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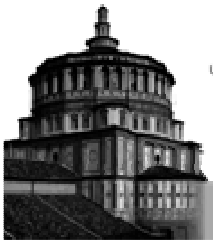

Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME)

under the auspicies of the

2nd International Scientific Meeting
STANDARDIZATION IN CLINICAL ENZYMOLOGY:
A CHALLENGE FOR THE THEORY OF METROLOGICAL TRACEABILITY
Milano, 25 Novembre 2008

Standardization in Clinical Enzymology: the IFCC efforts

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Chair, IFCC Scientific Division

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Standardisation – why bother?

- ★ Result today will be the same as tomorrow
- ★ Result in Milan will be the same as the result in London
- ★ We can set common reference limits and clinical cutpoints for intervention
- ★ We all measure to the same set of rules

.....so we can diagnose, monitor and treat patients appropriately.

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Why standardization in clinical enzymology?

- ✓ The determinations of some enzymes (CK, LDH, AST, ALT, ALP, γGT, amylase & lipase) are among the 20 most frequently ordered tests in clinical laboratories.
- ✓ These enzymatic determinations are important biochemical parameters for the diagnosis and monitoring of diseases of liver, pancreas, skeletal muscle, bone, etc.

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SPECIAL ARTICLE HEPATOLOGY, April 2008

Serum Activity of Alanine Aminotransferase (ALT) as an Indicator of Health and Disease

W. Ray Kim,[1] Steven L. Flamm,[2] Adrian M. Di Bisceglie,[3] and Henry C. Bodenheimer, Jr.,[4]
On behalf of the Public Policy Committee of the American Association for the Study of Liver Disease

This document presents the official position of the American Association for the Study of Liver Diseases (AASLD) on the application of serum alanine aminotransferase (ALT) activity, based upon an analysis of the currently available scientific data. Its authorship was selected by the Public Policy Committee. The document is fully endorsed by the AASLD Governing Board

Conclusions

ALT is an integral part of the evaluation of patients with liver disease. Its importance as a screening test for liver disease is highlighted by the fact that most patients with common liver diseases such as viral hepatitis B and C and non-alcoholic fatty liver disease have elevated ALT, even though they remain without symptoms to prompt a medical evaluation. Thus, although the interpretation and practical use of ALT analysis may differ across specific liver disease categories, ALT is a sensitive test to detect individuals with liver disease. The importance of ALT activity as an indicator of liver disease has recently been demonstrated in population-based studies which documented a strong association between ALT and subsequent mortality from liver disease.

Overall, although measurement of ALT is commonly performed as a part of the hepatic panel, the significance of this test may have been underestimated. In examining ALT as a screening tool for the population, we found that ALT meets most of the accepted criteria for a screening test. However, additional data will strengthen the rationale and inform optimal implementation of ALT screening. These include determination of the optimal schedule for ALT screening and assessment of the practical impact of its implementation as well as its cost-effectiveness. While we wait for these data, we highlight that ALT is an excellent screening test in individuals at risk of liver disease. Subsequently, an abnormal ALT result, as determined by a properly defined normal range, must trigger an appropriate clinical evaluation.

AGA Institute Medical Position Statement on Acute Pancreatitis

This document presents the official recommendations of the American Gastroenterological Association (AGA) Institute on "Management of Acute Pancreatitis." It was approved by the Clinical Practice and Economics Committee on February 14, 2007, and by the AGA Institute Governing Board on March 15, 2007.

Recommendations

Diagnosis

- The diagnosis of acute pancreatitis should be established within 48 hours of admission. The diagnosis should be based on compatible clinical features and elevations in amylase or lipase levels. Elevations in amylase or lipase levels greater than 3 times the upper limit of normal, in the absence of renal failure, are most consistent with acute pancreatitis. Elevations in amylase or lipase levels less than 3 times the upper limit of normal have low specificity for acute pancreatitis and hence are consistent with, but not diagnostic of, acute pancreatitis. Elevation of lipase levels is somewhat more specific and is thus preferred.

