The importance of metrological traceability on the validity of creatinine measurement as an index of renal function

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)

IFCC Scientific Division, Working Group on Standardization of Glomerular Filtration Rate Assessment (WG-GFRA)
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Abstract
The glomerular filtration rate (GFR) is currently considered the best overall index of kidney function. The possibility that laboratories might routinely report an estimated GFR has become practically feasible with the development of a formula, the “four-variable” Modification of Diet in Renal Disease study (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. The only way to achieve universal implementation of the GFR prediction equation, with the associated clinical benefits for patients, is, therefore, to promote worldwide standardization of methods to determine creatinine, together with the introduction of a revised GFR-estimating equation appropriate for use with standardized creatinine methods.

Keywords: calibration; creatinine; glomerular filtration rate; kidney function tests; reference standards; traceability.

Introduction
Worldwide, chronic kidney disease (CKD) is a major public health problem (1). 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 (2). In 2003, Coresh et al. (3) estimated that the number of people in the US with earlier stages of CKD was approximately 19 million, including approximately eight million people with a reduced glomerular filtration rate (GFR). 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 (1). The US National Kidney Foundation has recently defined CKD as either structural or functional kidney damage or a GFR < 60 mL/min 1.73 m2 for 3 months or more, irrespective of cause (4). The threshold of GFR < 60 mL/min 1.73 m2 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 (5). The National Kidney Foundation also classified stages of CKD severity based predominantly on GFR estimation (4). GFR is, therefore, currently considered the best overall index of kidney function.

GFR estimates
GFR can be assessed by measuring the urinary clearance of exogenous filtration markers such as inulin, iothexol, 125I-labeled iothalamate, 51Cr-labeled EDTA, or 99Tc-labeled diethylenetriaminepentaacetic acid (6, 7). However, because of difficulty in use, special spec-
imen handling, cost, radiation exposure, and radio-
uclide regulatory requirements, these methods have
limited use in clinical practice and are typically con-
fined to specialized settings (8). 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 inaccurate, making the test unsuitable
for widespread clinical application (9). Thus, GFR
is often estimated clinically from serum concentra-
tions of endogenous creatinine or cystatin C (10).
However, serum cystatin C has not yet been ade-
quately evaluated as an index of GFR (11). Serum cre-
atinine is frequently ordered to assess kidney
function, but the sensitivity of serum creatinine alone
for the detection of CKD is poor because it is affected
by the GFR and by factors independent of GFR,
including age, sex, race, muscle mass, diet, and cer-
tain drugs (9). The serum creatinine alone fails to
identify half of patients with stage 3 CKD, having GFR
between 30 and 59 mL/min 1.73 m², and performance
is even worse in certain patient groups, e.g., older
subjects (12).

More accurate and precise 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
(13). Theoretically, estimating equations for GFR
should be: (a) developed in a large cohort, including
a variety of racial and ethnic groups for international
comparisons; (b) evaluated in an independent cohort;
(c) validated to have acceptable bias against a “gold
standard” measure of GFR; and (d) practical to imple-
ment, taking into consideration cost, required data
elements, generalizability, and reliability of the cre-
atinine measurement procedure (5).

There are now at least 25 different equations for
estimating GFR, but most (including the Cockcroft-
Gault equation) require additional information, such
as a measure of body surface (based on height and/or
weight measurements), that is not readily availa-
bile, thus limiting the wider use of this approach. The
possibility that clinical laboratories might routinely
report an estimated GFR derived from the serum cre-
atinine concentration has become practical with the
development of a formula with only the variables age,
sex, race, and serum creatinine (14). This formula, the
“four-variable” MDRD equation (developed from the
US Modification of Diet in Renal Disease study
(MDRD)), is based on GFR values measured by 125I-
iothalamate clearance in 1628 adults and subsequent-
ly validated in another 1775 adults in the African
American Study of Kidney Disease, with 91% of sub-
jects having a GFR estimate within 30% of the meas-
ured value (15, 16). The MDRD equation does not
require a body weight variable because it normalizes
GFR for a standard body surface area of 1.73 m².

This equation has been demonstrated to be useful
for CKD patients and performs similarly in type 2 dia-
abetics and kidney transplant recipients, but its use is
still unclear for people with low values for serum cre-
atinine and high values for GFR, including healthy
individuals, children and pregnant women (5). Vali-
dation studies are in progress to evaluate the MDRD
equation for other ethnic groups (in addition to Cau-
casians and African-Americans) and various disease
conditions.

