Enzymatic assays for creatinine: time for action

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ABSTRACT

Accurate serum creatinine measurements in glomerular filtration rate estimation (eGFR) using equations are critical to ongoing global public health efforts to improve the diagnosis and treatment of chronic kidney disease. There is now an ongoing activity to promote worldwide standardization of methods to determine creatinine together with the introduction of a revised eGFR equation appropriate for use with standardized creatinine methods. Standardization of calibration, i.e. implementation of calibration traceability to high-order reference measurement procedures and reference materials, does not, however, correct for analytical interferences of field methods (non-specificity bias). To account for the sensitivity of alkaline picrate-based methods to non-creatinine chromogens, some manufacturers have adjusted the calibration to minimize the pseudo-creatinine contribution of plasma proteins, producing results more closely aligned to the reference method (isotope dilution-mass spectrometry), but this strategy makes an assumption that the non-creatinine chromogen interference is a constant among samples, which is an oversimplification. Thus, analytical non-specificity for substances found in individual patient samples can affect the accuracy of eGFR computed from serum creatinine values for any alkaline picrate method including the so-called "compensated" Jaffé methods. The use of assays that are more specific for serum creatinine determination, such as those based on enzymatic reactions, may provide more reliable eGFR values. Supporting the choice of more specific assays by clinical laboratories represents one of the main tasks of our profession in order to achieve the ultimate clinical goal, which is to routinely report an accurate eGFR in all the pertinent clinical situations.

INTRODUCTION

A major barrier to the general implementation in healthcare of equations for glomerular filtration rate estimation (eGFR) has been the use of different creatinine measurement procedures among laboratories. Lacking standardization for creatinine measurement, assays not calibrated in agreement with the method used in the core laboratory to develop and validate a specific equation may introduce an additional source of error into the mathematical prediction of GFR (1). Importantly, calibration bias contributes to larger uncertainty in eGFR at serum creatinine concentrations within or just outside the physiologic range that are clinically crucial for detecting silent kidney disease (2). Thus, the universal implementation of the serum creatinine-based eGFR prediction equation, with the associated clinical benefits for patients, requires worldwide standardization of creatinine measurement procedures, together with revalidation of the recommended eGFR equation using standardized creatinine results (3, 4).

Although the creatinine determination in clinical practice is more than 100 years old, there is still much debate regarding its accuracy (2, 5). There is now international agreement that the implementation of calibration traceability to higher-order reference methods and materials is the best approach to achieve the needed comparability in biochemical measurement results, regardless of the method used and/or the laboratory where the analyses are performed (6, 7). Particularly, achievement of improved trueness for creatinine measurements requires that the values assigned by manufacturers to calibrators and control materials supporting routine measurement procedures are traceable to available higher-order reference measurement procedures and reference materials (3). In the European Union (EU) the implementation of calibration traceability in Laboratory Medicine to higher-order standards is already mandatory by law. The EU directive 98/79/EC on in vitro diagnostic (IVD) medical devices explicitly requires manufacturers to ensure metrological traceability of their products (8). Internationally, we are in a transition period in which very different levels of implementation are apparent (3). Some manufacturers have already recalibrated their creatinine assays to isotope dilution-mass spectrometry (IDMS) reference method worldwide. However, some manufacturers sell kits with different calibrations in Europe compared to other parts of the world, and some manufacturers still maintain old calibrations and will recalibrate sooner or later with the introduction of new reagent lots. This confounding situation clearly emerges upon examination of data from recently performed international and national external surveys (9-12). Collectively, these

observations suggest that a number of routine analytical systems for serum creatinine are still significantly biased and that further work is needed to achieve substantially improved trueness in creatinine results with routine methods.

