Letter to the Editor

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Alignment of the new generation of Abbott Alinity γ-glutamyltransferase assay to the IFCC reference measurement system should be improved

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To the Editor,

Serum enzymes are important for the diagnosis and monitoring of many organ-related diseases and therefore the standardization of their measurements is essential [1]. Among commonly employed enzymes, γ-glutamyltransferase (GGT) is a sensitive indicator of hepatobiliary disease [2]. In our laboratory, GGT catalytic activity has been assayed for more than three years by using a measuring system provided by Abbott Diagnostics on the Alinity c platform (code no. 07P73; hereafter termed GGT1). The calibration of this system is based on the use of a calibration factor, which was experimentally derived by a comparison on native clinical samples with the IFCC reference measurement procedure (RMP) [3]. In a previous paper, we showed the optimal traceability of GGT1 to the IFCC RMP associated with a clinically negligible bias and a measurement uncertainty (MU) fulfilling the optimal analytical performance specification (APS) [4].

In July 2021, Abbott marketed for use with Alinity a new generation GGT assay (code no. 04T96; hereafter termed GGT2), which introduces the use of a multiparametric calibration material [Consolidated Chemistry Calibrator (ConCC), code no. 04V6201] in place of the factor-based calibration. The manufacturer declares the traceability of ConCC GGT assigned value to the ERM-AD452/IFCC reference material (European Commission, Joint Research Centre), which in turn is traceable to the IFCC RMP. Before to start its use in clinical practice, as per laboratory accreditation duties, we verified the comparability of GGT2 with GGT1 by following the Clinical and Laboratory Standards Institute EP09-A3 guideline protocol [5]. Thirty-six leftover native serum samples, with GGT1 values ranging from 9 to 777 U/L, were measured in duplicate in the same run by GGT1 (reagent lot no. 32164UN21) and GGT2 (reagent lot no. 35909UD00, calibrator lot no. 0001198UE) systems. The system alignment to the manufacturer's specification was verified by measuring, before and after the analytical run, the three-level control material (Multichem S Plus Technopath, code no. 08P88) offered by Abbott as part of the CE-marked measuring system. The measuring systems were considered aligned if the control material results fell within the ±10% range around the target value provided by the manufacturer. As GGT1 was previously verified unbiased, a Passing-Bablok regression analysis was performed by considering GGT1 as reference. Surprisingly enough, the regression equation [GGT2=1.061 [95% confidence interval (CI): 1.050–1.069] GGT1 + 0.9 (CI: 0.1–1.6) U/L] showed a slope significantly different from 1 and the difference plot analysis confirmed a positive GGT2 proportional bias [mean absolute bias, 10.6 U/L (CI: 7.4–13.8); mean percentage bias, 6.88% (CI: 6.03–7.74)] (Figure 1). Although this bias was still inside the desirable APS of ±8.4% [6], it raised our concerns about a possible negative influence of the detected bias on the estimation of MU of clinical samples [7].

Given the above results, we repeated for GGT2 the traceability validation study previously performed for GGT1 [4]. We employed the same three frozen human serum pools [low (L), medium (M) and high (H) GGT catalytic concentrations], aliquoted and stored in polypropylene cryovials at −80 °C until use. Pools were value assigned, together with their corresponding MU, by the CIRME

