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Traceability validation of six enzyme measurements on the Abbott Alinity c analytical system

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Abstract

Background: Laboratory professionals should independently verify the correct implementation of metrological traceability of commercial measuring systems and determine if their performance is fit for purpose. We evaluated the trueness, uncertainty of measurements, and transferability of six clinically important enzyme measurements (alanine aminotransferase [ALT], alkaline phosphatase [ALP], aspartate aminotransferase [AST], creatine kinase [CK], γ-glutamyltransferase [γGT], and lactate dehydrogenase [LDH]) performed on the Abbott Alinity c analytical system.
Methods: Target values and associated uncertainties were assigned to three pools for each enzyme by using the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference measurement procedures (RMPs) and the pools were then measured on the Alinity system. Bias estimation and regression studies were performed, and the uncertainty associated with Alinity measurements was also estimated, using analytical performance specifications (APS) derived from biological variability of measurands as goals. Finally, to validate the transferability of the obtained results, a comparison study between two Alinity systems located in Milan, Italy, and Bydgoszcz, Poland, was carried out.
Results: Correct implementation of traceability to the IFCC RMPs and acceptable measurement uncertainty fulfilling desirable (ALP, AST, LDH) or optimal APS (ALT, CK, γGT) was verified for all evaluated enzymes. An optimal alignment between the two Alinity systems located in Milan and Bydgoszcz was also found for all enzyme measurements.
Conclusions: We confirmed that measurements of ALT, ALP, AST, CK, γGT, and LDH performed on the Alinity c analytical system are correctly standardized to the IFCC reference measurement systems and the system alignment is consistent between different platforms.

Keywords: enzymes; standardization; traceability; trueness; uncertainty.

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Introduction

Serum enzymes are important biomarkers for the diagnosis and management of many organ-related diseases and are among the most requested tests in medical laboratories [1]. Standardization in clinical enzymology is therefore essential in order to provide global equivalence of results in clinical samples, independently of the employed measuring system (MS) [2, 3]. To pursue this objective, the ‘reference system’ approach, based on the concepts of metrological traceability and a hierarchy of measurement procedures, should be applied [4]. In the standardization of enzyme measurements, a reference measurement procedure (RMP), which defines conditions under which a given enzyme catalytic activity is measured, occupies the highest level of the traceability chain [5, 6]. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has established RMPs for the most clinically important enzymes, which are now listed in the database of the Joint Committee on Traceability in Laboratory Medicine [7]. To fulfil the European Union 1998/79 Directive and the new 2017/746 Regulation on in vitro diagnostics (IVD) medical devices, manufacturers should align their commercial MS to these RMPs [8]. This will permit to obtain equivalent results in clinical samples, independently of the employed MS and the individual laboratory where measurements are carried out. The achievement of interlaboratory agreement of enzyme activity measurements allows the use of common reference intervals, thus

facilitating result interpretation and ultimately improving patient care [3, 6, 9–14].

Despite the aforementioned approach being now clear in terms of role, responsibilities, and goals to be achieved, the enzyme measurement standardization seems often incorrectly implemented. This is largely resultant from some manufacturers continuing to market assays with different analytical selectivity for the same enzyme and, consequently, with demonstrated insufficient quality for the fulfilment of requirements for standardization [6, 15]. A sizeable bias toward the RMP measured values is sometimes observed in clinical results, suggesting the need for improvement of traceability implementation to higher-order references [15, 16].

In addition to the manufacturers' responsibility, laboratory professionals should independently verify the correct implementation of traceability to higher-order references and determine if the performance of the evaluated MSs is fit for purpose [17, 18]. In doing this work, the definition of analytical performance specifications (APS) becomes essential to check if the evaluated determination is clinically usable and to ensure that the measurement error does not prevail on the result. In 2014, the 1st European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Strategic Conference redefined the approaches for establishing APS [19, 20]. Among proposed models, the one based on the biological variability has been used to derive APS for enzymes [6, 21].

The aim of our study was to validate the trueness and to estimate the uncertainty of measurements of six clinically important enzyme measurements (alanine aminotransferase [ALT], alkaline phosphatase [ALP], aspartate aminotransferase [AST], creatine kinase [CK], γ-glutamyltransferase [γGT], and lactate dehydrogenase [LDH]) performed on the Alinity c analytical system (Abbott Diagnostics), using APS derived from biological variability of measurands as goals. The transferability of the obtained results in two different European laboratories was also evaluated.