STANDARDIZATION DOES NOT CORRECT FOR ANALYTICAL NON-SPECIFICITY PROBLEMS

Analytical non-specificity, i.e. inability to measure solely creatinine, of some routine methods must also be addressed. Traceability implementation does not solve the analytical interferences related to an assay's non-specificity and, if the reference measurement procedure and corresponding lower order routine methods have not identical, or at least very similar, specificities for the measurand, traceability cannot be obtained (13). Establishing calibration traceability to the creatinine reference system will align the average performance of methods to each other, but will not substitute for improvement of suboptimal routine methods. Several studies indicate that the use of assays that are specific for serum creatinine determination, such as those based on enzymatic reactions, produce results that agree closely with IDMS (4, 9, 11, 14). On the other hand, it is well known that as a result of reaction with plasma pseudo-creatinine chromogens, including proteins, ketones and glucose, methods based on alkaline picrate reaction overestimate true serum creatinine concentrations, inducing proportionally greater errors at values lower than 2.00 mg/dL (177 µmol/L). This still remains true even after potential elimination of the calibration error by alignment to IDMS (Figure 1) (15).

To account for the sensitivity of alkaline picrate-based methods to non-creatinine chromogens, some manufacturers have recently adjusted the calibration to minimize the pseudo-creatinine contribution of plasma proteins by introducing a negative offset to "compensate" the positive intercept found in the correlation (16). For example, in the Roche Integra compensated Jaffé assay, 0.204 mg/dL (18 µmol/L) is automatically subtracted from each result. This numerical term reflects the average contribution of the creatinine-free serum matrix to the alkaline picrate reaction as estimated in the correlation studies (17). This strategy makes, however, an assumption that the non-creatinine chromogen interference is a constant among samples, which is an oversimplification, so that in the everyday practice the unspecificity bias from individual sample matrices can not be completely eliminated (18). For children, who generally present with higher non-creatinine cromogens and very low serum creatinine concentrations, as well as for adults who have low protein and low creatinine concentrations in serum, such as elderly, pregnancy women or cancer patients, then poor trueness for compensated assays is to be expected. Furthermore, at least for some commercial systems, the manufacturers' recommended offsets appear to paradoxically result in an average negative bias, with results falling below the acceptable error range at clinically important concentrations, as shown in a survey recently performed in Australia (11). This may result in eGFR that are positively biased even when the IDMS-traceable equation is used (19).

