Introduction
The best indicator of kidney function is considered to be the rate that blood is filtered at the glomerulus, or glomerular filtration rate (GFR). Chronic kidney disease can then be quantitatively defined as a GFR <60 mL/min/1.73m^2 for three months or more, irrespective of the cause. While the GFR can be measured directly by clearance studies of exogenous markers, such as inulin, iohexol, iothalamate, and Cr^51-EDTA, these procedures are costly and time consuming and are not suited to the routine detection of kidney disease. Even to measure the clearance of endogenous substances, such as urea and creatinine, requires both serum and an accurately timed urine collection, so efforts have been directed at more convenient “urine-free” estimates of GFR. The abbreviated Modification of Diet in Renal Disease (MDRD) equations were developed in 1999 to allow the estimation of eGFR based on routine measurement of serum creatinine, along with the readily available demographic variables age, gender and race. To improve the accuracy of these equations, a Laboratory Working Group of the National Kidney Disease Education Program (NKDEP) of the USA has published detailed recommendations for improving the measurement of serum creatinine. These recommendations cover important issues, such as the effect of creatinine method bias on the calculated GFR value. Because of the inverse relationship between serum creatinine concentration and GFR, the impact of bias and imprecision of creatinine measurement is significant at low serum creatinine concentrations corresponding to GFR values near the 60 mL/min/1.73m^2

Analytical Commentary
Measurement of Serum Creatinine – Current Status and Future Goals

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“A review of the literature on methods of creatinine determination in blood cannot but leave one with the impression that each investigator, using a new technique and one seemingly accurate, is able to get figures quite different from those obtained by any previous method” (Behre and Benedict, 1922).

Abstract
The first methods for the measurement of creatinine in serum and plasma were published over a century ago. Today, the Jaffe reaction using alkaline picrate remains the cornerstone of most current routine methods, after continuous refinements attempting to overcome inherent analytical interferences and limitations. With the recent introduction of the reporting of estimated glomerular filtration rate (eGFR), inter-laboratory agreement of serum creatinine results has become an important international priority. Expert professional bodies have recommended that all creatinine methods should become traceable to a reference method based on isotope dilution-mass spectrometry (IDMS).

It is important that clinical biochemists have a good understanding of the relative performance of routine creatinine methods. Using a new commutable IDMS-traceable reference material (SRM 967), and a validated tandem IDMS assay developed in our laboratory, we assessed the accuracy of nine routine creatinine methods with assistance from other laboratories in our region. Three methods appeared to have patient sample bias that exceeded 5% in the range of creatinine concentrations where eGFR estimations are most important.

Companies are currently recalibrating their creatinine assays. This task should be complete in 2007, and then creatinine results for eGFR calculations will require the use of a modified eGFR equation. Laboratories considering calibration changes before this time can seek advice from the Australasian Creatinine Working Group.
threshold for identifying kidney disease, but is less important at high creatinine-low GFR values characteristic of severe kidney disease. The NKDEP recommended not to report calculated GFR values above 60 mL/min/1.73 m² because of concerns that creatinine method error would compromise the clinical utility of the GFR estimates. In Australia, a Creatinine Consensus Working Group has published a Position Statement on the automatic reporting of eGFR, with recommendations which complement those of the NKDEP.

To improve the performance of calculated GFR values, a number of initiatives for clinical laboratories have been suggested by the NKDEP. These include:

1. Recalibration of routine serum creatinine methods to be traceable to the reference method for creatinine, which is IDMS. This initiative is proposed to remove bias between methods and laboratories, and requires the use of a revised MDRD equation with a 6% change in calculation factor from a value of 186 to 175.7,8

2. After recalibration to an IDMS method, to set a total error goal for creatinine measurement to produce a maximum 10% error in eGFR. It has been calculated that routine laboratory methods need to achieve an analytical bias, compared to an IDMS reference measurement procedure, of <5% and analytical imprecision of <8% (including between-laboratory calibration variability) at serum creatinine concentrations >88 µmol/L to achieve this goal.5

Before examining the accuracy of a range of routine creatinine methods in current use in the Australasian region relative to an IDMS method, it is of interest to review how creatinine methods have developed over time.

