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Trueness verification of actual creatinine assays in the European market demonstrates a disappointing variability that needs substantial improvement

An international study in the framework of the EC4 creatinine standardization working group

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Abstract

Background: The European In Vitro Diagnostics (IVD) directive requires traceability to reference methods and materials of analytes. It is a task of the profession to verify the trueness of results and IVD compatibility.
Methods: The results of a trueness verification study by the European Communities Confederation of Clinical Chemistry (EC4) working group on creatinine standardization are described, in which 189 European laboratories analyzed serum creatinine in a commutable serum-based material, using analytical systems from seven companies. Values were targeted using isotope dilution gas chromatography/mass spectrometry. Results were tested on their compliance to a set of three criteria: trueness, i.e., no significant bias relative to the target value, between-laboratory variation and within-laboratory variation relative to the maximum allowable error.
Results: For the lower and intermediate level, values differed significantly from the target value in the Jaffe and the dry chemistry methods. At the high level, dry chemistry yielded higher results. Between-laboratory coefficients of variation ranged from 4.37% to 8.74%. Total error budget was mainly consumed by the bias. Non-compensated Jaffe methods largely exceeded the total error budget. Best results were obtained for the enzymatic method. The dry chemistry method consumed a large part of its error budget due to calibration bias.
Conclusions: Despite the European IVD directive and the growing needs for creatinine standardization, an unacceptable inter-laboratory variation was observed, which was mainly due to calibration differences. The calibration variation has major clinical consequences, in particular in pediatrics, where reference ranges for serum and plasma creatinine are low, and in the estimation of glomerular filtration rate.
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Keywords: analytical error; bias; calibration; creatinine; external quality assessment; glomerular filtration rate; reference methods.

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Introduction

Standardization of laboratory results is important for comparability of patient data, particularly when a patient is treated on the basis of formal medical decision levels (1). In the case of serum creatinine, standardization is of particular importance because of its central role in the assessment of renal function and the use of creatinine values for estimation of glomerular filtration rate (eGFR) (2). More accurate and precise estimations of GFR can be obtained using equations that empirically combine all of the average effects from factors that affect serum creatinine other than GFR (3). The currently recommended estimating equation has been developed from the Modification of Diet in Renal Disease (MDRD) Study (4). In particular, the ''four-variable'' MDRD Study equation uses age, sex, race, and serum creatinine (5). Owing to the current variability in calibration of serum creatinine assays, assays not calibrated in agreement with the

kinetic alkaline picrate method, used in the original MDRD Study, introduce a source of error into GFR estimates. This calibration error is relatively greater and contributes to larger uncertainty in GFR estimates at physiological creatinine values (6). This limitation applies to all estimating equations based on serum creatinine, not only the MDRD Study equation (7). Variability in creatinine calibration and measurement imprecision also contributes to substantial uncertainty in estimating GFR in children, who usually have lower serum creatinine concentrations than adults.

Since December 2003, The European In Vitro Diagnostics (IVD) directive (8, 9) has required traceability for approximately 80 category A analytes. The traceability should be certified by a reference system (10), which covers the chain from reference method and primary certified reference material to testing instrument and instrument specific calibrators. An international committee, the Joint Committee on Traceability in Laboratory Medicine (JCTLM) defines the reference methods, reference laboratories, and reference materials needed in the reference systems. Industry should make their methods traceable to the reference methods and materials. Only recently did the National Institute of Standards and Technology Standard Reference Material (NIST SRM) 967 standard become available for the IVD industry (11).

Calibration by individual laboratories using secondary certified reference material would be very costly and practically not feasible owing to the large batches of materials needed. Since Jaffe (12) only observed a complexation between picric acid and creatinine in alkaline environment and never described an analytical method, variation among Jaffe method formulas is broad (13, 14). The reference methods and materials included in the reference systems required in the IVD directive should reduce inter-laboratory variation. However, it is the responsibility of the profession to verify the IVD trueness and compatibility of the commercial methods and to perform trueness verification studies of the instruments and methods used in daily practice. Trueness verification is defined as the extent to which a method measures the true or target value as obtained in a reference system. This should be a task for the External Quality Assessment (EQA) organizers and commutable trueness verification materials should be used.