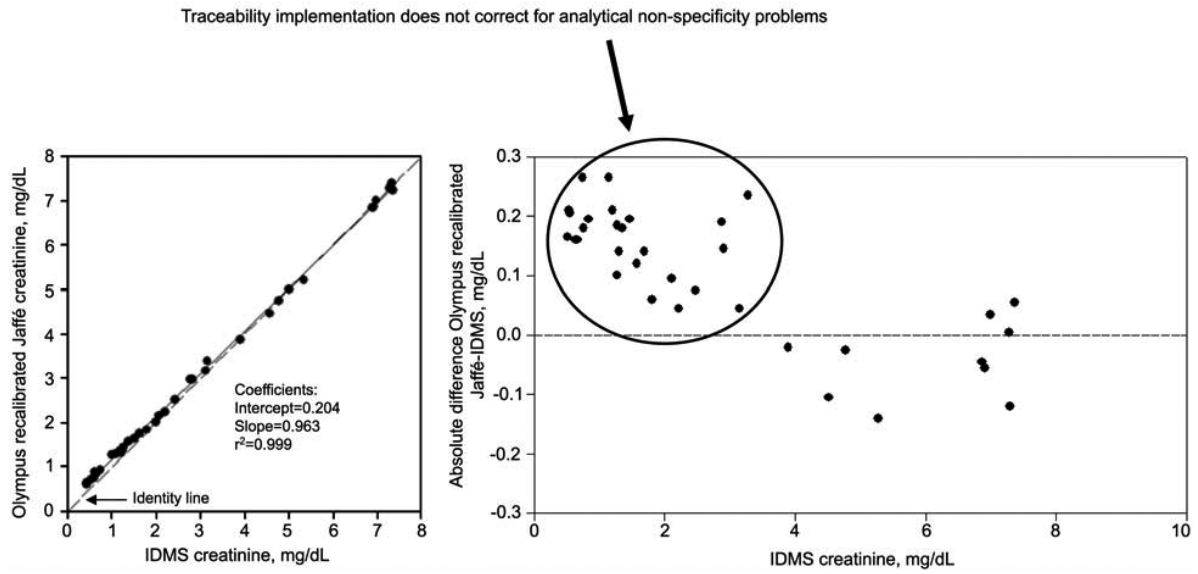

Figure 1

Regression analysis and bias plot comparing serum creatinine results obtained with Olympus alkaline picrate (Jaffé) assay (AU2700 platform) and gas chromatography-isotope dilution mass spectrometry (IDMS) reference method. The Olympus Jaffé assay is reported by the manufacturer's package insert to have been standardized ("recalibrated") against the National Institute of Standards and Technology SRM 909b, a secondary reference material with creatinine values assigned by IDMS. Note the positive intercept [0.204 mg/dL] of the regression analysis indicating difference in analytical specificity between the two methods. To express creatinine values in µmol/L, multiply the values by 88.4.

In addition, as there are no non-creatinine chromogens present in urine, which interfere with the alkaline picrate reaction, compensation is basically not necessary with creatinine measurements in urine. Thus, if serum and urine are measured on the same instrument channel using a compensated method, the results for urine will show a basic negative bias due to the automatic subtraction of the offset. If an accurate measurement of urinary creatinine is needed, e.g. in the estimate of the albumin:creatinine ratio or for creatinine clearance determination, a separate channel with no subtraction mode has to be used.

ARE ALKALINE PICRATE ASSAYS STILL SUITABLE FOR CLINICAL USEFULNESS?

Not only trueness, but also the precision of creatinine measurements may significantly improve when enzymatic methods are employed. In a recent study the imprecision of daily creatinine measurements of a liquid-frozen material, obtained using two assays (a kinetic alkaline picrate "compensated" assay and an enzymatic assay, both from Roche Diagnostics) on two different instruments, was evaluated during two consecutive eight-month working periods (20). The imprecision of creatinine measurements decreased in both analytical systems when the enzymatic assay replaced the alkaline picrate assay (Figure 2). In particular, while only one out 16 monthly CVs by enzymatic method was higher than the desirable goal for imprecision derived from biological variation of creatinine in blood (≤2.2%), six monthly CVs by alkaline picrate assay (37.5% of total) surpassed this limit. These data could not be comprehensive enough to draw a sweeping conclusion that all enzymatic methods are more precise than alkaline picrate methods, but they represent important evidence supporting the usefulness of enzymatic assays in clinical practice.

Access to enzymatic assays can also be useful when interference from substances such as bilirubin and hemolysis is suspected (Table 1) (11). On the other hand, a very few compounds may interfere with enzymatic procedures. Interference for enzymatic assays has been reported in case of intravenous fluid contamination of plasma samples from dopamine or dobutamine solutions (21). Steady-state plasma concentrations of dopamine or dobutamine in vivo, even when multiple catecholamine agents are administered simultaneously, does not, however, affect the enzymatic methods (21). The only drug reported to interfere with currently available enzymatic assays at borderline therapeutic concentrations is calcium dobesilate, used to reduce capillary permeability in diabetic retinopathy (22).

The enzymatic creatinine methods appear to be the only assays giving reliable results when specimens take time to reach the laboratory and blood centrifugation is delayed for 24 h or more. In a recently published study, delays in sample centrifugation caused false increases in measured creatinine by three alkaline picrate assays due to the possible interference effect of some metabolites built up in vitro, such as pyruvate or ketones (23). Note that even seemingly minimal shifts in creatinine results can actually cause major alterations in the number of subjects classified as having different grades of reduced kidney function. Klee et al. (24) recently showed that a positive shift of 0.23 mg/dL (20 µmol/L) creatinine approximately triples the number of individuals with eGFR value of 60 mL/min 1.73 m^2 in a typical outpatient population (Figure 3).