Creatinine Methods - Past and Present
Most routine serum creatinine assays in current use have evolved from the reaction first described by Jaffe in 1886.9 Over the years, the Jaffe assay has progressed through many phases. Early methods used deproteinised blood. To improve specificity, creatinine was isolated from common interfering substances by adsorption on to aluminium silicates such as Lloyd’s reagent, followed by elution into alkaline picrate after centrifugation and decanting.10 Cation exchange resins were also used for this purpose. Other strategies for improving specificity involved performing the Jaffe assay at both very alkaline pH, and after acidification to a more neutral pH. Only interfering substances react at neutral pH, and by difference a more accurate result could be obtained.11

The era of automation began in 1957 with the introduction of the Technicon continuous flow AutoAnalyzer invented by Skeggs. This system provided the first opportunity to efficiently process increasing workloads, and incorporated on-line dialysis to remove protein, an important interfering substance in the Jaffe assay (Table 1). An added advantage was that protein-bound interfering substances such as bilirubin were also removed, but smaller molecules such as glucose, pyruvate, acetoacetate and cephalosporins were still able to cross the dialysis membrane and cause falsely increased results.

With the introduction of random access, centrifugal, and other discrete analysers, the challenge to maximise specificity in the absence of dialysis was achieved by careful investigation of the kinetics of the reaction of creatinine and interfering substances with alkaline picrate. Centrifugal analysers, with multiple reading points, were ideal for this purpose. Investigators such as Cook, Bowers and Wong and Pardue et al. found that the reaction of creatinine with alkaline picrate progressed in the presence of both fast- and slow-reacting interfering substances.12,13 Acetoacetate, formed during ketogenesis in conditions such as diabetes mellitus, is an important substance that reacts rapidly with alkaline picrate. This compound caused significant overestimation of creatinine on an early STAT analyser, the Beckman Astra, which could measure a range of important analytes within a minute. The problem was solved on the Beckman Synchro CX3 by increasing the temperature in the creatinine reaction cup from 37 °C to 42 °C so that acetoacetate interference was minimal by the time that the main creatinine reaction was monitored at 25.6 seconds. Overestimation of creatinine was reduced from 33 to 6 µmol/L per mmol/L of acetoacetate.16 This Beckman systems were used by Levey et al. to develop both the initial MDRD equation and the revised IDMS traceable equation, the latter using a calibration based on the Roche enzymatic assay which has been shown to provide IDMS compatible results.7,17,18

For analysers not designed for STAT assays, the flexibility of longer reaction times and the ability to add reagents separately presents both opportunities and problems. Slow-reacting substances such as protein, glucose and ascorbic acid can cause falsely high results by reducing alkaline picrate to picramate. In addition, the positive interference seen with cefoxitin, a first generation cephalosporin, can be seen to varying degrees with more recent cephalosporins in all Jaffe assays (Table 1).

Boyne and a group of innovative Australian biochemists devised an interesting solution to detect falsely high results produced by the Jaffe assay. Their procedure involved incubating approximately 100 µL of serum for 1 hour in a sample cup containing a mixture of a dried solution of
creatinase and creatinase. By assaying the sample before and after enzymatic treatment, they found they could obtain similar accuracy to HPLC. The assay was reliable up to 1800 µmol/L, with coefficients of variation of 7.8% and 3.4% at 70 and 470 µmol/L respectively.