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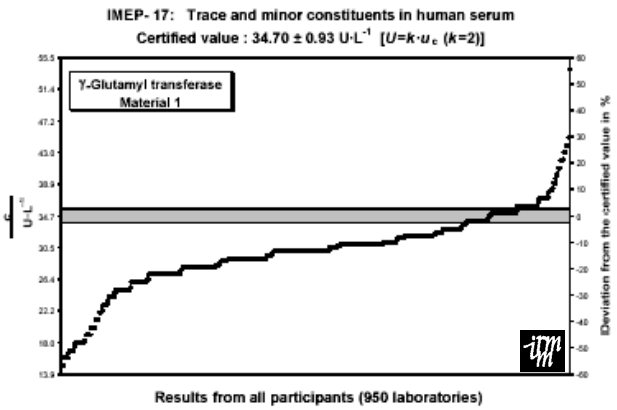
IMEP®

International Measurement Evaluation Programme - 17

Fig. 15



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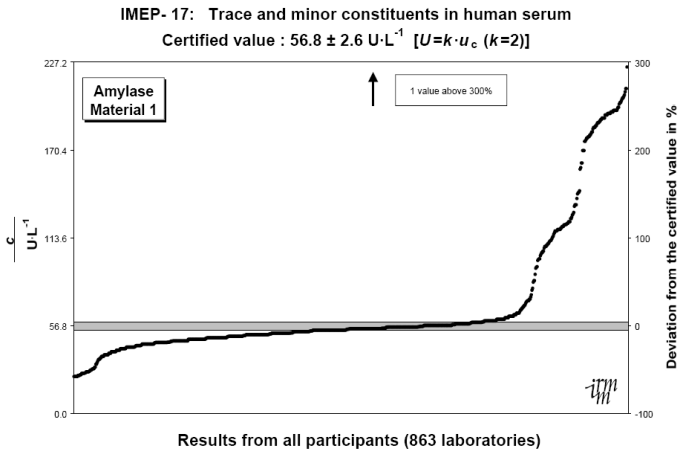
International Measurement Evaluation Programme - 17

Fig. 12



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Result expression as multiples of the URL should be discouraged!

Table 2 Inter-laboratory variation of patient results and of results expressed in multiples of the upper reference limit for routine procedures.

Laboratory	Mean, U/L (range) AST	URL[a] Female	URL[a] Male	Multiples of URL[a]
Laboratory 1[b]	40.7 ± 27.9 (12–119)	31	31	0.94
Laboratory 2	51.6 ± 36.8 (15–159)	31	31	1.18
Laboratory 3[b]	42.6 ± 28.6 (15–122)	35	40	0.64
Laboratory 4	43.2 ± 34.8 (18–143)	35	40	0.75
Laboratory 5	47.0 ± 33.5 (12–143)	31	31	0.97
Laboratory 6	49.4 ± 33.8 (15–146)	31	31	1.02
Laboratory 7	50.3 ± 35.7 (20–149)	30	40	1.60
Laboratory 8	46.0 ± 34.9 (12–143)	31	35	1.08
Laboratory 9	50.2 ± 33.5 (16–147)	32	35	1.03
Laboratory 10	50.3 ± 35.7 (16–148)	35	45	0.94
Laboratory 11	52.0 ± 35.6 (19–158)	32	35	0.89
Laboratory 12	55.8 ± 37.2 (18–166)	27	32	1.26
Laboratory 13	54.6 ± 36.9 (17–162)	27	32	1.27
Mean, U/L	46.7			0.99
Number of procedures	13			13
SD, U/L	4.60			0.18
CV, %	9.85			18.5

[a] URL: upper limit of reference values used by the laboratory. [b] Performed with reagents without pyridoxal-5′-phosphate. Results were significantly lower ($p < 0.0001$) when compared to those obtained in laboratories 2 and 4.

