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ORIGINAL ARTICLE

Enzymatic assays for creatinine: Time for action

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Estimation of glomerular filtration rate (eGFR) on the basis of serum creatinine concentration measurements using equations is critical to ongoing global public health efforts to improve the diagnosis and treatment of chronic kidney disease. There is now ongoing activity to promote world-wide standardization of methods to measure creatinine concentrations, together with the introduction of a revised eGFR equation appropriate for use with standardized creatinine methods. Standardization of calibration, i.e. implementation of calibration traceable to higher-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, thereby producing results more closely aligned with the reference method (isotope dilution-mass spectrometry), but this strategy makes the assumption that the non-creatinine chromogen interference is constant among samples, which is an oversimplification. Thus, analytical non-specificity for substances found in individual patient samples affects the accuracy of eGFR computed from serum creatinine concentrations for any alkaline picrate method, including the so-called "compensated" Jaffe methods. Using assays that are more specific for serum creatinine, such as those based on enzymatic reactions, may provide more reliable eGFR values. Supporting the choice of more specific assays by clinical laboratories is one of the main tasks of our profession in achieving the ultimate clinical goal, which is to routinely report an accurate eGFR in all pertinent clinical situations.

Keywords: Calibration; glomerular filtration rate; isotope dilution-mass spectrometry; kidney function tests; reference standards; traceability

Introduction

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

Although the creatinine concentration measurement 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 towards achieving the needed comparability of biochemical measurement results, regardless of the method used and/or the laboratory where the measurements are performed [6,7]. In particular, achievement of improved trueness for creatinine concentration measurements requires that the values assigned by manufacturers to the calibrators and control materials used in routine measurement procedures be traceable to 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 mandatory. The EU

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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 with very different levels of implementation [3]. Some manufacturers have already recalibrated their creatinine assays to the isotope dilution-mass spectrometry (IDMS) reference method world-wide. However, others sell kits with different calibration in Europe compared to other parts of the world, and some manufacturers still maintain old calibrations and will recalibrate later with the introduction of new reagent lots. This confusing 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 concentration measurements are significantly biased and that further work is needed to substantially improve the trueness.

Standardization does not correct for analytical non-specificity problems

Analytical non-specificity, i.e. inability to measure creatinine concentrations solely, of some routine methods must also be addressed. Traceability implementation does not solve the analytical interferences caused by assay non-specificity and, if the reference measurement procedure and corresponding lower-order routine methods are not identical, or at least very similar, specificities for the measurand, traceability cannot be achieved [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 specific for serum creatinine concentration measurements, 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 177 μmol/L (2.00 mg/dL). This problem is not eliminated by alignment to IDMS [15].

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

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 concentration 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 a non-biased measurement of urinary creatinine is needed, e.g. for estimation of the albumin:creatinine ratio or for creatinine clearance determination, a separate channel with no subtraction mode has to be used for the measurements of urinary creatinine concentrations.

Are alkaline picrate assays still suitable for clinical use?

Not only trueness, but also precision of creatinine concentration measurements is likely to improve when enzymatic methods are employed. A recent study has shown that the imprecision of daily creatinine concentration measurements, obtained using two assays (a kinetic alkaline picrate "compensated" assay and an enzymatic assay, both from Roche Diagnostics), decreased when the enzymatic assay replaced the alkaline picrate assay [20]. These data were not comprehensive enough for the sweeping

Table I. Major interferences with kinetic alkaline picrate and enzymatic assays for creatinine concentration measurements. Adapted from [11].

		Measured bias, μmol/L[a] (basal creatinine, 71)	
Interferent	Added concentration	Alkaline picrate	Enzymatic
Albumin	40 g/L	+21	No bias
Glucose	50 mmol/L	+19	No bias
Bilirubin	495 μmol/L	−18	−11
Haemoglobin (neonates)	10 g/L	−66	No bias
Pyruvate	2 mmol/L	+31	No bias

[a]To express creatinine values in mg/dL, multiply by 0.0113.

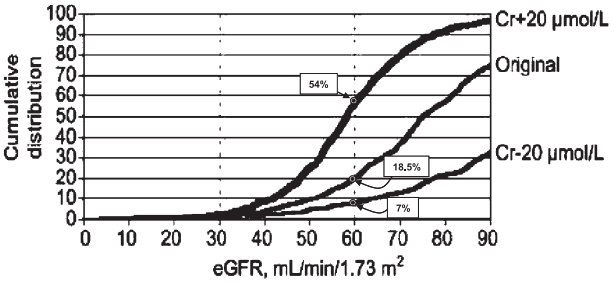

Figure 1. Effect of analytic shifts in serum creatinine concentrations on the distribution of estimated glomerular filtration rate (eGFR) values on a typical outpatient group. Estimated percentages of individuals with eGFR value of 60 mL/(min × 1.73 m^2) are shown. To express creatinine values in mg/dL, multiply by 0.0113. Reprinted with permission from [24].

conclusion to be drawn 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.