The enzymes used in this assay now form the basis of the most specific routine assays available today, which use the reaction sequence shown below:

\[
\text{Creatinine} + \text{H}_2\text{O} \xrightarrow{\text{creatinase}} \text{creatinine} \\
\text{Creatine} + \text{H}_2\text{O} \xrightarrow{\text{creatinase}} \text{sarcosine} + \text{urea} \\
\text{Sarcosine} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{sarcosine oxidase}} \text{formaldehyde} + \text{glycine} + \text{H}_2\text{O}_2
\]

The hydrogen peroxide generated in the above reaction sequence can be measured spectrophotometrically using a Trinder’s reaction acceptor, producing a quinoneimine with high molar absorptivity (eg Roche Creatinine Plus assay), using a leuco dye (Vitros dry chemistry system), or with blood gas analyser electrodes (eg new Radiometer dual electrode system which corrects for creatine). Other enzymatic systems using creatinine deaminase, which converts creatinine to N-methylhydantoin and ammonia (with various options for measuring ammonia), and creatininase, (with NADH measured at 340 nm after a creatine kinase reaction sequence), have found little acceptance in routine laboratories.

Enzymatic methods for measuring creatinine, although theoretically more specific, can have their own interference problems. The first version of the Roche assay showed significant interference from bilirubin due to competition between bilirubin and the assay substrate for the \(\text{H}_2\text{O}_2\) produced in the adjacent reaction. Crocker et al. demonstrated that the underestimation of creatinine depended not only on the bilirubin concentration, but also on the creatinine concentration itself. Thus the assay was very unreliable when both substances were present in increased amounts. This problem has been largely overcome in the current assay, which uses a more efficient \(\text{H}_2\text{O}_2\) acceptor (triiodo-hydroxy-benzoic

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Table 1. Substances added to serum (70 µmol/L creatinine) to test for interference with Roche Jaffe and enzymatic creatinine assays on an Hitachi 917 analyser (data from authors’ laboratory).

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Concentration</th>
<th>Measured Bias (µmol/L)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Jaffe Rate Blank Method</td>
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<tr>
<td>Ascorbate</td>
<td>10 mmol/L</td>
<td>+ 37</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>2 mmol/L</td>
<td>+ 31</td>
</tr>
<tr>
<td>Albumin</td>
<td>40 g/L</td>
<td>+ 21</td>
</tr>
<tr>
<td>Glucose</td>
<td>50 mmol/L</td>
<td>+ 19</td>
</tr>
<tr>
<td>Creatine</td>
<td>2 mmol/L</td>
<td>+ 16</td>
</tr>
<tr>
<td>Bilirubin ditarate</td>
<td>500 µmol/L</td>
<td>- 18</td>
</tr>
<tr>
<td>Haemoglobin F</td>
<td>10 g/L</td>
<td>- 66</td>
</tr>
<tr>
<td>Haemoglobin adult</td>
<td>10 g/L</td>
<td>n.s.</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>2 mmol/L</td>
<td>n.s.</td>
</tr>
<tr>
<td>Intralipid</td>
<td>12 g/L triglyceride</td>
<td>n.s.</td>
</tr>
<tr>
<td>Dopamine</td>
<td>1 mmol/L</td>
<td>- 15</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td></td>
<td></td>
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<tr>
<td>Cefpirome</td>
<td>1 mmol/L</td>
<td>+ 213</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1 mmol/L</td>
<td>+ 47</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>1 mmol/L</td>
<td>+ 24</td>
</tr>
<tr>
<td>Cephazolin</td>
<td>1 mmol/L</td>
<td>+ 20</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1 mmol/L</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>1 mmol/L</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1 mmol/L</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s.= not significant
acid), and includes potassium ferrocyanide and detergents to further reduce bilirubin interference.

Over the years, bilirubin has also caused significant problems in direct Jaffe assays, but not in methods involving dialysis. The Jaffe assay system is very alkaline, and bilirubin is oxidised to biliverdin during the assay period, which offsets the increase in colour formed by creatinine reacting with alkaline picrate. Different approaches have been taken to minimise this interference, with varying degrees of success. Beckman Astra and Synchron CX3 reagent systems use sodium dodecyl sulphate to minimise protein interference and prevent the oxidation of bilirubin to biliverdin. This reagent is not soluble when cold, and lithium dodecyl sulphate can be substituted to avoid precipitation in the refrigerated reagent compartments of high-throughput analysers.