The present study was performed in autumn 2005 to experimentally assess trueness and compatibility of the most frequently used systems for serum creatinine measurement across Europe. A commutable serum-based material was used (15, 16) and serum creatinine was analyzed at three different concentrations. This material was targeted by a reference laboratory using the JCTLM-approved reference method for creatinine and assayed by a total of 189 laboratories in six European Union member states (Belgium, Finland, France, Germany, Italy, and The Netherlands). In addition to the trueness verification, the analytical performance of the methods was assessed using a system based on the biological variation model, already employed to assess traceability of enzyme measurements (15).

Materials and methods

Trueness verification material

To assess IVD conformity, selected trueness verification material (level 1) was prepared from fresh human serum, based on the National Committee for Clinical Laboratory Standards (NCCLS) C37-A guideline (17). Levels 2 and 3 were supplemented with the NIST SRM 914a, crystalline creatinine, which is intended for use in calibration of reference measurement procedures. The preparations were divided into 1-mL vials and stored at –80°C.

The commutable trueness verification materials were analyzed in the reference laboratory of Prof. L. Thienpont, Ghent, Belgium, using an isotope dilution gas chromatography/mass spectrometry (ID-GC/MS) method, which has been previously described and which has been approved by the JCTLM as a reference measurement procedure for serum creatinine (18, 19).

The measurement protocol used for target value assignment consisted of analysis of each sample in duplicate on three independent occasions ($n=6$). The latter means that on each occasion of measurement separate sampling, sample preparation, calibration, and internal quality control (via NIST SRM 909b1 and b2) were carried out. Calibration mixtures were taken from three independently prepared working solutions. The expanded uncertainty of the methods is estimated to be of the order of 1.5% to 2%. Target values for the three creatinine levels were 76.0 μmol/L [level 1, coefficient of variation (CV) 0.5%], 153.4 μmol/L (level 2, CV 0.6%), and 305.7 μmol/L (level 3, CV 0.4%), respectively.

Through the national EQA coordinators of the six participating countries (Dr. J.C. Libeer, Belgium; Dr. M. Loikkanen, Finland; Prof. M.-M. Galteau, France; Dr. R. Kruse, Germany; Prof. F. Ceriotti, Italy; Dr. H. Baadenhuijsen, The Netherlands), the trueness verification material was distributed to a total of 189 representative laboratories, 23 in Belgium, 17 in Finland, 45 in France, 27 in Germany, 40 in Italy, and 37 in The Netherlands, applying various methods and instruments. The selection of the laboratories was carried out by the national coordinators on the basis of the methods and instruments used for the determination of serum creatinine. In total, 29 laboratories (15%) used a compensated Jaffe method, and 103 laboratories (54%) used a non-compensated Jaffe method. Furthermore, 33 laboratories (17%) used a creatininase-based enzymatic method, 19 laboratories (10%) a creatinine iminohydrolase-based dry chemistry method, and in five laboratories (3%) an enzymatic UV method was used. Companies and instruments included Abbott (Abbott Park, IL, USA; Aeroset, Architect), Bayer (Tarrytown, NY, USA; Advia 1650), Beckman (Brea, CA, USA; Synchron), Dade Behring (Deerfield, MA, USA; Dimension), Olympus (Tokyo, Japan; AU 640, 2700, 5400), Ortho Clinical Diagnostics (Rochester, NY, USA; Vitros), Roche Diagnostics (Mannheim, Germany; Integra, Hitachi, Modular) and Konelab (Vantaa, Finland). The laboratories were instructed to analyze the material exactly according to the manufacturer's instructions (excluding any correction factors) and were asked to provide information on reagents, wavelength, calibrator, and any factors used. The three level materials were analyzed in each laboratory in five-fold in one run for three serum creatinine levels. The five replicates per laboratory per level were inspected for outliers. The results were sent to the organizers.

