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Evaluation of commutability of several materials for harmonization alkaline phosphatase catalytic concentration measurements ☆

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ABSTRACT

Background: The International Standard ISO 18153 establish that one of the requirements to assure the metrological traceability of values for catalytic concentration of enzymes is the commutability of calibrator and control materials used in the reference measurement systems. This approach was applied to verify the commutability of several commercial stabilized materials using the recently published alkaline phosphatase IFCC primary reference procedure and two routine procedures.
Methods: ALP catalytic activity was measured in 50 serum samples and 16 commercial materials, including control materials from EQAS programs, using primary reference measurement procedure and two routine measurement procedures with AMP and DEA as buffers. Calibration materials with a value assigned by reference procedure which were proved to be commutable were used to recalculate the serum values obtained by routine procedures.
Results: All commercial materials showed a similar behaviour to the patient specimens when AMP vs IFCC procedures were compared. For DEA vs IFCC comparison only one calibration material and two quality control materials were commutable. Recalculation of serum results with a commutable common calibrator improves the agreement between methods changing the ratio AMP vs IFCC from 1.44 to 1.04 and DEA vs IFCC from 3.02 to 1.05.
Conclusions: The use of a common commutable calibration material allows harmonizing ALP measurements and made traceable patient results to reference procedure.

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1. Introduction

As described in the International Organization for Standardization (ISO) standard 18153 [1] one of the requirements to assure the metrological traceability of values for catalytic concentration of enzymes is the commutability of the calibrator and control materials used in the reference measurement systems. Briefly, the reference systems consist on a hierarchical order of measurement procedures and calibration materials. On top of the calibration hierarchy there are primary reference procedures and certified reference materials. To ensure the traceability of results, manufacturers of analytical systems and calibrator and control materials have to recognize these higher-order references. Clinical laboratories, as end-users of the system, have to rely on manufacturers who ensure traceability of the values assigned to their calibrator materials to the highest level. By this way, the standardization of enzyme measurements will be achieved obtaining comparable results among laboratories independently of the analytical system used [2,3].

On the other hand, harmonization of results and methods among laboratories is an important objective of External Quality Assessment (EQA) programs. When a reference measurement system is available for an enzyme, it is necessary to validate that routine procedures produce traceable results to reference procedure. In addition control materials used in the programs should be commutable to allow transferability to patient samples [4–6].

The measurement of the catalytic concentration of alkaline phosphatase (ALP, ortophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1) in serum is relevant in the diagnosis, prognosis and treatment of hepatobiliary and bone diseases. There are several isoenzymes and multiple molecular forms of the enzyme in serum. In healthy adults, ALP activity in serum comes from the liver and

Abbreviations: ISO, International Organization for Standardization; EQA, external quality assessment; ALP, alkaline phosphatase; AMP, 2-amino-2-methyl-1-propanol; DEA, diethanolamine; C-RSE, Committee on Reference Systems for Enzymes; SEQC, Sociedad Española de Bioquímica Clínica y Patología Molecular; HEDTA, N-(2-hydroxyethyl)ethylenediamine-N,N′,N′-triacetic acid.

☆ LREC is accredited according to ISO 17025 and ISO 15195 standards (ENAC accreditation number 195/LC524) and is a member of reference laboratories network of the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

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bone in similar proportions and from the small intestine in smaller quantity. Placental isoenzyme is present only in serum of pregnant women [7,8].

The measurement of ALP activity depends strongly on the measurement conditions and particularly on the buffer selected. The type of buffers used acting as phosphate group acceptors greatly increases the rate of enzyme reaction. Mainly two buffers are used at the present time in routine procedures for ALP measurements in human serum: 2-amino-2-methyl-1-propanol (AMP) [9] and diethanolamine (DEA) [10]. The Committee on Reference Systems for Enzymes (C-RSE) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has recently published the primary reference procedure for ALP catalytic concentration measurements in serum, which is based on the use of 4-nitrophenyl phosphate as substrate and AMP as buffer at 37 °C [11]. The ALP reference measurement procedure will provide the basis to diagnostic industry to assign traceable values to their calibrator and control materials and to clinical laboratories to produce traceable measurement results. When ALP certified reference material will be available, the reference measurement system for ALP will be completed assuring the standardization of this enzyme measurement.

The aim of this work is to demonstrate how the ALP reference system can be used to harmonize catalytic concentration results in routine independently of the method used by following the approach proposed by ISO 18153 [1]. The experimental work was focused to verify the commutability of several stabilized commercial calibrator and control materials with a traceable value assigned by IFCC primary reference procedure, and how to improve the comparability of results by routine procedures after the recalibration with these commutable materials.