An alternative approach to manage bilirubin interference is to oxidise bilirubin to biliverdin using potassium ferricyanide prior to the Jaffe reaction. Bilirubin oxidase has also been used for this purpose. It has been reported, however, that ferricyanide is destroyed over time if the reagent contains sodium dodecyl sulphate. Thus, ferricyanide must be separated from detergent to ensure protection from bilirubin interference. Another approach to minimise interference has been to use “rate-blanking”, where the rate of colour change in the sample with NaOH alone is used as a correction factor for the rate observed after the addition of picric acid. This strategy provides only a partial correction for bilirubin interference, due to the curvilinear nature of the oxidation.

For neonates and adults with hyperbilirubinaemia, creatinine can be significantly under-estimated at low concentrations of bilirubin with some commonly used reagent systems. It is therefore important that laboratories understand the limitations of their methods. For liver transplant patients, we have found that serum bilirubin concentrations can exceed 500 µmol/L, which is above the recommended limits for bilirubin interference for our Hitachi enzymatic and Jaffe creatinine assays. In this situation, we place a small amount of sample in a commercially-available Eppendorf tube containing a 30,000 MW cut-off filter to produce a bilirubin-free filtrate after 15 minutes centrifugation. Because the filtrate does not contain protein, the 26.5 µmol/L offset in the Hitachi Jaffe assay becomes inappropriate and no offset is used.

One other interference that is often overlooked is seen with haemolysed samples which contain foetal haemoglobin (Hbf), for example in neonates <6 months old. If enough Hbf is present, negative creatinine results can be obtained. Unlike adult haemoglobin, which immediately turns brown in the presence of NaOH, Hbf is alkali resistant, and slowly changes colour during the course of the Jaffe assay. Rate-blanking can only partially correct for this interference. Because of the problems of interference from bilirubin and Hbf, we assay all samples from babies aged <1 year using the Roche enzymatic assay. Access to this assay can also be useful when interference from substances such as cephalosporins is suspected in our routine Jaffe system.

For biochemists interested in further reading on creatinine methodology, a review by Spencer is strongly recommended. A bibliography by Goodall and a report prepared by Boyne on behalf of an Australian Creatinine Interferences Working Party provide useful information on some of the reagent systems still in use today, even though these reports are somewhat historical. For very recent information on eGFR, methodology and interferences, a report this year by the NKDEP Laboratory Working Group is a valuable resource.

To investigate measurement bias relative to IDMS targets, the IMEP-17 International Measurement Programme multi-analyte survey conducted in 2002 in over 1,000 international laboratories found that many creatinine methods overestimated the concentration of a fresh frozen human serum (FFS) sample by 10-15% (IDMS target 74.6 µmol/L). The Roche enzymatic assay was a notable exception. Both positive and negative bias was seen for a sample with a higher creatinine concentration (168.8 µmol/L). Jones has summarised data for the 56 Australasian laboratories participating in this survey (Jones G. Creatinine measurement and reporting: evaluation of data from IMEP-17. Personal communication. June 2004). Similar findings were found when a commutable FFS was distributed by the College of American Pathologists (CAP) in 2003 (IDMS target 79.7 µmol/L). Thirty of 50 peer groups had significant bias for creatinine. An important finding from this survey was that bias was related to instrument manufacturer, rather than the type of method used. It was also found that conventional quality assurance specimens had bias profiles that were significantly different from the commutable FFS for 68% of the peer groups.