Materials and methods

Preparation and value assignment of serum pools

For each of the six enzymes, three fresh-frozen human serum pools with different catalytic concentrations were prepared using leftover samples, following recommendations from the Clinical Laboratory and Standards Institute (CLSI) C37-A guideline and its recently released update [22, 23]. Catalytic concentrations of pools were distributed across the reportable range for each enzyme measurement, i.e. with concentrations around the upper reference limit, slightly elevated, and markedly abnormal. Each pool was then divided into 1-mL aliquots and stored in polypropylene cryovials at −80 °C until use.

Enzyme target values (and corresponding uncertainties) were assigned to each pool in the CIRME reference laboratory (University of Milan), by using the corresponding IFCC RMP [24–29]. In particular, the concentration of catalytic activity of each pool was measured in triplicate for 3 consecutive days and the target value for each enzyme was calculated as an average of the averages of the daily results. The uncertainty of the assigned value was calculated as a combined standard uncertainty, expanded with a coverage factor of 2 (95.45% level of confidence), taking into account the relative standard uncertainty of the repeated measurements for each enzyme (CV of the averages of the daily results divided by the square root of the number of determinations) and other components of uncertainty of the RMP arising from systematic effects, previously estimated by the CIRME laboratory from specific information and investigations related to calibration procedures for spectrometry, gravimetry, volumetry, potentiometry, and thermometry [3, 30, 31].

Verification of Alinity traceability

The traceability toward the IFCC RMPs for the six evaluated enzymes was verified using an Alinity c platform installed in the core laboratory of the 'Luigi Sacco' academic hospital in Milan. Table 1 reports the characteristics of six enzyme assays evaluated in this study. The system alignment to the manufacturer's specifications was checked by measuring, before and after each analytical run, the three-level control material offered by Abbott as part of their CE-marked MS (Multichem S Plus Technopath, ref. 08P88) and by verifying that results for all enzymes were well within the ±10% range from the control target value declared by the manufacturer. On the day of measurements, aliquots of each pool were gradually thawed and then measured in triplicate. Correlations between Alinity and RMP results were assessed using Passing-Bablok regression and the bias was estimated on each of the three pools for each enzyme by comparing the mean of Alinity triplicates with the target values assigned to pools by the IFCC RMP. The traceability of Alinity assays to the RMP was considered rightly implemented if the mean percentage bias on the three pools was less than the bias specifications, derived by using the classical Fraser's approach [32] and the biological variation data retrieved from the EFLM database [33] (Table 2). As biological variability data for total ALP are not available in this database, APS for ALP measurements were derived from previously published studies [34, 35].

Estimate of measurement uncertainty of Alinity MS

The uncertainty associated with the enzyme measurements carried out by the Alinity MS was estimated according to the ISO 20914:2019 technical specification [36]. In particular, the relative standard uncertainty (u_{result}) was estimated by combining the uncertainty due to random effects (u_{Rw}) to the standard uncertainty of bias (u_{bias}), using the formula $\sqrt{u_{bias}^2 + u_{Rw}^2}$. The u_{result} was then multiplied by a coverage factor of 2 to obtain the expanded uncertainty (U). Three components contributed to u_{bias}: (a) the average difference between the obtained mean for the pool with enzyme concentration closer to that of control material employed for estimating intermediate reproducibility (see below) and the corresponding RMP target value, (b) the

Table 1: Characteristics of Abbott Alinity c enzyme assays evaluated in the study.

Enzyme	Code no.	Method principle	Calibration principle	Declared traceability	Employed reagent lot
ALT	08P1820	Kinetic spectrophotometric (NADH with pyridoxal-5′-phosphate)	Calibration factor (7658)	IFCC RMP	75228UN18
ALP	08P2020	Kinetic spectrophotometric (para-nitrophenyl phosphate)	Calibration factor (2290)[a]	IFCC RMP	86495UN18
AST	08P2320	Kinetic spectrophotometry (NADH with pyridoxal-5′-phosphate)	Calibration factor (6835)	IFCC RMP	61147UN18
CK	08P4220	Kinetic spectrophotometric (N-acetyl-L-cysteine)	Calibration factor (9081)	Not declared[b]	00675UN19
γGT	07P7320	Kinetic spectrophotometric (L-gamma-glutamyl-3-carboxy-4-nitroanilide)	Calibration factor (8372)[a]	IFCC RMP	68895UN18
LDH	07P7420	Kinetic spectrophotometric (lactate to pyruvate)	Calibration factor (11180)	IFCC RMP	61976UN18

