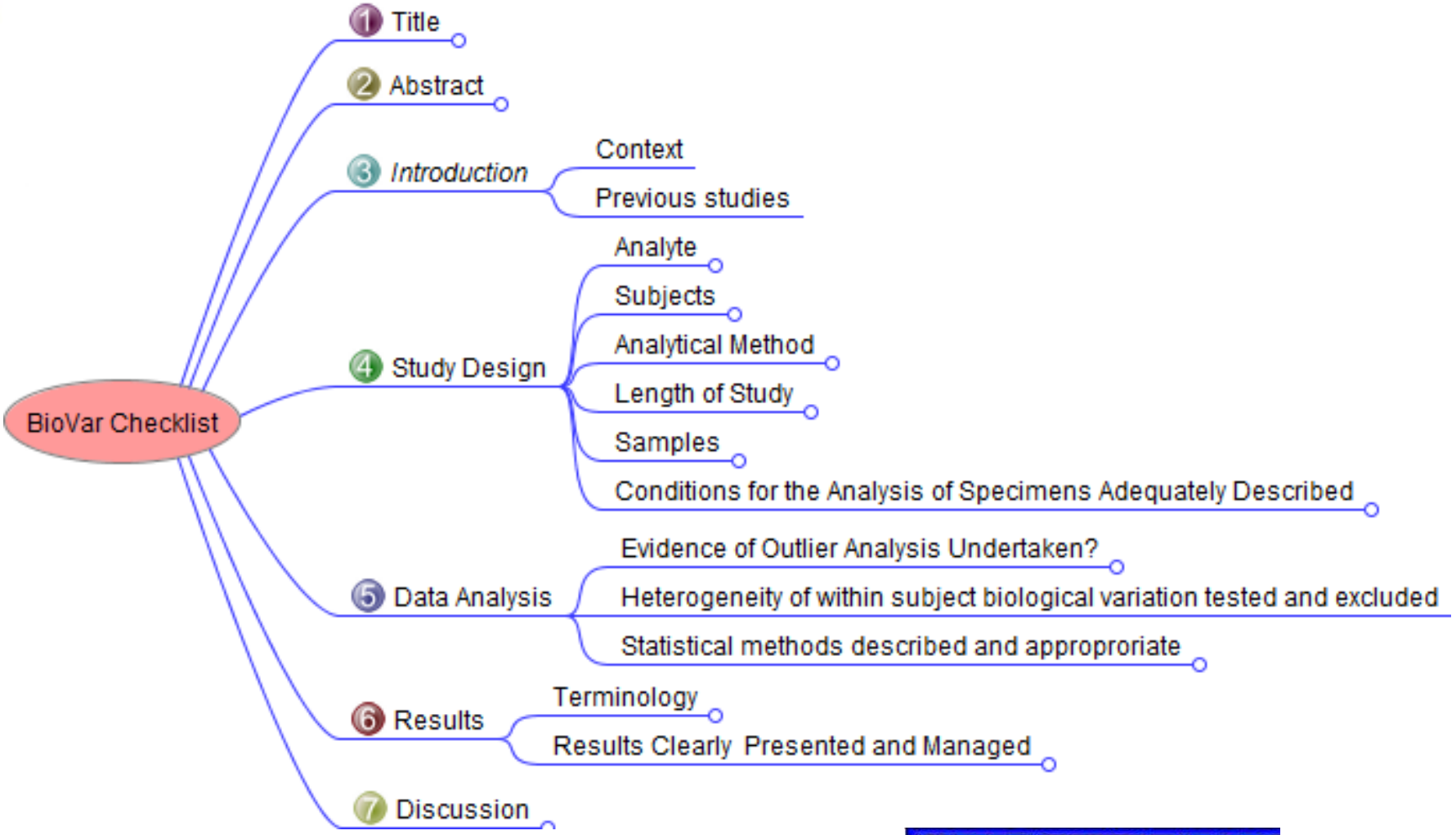
A checklist for critical appraisal of studies of biological variation



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Bartlett WA et al. Clin Chem Lab Med 2015;53: 879

Biological Variation Working Group

EFLM EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

Allowable maximum uncertainty for clinical measurements of enzymes

	Quality level		
	Minimum	Desirable	Optimum
AST	±9.3%	±6.2%	±3.1%
ALT	±14.6%	±9.7%	±4.9%
γGT	±5.6%	±3.7%	±1.9%
LDH	±6.5%	±4.3%	±2.2%
CK	±17.1%	±11.4%	±5.7%
ALP	±4.5%	±3.0%	±1.5%
AMY	±6.6%	±4.4%	±2.2%

Adapted from Panteghini M, Bais R. Tietz Textbook of Clinical Chemistry & Molecular Diagnostics, 6th ed.