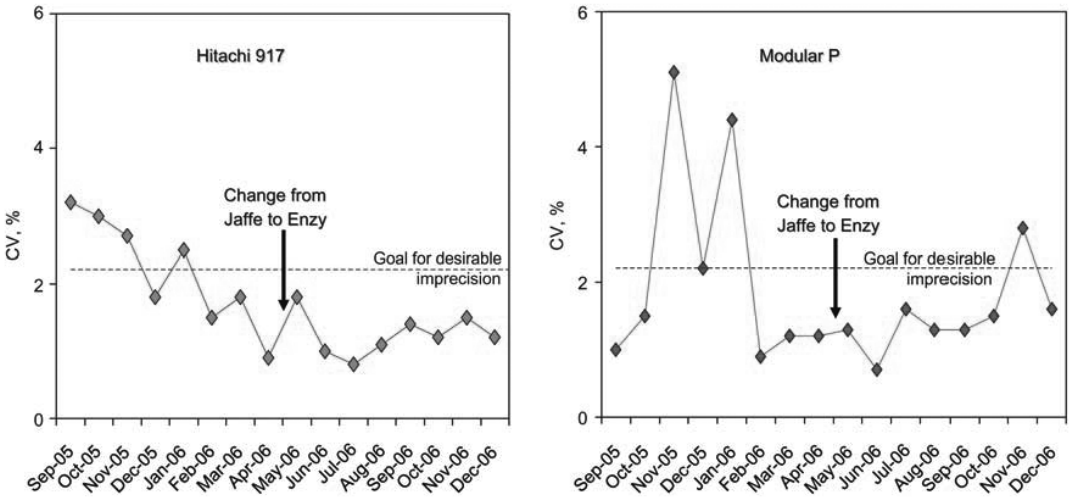

Figure 2
Imprecision of daily creatinine measurements (expressed as monthly CV) on two analytical systems over two eight-month periods using a kinetic alkaline picrate "compensated" assay and an enzymatic assay. A total of 624 measurements were performed on each platform during the evaluation period. The arrows indicate the change of creatinine method from alkaline picrate to enzymatic assay. The dashed line indicates the desirable imprecision limit (CV, 2.2%).

Table 1
Major interferences with kinetic alkaline picrate and enzymatic assays for creatinine determination. Adapted from Peake M, Whiting M. Clin Biochem Rev 2006;27:173

Interferent	Added concentration	Measured bias, mg/dL[a] (basal creatinine, 0.80)	
		Alkaline picrate	Enzymatic
Albumin	40 g/L	+0.24	No bias
Glucose	900 mg/dL	+0.21	No bias
Bilirubin	29 mg/dL	-0.20	-0.12
Hemoglobin (neonates)	1 g/dL	-0.75	No bias
Pyruvate	2 mmol/L	+0.35	No bias

[a] *To express creatinine values in µmol/L, multiply the values by 88.4*

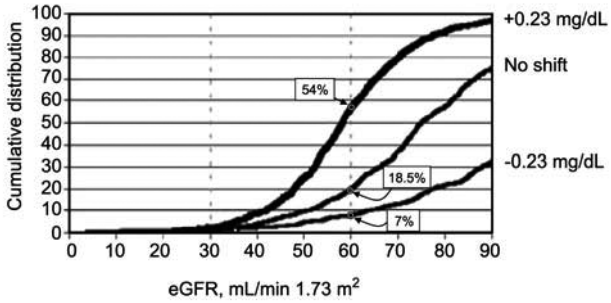

Figure 3
Effect of analytic shifts in serum creatinine results on the distribution of estimated glomerular filtration rate (eGFR) values on a typical outpatient group. Estimated percentages of individuals with eGFR value of 60 mL/min 1.73 m^2 are shown. To express creatinine values in µmol/L, multiply the values by 88.4. Reprinted with permission from Klee GG et al., Clin Chem Lab Med 2007;45:737.