Statistical methods

The five intra-laboratory measurements for each creatinine level were first inspected for outliers (truncated at ±3 SD

level). The overall mean and SD were calculated from all measurements obtained for each level. For each laboratory, the average value for each level was calculated. These laboratory mean values were grouped per method group. Five method groups were selected: the conventional Jaffe group (20), the compensated Jaffe group (20), the enzymatic method based on creatininase (21), and two creatinine iminohydrolase groups (dry and wet chemistry) (22, 23). Since the trueness verification material was targeted using reference methods, plots against the target value could be constructed for each creatinine level. Analytical performance was assessed at three levels: deviation of the mean value from the target value, between-laboratory (SD_{bl}) and within-laboratory variation (SD_{wl}). The deviation of the mean value from the target value was tested using the t-test. The pooled SD_{wl} was tested against the maximum allowable within-laboratory variation (SD_{wl} max) and the SD_{bl} against the maximum allowable between-laboratory variation (SD_{bl} max), as described earlier (15).

The SD_{bl} max and SD_{wl} max were computed from the biological variation-based model by Fraser (24). The desirable analytical variation parameters for an individual laboratory as published by Ricos et al. (25) were used. In this model, for each individual laboratory the allowable analytical bias (B) is $<0.25\ (SD_w^2+SD_b^2)^{0.5}$ and the desirable analytical variation (SD_d) is $<0.5SD_w$, where SD_w is the within-subject biological variation and SD_b is the between-subject biological variation. The total allowable error (TAE) for a single laboratory is $1.65SD_d+AB$.

SD_{bl} max is defined (15) as the between-laboratory variance at which the laboratory mean values of 95% of the laboratories are within the AB limit. In the presence of significant bias of the method mean, SD_{bl} max is computed from a one-sided probability, in the absence of bias from a two-sided probability. In the presence of a bias (one-sided approach), SD_{bl} max is $(AB-|X-T|)/1.65$, where X is the method mean, and T is the target value. In the absence of a bias (two-sided), X is not significantly deviating from T, and therefore SD_{bl} max is AB/1.96.

The SD_{wl} max was computed from $(TAE-1.96\ SD_{bl}-|X-T|)/1.96$ if X is deviating significantly from T, and $(TAE-1.96\ SD_{bl})/1.96$ if X is not significantly deviating from T. SD_{bl} and SD_{wl} were tested statistically against SD_{bl} max and SD_{wl} max using the F-test.

In addition, method group performance was investigated by plotting systematic bias and imprecision of the method groups into the parabolic curve developed by Myers et al. (26). Hence, the performance of the method groups is compared to the upper bounds which limit the total error budget for creatinine measurements in such a way that the combination of systematic bias and imprecision would increase the root mean square error in eGFR by no more than 10%.

Results

The target values (±SD) for the three creatinine materials investigated were 75.9 μmol/L (CV 0.5%), 153.4 μmol/L (CV 0.6%), and 304.9 (CV 0.4%) μmol/L, respectively. No gross errors were present in the sets of results per level per laboratory. Overall means, SD_{bl} and SD_{wl} per creatinine level and per method group were calculated. These were tested, respectively, against the target value and the SD_{bl} max and SD_{wl} max. Since the laboratories were asked to measure the material in five-fold in one run, the SD_{wl} was the within-run SD, thus excluding within-laboratory between-batch and between-calibration variation. As the laboratories applied their methods exactly according to the manufacturer's instructions, the SD_{bl} was expected to be influenced predominantly by between-batch and between-calibration variance.

Mean values, between- and within-laboratory SDs, and maximum allowable between- and within-laboratory SDs (μmol/L) for creatinine according to method and to vendor are summarized in Table 1.

Table 1 includes the allowable error components based on the biological variation model for a single laboratory, and the theoretical SD_{bl} max and SD_{wl} max per method group. For the lower creatinine level, the mean values per method group differed significantly from the target value in the Jaffe and the dry chemistry method group. The same phenomenon was observed at the intermediate level, whereas at the high concentration level, only dry chemistry yielded significantly higher results. For the three levels, the SD_{bl} was significantly ($p=0.05$) larger than the SD_{bl} max in all method groups. For the heterogeneous Jaffe group, values were further studied according to the vendors. Variation between vendors was broad. Overall between-laboratory variation among European clinical laboratories was high: 8.74% CV (lower level), 4.84% CV (intermediate level), and 4.37% CV (higher level).