Other studies have used patient samples to investigate the bias of selected routine methods versus IDMS. An important conclusion from these studies is that routine creatinine methods can have significant degrees of bias. To extrapolate conclusions from these studies requires additional information regarding the specific details of the routine methods used. Reference material data would also be useful to support the accuracy of the IDMS method. Further studies of this type are essential to assess the latest attempts at method harmonisation and alignment with IDMS.

We decided to assess the current accuracy of commonly used serum creatinine measurements in the Australasian region by
distributing a range of patient samples, reference materials, and controls for assay by different laboratories in South Australia and Victoria. Before this work could commence, however, it was necessary to develop and validate an IDMS method for serum creatinine to act as the reference base. This decision follows a recommendation of the Laboratory Working Group of the NKDEP that a network of reference laboratories should be established in the USA and other countries to perform high-throughput measurement of serum creatinine using an IDMS reference procedure.⁵

**Creatinine Reference Materials and Methods**

For the purpose of traceability, both routine and reference methods should be regularly checked against certified reference materials. A number of these materials are available for creatinine, and those that can be purchased from the National Institute of Standards and Technology (NIST) of the USA and CAP (LN-24) are summarised in Table 2. Standard Reference Materials (SRM) are intended for use in evaluating the accuracy of higher order clinical methods or to validate working or secondary reference materials. They can also be used to assess the accuracy of routine laboratory methods, providing they are commutable with patient samples. SRM 967 is a newly released commutable frozen human serum that is currently being used by in vitro diagnostic (IVD) manufacturers to achieve IDMS traceability for routine creatinine methods. It is important to note that two of the reference materials listed in Table 2 have not been validated for commutability with Jaffe methods (SRM 909b 1 and 2).³ Calibrations based on non-commutable materials can cause significant errors in patient results.

Until recently, the reference method for creatinine quantitation was gas chromatography (GC) coupled to mass spectrometry with isotope dilution.³³ Three such methods in laboratories in Europe and the USA have been approved by the Joint Committee on Traceability in Laboratory Medicine.⁵ All these methods require a separation step to remove creatine, as this molecule is derivatised into the same chemical species as creatinine prior to gas chromatography. The method is therefore long and laborious, and interest has been raised in liquid chromatography-mass spectrometry (LCMS) as a better alternative to avoid derivatisation procedures. The method of Stokes and O’Connor³⁶ has been proposed as a candidate reference method which is simple and capable of high throughput without compromising accuracy.³ An assessment of different sample preparation methods revealed that simple solvent protein precipitation, as used extensively in LC-MS analytical work-up, was satisfactory for sample clean-up.³⁶ Deuterated creatinine was added to samples prior to the addition of ethanol to compensate for incomplete recoveries during the preparation procedure. The use of isotopic ratio measurements in this method produced quantitative results for reference materials with accuracy and uncertainty comparable to GC-IDMS methods.³⁶

Due to reducing costs and easier operator training, triple quadrupole mass spectrometers (tandem MS) are now within the reach of routine biochemistry laboratories. This is reflected in the increasing number of publications describing tandem MS methods in clinical chemistry journals. Using an API-Sciex 3200 bench-top Q-trap mass spectrometer, we have established the LC-MS method of Stokes and O’Connor³⁶ for serum creatinine analysis, but with the addition of multiple reaction monitoring (MRM) instead of selected ion recording as the mode of ion detection. MRM was used to record the ion transitions \( m/z 114 \rightarrow 44 \) for creatinine, and \( m/z 117 \rightarrow 47 \) for tri-deuterated creatinine, respectively, to give the mass spectral analysis increased specificity. This approach has also been used recently by Owen et al.³⁷

To investigate the performance characteristics of our candidate reference tandem MS method for serum creatinine, results and uncertainties of measurement were calculated

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**Table 2.** Reference materials available from NIST and CAP with IDMS-assigned creatinine values.

