

# Estimating Glomerular Filtration Rate from Serum Creatinine Measurements: Analytical Issues and Standardization Programs

M. Panteghini

*Centre for Metrological Traceability in Laboratory Medicine (CIRME) and  
Department of Clinical Sciences "Luigi Sacco", University of Milan*

## Summary

Reliable serum creatinine measurements in glomerular filtration rate estimation (eGFR) are critical to ongoing global public health efforts to increase the diagnosis and treatment of chronic kidney disease. It is accepted that use of serum creatinine concentration alone as a GFR marker is inadequate. International recommendations favour the reporting of creatinine-based eGFR using equation that was developed from the Modification of Diet in Renal Disease (MDRD) study, i.e. the "four-variable" MDRD equation that uses age, sex, race, and serum creatinine parameters. However, a limitation of this equation for general implementation in healthcare is related to the use of differently calibrated creatinine measurement procedures among laboratories. In particular, creatinine results which were used to generate the clinical basis for the eGFR MDRD equation were not traceable to high-order reference measurement procedures and reference materials. Consequently, the eGFR is very dependent on the accuracy of the creatinine method in use. The only way to achieve universal implementation of the eGFR prediction equation, with the associated clinical benefits for the patients, is, therefore, 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 does not,

however, correct for analytical interferences of methods (non-specificity bias). 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.

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. 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" Jaffe 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.

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## Chronic kidney disease

Worldwide, chronic kidney disease (CKD) is a major public health problem. In the United States (US), the incidence and prevalence of end-stage renal disease, kidney failure treated by dialysis, and transplantation have more than quadrupled over the last two decades. In Europe, the annual incidence of end-stage renal disease has doubled over the past decade to reach approximately 135 new patients per million of population<sup>1</sup>.

The US National Kidney Foundation has recently defined CKD as either structural or functional kidney damage or a glomerular filtration rate (GFR)  $<60$  mL/min  $1.73$  m<sup>2</sup> for three months or more, irrespective of cause<sup>2</sup>. The threshold of GFR  $<60$  was selected as the definition of CKD because at this value approximately half of an adult's normal kidney function is lost, leading to several possible complications. In addition, the National Kidney Foundation classified stages of CKD severity based predomina-

tly on GFR estimation<sup>2</sup>. Measures to reduce kidney toxicity associated with radiographic contrast procedures are now also based on GFR estimation.

### GFR assessment

GFR is, therefore, currently considered the best overall measure of kidney function and an accurate, inexpensive, and widely available method that estimates GFR is of paramount importance. GFR can accurately be assessed by measuring the urinary clearance of exogenous filtration markers such as inulin, iothexol, iohalamate, or <sup>51</sup>chromium-labeled EDTA. However, because of difficulty in use, special specimen handling, cost, radiation exposure, and radionuclide regulatory requirements, these methods are incompatible with clinical practice and are typically confined to specialized setting.

Creatinine clearance may be a useful alternative when exogenous filtration markers are not available. However, it requires a timed (usually 24 h) urine collection, which is often problematic and susceptible to error, making the test unsuitable for widespread clinical application. Thus, kidney function is often assessed clinically from serum concentrations of endogenous creatinine, but its sensitivity for the detection of CKD is poor because it is affected by the GFR and by factors independent of GFR, including age, gender, race, muscle mass, diet, and certain drugs. Particularly, the use of a single reference interval for serum creatinine to distinguish between a physiological GFR and an abnormal one can be misleading and serum creatinine alone fails to identify half of the patients with stage 3 CKD, having GFR between 30 and 59, and performance is even worse in certain patient groups, such as older subjects.

More accurate estimations of GFR can be obtained with prediction equations that empirically combine all of the average effects from confounding variables that affect serum creatinine other than GFR, therefore overcoming some of the limitations of the use of serum creatinine alone<sup>3</sup>. Practically, GFR estimation (eGFR) is a mechanism to standardize reporting, which will hopefully provide more uniform interpretation and follow-up by clinicians. There are now at least 25 different proposed equations for eGFR, but most require additional information, such as a measure of body surface (based on height and/or weight measurements), that is not readily available, thus limiting the wider use of this approach.