RMP, reference measurement procedure. [a]Note that for ALP and γGT Abbott Diagnostics offers two different calibration options, one declared traceable to the respective IFCC RMP and another derived from an optimized enzymatic reaction developed internally by the manufacturer. [b]No information about traceability to higher-order references is reported by the manufacturer. However, according to the Abbott Diagnostics package insert (Alinity c. Creatine kinase reagent kit, G71241R03B8P420, revised February 2018), this method correlates well with the Architect CK measuring system (Alinity = 1.02 Architect−1 U/L; n = 124, activity range, 14–3844 U/L), which, in turn, is standardized to the IFCC RMP.

Table 2: Analytical performance specifications for bias and measurement uncertainty employed in this study.

Enzyme	Allowable bias[a]		Allowable measurement uncertainty[b]	
	Desirable	Optimum	Desirable	Optimum
ALT	±7.7%	±3.9%	±10.1%	±5.1%
ALP	±5.5%	±2.8%	±6.0%	±3.0%
AST	±5.7%	±2.8%	±9.8%	±4.9%
CK	±8.8%	±4.4%	±15.4%	±7.7%
γGT	±10.5%	±5.3%	±8.6%	±4.3%
LDH	±3.4%	±1.7%	±5.2%	±2.6%

All biological variability information (i.e. within-subject CV [CV_I] and between-subject CV [CV_G]) is derived from the EFLM database (https://biologicalvariation.eu), except for ALP derived from refs. [34] and [35]. [a]Calculated as $0.250\,(CV_I^2+CV_G^2)^{0.5}$ (desirable) and $0.125\,(CV_I^2+CV_G^2)^{0.5}$ (optimum). [b]Calculated as standard uncertainty as 0.50 CV_I (desirable) and 0.25 CV_I (optimum), and expanded by multiplying by a coverage factor of 2 (95.45% level of confidence). Note that for measurement uncertainty, the relevant goal that should be fulfilled is that related to the allowable random variability of patient results, as the correct trueness transfer along the metrological traceability chain should allow the achievement of unbiased (or negligibly biased) results.

bias variability (expressed as relative SD of individual bias divided by the square root of the number of measurements), and (c) the relative standard uncertainty of the target value assigned to the pool by the RMP. The u_{Rw} was estimated as intermediate reproducibility from 5-month (July–November 2019) consecutive measurement data (n = 120) of a serum-based fresh-frozen control material (Liquichek Unassayed Chemistry Control Level 2, Bio-Rad), randomly analyzed daily during the ordinary laboratory activity in Milan. This material has the characteristics previously recommended for correctly deriving the uncertainty of MS due to random effects [37]. The obtained U values were finally compared with the respective APS reported in Table 2.

Transferability of the Alinity performance across laboratories

To verify if the traceability of enzyme results provided by the Alinity c located in Milan was extendable to other laboratories using the same MS, a comparison study was carried out with another Alinity c located in the Department of Laboratory Medicine of the Collegium Medicum in Bydgoszcz. Similar to what described earlier, fresh-frozen human serum pools at three clinically relevant concentrations for each enzyme were prepared in Bydgoszcz, aliquoted, and frozen at −80 °C. One aliquot for each pool was then sent to Milan in dry ice. After delivery, simultaneously in Milan and Bydgoszcz, pools were gradually thawed and assayed on the same day in triplicate by using the Alinity MSs, with each system alignment checked as described earlier. On each of the three pools for each enzyme, we compared the mean of triplicates obtained in Bydgoszcz with the mean value obtained in Milan (used as reference because previously validated for traceability). The difference between the two MSs was considered acceptable if the mean percentage bias on the three pools (Bydgoszcz vs. Milan) was less than 1/3 of desirable bias goals reported in Table 2 [38].

Because the study involved anonymized leftover samples, it did not require approval by an Ethics Committee.

Results

Table 3 displays the enzyme target values assigned to the pools by the CIRME reference laboratory, together with

Table 3: Results for the six evaluated enzymes on serum pools obtained by the IFCC reference measurement procedure (RMP) and Alinity c measuring system in Milan.

