Mini Review

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

Progress and impact of enzyme measurement standardization

DOI 10.1515/cclm-2016-0661
Received July 25, 2016; accepted August 29, 2016; previously published online October 8, 2016

Abstract: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has established reference measurement procedures (RMPs) for the most popular enzymes. Manufacturers should assign values to commercial calibrators traceable to these RMPs to achieve equivalent results in clinical samples, independent of reagent kits, instruments, and laboratory where the measurement is carried out. The situation is, however, far from acceptable. Some manufacturers continue to market assays giving results that are not traceable to internationally accepted RMPs. Meanwhile, end-users often do not abandon assays with demonstrated insufficient quality. Of the enzyme measurements, creatine kinase (CK) is satisfactorily standardized and a substantial improvement in performance of marketed γ-glutamyltranspeptidase (GGT) assays has been demonstrated. Conversely, aminotransferase measurements often exceed the desirable analytical performance because of the lack of pyridoxal-5-phosphate addition in the commercial reagents. Measurements of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and α-amylase (AMY) still show major disagreement, suggesting the need for improvement in implementing traceability to higher-order references. This is mainly the result of using assays with different analytical selectivities for these enzymes. The definition by laboratory professionals of the clinically acceptable measurement uncertainty for each enzyme together with the adoption by EQAS of commutable materials and use of an evaluation approach based on trueness represent the way forward for reaching standardization in clinical enzymology.

Keywords: enzymes; reference procedures; reference values; traceability; uncertainty.

***Corresponding author: Ilenia Infusino,** Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), Via GB Grassi 74, University of Milan, 20157 Milan, Italy,
Phone: +390239042745, Fax: +390239042364,
E-mail: infusino.ilenia@asst-fbf-sacco.it
Erika Frusciante, Federica Braga and Mauro Panteghini: Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy

Introduction

Serum enzymes are among the 20 most frequently ordered tests in clinical laboratories, as they represent important biomarkers for the diagnosis and monitoring of diseases of the liver, pancreas, skeletal muscle, bone, etc. [1]. Consequently, the analytical validity and standardization of their measurements have a central role and may become a matter of patient safety, thus potentially affecting the way enzyme tests should be used to provide optimal care [2]. It is now globally recognized that to be accurate and equivalent for long term, laboratory results should be traceable to higher-order references (methods and/or materials) [3]. In this regard, it is essential to build an unbroken metrological traceability chain that starts from the definition of the measurand and ends, through a calibration hierarchy, at the level of the patient's result [4]. Through a suitable metrological traceability chain, the in vitro diagnostics (IVD) manufacturers can reliably transfer the measurement trueness from the highest level of the metrological hierarchy to the calibrators of commercial analytical systems used in clinical laboratories to obtain unbiased results on clinical samples.

Previously, we discussed how standardization in clinical enzymology and the achievement of interlaboratory agreement of enzyme catalytic activity measurements may represent a challenge for the theory of metrological traceability in laboratory medicine [5, 6]. Furthermore, it is now clear that having all traceability tools in place is not often enough to ensure that patient care remains consistent, as the efficacy of traceability implementation should also be considered [4, 7]. The aim of this article is to update the knowledge in the field 6 years since our last review on this journal [6], with special reference to the progress and impact of enzyme measurement standardization.

Establishing a reference system in clinical enzymology

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 [8]. As a consequence, the numerical results of enzyme catalytic activity are method-dependent, i.e. they depend entirely on the experimental conditions under which measurements are made (pH and nature of buffer, nature and concentration of substrate, presence of activators and inhibitors, measurement temperature). In the standardization of enzyme assays, a reference measurement procedure (RMP), which defines the conditions under which a given enzyme activity is measured, occupies therefore the highest level of the traceability chain [9]. In performing RMPs for enzymes, technical aspects related to gravimetry, volumetry, pH, reaction temperature, and photometry must be carefully controlled to achieve a level of measurement uncertainty as lower as possible; meanwhile, a comprehensive list of relevant sources of uncertainty should be assembled in order to identify the most relevant ones (Figure 1) [10, 11].