Figure 1 depicts the total error budget for creatinine measurements for levels 1, 2, and 3. The limit of systematic biases and random imprecisions produce a relative increase of <10% in the root mean square error when estimating GFR using the MDRD Study equation. The total error budget was mainly consumed by the bias and to a much lesser extent by the within-laboratory variation. The non-compensated Jaffe methods largely exceed the total error budget and the compensated Jaffe method yielded satisfactory results at levels 1 and 2. The best results were obtained for the enzymatic method; the dry chemistry enzymatic method consumes a large part of its error budget due to calibration bias.

Figure 2 shows the creatinine results obtained for the various method groups in comparison with the target value (full line) and the total allowable error margins (dashed lines in Figure 2) at the various levels.

Discussion

In the present study, disappointing results were obtained for the analytical bias of current creatinine methods. At the lower level, the liquid enzymatic-based method and the compensated Jaffe method showed a minor but significant positive bias, whereas a major positive bias was observed for the creatinine iminohydrolase dry chemistry method and the uncompensated Jaffe method. At the intermediate and higher concentration levels, relative magnitude of the bias tended to decrease. This bias is likely due to the analytical interference by pseudochromogens for the Jaffe group (20) and the calibration used in the

Table 1 Mean values, between- and within-laboratory SDs, and maximum allowable between- and within-laboratory SDs (μmol/L) for creatinine.

(A) Level 1	Target	SD_d	AB	TAE	
Method	75.9	1.63	2.58	5.27	
	Mean	SD_{bl}	SD_{bl} max	SD_{wl}	SD_{wl} max
Overall (n=189)	83.86*	7.39	0.00	1.36	0.00
Compensated Jaffe (n=29)	77.32	4.59	0.00	1.70	0.27
Enzymatic (n=33)	76.31	2.45	0.03	0.99	1.32
Enzymatic dry chemistry (n=19)	84.10*	3.03	0.00	0.86	0.00
Enzymatic UV (n=5)	78.72	1.62	0.00	2.11	1.32
Jaffe (n=103)	88.20*	6.32	0.00	1.42	0.00
Jaffe group according to vendor					
Abbott (n=20)	85.71*	2.19	0.00	1.15	0.00
Bayer (n=17)	92.55*	10.39	0.00	1.25	0.00
Beckman Coulter (n=16)	85.38*	3.44	0.00	2.30	0.00
Dade Behring (n=16)	84.40*	5.40	0.00	1.43	0.00
Konelab (n=2)	84.50*	2.69	0.00	1.33	0.00
Olympus (n=23)	91.37*	3.29	0.00	0.91	0.00
Roche (n=9)	93.00*	4.69	0.00	1.55	0.00
(B) Level 2	Target	SD_d	AB	TAE	
Method	153.4	3.30	5.21	10.66	
	Mean	SD_{bl}	SD_{bl} max	SD_{wl}	SD_{wl} max
Overall (n=189)	159.41*	7.66	0.00	2.09	0.00
Compensated Jaffe (n=29)	153.23	6.71	0.00	2.47	2.66
Enzymatic (n=33)	153.28	4.21	1.16	1.55	2.66
Enzymatic dry chemistry (n=19)	165.09*	5.14	0.29	1.52	0.00
Enzymatic UV (n=5)	155.63	1.50	0.00	3.00	2.66
Jaffe (n=103)	162.09*	7.02	0.00	2.19	0.00
Jaffe group according to vendor					
Abbott (n=20)	159.67*	3.47	1.96	1.19	0.00
Bayer (n=17)	161.15*	10.72	0.00	3.11	0.00
Beckman Coulter (n=16)	164.51*	5.57	0.00	3.44	0.00
Dade Behring (n=16)	160.11*	4.30	1.14	1.66	0.00
Konelab (n=5)	146.10*	5.80	0.00	1.03	0.00
Olympus (n=23)	163.73*	5.26	0.17	1.72	0.00
Roche (n=9)	168.49*	8.15	0.00	2.36	0.00
(C) Level 3	Target	SD_d	AB	TAE	
Method	304.9	6.56	10.36	21.18	
	Mean	SD_{bl}	SD_{bl} max	SD_{wl}	SD_{wl} max
Overall (n=189)	307.47	13.50	0.00	2.69	5.29
Compensated Jaffe (n=29)	301.09	12.33	0.00	3.26	5.29
Enzymatic (n=33)	307.09	7.29	2.40	2.42	5.29
Enzymatic dry chemistry (n=19)	317.21*	23.22	0.00	2.88	0.00
Enzymatic UV (n=5)	303.92	5.56	2.75	2.32	5.29
Jaffe (n=103)	307.65	12.29	0.00	2.56	4.62
Jaffe group according to vendor					
Abbott (n=20)	304.53	5.91	4.89	1.51	5.29
Bayer (n=17)	298.28	11.68	0.00	3.55	2.27
Beckman Coulter (n=16)	320.89	4.90	5.91	2.46	0.00
Dade Behring (n=16)	309.68	5.55	5.26	2.68	3.38
Konelab (n=5)	268.50*	14.00	0.00	2.11	0.00
Olympus (n=23)	304.93	6.84	3.97	2.63	5.29
Roche (n=9)	316.14	15.93	0.00	3.07	0.00

