Letter to the Editor

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Traceability of alkaline phosphatase measurement may also vary considerably using the same analytical system: the case of Abbott Architect

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To the Editor,

Standardization in clinical enzymology should be globally pursued to obtain equivalent results for serum enzyme activities measured on clinical samples independently of which commercial measuring system is used [1]. Due to the strong dependence of catalytic activity measurements on the conditions under which they are performed, the highest level of the traceability chain of an enzyme measurement is occupied by the reference measurement procedure (RMP) that defines the measurand [2]. In 2011, the IFCC published the primary RMP for serum alkaline phosphatase (ALP), which became the highest order reference available for ALP measurements in laboratory medicine [3]. Despite the availability of an internationally accepted reference measurement system, a recent post-marketing quality investigation has shown that standardization of ALP assays is substantially poor and the ability to meet the analytical performance specifications was clearly dependent on the measuring system used [4]. Particularly, the Cobas systems (Roche Diagnostics) significantly underestimated ALP values, the AU systems (Beckman Coulter) tended to overestimate them, whereas the Architect system (Abbott Diagnostics) (even if used in only one participating center, i.e. our clinical laboratory of the 'Luigi Sacco' academic hospital in Milan) showed a perfect alignment to the RMP [4].

To fulfill the European Directive 98/79 on *in vitro* medical diagnostics (IVD), manufacturers are requested to assure metrological traceability of values assigned to calibrators of their measuring systems to higher order references. To comply with this demand, since 2015 Abbott Diagnostics correctly validates the traceability of the ALP assay for the Architect c series systems by comparison to results from IFCC RMP and, to ensure agreement with it, the manufacturer provides the users with an 'experimental' calibrator factor (eCF, 2290) [5]. This is offered as an optional alternative to the 'theoretical' calibrator factor (tCF, 2150), derived from the p-nitrophenol molar extinction coefficient of an optimized reaction for ALP developed by the manufacturer. To assure standardization in our clinical laboratory practice and comparability of patient ALP results, we implemented eCF as soon as it was available. Using this eCF, however, we observed a constant positive error, sometimes higher than the desirable goal derived from ALP biological variation (±12%), during the participation to the regional external quality assessment scheme (EQAS) when our ALP results were compared with the median of the Architect users' group. This performance was completely unexpected having verified the perfect alignment of our Architect ALP assay to the IFCC RMP during the study mentioned above [4]. As the average imprecision of our Architect system was optimal (January–August 2017 average CV, 1.5% [n = 226]; mean ALP activity, 416.5 U/L), we decided to investigate once again the trueness of the Architect ALP assay calibrated with eCF employed in our laboratory by performing a correlation experiment with the IFCC RMP.

Three fresh-frozen serum pools with low (L), borderline (M) and high (H) ALP activities were prepared, aliquoted and stored at −80 °C, according to Braga et al. [4]. The ALP target values (and corresponding uncertainties) were assigned with the IFCC RMP by the CIRME reference laboratory accredited according to ISO 17025 and 15195 standards

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Table 1: Results for alkaline phosphatase and bias of the Abbott Architect assay calibrated with the 'experimental' calibration factor on the platform Architect c16000.

Pool	RMP			Architect replicates, U/L			Mean of replicates, U/L	Bias, U/L	Bias, %
	Mean, U/L	CV, %	U, %	I	II	III			
L	55.6	1.61	3.1	56	56	56	56.0	+0.4	+0.72
M	157.2	0.40	2.5	155	155	155	155.0	−2.2	−1.40
H	401.5	0.11	2.5	398	399	400	399.0	−2.5	−0.62

The bias was estimated by comparing Architect results with target values assigned to pools by the IFCC reference measurement procedure (RMP). U, expanded uncertainty.