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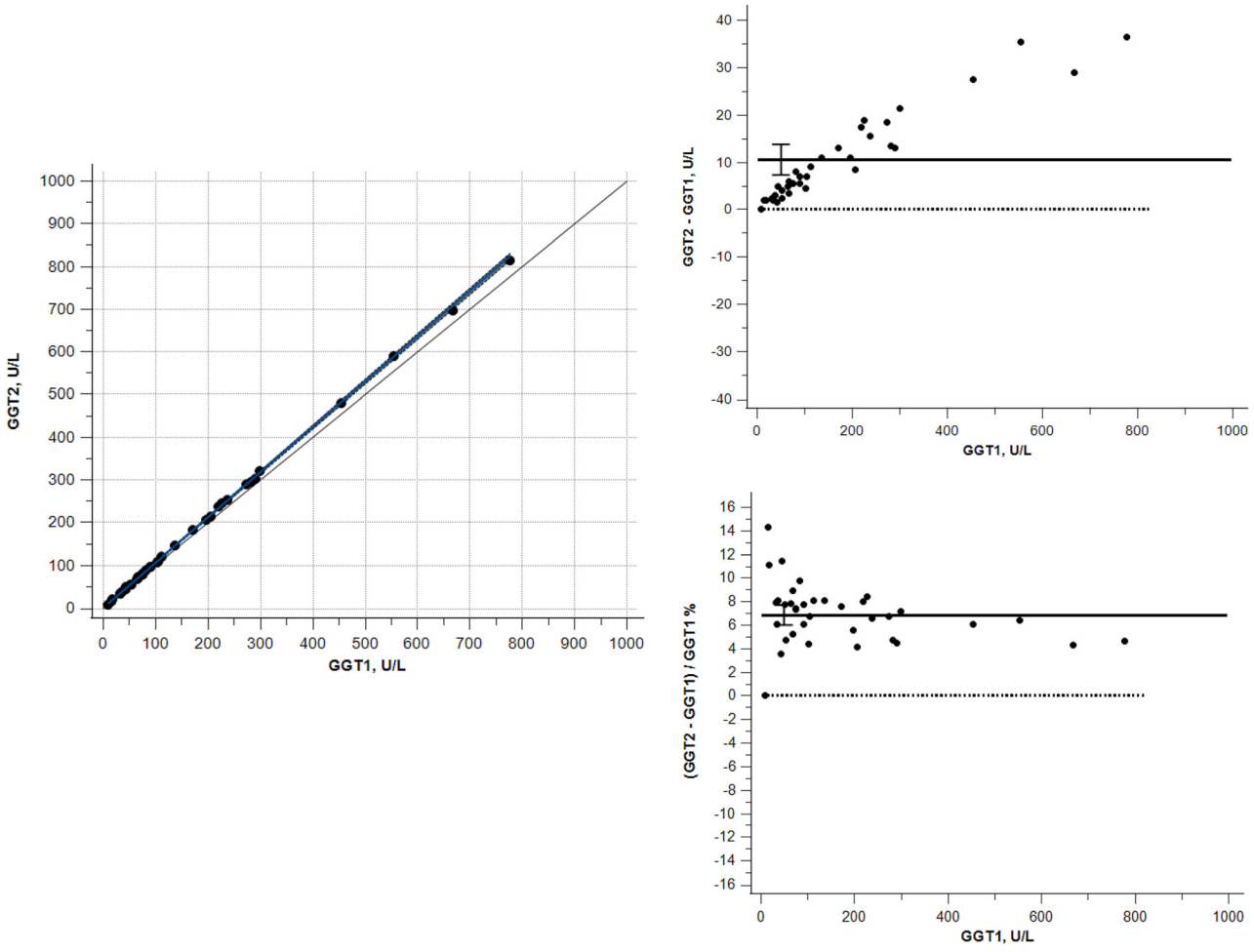

Figure 1: Comparison study between Alinity c GGT2 and GGT1 assays.
Left: Passing-Bablok regression analysis; regression line: *y*=1.061 (CI: 1.050–1.069) *x* + 0.9 (CI: 0.1–1.6) U/L. Top right: absolute difference plot; continuous line depicts mean bias and error bars show its 95% CI (10.6 U/L and 7.4–13.8 U/L, respectively). Dashed line corresponds to the zero bias. Bottom right: difference plot in percentage; continuous line depicts mean bias and error bars show its 95% CI (6.88% and 6.03–7.74%, respectively). Dashed line corresponds to the zero bias. The 95% CI of the mean difference illustrates the magnitude of the systematic difference. If the line of equality (0 bias) is not in the interval, there is a statistically significant systematic difference.

reference laboratory by using the IFCC RMP. The three pools were gradually thawed, manually mixed, centrifuged, and measured in triplicate with GGT2 assay, with the measuring system alignment verified as previously described. Table 1 reports means of triplicate results obtained on Alinity by GGT2, the estimated bias between Alinity and RMP results, and Passing-Bablok regression parameters obtained by comparing the Alinity means with the expected values. The mean percentage positive bias (7.41%) was quite similar to that obtained in the GGT2 vs. GGT1 comparison experiment. The obtained bias for each pool was statistically evaluated by considering it significant if: $(x_{ref} - x_{GGT2}) > 2^{*}u_{bias}$, where x_{ref} is the GGT certified CIRME value, x_{GGT2} is the mean of triplicates obtained for each pool by Alinity GGT2 measuring system, and u_{bias} is equal to $\sqrt{(u_{ref}^2 + SD_{mean}^2)}$, where u_{ref} is the standard uncertainty of the value assigned by CIRME laboratory and SD_{mean} is the standard deviation of the mean value of each pool measured on Alinity calculated as $SD/\sqrt{n}$, in which SD is the standard deviation of the triplicate measurement of the pool and n=3. For all three pools, the detected bias was statistically significant (Table 1).