Very few compounds interfere with enzymatic procedures, which are valuable when interference from substances such as bilirubin and haemolysis is suspected (Table I) [11]. Interference in enzymatic assays has been reported for intravenous fluid contamination of plasma samples from dopamine or dobutamine solutions [21]. However, steady-state plasma concentrations of dopamine or dobutamine in plasma do not affect the enzymatic methods, even when multiple catecholamine agents are administered simultaneously [21]. The only drug reported to interfere with currently available enzymatic assays at borderline therapeutic concentrations is calcium dobesilate, which is used to reduce capillary permeability in diabetic retinopathy [22].

The enzymatic creatinine concentration measurement methods appear to be the only assays giving reliable results when blood centrifugation is delayed for 24 h or more. In a recently published study, delays in sample centrifugation caused false increases in measured creatinine concentration by three alkaline picrate assays due to the interference by metabolites forming *in vitro*, such as pyruvate or ketones [23]. It is of note that even seemingly minimal shifts in creatinine concentration results can 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 20 μmol/L (0.23 mg/dL) creatinine approximately triples the number of individuals with an eGFR value of 60 mL/(min × 1.73 m^2) in a typical outpatient population (Figure 1).

Post-market surveillance confirms the better performance of enzymatic methods

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

Using this approach, results of the 2007 German EQA programme have shown that a large number of laboratories using alkaline picrate-based assays are still significantly biased, particularly at lower creatinine concentrations. For a sample with an IDMS target value of 95 μmol/L (1.07 mg/dL), the median value of participants using enzymatic assays (n=136) was 95 μmol/L (1.08 mg/dL), whereas the median value of participants using assays based on the Jaffe reaction (n=478) was 110 μmol/L (1.24 mg/dL) (for more details, visit www.dgkl-rfb.de).

Trueness as a prerequisite for use of common reference intervals for serum creatinine

A further difficulty associated with standardization efforts is the need to develop scientifically sound and

Table II. Common reference intervals for creatinine concentrations in serum. Adapted from [28].

	Percentile value, μmol/L[a]	
Age (gender) group	2.5th	97.5th
Cord blood	46	86
Preterm neonates 0–21 d	28	87
Term neonates 0–14 d	27	81
2 m–<1 y	14	34
1 y–<3 y	15	31
3 y–<5 y	23	37
5 y–<7 y	25	42
7 y–<9 y	30	48
9 y–<11 y	28	57
11 y–<13 y	37	63
13 y–<15 y	40	72
Adult (males)	64	104
Adult (females)	49	90

[a]To express creatinine values in mg/dL, multiply by 0.0113.

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 to establish reference intervals.

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

Are costs a major obstacle in applying enzymatic assays?

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 will 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. Otherwise, a cent saved in the laboratory can, paradoxically, cost Euros in the clinic [29]!

Conclusions

Any choice or change in clinical practice must be based on evidence. Currently, numerous pieces of evidence support the substitution of alkaline picrate methods with enzymatic ones for suitable clinical usefulness of creatinine concentration measurements. Reporting a non-biased eGFR in all pertinent clinical situations requires the use of more specific assays. It is argued that increased use of enzymatic measurement procedures will make wide use of such methods economically possible.

Key points from the discussion

- There is a publication providing data indicating that the Roche modified Jaffe method exhibits less interference by bilirubin than the enzymatic method. This has not been confirmed by other groups. The overwhelming view is that less interference is shown by the enzymatic method.
- The Lab Working Group of the NKDP is to collaborate with the IFCC Working Group on GFR to undertake systematic evaluation of interferences with the most widely used creatinine measurement methods. It is anticipated that concrete recommendations will be available later in 2008. Manufacturers are reluctant to accept that Jaffe methods cannot function satisfactorily. However, it is anticipated that, in time, enzymatic methods will gain acceptance. Thought needs to be given to the gold standard that should be used to evaluate GFR.
- It is important that clinicians know what the reference values mean. If we do not use a population-based reference interval then we are giving a decision limit and the evidence for defining that decision limit needs to be communicated.

Contributing to the discussion

J. Smith, A. Kallner, J. Kytzia, G. Miller, A. Grubb, G. Jones, J. Coresh.

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