*Result exceeding total allowable error.

dry chemistry method. The marked bias differences between the liquid and dry chemistry versions of the same creatinine iminohydrolase-based method prove that the positive bias in the dry chemistry group is attributable to differences in calibration.

In the earliest manual methods, serum creatinine was assayed by the Jaffe reaction after deproteinization, eliminating the pseudochromogen effect of proteins (2). Similarly, the first automated methods used dialysis membranes to prevent interference from plasma proteins. Today, however, analyzers use undiluted serum and plasma, making them prone to the so-called ''protein error'' in the alkaline picrate reaction (20). On average, this effect produces a positive difference of 27 μmol/L creatinine compared with HPLC or enzymatic methods (20). Because urine contains relatively little or no protein, the protein error affects only creatinine determinations in serum or plasma. Therefore, the creatinine clearance is underestimated when creatinine methods affected by

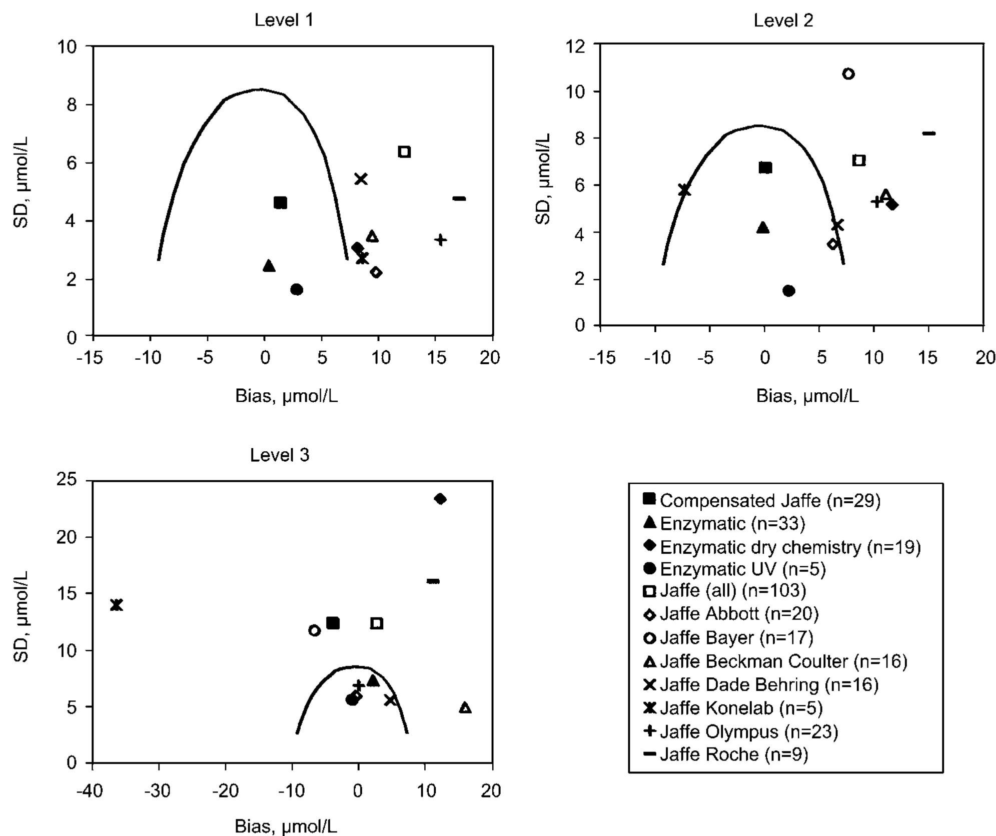

Figure 1 Total error budget for creatinine measurements for levels 1, 2 and 3.
The line represents the limit of systematic biases and random imprecisions that produce a relative increase of $<10\%$ in the root mean square error when estimating GFR using the MDRD Study equation.

protein error are used. For calculating GFR, this systematic positive bias has been stated to be compensated by the overestimation attributable to tubular secretion of creatinine.

Serum creatinine concentration having relatively small biological variation, the combination of bias and significant between-laboratory variance caused exceeding of the total error budget and caused violation of the allowable maximum within- and between-laboratory variance criteria.

The disappointing results of this study do not show any improvement in comparison to other studies in the recent past. The two largest studies that used a commutable serum sample reported method group SDs of 0.088–12 μmol/L with a median SD of 5.1 μmol/L (27) and 2.6–11 μmol/L (28), and median CVs of 6.4% at a creatinine concentration