



Letter to the Editor

The calibrator value assignment protocol of the Abbott enzymatic creatinine assay is inadequate for ensuring suitable quality of serum measurements



To the Editor,

The measurement of creatinine in serum is a key indicator of glomerular function of the kidney and its pivotal role in this evaluation has been further increased by recommendations issued by nephrology societies for using equations to estimate the glomerular filtration rate (GFR) [1]. In 2006 the reference system for the measurement of serum creatinine, using suitable higher-order reference materials and reference methods, was completed and internationally promoted [2]. Particularly, the U.S. National Institute for Standard and Technology (NIST) developed the standard reference material (SRM) 967 (“Creatinine in frozen human serum”), in which there are two concentration levels with values assigned by isotope dilution-mass spectrometry (IDMS) and with proven commutability with native serum samples for most of the available commercial creatinine methods [3]. In vitro diagnostic (IVD) manufacturers (re)calibrated their creatinine measurement systems to the internationally agreed reference system to implement the SI traceability of blood creatinine results, with the consequence that for clinical laboratories only standardized assays should remain available. IVD manufacturers may employ different metrological chains and spend substantially different amounts of the total budget of measurement uncertainty just by selecting one or the other of the available traceability chains [4]. This is a central issue as the selection and application of a reference measurement system for calibration of routine methods should be associated with the fulfilment of measurement uncertainty goals based on medical relevance, so that results are suitable for patient management [5]. Using the information on the biological variation of the analyte [www.westgard.com/biodatabase1.htm], to be acceptable, the degree of expanded uncertainty (U) of creatinine measurements for clinical laboratory using unbiased assays on patient samples should stay within approximately $\pm 6.0\%$ or $\pm 9.0\%$ (desirable or minimum quality level, respectively, for imprecision multiplied by a coverage factor of 2), when both the accumulated uncertainty of the corresponding traceability chain and the uncertainty due to the random effects of measurement are included.

In our laboratory the serum creatinine measurement is performed with the enzymatic (creatinine hydrolysis to creatine by creatininase) assay (cod. 8L24) on the Abbott Diagnostics Architect c16000 platform. According with the IVD EU Directive 98/79/EC [6], the analytical system is CE marked and the calibrator (Multigent Clin Chem Calibrator, cod. 6K30) (CAL) is traceable to the NIST SRM 967, with a U of 0.059 mg/dL (1.48%) at

a creatinine nominal value of 4.00 mg/dL [7]. After the introduction of a new lot of CAL (lot no. 40043Y600, CAL1), we observed a constant over-estimation of creatinine results during the participation in the Regional External Quality Assessment Scheme (EQAS), which in 6 exercises carried out monthly between September 2014 and February 2015 spanned between $+5.5\%$ and $+10.3\%$ (mean, $+8.1\%$). As the analytical imprecision of the method during that period was optimal (total CV on two different instruments, 0.8%; mean concentration, 6.35 mg/dL, $n = 287$), we aimed to verify the trueness of the analytical system by measurements of NIST SRM 967a. Particularly, the two levels of SRM 967a (certified values: L1, 0.847 mg/dL and L2, 3.877 mg/dL, U of 2.12% for both levels) were analysed in triplicate for four consecutive days on two identical c16000 platforms using the same reagent lot and by calibrating the two systems with CAL1 and with a more recently released different lot of CAL (lot no. 40496Y600, CAL2), respectively. The same experiment was repeated on the next week just inverting the two calibrator lots on the two instruments. The analytical systems were used in accordance with the manufacturer's instructions and their alignment in each run was checked according to the manufacturer's established parameters, i.e. the acceptable range of Technopath Multichem S plus control materials (three concentration levels). To estimate the uncertainty of creatinine measurement we applied the “top-down” model of Magnusson et al. [8] by employing the imprecision and bias data obtained by measurements of NIST SRM 967a.