Enzyme	Pool ID	RMP		Mean of three Alinity replicates, U/L	Bias, U/L	Bias, %	Mean bias, %	Regression parameters
		Target[a], U/L	U[b], %					
ALT	L	38.9	5.12	40.0	1.1	2.83	0.44	$y=0.98x+1.9$ U/L,
	M	78.1	3.43	77.7	−0.4	−0.51		$R^2=1.0000$
	H	201.3	2.64	199.3	−2.0	−0.99		
ALP	L	95.9	3.00	97.0	1.1	1.15	1.78	$y=1.05x-3.5$ U/L,
	M	310.1	2.57	310.3	0.2	0.06		$R^2=0.9991$
	H	504.9	2.64	525.7	20.8	4.12		
AST	L	30.0	2.53	32.3	2.3	7.67	4.44	$y=1.01x+2.0$ U/L,
	M	70.4	2.52	72.7	2.3	3.27		$R^2=1.0000$
	H	172.2	2.60	176.3	4.1	2.38		
CK	L	108.6	3.98	110.7	2.1	1.93	0.61	$y=1.02x+0.1$ U/L,
	M	243.0	3.21	238.3	−4.7	−1.93		$R^2=0.9992$
	H	461.6	2.55	470.0	8.4	1.82		
γGT	L	43.0	3.47	41.3	−1.7	−3.95	−1.83	$y=1.01x-2.1$ U/L,
	M	84.3	2.68	83.0	−1.3	−1.54		$R^2=1.0000$
	H	211.3	2.72	211.3	0.0	0.0		
LDH	L	220.6	2.70	224.0	3.4	1.54	0.83	$y=0.99x+5.2$ U/L,
	M	361.3	2.57	363.5	2.2	0.60		$R^2=1.0000$
	H	442.7	2.66	444.3	1.6	0.36		

[a]Measurements in triplicate for 3 consecutive days: the target value was calculated as an average of the averages of the daily results.
[b]Expanded uncertainty (by a coverage factor of 2). Bias (estimated by comparing the Alinity results with the target values assigned to pools by the RMP) and regression data (y = Alinity; x = RMP) are also reported.

Table 4: Measurement uncertainties of enzyme results obtained with the Alinity c measuring system.

Enzyme	Catalytic activity concentration (order of magnitude compared to the upper reference limit [URL] in adult white males[a])	u_{bias}	u_{Rw}	u_{result}	U[b]
ALT	88 U/L (1.5 × URL)	1.9%	1.1%	2.2%	4.4%
ALP	345 U/L (3.0 × URL)	1.3%	1.3%	1.9%	3.8%
AST	204 U/L (6.0 × URL)	2.7%	0.7%	2.8%	5.6%
CK	513 U/L (3.0 × URL)	2.2%	0.6%	2.3%	4.6%
γGT	184 U/L (2.7 × URL)	1.4%	1.4%	2.0%	4.0%
LDH	374 U/L (1.7 × URL)	1.6%	1.4%	2.1%	4.2%

[a]URL derived from Ref. [6]. [b]Expanded uncertainty (by a coverage factor of 2).

corresponding U. Means of triplicate results obtained on Alinity MS, the estimated bias between Alinity and RMP results, and regression parameters obtained by comparing the Alinity means with the expected values are also reported. The mean bias was within the optimum quality goal for ALT, ALP, CK, γGT, and LDH, while for AST the mean bias (+4.4%) fulfilled the desirable APS.

The uncertainties associated with enzyme measurements carried out on the Alinity c system are shown in Table 4. U for ALP, AST, and LDH met the corresponding desirable APS, whereas ALT, CK, and γGT fulfilled optimal U goals, as reported in Table 2.

Results about the transferability between Milan and Bydgoszcz Alinity platforms are presented in Table 5. As can be seen, the inter-platform bias for all enzymes was less than 1/3 of desirable bias goals reported in Table 2. According to this criterion, we considered enzyme results obtained on the two platforms negligibly biased and, consequently, the Alinity MS standardization to IFCC RMPs, shown for the Milan system, was considered perfectly transferable to the Bydgoszcz platform.

Discussion

Verification of metrological traceability of IVD medical devices in order to demonstrate the standardization of results obtained on clinical samples is an essential task to guarantee the quality of medical laboratory activities, in which laboratory professionals play a key role [17, 18]. To make standardization effective, the laboratory results should be unbiased (or negligibly biased according to the clinically defined APS) and their measurement uncertainty, obtained by combining the uncertainty accounting for random sources and the uncertainty

Table 5: Results obtained by measuring enzymes with the Alinity c system in Milan, Italy, and Bydgoszcz, Poland.