POST-MARKET SURVEILLANCE CONFIRMS THE BETTER PERFORMANCE OF ENZYMATIC METHODS

The pivotal importance of creatinine measurement assumes that laboratories are prepared to carefully monitor the performance of their methods through a very tight quality control. Unfortunately, the materials typically used for most External Quality Assessment (EQA) programs are noncommutable and cannot be used to evaluate accuracy for an individual participating laboratory (25). Therefore, the introduction of regularly recurring EQA programs that use commutable control materials with target values assigned using the IDMS reference method for creatinine together with a clear definition of the clinically allowable total error of measurements is required (2, 26). True value assignment to commutable EQA materials provides a unique resource to allow an objective evaluation of the performance of IVD devices, together with an accuracy-based (instead of inferior consensus-group) grading of the competency of participating clinical laboratories.

Using this approach, on samples distributed in 2007 the German EQA program has been able to show that a large number of laboratories using alkaline picrate-based assays are still significantly inaccurate, particularly at lower creatinine concentrations. For a sample with an IDMS target value of 1.07 mg/dL (95 µmol/L) the median value of participants using enzymatic assays (n=136) was 1.08 mg/dL (95 µmol/L), whereas the median value of participants using assays based on Jaffé reaction (n=478) was 1.24 mg/dL (110 µmol/L) (for more details, www.dgkl-rfb.de).

ACCURACY AS A PREREQUISITE FOR USE OF COMMON REFERENCE INTERVALS FOR SERUM CREATININE

A further difficulty associated with the standardization efforts is the need of development of scientifically sound and globally useful reference intervals for serum creatinine concentrations. As the most widely used eGFR formulas are validated only for adults and in persons with impaired renal function, there is still the need for these reference intervals.

For the production of common reference intervals the method specificity is paramount (27). Thus, only serum creatinine reference intervals obtained with standardized, specific assays, such as those based on some enzymatic principles, should be considered for the establishment of reference values, as these methods have the unique analytical specificity to guarantee traceability of each reference individual result to the reference measurement system for creatinine measurement, especially at the low serum creatinine concentrations found in children (Table 2) (28). Clinical laboratories using these

Table 2
Common reference intervals for creatinine concentrations in serum. Adapted from Ceriotti F et al. Clin Chem 2008;54:559

	Percentile value, mg/dL[a]	
Age (gender) group	2.5th	97.5th
Cord blood	0.52	0.97
Preterm neonates 0 - 21 d	0.32	0.98
Term neonates 0 - 14 d	0.31	0.92
2 m - <1 y	0.16	0.39
1 y - <3 y	0.17	0.35
3 y - <5 y	0.26	0.42
5 y - <7 y	0.29	0.48
7 y - <9 y	0.34	0.55
9 y - <11 y	0.32	0.64
11 y - <13 y	0.42	0.71
13 y - <15 y	0.46	0.81
Adult (males)	0.72	1.18
Adult (females)	0.55	1.02

[a]*To express creatinine values in µmol/L, multiply the values by 88.4*

methods for serum creatinine measurement can finally adopt the selected reference intervals in evaluating their own population.

CONCLUSIONS

Nowadays, in clinical practice any choice or change must be based upon robust and widely accepted evidence. Numerous pieces of evidence are now available to support the routine substitution of alkaline picrate methods with the enzymatic ones for suitable clinical usefulness of creatinine measurements. Supporting the choice of more specific assays by clinical laboratories represents one of the main tasks of our profession in order to report an accurate eGFR in all the pertinent clinical situations. The frequently raised issue of reagent costs is a false problem. First, several of the larger IVD companies that have historically provided only alkaline picrate-based creatinine reagents are now actively engaged in product development programs to introduce enzymatic assays in the near future. Consequently, as more and more vendors begin providing commercial enzymatic assays for creatinine, it is likely that there will be a more competitive situation in the marketplace, and ultimately, prices may be driven lower. More importantly, the cost aspects in clinical laboratories must be considered in the wider overall context of health economics and not within the more blinkered area of pure laboratory economics where, almost by definition, every test represents a cost, and its value is outside the scope of the laboratory service (29).

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