of 80 μmol/L (27) and 5% at a creatinine concentration of 74 μmol/L (28). Notwithstanding the stricter regulations and the changing clinical needs, between-laboratory variation of Jaffe-based methods has not decreased over the last decade, despite technical progress in laboratory automation. The data of the present study (Figure 1) clearly show that excessive analytical bias in creatinine assays is the major hurdle on the road toward a better creatinine assay. Next to the standardization issue, correcting for analytical interferences (non-specificity bias) should be improved. Vendors should reduce the bias of their methods by better calibration to the reference systems. In addition, the between reagents and calibrator variations, which cause the between-laboratory variances, should be reduced.

In the case of creatinine, the standardization issue has major clinical consequences which are far beyond the significance of the parameter itself. In clinical practice, serum creatinine is often used as a parameter for estimating GFR. Apart from the conventional calculation of the creatinine clearance, also the calculation of the clearance using derived formulas is a key element in the assessment of renal function and the calculation of the correct dose of many drugs which are characterized by a narrow therapeutic index and a renal elimination mechanism (29).

In particular, the bias in serum creatinine concentration in the lower range should be a major concern in pediatric medicine, due to the much lower reference ranges for serum creatinine in infants and children (30). For estimating GFR in children and infants, the Schwartz and the Counahan-Barratt equations are recommended (31–34). Both provide GFR estimates based on a constant multiplied by the child's height divided by the measured serum creatinine concentration. It is obvious that the observed inter-laboratory variation for serum creatinine leads to an unacceptable variation in the estimation of kidney function. Since these formulas were validated 30 years ago, reassessment of classical formulas for estimating creatinine clearance and GFR using modern creatinine assays is strongly recommended.