(Accredia accreditation certificate no. 217). At the same time, aliquots of the three pools were gradually thawed and assayed in triplicate using the Abbott Architect ALP assay (cod. 7D55, reagent lot no. 36330UN16) calibrated with eCF, carried out on the Abbott Architect c16000 platform. The system alignment to the manufacturer's specifications was checked by measuring, before and after the analytical run, the 3-level quality control material Multichem-S Plus (cod. 05P78, lot no. 15006150), produced by Tecnopath and offered by Abbott as part of their CE-marked measuring system. Correlation between RMP and Architect results was assessed using Passing-Bablok regression and the bias was estimated on each of the three pools.

The ALP target values obtained for L, M and H pools were 55.6, 157.2 and 401.5 U/L, respectively, with corresponding relative uncertainties (expanded with a coverage factor of 2) of 3.1%, 2.5% and 2.5%. Results obtained on Architect are reported in Table 1. The regression analysis gave the following equation: Architect = 0.992 RMP + 0.87 U/L, $R^2 = 0.9999$, with the Architect assay showing a negligible average bias, i.e. −0.43%. Using the available information, we were able to estimate the relative standard uncertainty (u_c) associated with the ALP measurement carried out by the Architect system, by combining the uncertainty due to random effects (average long-term imprecision, 1.5%) to the standard uncertainty of bias (1.3%), derived from the following three components: the average difference between the obtained mean for pools and the target value, the bias variability and the relative standard uncertainty of the certified value of pools. The estimated u_c was 2.0%, well within the desirable performance specification of ±3% [2]. These results confirmed that, despite our EQAS results, the Architect ALP assay calibrated with eCF is optimally standardized to the IFCC RMP. However, this is probably not true when the assay is calibrated with tCF, as the manufacturer itself reports a non-negligible bias (~7%) between results obtained with different calibrator factors. Considering our participation to an EQAS in which the performance evaluation is based on a system-specific consensus value, it was therefore reasonable to suspect that, if the majority of participating laboratories in the Architect group use the tCF, this can significantly influence the average value used as a reference, thus providing an explanation for the "biased" results of laboratories using eCF, like ours.

In order to test this hypothesis, a survey was issued in collaboration with the EQAS provider to assess, among participating laboratories using the Architect system, which calibration factor was used. The survey was issued to the 49 participating laboratories who measure ALP with the Architect system, and 39 answers were returned. The great majority of laboratories (34, 87%) declared to using tCF. The 'peer-group' consensus value used in the EQAS was therefore expected to be strongly influenced by the type of calibration adopted by the majority of laboratories, i.e. tCF. Consequently, we assume that this significantly lowers the EQAS value used as a reference for evaluating the performance of individual participating laboratories and may explain our (apparent) positive error.

Even if traceability of measuring systems to the highest available reference has to be guaranteed by IVD manufacturers, the post-marketing verification of the consistency of the declared performances should be a priority of the laboratory profession, including EQAS organizers [6, 7]. In doing this, EQAS should provide an ultimate assessment of the quality of measurements in terms of standardization and equivalence of results among different laboratories and commercially available measuring systems. However, this can be accomplished only when (commutable) EQAS materials are value assigned by RMP, allowing the correct evaluation of analytical performance through a trueness-based (instead of consensus-based) grading of the competency of laboratories. Unfortunately, properly structured EQAS are still not widely available due to technical, practical, economical and cultural constraints [8]. The experience presented in this study highlights that the EQAS grading based on 'peer group' consensus values can be misleading and may contribute in highlighting

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false problems and keeping true issues hidden [9]. In this case, an EQAS that does not categorize different measuring systems performed on the same measuring platform requires logistic support and more scientific input by professionals.

Based on the results of this study, we expect that Abbott Diagnostics will withdraw as soon as possible the so-called tCF from the Architect ALP package insert, recommending users to only employ eCF, which was obtained by correlation results using clinical samples with RMP-assigned values, allowing optimal standardization [4, 5]. This would certainly be a relevant step towards standardization of ALP activity measurements, permitting to use common reference intervals and decision limits for improving the test interpretation and, consequently, the patient's outcome [10]. Overall, diagnostic companies that have not yet recalibrated their measuring systems for IFCC RMP need more pressure by professionals to do so.

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