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Ferard et al. CCLM 2005;43:549

Measurement of enzyme catalytic activity

⇨ The results are method-dependent

Variables:

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

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Standardization of measurement of enzyme catalytic activities

Traditional approach →

"Method globalization"

1. Carefully define the characteristics of recommended procedure
2. Support the widespread use of the selected procedure

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"Method globalization" approach: fundamental steps

- @ National level:
  - Recommendations of the German Society for Clinical Chemistry (1972)
  - Società Italiana di Biochimica Clinica (SIBioC), Enzyme Commission. Recommended methods for determination of four enzymatic activities (1980)
  - Société Francaise de Biologie Clinique, Commission Enzymologie (1982)
- @ Regional level:
  - Scandinavian standardization of enzyme determinations (1974)
  - ECCLS Documents no. 3/4 (1988)
- @ International level:
  - IFCC recommended methods for the measurement of catalytic concentrations of enzymes at 30 °C (1983-1998)

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"Method globalization" approach: problems

- ✓ Reference methods are generally not appropriate for direct routine use in clinical labs (temperature, sample blank, reaction times, etc.)
- ✓ Reference methods could not respond to the continuous development and improvement of technology
- ✓ The goal of a single, universally used method cannot therefore be achieved

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Development of a reference system

- ✓Objective is the comparability of the results *(read medical meaning)* by different laboratories with a free choice of measurement procedures and analytical instruments.
- ✓The traceability of values assigned to calibrators and controls must be assured through available reference measurement procedures and reference materials of higher order.

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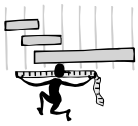

What is metrological traceability?

Property of the result related to national or international standards through an unbroken chain of comparisons all having stated uncertainties

Objective → 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

NOTES
1. The concept is often expressed by the adjective "traceable"
2. The unbroken chain of comparisons is called a "traceability chain"

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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.

Definition of Enzyme Catalytic Activity

Conversion rate of an indicator substance in a specified system according to a given measurement procedure expressed in “katal” which is *practically* equivalent to “mol/s”.

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:

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

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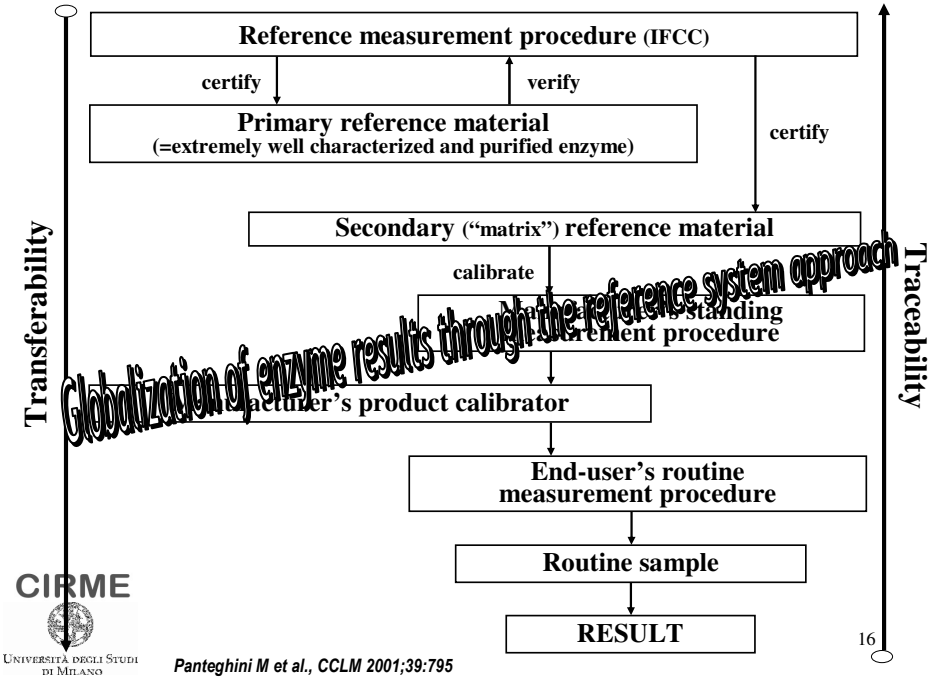
ISO 18153:2003. In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials.