The possibility that clinical laboratories might routinely report an eGFR derived from the serum creatinine concentration has become practical with the development of a formula with only the variables age, sex, race, and serum creatinine. This formula, the "four-variable" MDRD equation (developed from the US Modification of Diet in Renal Disease study), is based on GFR values measured by iohalamate clearance on a non-hospitalized cohort of individuals with CKD, with 91% of the evaluated subjects having an eGFR within 30% of the measured value<sup>2</sup>. Particularly, this approach was more accurate than either the use of the Cockcroft-Gault equation or the measurement of creatinine clearance. Furthermore, the MDRD study equation does not require a body weight variable because it normalizes GFR for a standard body surface area of 1.73 m<sup>2</sup>. This equation has been demonstrated to be useful

for CKD patients and performs similarly in type 2 diabetics and kidney transplant recipients, but its performance may be limited for people with low values for serum creatinine and high values for GFR, including healthy individuals, children and pregnant women.

### Susceptibility of eGFR to variations in creatinine methodology

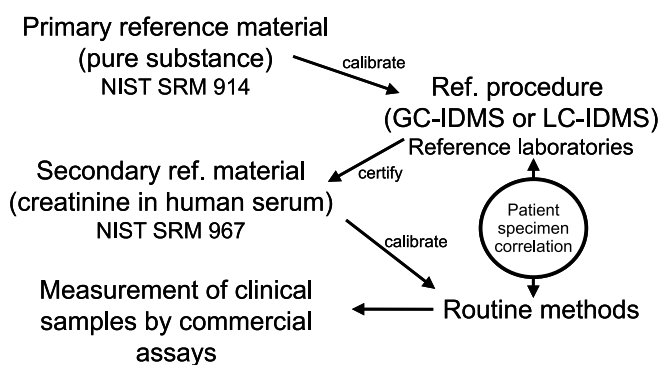
A major barrier to the general implementation in healthcare of equations for eGFR is the use of different creatinine measurement procedures among laboratories. Lacking standardization for creatinine measurement, assays not calibrated in agreement with the method used in the core laboratory to develop and validate a specific equation (e.g., laboratory at the Cleveland Clinic for development of the MDRD equation) introduce an additional source of error into the mathematical prediction of GFR. Care should be taken concerning clinical consequences of differences in calibration compared to the core laboratory, especially considering that the relationship of an individual laboratory's assay to that provided by the core laboratory is generally unknown and that a widespread calibration effort of all clinical laboratories to one research laboratory is not feasible.

Although the US National Kidney Foundation clearly advised in its 2002 guidelines the use of only corrected creatinine values in eGFR with the MDRD formula, many laboratorians, practitioners, and nephrologists do not seem to be aware of this important source of error in routine clinical practice, when equations are applied globally with a single, universal decision limit of 60 mL/min 1.73 m<sup>2</sup>.

Calibration bias contributes to larger uncertainty in eGFR at lower creatinine values within the physiologic concentrations. Myers et al.<sup>4</sup> have shown the effect on eGFR by the MDRD equation of different calibration biases of creatinine methods. In their example, for a 60-year-old Caucasian female, for whom the eGFR was 60 mL/min 1.73 m<sup>2</sup> at a creatinine of 1.00 mg/dL (88 μmol/L), a calibration difference of 0.12 mg/dL (11 μmol/L) was associated with an error in eGFR of -12%. The error in eGFR over the range of biases examined [-0.06 mg/dL to +0.31 mg/dL (-5 μmol/L to 27 μmol/L)] was from +7.5% to -27% and, of relevance here, data from External Quality Assessment Schemes (EQAS) suggest that this amount of systematic variation across laboratories is common.