Using CAL1 the obtained mean of means of replicate measurements on the two instruments ($n = 24$) of SRM 967a (L1, 0.876 mg/dL and L2, 4.147 mg/dL) showed a positive bias, mainly at abnormal creatinine concentration ($+3.3\%$ and $+7.0\%$, respectively). Conversely, after calibrating the platforms with CAL2 the mean of means (L1, 0.815 mg/dL and L2, 3.825 mg/dL) showed a negative bias mostly at the physiological concentration (-3.8% and -1.3% , respectively). The relative standard uncertainties from each contribution referred to results obtained with the two CAL are presented in Table 1. The uncertainty due to random effects (u_{RW}) was obtained by the mean of SDs obtained from the replicate of each analytical run for two SRM levels divided by the respective mean of means. Three components contributed to the standard uncertainty of bias (u_{bias}): the difference between the obtained mean of the means for two SRM levels and the target value, the bias variability, expressed as SD of individual bias at each level divided by the square root of number of measurements, and the relative standard uncertainty of the certified value of reference material. As can be seen, the U [obtained by multiplying the relative combined standard uncertainty by a coverage factor of 2 (95% level of confidence)] obtained in measuring the SRM using CAL1 was higher than the desirable performance goal derived from the biological variability of creatinine in serum ($\pm 6.0\%$), neither able to fulfil the minimum quality goal ($\pm 9.0\%$) at the concentration level 2. This was clearly due to the large bias, as the assay imprecision was extremely good. The use of CAL2 significantly reduced U at abnormal creatinine concentration (widely fulfilling quality specifications), but still resulted in a too high U at the physiological level if the desirable goal for U is applied.

Table 1

Uncertainties for each contributing factor in determination of serum creatinine with Abbott enzymatic assay on Architect c16000 platform after calibration with two different lots of system calibrator. Data obtained by measurements of NIST SRM 967a reference material (certified value \pm expanded uncertainty: L1, 0.847 mg/dL \pm 0.018 mg/dL and L2, 3.877 mg/dL \pm 0.082 mg/dL).

	SRM 967a level 1	SRM 967a level 2
<i>Multigent Clin Chem Calibrator lot no. 40043Y600</i>		
Imprecision (u_{Rw})	0.47%	0.40%
Bias (u_{bias})	3.57%	7.05%
Relative combined standard uncertainty [$u_c = (u_{bias}^2 + u_{Rw}^2)^{0.5}$]	3.60%	7.06%
Expanded uncertainty ($U = k \times u_c$)	7.20%	14.12%
<i>Multigent Clin Chem Calibrator lot no. 40496Y600</i>		
Imprecision (u_{Rw})	0.53%	0.42%
Bias (u_{bias})	4.02%	1.71%
Relative combined standard uncertainty [$u_c = (u_{bias}^2 + u_{Rw}^2)^{0.5}$]	4.05%	1.76%
Expanded uncertainty ($U = k \times u_c$)	8.10%	3.52%

The data obtained in this evaluation clearly show some important issues in the CAL value-assignment protocol transferring trueness from higher-order references to the Abbott calibrator of creatinine enzymatic assay. Bais et al. [9] previously highlighted that if SRM 967 is used to transfer trueness to manufacturer's calibrators, there is still 30% (if desirable goal is used) or approximately 53% (if minimum goal is used) of the total uncertainty budget available for the remainder of the chain. Abbott Diagnostics in a document released on August 2014 informed customers that the internal release specification for CAL was $\pm 5\%$ from the target value of SRM 967a L1, which is more than two times higher than the SRM U. Our study shows that this validation criterion for traceability of different CAL lots adopted by the manufacturer is however too large to comply with the U goal for creatinine measurements in biological samples with an acceptable confidence. Moreover, it is also unclear why SRM 967a L1 is used for the CAL value-assignment instead of L2, whose certified value is closer to the CAL nominal value, thus increasing the risk of misalignment of the analytical system to the higher-order metrological references and to result in an unacceptable systematic error in serum creatinine measurements.

In conclusion, our results demonstrate that, despite standardization efforts, some problems still exist in the implementation of metrological traceability by IVD manufacturers. In the case presented in this study, this could be solved through an enhancement to the value assignment process to manufacturer's commercial calibrators, with a better correction of bias and a subsequent reduction of combined uncertainty, without any further technical developments. As recently discussed [10], IVD manufacturers should define a calibration hierarchy to assign traceable values to their system calibrators and to fulfil during this process uncertainty limits, which represent a proportion of the uncertainty budget allowed for clinical laboratory results. Clinical laboratories need to rely on the

manufacturers for this, but it remains an essential task of our profession to continue to verify the correctness of the alignment process.

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