In the last 14 years, the IFCC has recommended RMPs for seven enzymes [12–18], which are now listed in the database of the Joint Committee on Traceability in Laboratory Medicine (JCTLM) [19]. Few enzyme reference materials (ERM) are also listed (for aspartate aminotransferase [AST], γ-glutamyltranspeptidase [γGT], and α-amylase [AMY]), whereas reproduction and characterization of new ERMs for alanine aminotransferase (ALT), creatine kinase (CK) and lactate dehydrogenase (LDH) are ongoing [20]. It should be noted that the lack of ERMs is not a major issue for the implementation of enzyme measurement standardization, as manufacturers may better approach it by splitting a panel of native clinical samples with an accredited reference laboratory performing RMP and then calibrate their commercial systems in accordance with correlation results obtained using the RMP-assigned values of clinical samples [5]. To perform this exercise, nine accredited laboratories providing a reference service for enzyme measurements are currently present on the JCTLM database, four from Europe and five from China (http://www.bipm.org/jctlm/home.do).

Meanwhile, it has been noted that the lack of proper reference intervals may hamper the implementation of standardization in enzymology [6]. Standardization can indeed modify enzyme results and without adequate reference intervals this situation can impair the interpretation of the results and, paradoxically, worsen the patient's outcome [2, 21]. The introduction of enzyme reference measurement systems to provide traceable patient results have therefore been followed by the definition of traceable reference intervals to provide more congruent and effective information to clinicians. In particular, traceable reference intervals have been established for AST, ALT, γGT, LDH, CK, alkaline phosphatase (ALP), and AMY first in Caucasian adults and afterwards in Asian individuals [17, 18, 22–26]. Table 1 reports a synopsis of traceable reference intervals for enzymes obtained in European and Asian subjects. Note the slight difference in ALT and LDH values between the two groups and the marked increase in ALP, AMY, and CK (only in males) values in Asian adults.

Several examples in literature show that the application of the metrological traceability approach works well in harmonizing enzyme results in clinical samples [27, 28].

Fulfilling expectations

Traceability implementation by industry

Previously, we described what we consider the major steps in the achievement of standardization of enzyme measurements [6]. After the establishment of reference systems, we put on the top the implementation of

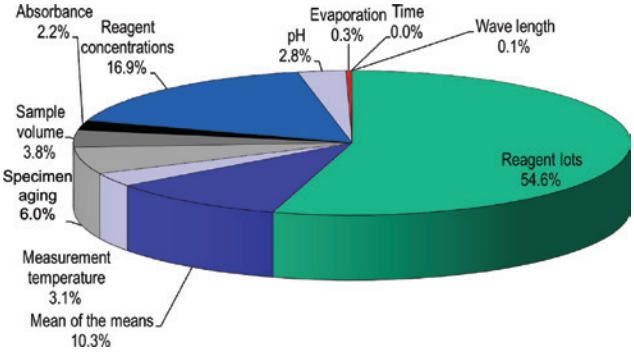

Figure 1: Example of uncertainty budget for the alanine aminotransferase (ALT) reference measurement procedure.
Adapted from ref. [10].

Table 1: Traceable reference intervals for enzymes with established reference measurement systems obtained in European and Asian adults.

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

Data from refs. [17, 18, 22–26].

traceability by diagnostic manufacturers to them. The European Union (EU) directive on IVD medical devices obliges manufacturers to ensure traceability of their measuring systems to recognized higher-order references [29]. However, the introduction of correctly standardized assays in enzymology appears not be occurring smoothly. In 2006, a first multicenter study involving 70 laboratories from three European countries assessed the traceability of enzyme results to RMPs using assays from six manufacturers [30]. Although for ALT results were relatively good, major drawbacks were shown for AST, CK, γGT, LDH, and AMY. More recently, the same group reassessed the traceability of enzyme measurements in four European countries [31]. Authors concluded that CK is now satisfactorily standardized and a substantial improvement in performance of marketed GGT assays is evident. Conversely, aminotransferases, LDH, and AMY still show major disagreement among assays, suggesting the need for improvement in implementing traceability. Interestingly, assay performance varied considerably also within users of instruments from the same manufacturer. This is mainly dependent on the availability on the same platforms of various reagent options having different analytical selectivities for the enzyme declared to be measured. The case of aminotransferases is very illustrative. Almost all manufacturers still market assays with or without the addition of pyridoxal-5-phosphate (P-5′-P), in both cases declaring to be traceable to RMP. However, it is impossible to calibrate procedures for aminotransferases that do not incorporate P-5′-P using a procedure that does, such as the RMP, because the ratio of pre-formed holoenzyme to apoenzyme differs among specimens. Another recent study confirmed that assays without P-5′-P activation are often unable to fulfill bias specifications when aminotransferase results are compared to RMPs [32].