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Panteghini M et al., CCLM 2001;39:795

Prerequisites for Applying Traceability to Clinical Enzymology

1. Analytical specificity of methods of measurement
2. Commutability of reference materials and calibrators

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COMMUTABILITY (def.):

③ "the ability of an enzyme [reference or calibrator] material to show interassay activity changes similar to those of the same enzyme in human serum."

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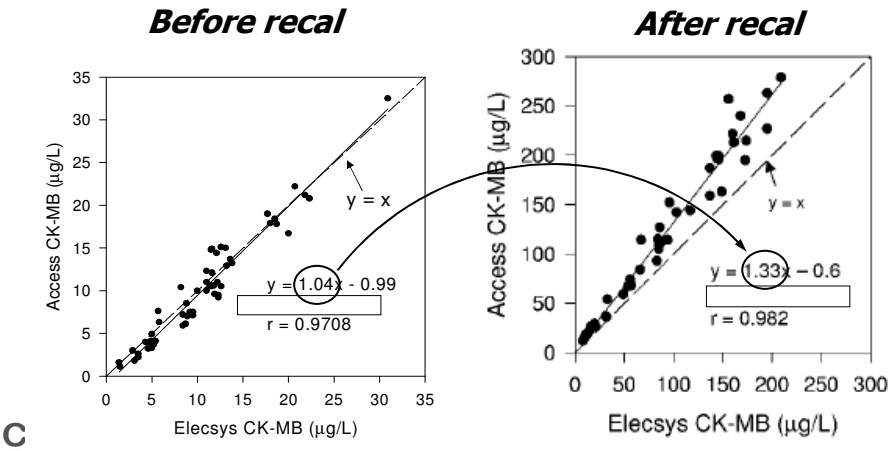
Rej R. Arch Pathol Lab Med 1993;117:352

The perverse effect of recalibrating CK-MB immunoassays with a non-commutable material



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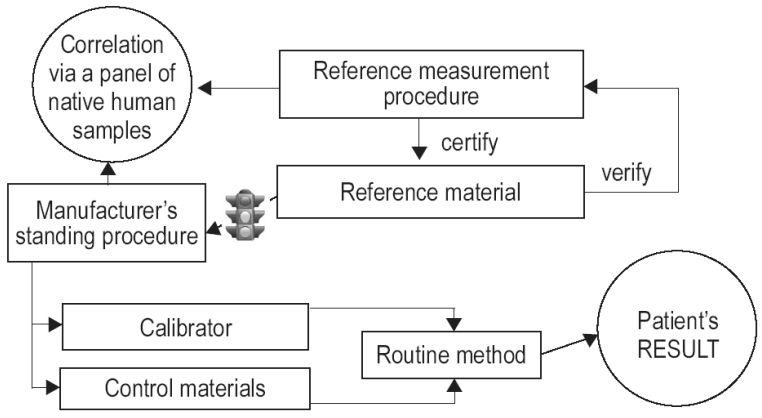
Panteghini M & Pagani F, Clin Chem 2004



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Infusino I et al, Clin Biochem Rev 2007;28:155

Components of a Working Reference Measurement System

The Reference Measurement System should comprise:

- a clear definition of the analyte to be measured in human samples
- reference measurement procedure(s) which specifically measures the analyte as defined
- primary and secondary (commutable) reference materials
- reference measurement laboratories, possibly collaborating in a network

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Panteghini M, Clin Biochem Rev 2007;28:97

IFCC Scientific Division

Committee on Reference Systems for Enzymes (C-RSE)