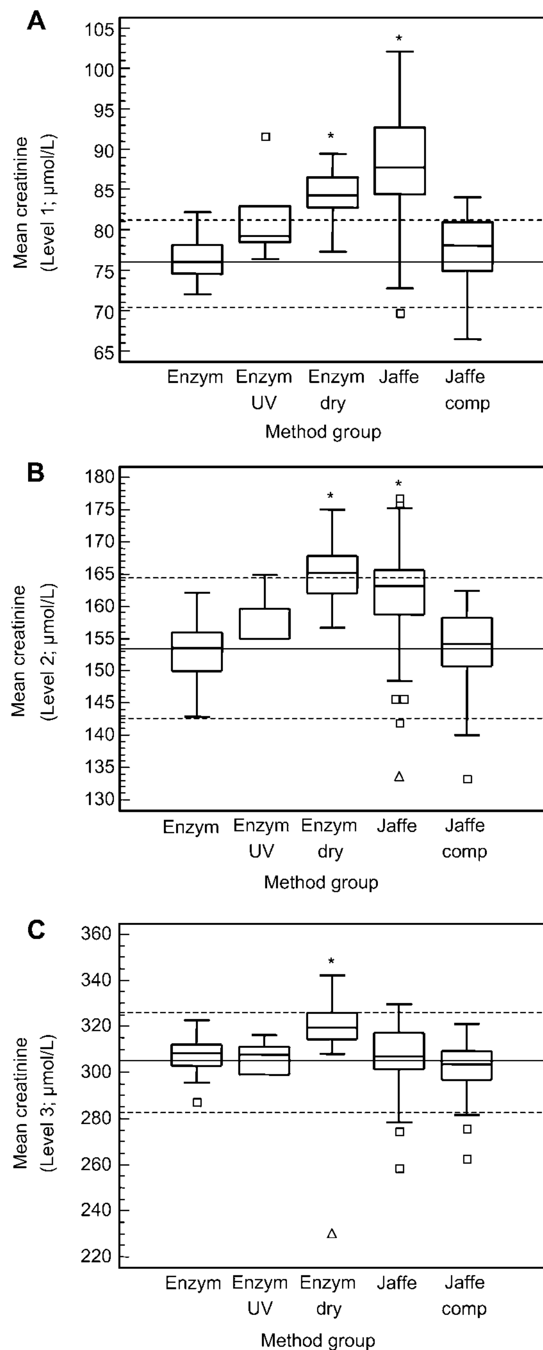

Figure 2 Creatinine results obtained for the various method groups in comparison with the target value (full line) and the total allowable error margins (dashed lines) at levels 1 (A), 2 (B), and 3 (C). *Result exceeding total allowable error.

For adults, an improved GFR-estimating equation based on serum creatinine values traceable to isotope dilution mass spectrometry (IDMS) reference measurement procedures has been recently presented (35). In transplantation medicine, the Model for End-Stage Liver Disease (MELD) score has been shown to be the best predictor of short-term mortality on the liver transplant waiting list (36). MELD score (in which creatinine is one of the contributing parameters) is used for assigning priority for transplantation. It is obvious that lack of standardization of creatinine prevents a correct estimation of the MELD score.

Establishing measurement traceability is an important tool to achieve the required comparability in serum creatinine measurement results regardless of the method used and/or the laboratory where the analyses are performed. This effort must involve international cooperation among the IVD manufacturers, clinical laboratories, professional organizations, government agencies, and external quality assurance services/proficiency testing (EQAS/PT) providers.

The National Kidney Disease Education Program (NKDEP), the College of American Pathologists (CAP), and NIST have collaborated to prepare a human serum-creatinine reference material with acceptable commutability with native clinical specimens in routine methods. This material is a fresh-frozen human serum pool prepared according to the Clinical and Laboratory Standards Institute (CLSI) guideline C-37A (11, 35). These materials are value-assigned by NIST with the GC-IDMS and LC-IDMS reference measurement procedures (11). The materials are designated as NIST SRM 967 and are commutable with native human sera. It can be expected that their recent availability (since November 2006) will lead to standardization improvements by IVD manufacturers.

Implementing traceability of serum creatinine assays to GC-IDMS or LC-IDMS will lead to changes in the clinical decision-making criteria currently used for serum creatinine and creatinine clearance and will compromise any clinical decisions based on the eGFR, unless the eGFR is calculated by use of the new MDRD equation based on creatinine values traceable to an IDMS reference method (35). When introducing revised serum creatinine calibration to be traceable to IDMS, laboratories will need to communicate the following to healthcare providers: the serum creatinine reference interval will change to lower values, calculations of eGFR used by pharmacies or other groups to adjust drug dosages will be affected by the decreased creatinine values, measured and calculated creatinine clearance values will increase, and the corresponding reference interval will be different.

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