A major barrier to the general implementation in
healthcare of equations for estimating GFR is the use
of different creatinine measurement procedures
among laboratories (17–19). Lacking standardization
for creatinine measurement, assays not calibrated in
agreement with the method used in the core labora-
tory 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 GFR estimates. Care should be taken con-
cerning clinical consequences of differences in cali-
bration compared to the core laboratory, especially
considering that the relationship of an individual labo-
rratory’s assay to that provided by the core laboratory
is generally unknown (20, 21). Although the US
National Kidney Foundation clearly advised in its 2002
guidelines the use of only corrected creatinine values
in GFR estimation 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 (22). Calibration bias contrib-
utes to larger uncertainty in GFR estimates at lower
creatinine values within the concentration range asso-
ciated with normal kidney function. Myers et al. (2)
recently showed the effect on estimated GFR by the
MDRD equation of different calibration biases of cre-
atinine methods. In their example, for a 60-year-old
Caucasian female, for whom the estimated GFR was
60 mL/min 1.73 m² 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 GFR esti-
mate of −12%. The error in GFR estimates over the
range of biases examined [−0.06 to +0.31 mg/dL
(−5 to +27 μmol/L)] was from +7.5% to −27% and,
of relevance here, data from External Quality Assess-
ment Schemes (EQAS) suggest that this amount of
systematic variation across laboratories is common
(23, 24).

Steps to improve GFR estimates by the MDRD
equation

From the previous considerations, it is clear that the
only way to achieve universal implementation of the
serum creatinine-based GFR prediction equation, with
the associated clinical benefits for patients, is to pro-
mote worldwide standardization of creatinine meas-
urement procedures, together with revalidation of the
MDRD equation using standardized creatinine results.

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 bio-
chemical measurement results, regardless of the
measurement procedures used and/or the laborato-
ries where the analyses are performed (25). This effort
must involve international cooperation among the in vitro diagnostic (IVD) manufacturers, clinical laboratories, professional organizations, government agencies, and EQAS providers. Achievement of improved accuracy for creatinine measurements requires that the values assigned by manufacturers to calibrators and control materials supporting all routine measurement procedures are traceable to higher-order reference measurement procedures and reference materials (2).

The National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 914, crystalline creatinine, is the available primary reference material. Solutions of SRM 914, prepared gravimetrically by dissolving SRM 914 in aqueous buffer, are intended for use in calibration of the high-order reference measurement procedures [i.e., gas chromatography-isotope dilution mass spectrometry (GC-IDMS) and liquid chromatography (LC-IDMS)] performed in reference laboratories. The GC-IDMS method requires a separation step to remove creatinine, which gives the same derivatization product as creatinine, before derivatization of the creatinine or the GC step, and is therefore a very time-consuming procedure with limited sample throughput (26–28). Conversely, the LC-IDMS method has much simpler and faster sample preparation and would be more amenable to assist IVD manufacturers in validating traceability of their systems and to promote a standardization program on a larger scale (29).

The NIST SRM 909b (two levels) and the Institute for Reference Materials and Measurements (IRMM) BCR 573/4/5 (three levels) are lyophilized secondary reference materials with values assigned by the reference method that could theoretically be used to calibrate routine methods. However, the matrix of these human serum-based materials has been modified by converting plasma to serum and by lyophilization, thus altering the recovery of creatinine in these fluids by routine methods (30). Because the commutability of these materials with native human sera has not been established for routine methods, they are in practice unsuitable for direct calibration. Reference material that is non-commutable with native human serum samples can cause significant errors in method calibration (31). In this situation, an alternative approach to standardizing results and establishing traceability to a reference measurement procedure is for IVD manufacturers to split fresh human samples with a laboratory performing a reference measurement procedure and use the results obtained from this comparison to align the calibration of commercial systems (32).

The availability of a secondary commutable reference material, intended for direct calibration of routine methods, is critical for effective implementation of creatinine standardization (Figure 1). With this aim in mind, NIST has quite recently prepared a new serum-matrixed creatinine reference material, designated SRM 967 (2). This material is a fresh-frozen pooled human serum prepared according to Clinical and Laboratory Standards Institute (CLSI) guideline

![Figure 1](image-url) The reference measurement system for creatinine.