→ Implementation of reference measurement procedures

→ Establishing of the reference procedures within a network of reference laboratories according to stringent metrological principles

→ Selection of suitable reference materials and certification by the network of reference laboratories

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Scientific Division

Committee on Reference Systems for Enzymes (C-RSE)

Design of new 37 °C IFCC Reference Procedures

- based on existing 30 °C IFCC procedures,
- optimised substrate concentration, pH, buffer concentration, lag phase, measuring time interval,
- fixed in exact protocols (standard operating procedures, SOP) prescribing all measurement conditions in detail,
- reporting uncertainty for all relevant steps of the analytical procedures.

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JCTLM

Reference Measurement Service Providers for Enzymes

CIRME (Centro Interdipartimentale per la Riferibilita' Metrologica in Medicina di Laboratorio - Universita' di Milano), Italy – Contact person: Prof. M Panteghini mauro.panteghini@unimi.it

DGKL (Reference Institut of the German Society of Clinical Chemistry and Laboratory Medicine), Germany – Contact person: Prof. G Schumann schumann.gerhard@mh-hannover.de

Instand e.V., Germany – Contact person: Prof. H Reinauer reinauer@instand-ev.de

KCHL HagaZiekenhuis (Klinisch Chemisch en Hematologisch Laboratorium HagaZiekenhuis), The Netherlands – Contact person: Dr. PFH Franck p.franck@hagaziekenhuis.nl

Laboraf (Diagnostica e Ricerca San Raffaele S.p.A.), Italy –
Contact person: Dr. F Ceriotti ceriotti.ferruccio@hsr.it

Odense University Hospital, Denmark –
Contact person: Dr. PJ Jorgensen poul.joergen.joergensen@ouh.regionsyddanmark.dk

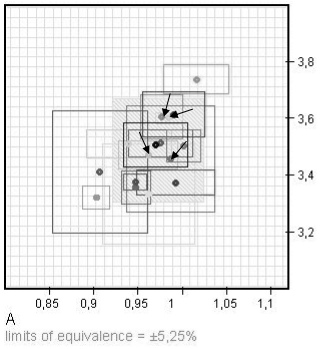
For use by (primarily):

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)

Arrows indicate results from 4 JCTLM-listed laboratories

ALT [ukat/l]

RELA 2007
13.06.2008



Lab	A	e.u.	B	e.u.	method
03	0,976	0,023	3,605	0,076	Kinetic spectroscopy
06	0,985	0,014	3,600	0,018	Kinetic spectroscopy
16	0,961	0,033	3,470	0,050	Kinetic spectroscopy
18	0,94	0,048	3,51	0,048	Kinetic spectroscopy
25	0,961	0,051	3,333	0,177	spectrophotometry
38	0,986	0,050	3,456	0,187	spectrophotometry
41	1,016	0,036	3,736	0,050	kinetic spectroscopy
46	0,903	0,015	3,321	0,039	Kinetic spectroscopy
47	0,993	0,044	3,373	0,044	Kinetic spectroscopy
48	0,99	0,035	3,613	0,079	Kinetic spectroscopy
54	0,948	0,016	3,356	0,056	Kinetic spectroscopy
55	0,976	0,036	3,512	0,047	Kinetic spectroscopy
61	0,907	0,053	3,410	0,215	spectrophotometry
63	1,002	0,019	3,503	0,057	Kinetic spectroscopy
64	0,97	0,036	3,506	0,077	Kinetic spectroscopy
65	0,947	0,013	3,375	0,029	Kinetic spectroscopy

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Results of IFCC ring trials are available at: http://www.dgkl-rfb.de:81

Table 1. Characteristics of the enzyme reference materials certified by the IFCC enzyme laboratory network in cooperation with the Institute for Reference Materials and Measurements (IRMM).