Abandonment of non-specific assays by end-users

The commercial availability of methods with different selectivities for an enzyme measurand points to the need for laboratories to take responsibility to move to assays displaying similar selectivity when compared to RMP. According to a simple market law, if users continue to ask for and buy non-specific assays, manufacturers will indeed continue to produce and market them.

The concept of the reference measurement system is working only if the RMP and corresponding commercial procedures have identical, or at least very similar, selectivity for the measured enzyme [9]. A recently performed survey of 73 Italian laboratories has checked the spread of different methods for enzymes measurements [33]. The percentage of laboratories declaring to use methods employing IFCC analytical principles was markedly increased for all common enzymes when compared to 2006. However, the central question is: "Those who believe to report enzyme traceable results, did they accurately recover the targets set by the RMP?" Using ALP as an example, while in the mentioned survey, 80% of laboratories declared to use methods based on the IFCC RMP analytical principle, a recent study evaluating the trueness of ALP measurements using serum pools with target values assigned by RMP showed that in a group of 13 first-rate laboratories, only three (23%) fulfilled the desired goal for bias at all three tested ALP concentrations and only one provided data with a dispersion always within the uncertainty of the target value set by the reference laboratory [34]. Interestingly, the ability to meet the goal was clearly dependent on the analytical system used.

External quality assessment schemes (EQAS) using commutable materials and trueness-based grading

We emphasized several times how the lack of properly structured EQAS prevents the objective evaluation of the reliability of measuring systems and of the quality of analytical measurements provided by clinical laboratories [4, 6, 35, 36]. Only true value assignment by RMP to EQAS commutable materials allows objective evaluation of the performance of enzyme measurements through a trueness-based (instead of inferior consensus-based) grading of the competency of participating clinical laboratories. The fulfillment of requirements for the applicability of EQAS results in the evaluation of the performance of participating laboratories in terms of traceability of their measurements involves both technical and economic efforts by EQAS organizers that are still limiting their introduction [37, 38]. It is, however, clear that these aspects have to be immediately solved since EQAS that meet metrological criteria have unique benefits that add substantial value to the practice of laboratory medicine [2]. This has been shown by Cobbaert et al. [39], who, using commutable and RMP-targeted EQAS materials, were able to demonstrate a significant improvement of enzyme standardization in the Netherlands from 2005 to 2010. The percentage of laboratories fulfilling the clinically allowable bias increased from ~10% to ~70% for LDH, from ~40% to ~60% for AST, and from ~70% to ~90% or more for ALT and γGT, respectively. By the way, the study has also shown, by comparing

the interlaboratory CV to the intralaboratory CVs, that enzymes are among the analytes with further harmonization potential.

Professionals establish analytical performance specifications (APS)

In addition to the definition of reference systems, the laboratory profession is expected to establish, in agreement to recognized models, APS for enzymes measurements to make their determination clinically usable and to ensure that the measurement error does not prevail on the result [35]. During the conference held in Milan in 2014, the hierarchy of models for deriving APS was revised [40]. In particular, three models to set APS have been recommended: model 1, based on the effect of analytical performance on clinical outcomes; model 2, based on components of biological variation of the measurand; and model 3, based on state-of-the-art of the measurement [40]. We strongly support the addition of APS derived from these models to the EQAS categorization previously published by Miller et al. [37] as criteria to evaluate the performance of laboratories participating to EQAS. Miller's categories 1 and 2, which fulfill the metrological requirements highlighted above, should be each split in two sub-categories: 1/2A, in which Milan high-order models 1 and 2 for APS are applied, and 1/2B, in which other low-order models are employed.