2. Materials and methods

2.1. Specimens

Human serum samples were obtained from local clinical laboratories. Icteric, lipaemic and haemolized samples were excluded. Approximately 60 specimens were selected covering ALP catalytic concentration values of IFCC reference measurement procedure: from 29.4 U/l to 680 U/l. Specimens were aliquoted and stored at −20 °C until use.

2.2. Calibrator and control materials

Ten commercial calibrator and control stabilized materials were assayed together with serum samples (6 lyophilized and 4 liquid). Materials were from Asahi Kasei Corporation (Japan), BioSystems (Spain), Roche Diagnostics (Germany), Wiener Laboratories (Argentina) and Bio-Rad Laboratories (USA). Control materials from EQAS programs were also assayed: 4 from Clinical Chemistry EQAS of the Spanish Society for Clinical Biochemistry and Molecular Pathology (SEQC) and 2 from IFCC/DGKL EQAS for reference laboratories (RELA). All the materials included in this study are listed in Table 1. At present, no reference material is available for ALP. Lyophilized materials were reconstituted and stored as recommended by the manufacturers and organizers.

2.3. Reagents

AMP (free of ALP inhibitors), magnesium acetate, zinc sulphate and sodium chloride reagents were purchased from Merck (Darmstadt, Germany). 4-Nitrophenyl phosphate disodium salt substrate and HEDTA trisodium salt were from Sigma-Aldrich Chemicals (St. Louis, MO, USA). ALP activity concentration was assayed by two commercial reagent kits using AMP (ref. 11598) and DEA (ref. 11597) as buffers and purchased from BioSystems (Barcelona, Spain).

2.4. ALP measurements

ALP measurements were performed according to IFCC primary reference procedure at 37 °C [11] and following an internal SOP. An Uvikon 860 spectrometer (Kontron Instruments, Zurich, Switzerland) was used for manual measurements under the conditions described in Table 2. All the commercial materials and serum samples were assayed with the primary reference procedure. One vial of lyophilized commercial material was reconstituted by adding 3.0 or 5.0 ml of distilled water at (20.0 ± 1.0) °C temperature using a balance able to measure 0.1 mg and adjusted with class E2 mass standards of precision. Frozen materials and serums were incubated in a water bath at room temperature for 10–15 min and mixed on a vortex mixer for 10 s. Triplicate measurements of each vial of commercial materials were performed, and the mean values from replicates were used for calculations. All the serum specimens were assayed one time.

ALP activity concentration in materials and serum specimens was also assayed by two commercial methods following manufacturer's instructions adapted to A25® analyzer (BioSystems). Table 2 indicates the ALP measurement conditions of each procedure adapted to the analyzer. Routine procedures were calibrated with theoretical factor provided by the manufacturer and calculated using a molar absorption coefficient (ε) at 405 nm of 1869 $m^2.mol^{-1}$ for 4-

Table 1
ALP catalytic concentration value assigned to commercial materials by IFCC primary reference measurement procedure and estimated expanded uncertainty of measurement with a coverage factor $k = 2$ (corresponding to a level of confidence of 95%) (mean of triplicate measurements) (1 to 5, calibration materials; 6 to 10, control materials; and 11 to 16, EQAS control materials).

No.	Manufacturer/material name	ALP assigned value (U/l)	Uncertainty (U/l) (%)	
1	Wiener Calibrator A plus (WC)	255.5	6.2	2.4
2	Roche Calibrator (RC)	256.1	3.8	1.5
3	Asahi Liquid Calibrator (AC)	163.2	3.0	1.8
4	BioSystems Bovine Calibrator (BBC)	342.5	1.6	0.5
5	BioSystems Human Calibrator (BHC)	278.6	4.6	1.7
6	Bio-Rad Lyphocheck Control 1 (LC1)	100.7	3.2	3.2
7	Bio-Rad Lyphocheck Control 2 (LC2)	453.2	14.0	3.1
8	Bio-Rad Multiqual liquid Control I (LA1)	40.5	2.4	5.9
9	Bio-Rad Multiqual liquid Control II (LA2)	151.0	4.8	3.2
10	Bio-Rad Multiqual liquid Control III (LA3)	293.1	10.0	3.4
11	RELA_A 2010 (RA)	192.2	0.6	0.3
12	RELA_B 2010 (RB)	140.6	4.1	2.9
13	SEQC control level 1 (S1)	42.4	2.4	5.7
14	SEQC control level 2 (S2)	92.5	3.1	3.4
15	SEQC control level 3 (S3)	143.7	1.6	1.1
16	SEQC control level 4 (S4)	274.5	10.1	3.7

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