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Clinical Chemistry 63:6
1141–1150 (2017)

Biological Variation Estimates Obtained from 91 Healthy Study Participants for 9 Enzymes in Serum

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

Allowable maximum uncertainty		
	Clin Chem 2017	Tietz 6th. ed
AST	±4.8%	±6.2%
ALT	±4.7%	±9.7%
γGT	±4.5%	±3.7%
LDH	±2.6%	±4.3%
CK	±7.3%	±11.4%
ALP	±2.7%	±3.0%
AMY	±3.4%	±4.4%

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- Summarizing considerations -

- Having all traceability tools in place is not enough.
- The IFCC standardization seems to be often declared but not soundly adhered to and/or correctly implemented.
- As a consequence, a sizeable bias of the analytical results vs. the reference method values is often observed.
- Some manufacturers continue to sell on the market assays giving results which are not traceable to the internationally accepted reference systems.

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Why are we still not there?

- Legislation
- IVD manufacturers
- Professionals

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Legislation

- EU IVD Directive 98/79 gives only generic indications on traceability
- The JCTLM database has no legal value
- ISO 15189 Accreditation does not specifically require traceability to JCTLM references
- 'Accuracy assessment' by existing EQA programs is based on consensus to peer groups using the same analytical equipment and not on the true value assignment. This has created a situation where clinical laboratories can meet governmental regulations despite consistently reporting biased test results.

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Limitations of CE mark

[stating compliance with legislation, mainly by means of European standards]

- Does ***not*** mean that manufacturer has transferred trueness successfully
- Does ***not*** mean that uncertainty of calibrator meets clinical needs
- Does ***not*** mean that comparators (e.g., similar assays) are also traceable

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[Adapted from G. Jones, JCTLM & IVD Industry Meeting – Los Angeles, USA 2012]

NEW EU regulatory framework

European Commission

Official Journal L 117

of the European Union

English edition

Legislation

Volume 60

5 May 2017

CIRME

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European Commission

GROWTH

Internal Market, Industry, Entrepreneurship and SMEs

European Commission > Growth > Sectors > Medical devices > Regulatory framework > Revisions of Medical Device Directives

Revisions of Medical Device Directives

The new Regulations on medical devices

On 5 April, 2 new Regulations on medical devices were adopted. These replace the existing Directives.

- Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC
- Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU

The new rules will only apply after a transitional period. Namely, 3 years after entry into force for the Regulation on medical devices (spring 2020) and 5 years after entry into force (spring 2022) for the Regulation on in vitro diagnostic medical devices.

To allow manufacturers and authorities to adapt, the new rules will only apply after a transitional period, namely 3 years after publication for the Regulation on medical devices and 5 years after publication for in the Regulation on vitro diagnostic medical devices.

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[http://ec.europa.eu/growth/sectors/medical-devices/regulatory-framework/revision_en]

NEW EU regulatory framework

- Supervision of Notified Bodies
- Post-market safety and surveillance activities, with enhanced involvement of healthcare professionals and patients
- Transparency
  - Summary of safety and performance data
  - Traceability of devices
- Access to external expertise (scientific experts, reference laboratories)

European Commission

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IVD Manufacturers

- Manufacturers may explicitly or implicitly object harmonisation for marketing or cost reasons: in absence of mandatory requirements and of clear requests from the profession they have no interest in new investments
- No perception of a competitive advantage in offering IFCC traceable enzyme results
- To fulfill the request of a global market most of them continue to offer different reagents for the same enzyme

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Adapted from F. Ceriotti, 7th CIRME International Scientific Meeting – May 2013

Professionals

- The advantages of enzyme standardization are not fully perceived, nor by laboratorians neither by clinicians
- Changes require efforts: new reference intervals, explanations to clinicians and patients, etc. Resistance of laboratorians and clinicians originates from common human conservatism.
- Instead of requesting manufacturers to change, most of us just waits for the new proposals from industry

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Modified from F. Ceriotti, 7th CIRME International Scientific Meeting – May 2013

Clin Chem Lab Med 2017; 55(3): 334–340 DE GRUYTER

Mini Review

Ilenia Infusino*, Erika Frusciante, Federica Braga and Mauro Panteghini

Progress and impact of enzyme measurement standardization

Standardization in clinical enzymology: the way forward

- **The definition by the laboratory professionals of the clinically acceptable measurement uncertainty for each enzyme together with the adoption by EQAS providers of commutable materials and use of an evaluation approach exclusively based on trueness represent the way forward to definitively reach the standardization in clinical enzymology.**

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Table 1: Unique benefits of External Quality Assessment Schemes meeting metrological criteria.

– Giving objective information about quality of individual laboratory performance
– Creating evidence about intrinsic standardisation status/ equivalence of the examined assays
– 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 systems to demonstrate the superiority of those products
– Identifying analytes that need improved harmonisation and stimulating and sustaining standardisation 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

Ferraro S, Braga F, Panteghini M. Clin Chem Lab Med 2016;54:523

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