Enzyme	Pool ID	Milan mean of triplicates, U/L	Bydgoszcz mean of triplicates, U/L	Bias, U/L	Bias, %	Mean bias, %	Goal[a]
ALT	1	39.7	40.3	0.6	1.5	1.23	±2.6%
	2	79.0	80.0	1.0	1.3		
	3	230.7	232.7	2.0	0.9		
ALP	1	113.3	113.3	0	0	−0.30	±1.8%
	2	515.7	512.7	−3.0	−0.6		
	3	811.3	809.0	−2.3	−0.3		
AST	1	38.0	36.7	−1.3	−3.4	−1.83	±1.9%
	2	82.3	81.3	−1.0	−1.2		
	3	193.7	192.0	−1.7	−0.9		
CK	1	95.0	96.0	1.0	1.1	0.97	±2.9%
	2	213.0	215.7	2.7	1.3		
	3	491.3	493.7	2.4	0.5		
γGT	1	37.0	37.0	0	0	−0.53	±3.5%
	2	72.0	70.3	−1.7	−2.4		
	3	226.0	227.7	1.7	0.8		
LDH	1	225.0	226.7	1.7	0.8	0.13	±1.1%
	2	370.7	368.0	−2.7	−0.7		
	3	878.7	881.3	2.6	0.3		

[a]1/3 of allowable desirable bias reported in Table 2.

associated with MS calibration, must fulfill the corresponding APS.

To the best of our knowledge, this is the first study aimed to independently verify the level of standardization of the procedures for the determination of the catalytic activity of the most clinically relevant enzymes, offered by Abbott Diagnostics on the new Alinity c platform. In our study, all evaluated enzyme assays achieved at least a desirable level of APS for both bias against the corresponding IFCC RMP and U of clinical samples. This confirms the correct implementation by the manufacturer of the Alinity enzymes of traceability to higher-order references. Furthermore, unbiased measurements were consistent between Alinity MSs located in two different European laboratories, suggesting the transferability of the MS performance. Importantly, these results were obtained by applying relatively stringent APS, derived from robust biological variation data recently made available by EFLM [33]. For evaluating the status of the standardization of enzyme measurements by Alinity MS, we tested their agreement against RMPs obtained for each of the three patient pools. We are aware that using pools instead of a panel of native samples may not represent an optimal approach and theoretically can result in non-commutability problems. However, having strictly followed the CLSI C37-A recommendations for their preparation [22, 23], we are confident that our pools may reasonably behave as individual clinical samples.

The catalytic activity of an enzyme is a property measured by the catalyzed rate of reaction, produced in a specific assay system and any variation in components of the reaction system may alter the magnitude of the measured activity [39]. The high stability of the Alinity MS, shown by the low values of u_{Rw} component (≤1.4% for all evaluated enzymes), allows the manufacturer to calibrate enzyme assays by constant calibration factors instead of using calibrator materials, which may in turn remove one of the major sources of variability, i.e. the calibration procedure. Often, the largest part of u_{result} is contributed by u_{Rw}, which depends on the MS stability over time and its variability when employed by individual end-users. However, for Alinity enzymes, the major contribution to U appears to be u_{bias}. From this point of view, it is extremely important to correctly select the right calibration factor, when the manufacturer offers alternative choices (ALP and γGT). A previous study, performed on the Architect platform using a quite similar protocol, revealed that the ALP assay calibrated with a calibrator factor derived from the p-nitrophenol molar extinction coefficient of an optimized reaction for ALP developed by the manufacturer, instead of using the calibrator factor standardized to the IFCC RMP, has a clinically significant positive bias [16]. Based on the results of our studies, Abbott Diagnostics should discontinue to offer in their package inserts the so-called 'theoretical' calibrator factors, recommending to end-users only factors obtained by correlation results using clinical samples with RMP-assigned values, which allow to achieve an optimal result standardization.

Calculating U for enzyme measurements using a single concentration level, as we did in this study, may

represent a limitation because it would be necessary to disseminate its estimates to more than one activity across the reportable range [40, 41]. However, it should be underlined that we performed this assessment at the most important enzyme concentrations near the decision cut-points employed in the medical application of the tests [1].

Traceability to higher-order references is essential to assure equivalency of clinical results across MSs and laboratories using them. Our study shows how measurements of the most clinically important enzymes on the Alinity c MS are correctly standardized to the IFCC RMPs and fulfill the APS for U on clinical samples. The transferability of the obtained results between two different European laboratories was also demonstrated.

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