It is still under discussion under which APS model different measurands should be placed [41]. Since valid outcome data are generally not available, the biological variability model is applied to enzymes. However, Carobene et al. [42], in assessing the validity of published biological variability data for ALT, AST and γGT, showed a wide range of values derived from inconsistent protocols and/or wrong statistical derivation. Therefore, there is a need for critical appraisal of such publications and only those that fulfill recommendations for biological variability data production should be considered in order to derive APS [43]. Table 2 reports performance specifications for allowable maximum uncertainty in enzyme measurements by clinical laboratories, obtained from studies using robust protocols to evaluate biological variability [1].

Table 2: Allowable maximum uncertainty for enzyme measurements performed by clinical laboratories.

Enzyme	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%

Summarizing considerations

The findings discussed in this review incontrovertibly show that having all traceability tools for the most important enzymes in place is not enough to obtain their standardization. The IFCC standardization seems to be often declared, but not soundly adhered to and/or correctly implemented. Furthermore, some manufacturers continue to sell on the market assays that are not traceable to internationally accepted reference systems. Therefore, a sizeable bias of the analytical results towards the RMP values is often observed. We can consider three main aspects that oppose the complete achievement of standardization in clinical enzymology: (a) legislation shortcomings, (b) manufacturing limitations, and (c) lack of a proactive role of professionals, who do not fully perceive the advantages of enzyme standardization.

Legislation

The EU directive on IVD medical devices gives only generic indications on traceability. Compliance with the directive is indicated through the CE ("Communautés Européennes") marking of conformity on diagnostic products, but at present, no normative verification by a third party of the manufacturer's statements is provided. CE mark does not mean that IVD manufacturers has transferred trueness successfully and that uncertainty of calibrators meets clinical needs [4]. Meanwhile, the JCTLM database has no legal value and the ISO 15189 accreditation standard does not specifically require traceability to JCTLM references. "Accuracy assessment" by existing EQAS is usually 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 enzyme results. The hope is that the forthcoming EU regulatory framework will require supervision of notified bodies, with access to external expertise (e.g. scientific experts, reference laboratories), well-defined post-market surveillance activities, with enhanced involvement

of laboratory professionals, and more transparency by manufacturers in providing performance data and traceability of IVD devices.

IVD manufacturers

Manufacturers may explicitly or implicitly object to standardization for marketing or cost reasons: in the absence of mandatory requirements and of clear requests from the profession, they have no interest in new investments. To fulfill the request of a global market, most continue to offer different reagents for the same enzyme, not having a perception of the competitive advantage in offering only RMP-traceable assays.

Professionals

The advantages of enzyme standardization are often not fully perceived, neither by laboratorians nor by clinicians. Resistance of professionals originates from common human conservatism because changes require efforts, such as establishment of new reference intervals and explanations to clinicians and patients. Instead of requesting manufacturers to change, most of us just waits for the new proposals from industry. As mentioned earlier [2], to become relevant in the healthcare environment, laboratory professionals have to change their attitude from one of being defensive to one that is outward looking and innovative.

The way forward

Fifteen years have passed from the publication on this journal of the first review promoting enzyme standardization by implementing traceability to higher-order RMPs [9]. Over this period, we saw (and we were part of) many efforts contributing to transfer the theory in the daily clinical practice. Quoting Sir Paul McCartney, "the road is still long and winding", but we should not despair. In particular, we strongly believe that the adoption of APS based on the clinically acceptable measurement uncertainty for each enzyme together with the provision by EQAS organizers of commutable materials and use of an evaluation approach exclusively based on trueness represents the way forward to reach the standardization in clinical enzymology. This will definitively help those manufacturers that produce superior products to demonstrate the superiority of those products and oblige users (and consequently industry) to abandon assays with demonstrated insufficient quality.