Even if a measurement bias is statistically significant, the final assessment of significance should be however based on its impact on clinical interpretation of measurement results. With this aim, the MU associated with the Alinity GGT2 measurement system was estimated and compared with APS for suitable clinical use. In particular, the relative standard MU of GGT results on clinical samples (u_{result}) was estimated by combining the uncertainty

Table 1: γ-Glutamyltransferase (GGT) results on serum pools obtained by IFCC reference measurement procedure (RMP) and Alinity c GGT2 measuring system.

Pool ID	RMP		Mean of three Alinity GGT2 replicates, U/L	Bias, U/L	$2*u_{bias}$, U/L[a]	Bias, %	Regression parameters
	Target, U/L	u_{ref}, U/L					
L	43.0	0.75	46.0	3.0	1.5	6.98	y=1.089x − 0.8 U/L,
M	84.3	1.13	89.7	5.4	2.4	6.41	R^2=1.0000
H	211.3	2.87	230.0	18.7	6.2	8.85	

[a]$u_{bias}=\sqrt{u_{ref}^2 + SD_{mean}^2}$, where SD_{mean} is the standard deviation of the mean value of each pool measured by GGT2 assay calculated as $SD/\sqrt{n}$, in which SD is the standard deviation of the triplicate measurement of the pool and n=3. The bias is statistically significant if $>2*u_{bias}$.

due to random effects (u_{Rw}) to the bias and its standard uncertainty (u_{bias}), estimated as described above, in addition to the calibrator uncertainty (u_{cal}) [7]. By assuming GGT2 u_{Rw} consistent with that of GGT1 assay previously estimated at a concentration close to the pool H (1.4%) [4], this uncertainty was combined with bias (8.85%) and u_{bias} (1.45%) obtained from measurements of this pool by GGT2, in addition to u_{cal} of ConCC declared by the manufacturer (1.06%), as follows: $u_{result}=\sqrt{(bias^2 + u_{bias}^2 + u_{cal}^2 + u_{Rw}^2)}$ =9.14%. This estimated MU on clinical samples was much higher than APS derived from the intra-individual biological variation (CV_I) of GGT (8.9%) [6], as 0.50 CV_I (4.45%, desirable quality) and 0.75 CV_I (6.68%, minimum quality), respectively [8]. The inability of Alinity GGT2 measuring system to fulfill MU APS was clearly due to the large bias introduced with the use of ConCC that significantly increased u_{result}.

The presence of a positive proportional bias when GGT2 is employed may denote some problems in the ConCC value-assignment protocol used for transferring trueness from higher-order references to ConCC. Apart from not understanding why the manufacturer decided to replace the use of a calibration factor (which was shown very stable in the daily use of the assay) with a calibrator (which has the challenge to continually ensure appropriate value assignment to subsequent lots), we previously showed that a significant bias may derive when a calibrator value-assignment protocol allows too wide validation criteria. As a matter of fact, the Abbott GGT2 instruction for use reports a slope of 1.08 in the comparison between the Architect GGT2 and GGT1 assays (the latter previously shown to be well aligned to the IFCC RMP), which is considered acceptable by Abbott. Commutability of reference materials is also fundamental to guarantee the correct transfer of trueness from higher-order references to commercial calibrators [9]. About this, we are not aware that the commutability of ERM-AD452/IFCC employed by Abbott to assure traceability of the GGT2 version was verified by the manufacturer, so that we cannot exclude that the use of this reference material may introduce a bias during the trueness transfer process to the measuring system, which, if present, should be corrected as recently recommended [10].

In conclusion, our results seem to demonstrate that problems exist in the correct implementation of metrological traceability of Alinity GGT2 assay by Abbott. It is the responsibility of the manufacturer to take an immediate investigation and eventually fix the problem with a corrective action. Theoretically, the end-user laboratory could introduce a correction factor for the detected proportional bias. However, as previously discussed [7], we are not supporting the individual use of bias correction factors in daily practice as this may alter the status of the measuring system, neglecting responsibilities from the manufacturer and interrupting the system (and, consequently, the produced results) originally provided through CE marking.

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