### Standardization of creatinine as a prerequisite for implementing eGFR

At a minimum, 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 MDRD equation using standardized creatinine results<sup>5</sup>. There is now international agreement that the implementation of calibration traceability to high-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. Particularly, achievement of improved accuracy for creatinine measurements requires that the values assigned



**Figure 1.** The reference measurement system for serum creatinine. Adapted from ref. 5. NIST, National Institute of Standards and Technology; SRM, standard reference material; GC-IDMS, gas chromatography-isotope dilution mass spectrometry; LC-IDMS, liquid chromatography-isotope dilution mass spectrometry.

by manufacturers to calibrators and control materials supporting routine measurement procedures are traceable to higher-order reference measurement procedures and reference materials (Fig. 1).

The National Institute of Standards and Technology (NIST) Reference Material 914, which is crystalline creatinine, is the available primary reference material. Solutions of SRM 914, prepared gravimetrically by dissolving this material in aqueous buffer, are intended for use in calibration of the high-order reference measurement procedures [gas chromatography-isotope dilution mass spectrometry (GC-IDMS) and liquid chromatography (LC)-IDMS] performed in reference laboratories. The IDMS is considered the method of choice for measurement of the true concentration of creatinine because of its high specificity and uncertainty lower than 0.3%. The NIST SRM 967 is a human serum-matrixed reference material with creatinine values assigned by the reference method that can be used to calibrate routine methods. The availability of this secondary reference material, intended for direct calibration of routine methods, is critical for effective implementation of creatinine standardization. Manufacturers then may use this material for calibration of a routine method, leading to traceable results for the end user's routine method.

Given the resources now available, it is time for all *in vitro* diagnostic (IVD) manufacturers to establish calibrations that are traceable to the creatinine reference system. Although this may seem easy in principle, implementation of a plan to introduce standardized creatinine measurement procedures can be complicated because the suggested steps must be recognized as sound by all those involved in measuring creatinine and eGFR. Particularly, this effort must involve international cooperation among the IVD manufacturers, clinical laboratories, professional organizations, government agencies, and EQAS providers. In the European Union (EU) the implementation of calibration traceability in Laboratory Medicine to available higher-order reference methods and materials is already mandatory by law. The EU 98/79/EC-IVD directive explicitly requires manufacturers to ensure metrological traceability of their products. Internationally, we are in a transition period in which very different levels of implementation are apparent. Some manufacturers have already

recalibrated their creatinine assays to IDMS 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 external surveys<sup>5</sup>.

### State of the art of creatinine measurement

In 2002, the International Measurement Evaluation Program (IMEP)-17 survey of more than 800 laboratories in 35 countries demonstrated almost universal overestimation of serum creatinine in a serum pool with an IDMS certified concentration of 0.84 mg/dL (74  $\mu$ mol/L). Of the fourteen method groups, eleven demonstrated significant positive bias compared with the reference value, typically varying between +10 and 15%. The Roche enzymatic assay was a notable exception. A more recent national proficiency study organized by the College of American Pathologists involving predominantly North American clinical laboratories demonstrated that, of the five major manufacturers in the US market, two had values aligned to IDMS and three were biased high at various concentration levels.

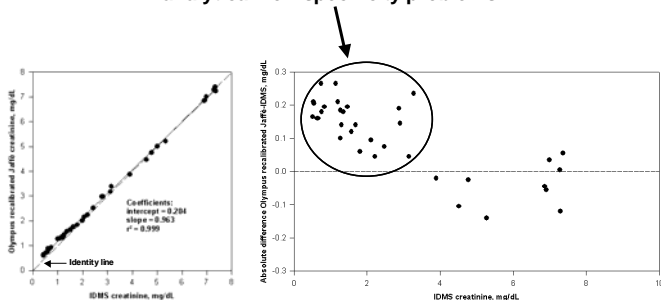
In spite of EU Directive on IVD medical devices, the situation appears to be no different in Europe. In a study involving more than 170 laboratories from six European countries, creatinine assays from at least four major manufacturers did not fulfill the traceability goal for results obtained in a human sample with creatinine concentration by IDMS of 0.85 mg/dL (75  $\mu$ mol/L). A similar situation was recently shown in a national survey performed in Italy, in which only two enzymatic assays performed close to the reference method<sup>6</sup>.