Acknowledgments: The authors acknowledge the long cooperation and the fruitful discussion with Prof. Ferruccio Ceriotti, Chair of the IFCC Committee on Reference Systems in Enzymology.
Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Panteghini M, Bais R. Serum enzymes. In: Rifai N, Horvat AR, Wittwer C, editors. Tietz textbook of clinical chemistry and molecular diagnostics, 6th ed. St. Louis: Elsevier Saunders, 2016.
2. Ferraro S, Braga F, Panteghini M. Laboratory medicine in the new healthcare environment. Clin Chem Lab Med 2016;54:523–33.
3. Panteghini M. Traceability as a unique tool to improve standardization in laboratory medicine. Clin Biochem 2009;42:236–40.
4. Braga F, Panteghini M. Verification of in vitro medical diagnostics (IVD) metrological traceability: responsibilities and strategies. Clinica Chimica Acta 2014;432:55–61.
5. Infusino I, Bonora R, Panteghini M. Traceability in clinical enzymology. Clin Biochem Rev 2007;28:155-61.
6. Infusino I, Schumann G, Ceriotti F, Panteghini M. Standardization in clinical enzymology: a challenge for the theory of metrological traceability. Clin Chem Lab Med 2010;48:301–7.
7. Panteghini M. Implementation of standardization in clinical practice: not always an easy task. Clin Chem Lab Med 2012;50:1237–41.
8. 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. ISO, Geneva, Switzerland.
9. Panteghini M, Ceriotti F, Schumann G, Siekmann L. Establishing a reference system in clinical enzymology. Clin Chem Lab Med 2001;39:795–800.
10. Frusciante E, Infusino I, Panteghini M. Stima dell'incertezza della misura della concentrazione di attività catalitica dell'alanina amminotransferasi (ALT) nel siero mediante il metodo di riferimento IFCC. Biochim Clin 2011;35:20–5.
11. Rami L, Canalias F. An approach to establish the uncertainty budget of catalytic activity concentration measurements in a reference laboratory. Clin Chem Lab Med 2015;53:743–51.
12. Schumann G, Bonora R, Ceriotti F, Clerc-Renaud P, Ferrero CA, Férard G, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 2. Reference procedure for the measurement of