Enzyme	Code	Origin	Form	Certified concentration	Uncertainty
GGT	ERM-AD452	Pig kidney	Light subunit	114.1 U/L	±2.4 U/L
LD	ERM-AD453	Human erythrocytes	LD1 isoenzyme	502.0 U/L	±7.0 U/L
ALT	ERM-AD454	Pig heart	-	186.0 U/L	±4.0 U/L
CK	ERM-AD455	Human heart	MB isoenzyme	101.0 U/L	±4.0 U/L

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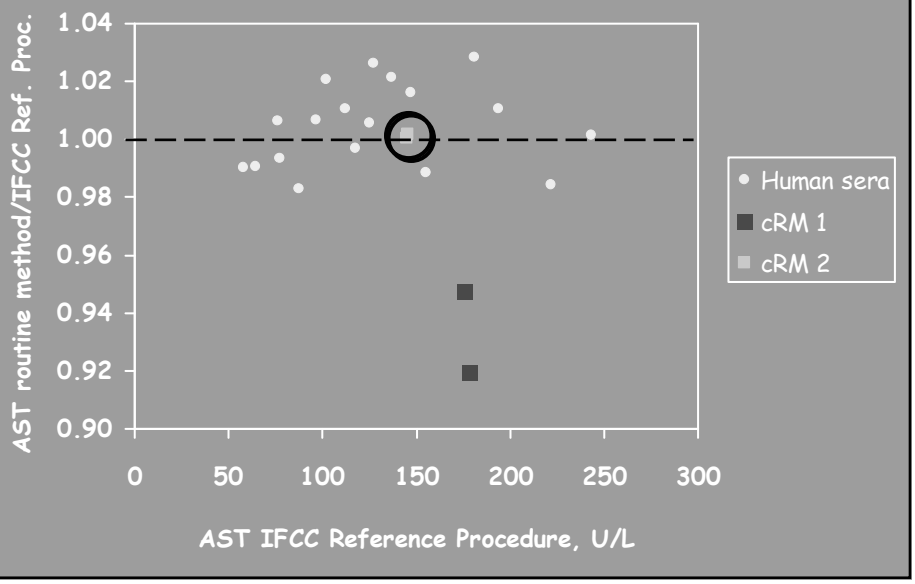
AST reference material: commutability



Existing Reference Systems for Enzymes

	Reference Method	Ref. Material
AST	Clin Chem Lab Med 2002;40:725-33	Released soon
ALT	Clin Chem Lab Med 2002;40:718-24	ERM-AD454 (IFCC)
γGT	Clin Chem Lab Med 2002;40:734-8	ERM-AD452 (IFCC)
LDH	Clin Chem Lab Med 2002;40:643-8	ERM-AD453 (IFCC)
CK	Clin Chem Lab Med 2002;40:635-42	ERM-AD455 (IFCC)
AMY	Clin Chem Lab Med 2006;44:1146-55	ERM-AD456 (IFCC)
ALP	Manuscript in preparation	Under evaluation

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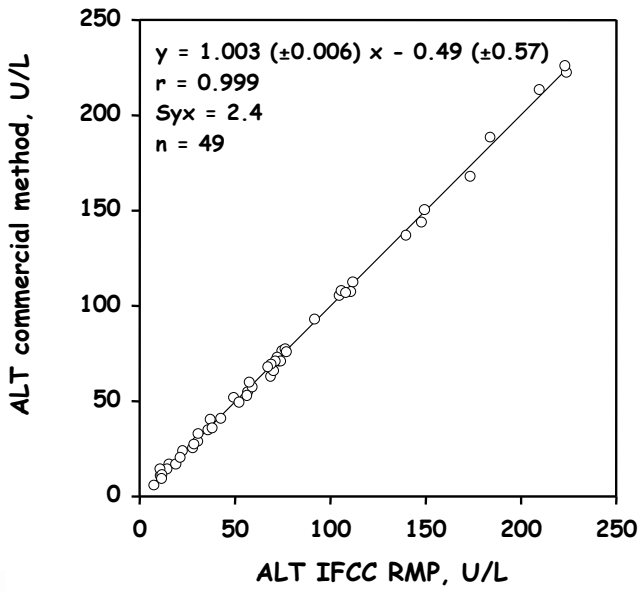