Collectively, these observations suggest that a large number of routine analytical systems for serum creatinine are still significantly biased and that further work is needed to achieve substantially improved accuracy in creatinine results with routine methods. The recent availability of the NIST SRM 967 is expected to provide manufacturers with an important practical tool to enable closure of this gap.

### Are alkaline picrate assays still suitable for clinical usefulness?

Analytical non-specificity of some routine serum creatinine methods must also be addressed. Standardization of calibration does not solve the analytical interferences related to an assay's non-specificity. 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. It is well known that as a result of reaction with plasma pseudo-creatinine chromogens, particularly proteins, alkaline picrate methods overestimate true serum creatinine by as much as 15% to 25%, inducing proportionally greater errors at values lower than 2.00 mg/dL (177  $\mu$ mol/L). This still remains true even after IDMS recalibration (Fig. 2). To account for the sensitivity of alkaline picrate-based methods to non-creatinine chromogens, some manufacturers have adjusted the calibration to mini-

### Traceability implementation does not correct for analytical non-specificity problems



**Figure 2.** 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 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 (18  $\mu$ mol/L)] of the regression analysis indicating difference in analytical specificity between the two methods.

mize the pseudo-creatinine contribution of plasma proteins by introducing a negative offset to “compensate” the positive intercept found in the correlation, but this strategy makes an assumption that the non-creatinine chromogen interference is a constant among samples, which is an oversimplification. Furthermore, at least in some cases, the manufacturer’s recommended offset appears to paradoxically cause a negative bias, with results falling below the acceptable range at clinically important concentrations, as shown in a study recently performed in Australia<sup>7</sup>.

Several studies indicate that the use of assays that are more specific for serum creatinine determination, such as those based on enzymatic procedures, produce results that agree closely with IDMS. The precision of creatinine measurements may also significantly improve when enzymatic methods are employed. In a study performed in our laboratory, 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<sup>8</sup>. The imprecision of creatinine measurements decreased in both analytical systems when enzymatic assay replaced alkaline picrate assay. In particular, while only one out 16 monthly CVs by enzymatic method was higher than the desirable goal derived from biological variation ( $\leq 2.2\%$ ), six monthly CVs by alkaline picrate assay (37.5%) surpassed this limit. Access to enzymatic assays can also be useful when interference from substances such as bilirubin and hemolysis is suspected<sup>7</sup>. Finally, enzymatic creatinine methods are the only assays giving reliable results when specimens take time to reach the laboratory and blood centrifugation is delayed for 24 h or more. Conversely, delays in sample centrifugation can cause false increases in measured creatinine by alkaline picrate assays due to the interference effect of some metabolites built up in vitro, such as pyruvate or ketones<sup>9</sup>.

For all the above reported reasons, it is therefore advisable that, for suitable clinical usefulness of creatinine mea-

**Table I.** Isotope dilution mass spectrometry-traceable MDRD study four-variable equation for estimating glomerular filtration rate (eGFR).

$$\text{eGFR (mL/min 1.73 m}^2\text{)} = 175 \times (\text{s-Creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if Female}) \times (1.210 \text{ if African American})$$

Note: Serum creatinine values are expressed in mg/dL. If creatinine values are expressed in  $\mu$ mol/L, divide the values by 88.4 before introducing them into the equation. This equation is only for use with creatinine results from methods that have been calibrated to the reference system for creatinine.

surements, laboratories should consider to definitively replace alkaline picrate methods with the enzymatic ones. 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 raised issue of reagent costs is a false problem. First, 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. 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.<sup>10</sup> recently showed that a positive shift of 0.23 mg/dL (20  $\mu$ mol/L) creatinine approximately triples the number of individuals with eGFR value of 60 mL/min 1.73 m<sup>2</sup> in a typical outpatient population.