C37-A (33). Two concentration levels have been prepared and the creatinine values [0.75 and 3.92 mg/dL (67 and 346 μmol/L)] were assigned by NIST with the GC-IDMS and LC-IDMS reference methods. Considering its characteristics, the new material is expected to be commutable with native human sera. Commutability of SRM 967 with native human serum samples using a variety of routine creatinine measurement procedures and reference methods will soon be experimentally validated.

Recently a re-stated MDRD equation based on creatinine values traceable to an IDMS reference method was developed (Table 1) (34). The new IDMS-traceable MDRD equation is to be used only with standardized creatinine results.

Given the resources now available, it is time for all IVD manufacturers to establish calibrations that are traceable to the reference system, and for clinical laboratories to implement these standardized assays together with the revised GFR-estimating equation appropriate for use with zero-biased routine methods.

### The current situation

Although standardization may seem easy in principle, implementation of a plan to introduce standardized creatinine measurement procedures and the IDMS-traceable MDRD GFR-estimating equation can be complicated because the suggested steps must be recognized as sound by all those involved in measuring creatinine and estimating GFR.

In the European Union the implementation of calibration traceability in Laboratory Medicine to available higher-order reference measurement procedures and reference materials is already mandatory by law.

### Table 1 Isotope dilution mass spectrometry-traceable MDRD study four-variable equation for estimating the glomerular filtration rate (GFR).

\[
\text{GFR (mL/min 1.73 m}^2\text{)} = 175 \times (\text{s-Creatinine})^{1.154} \times (\text{Age})^{0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})
\]

Serum creatinine values are expressed in mg/dL. If creatinine values are expressed in μmol/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.
International initiatives

The National Kidney Disease Education Program (NKDEP) (http://www.nkdep.nih.gov/index.htm) is an initiative of the US National Institutes of Health designed to reduce the morbidity and mortality caused by kidney disease and its complications. The NKDEP Laboratory Working Group recently published recommendations for improving serum creatinine measurement (2). Briefly, IVD manufacturers should ensure optimal performance at 1.00 mg/dL (88 μmol/L) for existing and new methods, and ensure that comparable trueness and imprecision extend throughout the analytical measurement range. A desirable total error goal for creatinine measurement is to contribute a maximum 10% increase in the relative error of the estimated GFR. A figure relating bias and imprecision combinations that are consistent with this total error was included in the report (2). For example, a routine creatinine method with imprecision (including between-laboratory calibration variability) of <8% CV and bias (compared to an IDMS reference method) of <5% at creatinine concentrations ≥1.00 mg/dL (88 μmol/L) would achieve this total error goal.

Finally, IVD manufacturers must address analytical non-specificity of routine serum creatinine methods. Standardization of calibration does not correct for 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. To account for the sensitivity of Jaffe-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 IDMS, but this strategy makes an assumption that the non-creatinine chromogen interference is a constant among samples, which is an oversimplification (37). Analytical non-specificity for substances found in individual patient samples can affect the accuracy of GFR estimates computed from serum creatinine values for any method, including the so-called “compensated” Jaffe methods (38).

The use of assays that are more specific for serum creatinine, such as those based on some enzymatic procedures, may provide more reliable estimated GFR values (13). Supporting the choice of more specific assays by clinical laboratories is one of the main tasks of the newly created IFCC Working Group on Standardization of GFR Assessment (WG-GGRA). Other major tasks are summarized in Table 2. All of these actions will coordinate to achieve the ultimate goal, which is to routinely report an accurate estimate of GFR in all pertinent clinical situations.

Table 2 Main tasks of the IFCC Working Group on Standardization of Glomerular Filtration Rate (GFR) Assessment (WG-GFRA).

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<tr>
<th>Task</th>
<th>Description</th>
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<td>Educate laboratory professionals regarding the importance of assessing risk of chronic kidney disease through the appropriate use of the GFR-estimating equation, with the international dissemination of relevant documents and educational materials</td>
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<td>In cooperation with the U.S. National Kidney Disease Education Program, develop guidelines to coordinate global introduction of standardized creatinine measurements in conjunction with the appropriate GFR-estimating equation</td>
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<td>Prepare a recommendation for use of specific assays for routine creatinine measurements</td>
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<td>Establish a reference laboratory network for creatinine to assist manufacturers in validating traceability of their methods and External Quality Assessment Scheme organizers in targeting commutable control materials</td>
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References