catalytic concentration of creatine kinase. Clin Chem Lab Med 2002;40:635–42.
13. Schumann G, Bonora R, Ceriotti F, Clerc-Renaud P, Ferrero CA, Férard G, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 3. Reference procedure for the measurement of catalytic concentration of lactate dehydrogenase. Clin Chem Lab Med 2002;40:643–8.
14. Schumann G, Bonora R, Ceriotti F, Férard G, Ferrero CA, Franck PF, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clin Chem Lab Med 2002;40:718–24.
15. Schumann G, Bonora R, Ceriotti F, Férard G, Ferrero CA, Franck PF, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med 2002;40:725–33.
16. Schumann G, Bonora R, Ceriotti F, Férard G, Ferrero CA, Franck PF, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 6. Reference procedure for the measurement of catalytic concentration of γ-glutamyltransferase. Clin Chem Lab Med 2002;40:734–8.
17. Schumann G, Aoki R, Ferrero CA, Ehlers G, Férard G, Gella F-J, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 8. Reference procedure for the measurement of catalytic concentration of α-amylase. Clin Chem Lab Med 2006;44:1146–55.
18. Schumann G, Klauke R, Canalias F, Bossert-Reuther S, Franck PF, Gella F-J, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. Clin Chem Lab Med 2011;49:1439–46.
19. Jones GR, Jackson C. The Joint Committee for Traceability in Laboratory Medicine (JCTLM)-its history and operation. Clin Chim Acta 2016;453:86–94.
20. Toussaint B, Ceriotti F, Schimmel H, Rej R, Besozzi M, Gella FJ, et al. Commutability study on candidate materials for three new enzyme certified reference materials. Clin Chem Lab Med 2014;52:S1657.
21. Panteghini M, Ceriotti F. Obtaining reference intervals traceable to reference measurement systems: is it possible, who is responsible, what is the strategy? Clin Chem Lab Med 2012;50:813–7.
22. Ceriotti F, Henny J, Queralto J, Ziyu S, Ozarda Y, Chen B, et al. Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) in serum: results from an IFCC multicenter. Clin Chem Lab Med 2010;48:1593–601.
23. Pagani F, Panteghini M. Reference interval for lactate dehydrogenase catalytic activity in serum measured according to the new IFCC recommendations. Clin Chem Lab Med 2003;41:970–1.
24. Schumann G, Klauke R. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin Chim Acta 2003;327:69–79.
25. Ichihara K, Ceriotti F, Tam TH, Sueyoshi S, Poon PMK, Thong ML, et al. The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes. Clin Chem Lab Med 2013;51:1429–42.
26. Han L, Wang J, Zhang Q, Ke P, Wu X, Wan Z, et al. Development of reference intervals for serum alkaline phosphatase among adults in Southern China traced to the new IFCC reference measurement procedure. Clin Chem Lab Med 2016;54:659–65.
27. Férard G, Piton A, Messous D, Imbert-Bismut F, Frairi A, Pyonard T, et al. Intermethod calibration of alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) results: application to Fibrotest and Actitest scores. Clin Chem Lab Med 2006;44:400–6.
28. Xia C, Tong Q, Wang Q, Tang Z, Qi L, Chi S, et al. Application of five frozen human-pooled serum samples assigned by the international federation of clinical chemistry and laboratory medicine reference procedure in a traceability investigation of gamma-glutamyltransferase catalytic concentration measurements in China. Ann Clin Biochem 2010;47:189–94.
29. Dati F. The new European Directive on in vitro diagnostics. Clin Chem Lab Med 2003;41:1289–98.
30. Jansen R, Schumann G, Baadenhuijsen H, Franck P, Franzini C, Kruse R, et al. Trueness verification and traceability assessment of results from commercial systems for measurement of six enzyme activities in serum. An international study in the EC4 framework of the Calibration 2000 project. Clin Chim Acta 2006;368:160–7.
31. Jansen R, Jassam N, Thomas A, Perich C, Fernandez-Calle P, Faria AP, et al. A category 1 EQA scheme for comparison of laboratory performance and method performance: an international pilot study in the framework of the Calibration 2000 project. Clin Chim Acta 2014;432:90–8.
32. Goossens K, Van Uytfanghe K, Thienpont LM. Trueness and comparability assessment of widely used assays for 5 common enzymes and 3 electrolytes. Clin Chim Acta 2015;442:44–5.
33. Cattozzo G. Survey of analytical methods and result reporting for the measurement of catalytic activity of serum enzymes. Biochim Clin 2015;39:575-84.
34. Braga F, Frusciante E, Infusino I, Aloisio E, Guerra E, Ceriotti F, et al. Evaluation of the trueness of serum alkaline phosphatase measurement in a group of Italian laboratories. Clin Chem Lab Med 2016.
35. Panteghini M. Application of traceability concepts to analytical quality control may reconcile total error with uncertainty of measurement. Clin Chem Lab Med 2010;48:7–10.
36. Braga F, Infusino I, Panteghini M. Performance criteria for combined uncertainty budget in the implementation of metrological traceability. Clin Chem Lab Med 2015;53:905–12.
37. Miller WG, Jones GR, Horowitz GL, Weykamp C. Proficiency testing/external quality assessment: current challenges and future directions. Clin Chem 2011;57:1670–80.
38. Ceriotti F. The role of external quality assessment Schemes in monitoring and improving the standardization process. Clin Chim Acta 2014;432:77–81.
39. Cobbaert C, Weykamp C, Franck P, de Jonge R, Kuypers A, Steigstra H, et al. Systematic monitoring of standardization and harmonization status with commutable EQA-samples – Five year experience from the Netherlands. Clin Chim Acta 2012;414:234–40.
40. Sandberg S, Fraser C, Horvath AR, Jansen R, Jones G, Oosterhuis W, et al. Defining analytical performance specifications: consensus statement from the 1st strategic conference of the European

Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem Lab Med 2015;53:833–5.
41. Ceriotti F, Fernandez-Calle P, Klee GG, Nordin G, Sandberg S, Streichert T, et al. Criteria for assigning laboratory measurands to models for analytical performance specifications defined in the 1st EFLM Strategic Conference. Clin Chem Lab Med 2017;55:189–94.
42. Carobene A, Braga F, Roraas T, Sandberg S, Bartlett WA. A systematic review of data on biological variation for alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transferase. Clin Chem Lab Med 2013;51:1997–2007.
43. Bartlett WA, Braga F, Carobene A, Coşkun A, Prusa R, Fernandez-Calle P, et al. A checklist for critical appraisal of studies of biological variation. Clin Chem Lab Med 2015;53:879–85.