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. Particularly, the introduction of a regularly recurring EQAS program that uses commutable serum materials with target values traceable to the IDMS reference method for creatinine can allow individual laboratories and IVD manufacturers, on an ongoing basis, to assess the performance of routine methods.

### Which eGFR equation should be used?

Since the creatinine results, which were used to generate the clinical validation for the original MDRD equation, were not traceable to the reference system, tracing back the calibration of routine tests to the reference system may invalidate the clinical value of GFR equation originally proposed. For this reason, a MDRD equation, sometimes referred to as ‘175’ formula, has now been re-expressed for eGFR with IDMS standardized serum creatinine results with the best approximation (Tab. I)<sup>11</sup>. By using this equation and a standardized creatinine assay, clinical laboratories can report eGFR more uniformly and accurately. This equation was, however, not developed de novo, but was created by doing a correlation study of specimens tested by both the original MDRD study creatinine method and an IDMS-aligned method. So that, it still needs to be vali-

**Table II.** Clinical situations in which the measurement of creatinine clearance is still recommended for assessing kidney function.

- Exceptional dietary intake (e.g., vegetarian, creatine supplements)
- Abnormal muscle mass or condition (e.g., muscle wasting)
- Rapidly changing kidney function
- Pregnancy
- Drug dosage
- In selected circumstances, creatinine clearance measurements can also be used as confirmatory test for possibly erroneous estimates of eGFR.

dated in other independent cohorts. On the other hand, following implementation of serum creatinine methods with calibration traceable to IDMS, other equations often used to estimate kidney function, such as Cockcroft-Gault or Schwartz, will give values that, in most cases, are higher than the values obtained using traditionally calibrated creatinine methods. This calibration change may, therefore, significantly affect interpretive criteria based on these estimates of kidney function and, in order to avoid this risk, a re-expression of these equations with standardized creatinine results will also be required. Recognizing the importance of these issues, the US National Institutes of Health have now founded a new collaborative study to develop and validate improved eGFR equations in an effort to resolve limitations associated with equations currently available for clinical practice. Until improved equations are developed, it may be appropriate to report a specific numeric results only for eGFR below 60 mL/min 1.73 m<sup>2</sup>, as is recommended by current guidelines<sup>4</sup>; higher values can be reported simply as “>60 mL/min 1.73 m<sup>2</sup>” in laboratory reports and not as an exact number.

### Creatinine clearance outlook

As the validity of the MDRD equation has been shown to be at least equal to creatinine clearance in most clinical situations, the importance of creatinine clearance measurement has remarkably declined. However, there are exceptions in which to obtain accurate GFR estimates, measurement of clearance using timed urinary collections should still be used (Tab. II). Once again, the effect on measured creatinine clearance will vary depending on the procedure used to calibrate serum and urine measurements, even when clearance results are corrected for systematic overestimation of GFR due to creatinine secreted by renal tubule.

### Conclusions

The goal of identifying persons with early CKD in the hopes of slowing progression is a worthy one. However, its implementation by providing an eGFR with every routine creatinine measurement (as recommended in some guidelines) seems to be a bit premature. In agreement with

Rainey's suggestion, “it would be more reasonable for laboratories to offer an eGFR when such a calculation is requested by a clinician. The clinician can determine whether the individual is an appropriate and willing candidate for eGFR calculation and can take responsibility for providing appropriate interpretation and follow-up of the results” by appreciating the inherent inaccuracy of the estimates in making clinical decisions<sup>12</sup>. This requires, however, education of clinicians about the interpretation of eGFR. Educational efforts are probably best initiated by a close working relationship between laboratorians, nephrologists, and other clinical colleagues practicing in renal medicine.

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