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Lack of proper reference intervals (R.I.) may hamper the implementation of standardization in enzymology

- The implementation of standardization can modify the 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 methods hampers their adoption
- A single clinical laboratory has not enough means to adequately produce the reference limits
- Manufactures have problems too

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Scientific Division

Committee on Reference Intervals & Decision Limits (C-RIDL)

→ Preparation of a protocol for collaborative experiments on the establishment of reference values using assays traceable to reference systems

→ Production of "standardized" reference intervals for AST, ALT, and γGT

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Common reference intervals as fourth pillar of the reference system: how a problem becomes a solution

Until today	**From today**
Method-dependent results	Standardized methods that provide traceable results
↓	↓
Method-dependent reference intervals	Common reference intervals (at least within homogeneous ethnic groups)

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Need of post-market vigilance of IVD systems

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

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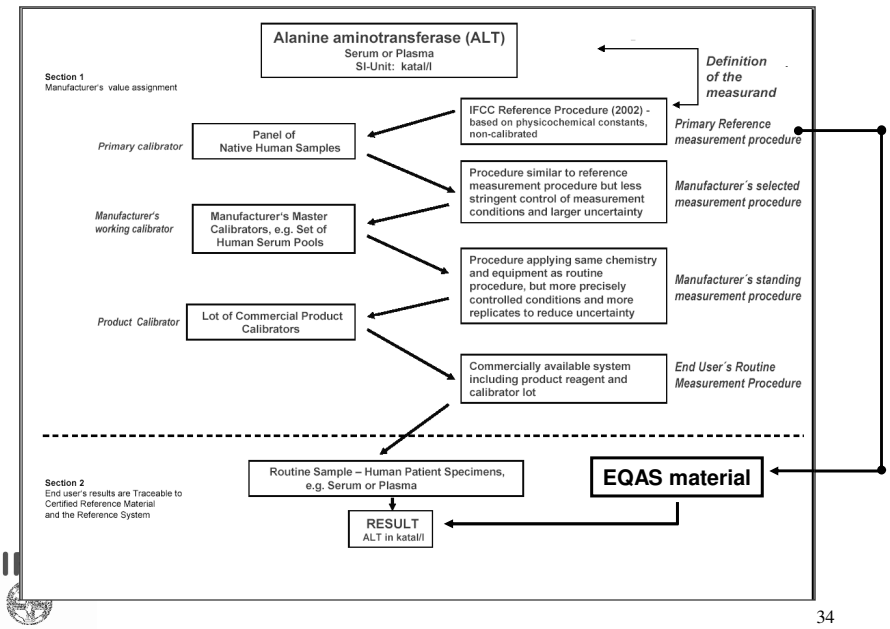

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Main features for the applicability of true value concept in EQAS

Feature	Aim
Values assigned with IFCC reference methods by an accredited reference laboratory	To check trueness as traceability to IFCC reference systems
Proved commutability of control material(s)	To allow transferability of results to patient samples
Definition of the clinically allowable total error of measurements	To permit reliable application of laboratory measurements in clinical setting

C

UNIVER

Clinically allowable total error for enzyme measurements

	Quality level		
	Minimum	Desirable	Optimum
AST	25.6	17.2	8.5
ALT	53.8	35.9	17.9
γGT	36.5	24.3	12.2
LDH	19.0	12.7	6.3
CK	50.8	33.8	17.0
AMY	23.9	16.0	8.0

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CONCLUSION

The reference system approach can give the clinical laboratory and medical community universal means of creating and ensuring result comparability without requiring disruptive changes in the existing working methods or in individual's preference for an analytical system.

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