<table>
<thead>
<tr>
<th>Name</th>
<th>Form</th>
<th>Creatinine Value</th>
<th>Pricing in 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRM 914a</td>
<td>Crystalline creatinine</td>
<td>99.7 ± 0.3 mass %</td>
<td>US$292 for 10 g</td>
</tr>
<tr>
<td>SRM 909b-1</td>
<td>Lyophilised human serum</td>
<td>56.18 ± 0.55 µmol/L</td>
<td>US$536 for set of 6x6</td>
</tr>
<tr>
<td>SRM 909b-2</td>
<td>Lyophilised human serum</td>
<td>467.4 ± 5.3 µmol/L</td>
<td>US$536 for set of 6x6</td>
</tr>
<tr>
<td>SRM 967-1</td>
<td>Frozen human serum</td>
<td>66.5 ± 1.9 µmol/L</td>
<td>To be released late 2006</td>
</tr>
<tr>
<td>SRM 967-2</td>
<td>Frozen human serum</td>
<td>346.2 ± 7.3 µmol/L</td>
<td>US$382 for set of 7</td>
</tr>
<tr>
<td>LN-24</td>
<td>Fresh frozen serum</td>
<td>Range from 44.3 to 354.9 µmol/L</td>
<td>US$382 for set of 7</td>
</tr>
</tbody>
</table>
Survey of the Accuracy of Current Routine Creatinine Methods

The most important creatinine measurement range for detecting silent kidney disease is between 85 and 150 µmol/L, corresponding to eGFR of approximately 60 mL/min/1.73m² (depends on age, sex, ethnicity and an IDMS-based calibration, see reference 38, Figure 3). This is because the impact of creatinine measurement bias and imprecision on the calculation of GFR is more pronounced at low creatinine concentrations. However, creatinine determinations with small total error are still required at pathological creatinine concentrations to assess the severity of renal failure, the need for dialysis, and the success of kidney transplantations.

Roche Hitachi 917 (H917) [Roche Diagnostics]
(a) Jaffé assay (IDMS traceable calibration).
- Uses a simple two-part reagent system with NaOH in the first reagent (rate-blanking) and picric acid in the second. Results are corrected with an “average” 26.5 µmol/L offset for non-creatinine chromogens.
- Has a limited ability to correct for icteric samples. IDMS calibration reference intervals have been published for both this assay and the Roche enzymatic assay. It has been reported that...

![Figure 1. Absolute difference in results between two Roche IDMS-aligned creatinine assays (rate-blanked Jaffé assay with offset minus enzymatic Creatinine Plus assay) compared to creatinine concentration. The lithium-heparin plasma samples were prepared from blood donors (“normal” samples) and pre- and post-dialysis patients. Reproduced with permission from the Editor of Clinical Laboratory (Clin Lab 2000;46:589-90)](image-url)
enzymatic and Jaffe creatinine results compare reasonably well for blood donor samples and for pre- and post-dialysis samples from CRF patients (Figure 1).\textsuperscript{41}

(b) Enzymatic creatinine assay.
- Widely accepted as one of the most accurate routine methods available at present and data in Table 3 indicates that this method produces results for patient samples that agree closely with IDMS.\textsuperscript{7,17,18}
- Cost is the main concern. The influences of some common interfering substances for both Hitachi assays are summarised in Table 1.

**Roche Integra 700 [Roche Diagnostics]**
- A simple assay system as for H917 (uses offset of -18 µmol/L), but lack of rate-blanking results in interference exceeding 10% at relatively low concentrations of bilirubin (85 µmol/L).

**Bayer Advia 2400 [Bayer HealthCare Diagnostics]**
- A rate-blanked assay having the largest recommended offset for non-creatinine chromogens for all assays discussed here (-35.4 µmol/L). Would like to see more useful references provided with this method.

**Abbott 8200 [Abbott Diagnostics]**
- Rate-blanking and offsets are not officially recommended and were not used with the current method, although Abbott have indicated that they plan to release an IDMS aligned method by quarter 1, 2007.

### Table 3. Inter-method creatinine results (µmol/L) for reference materials, controls and selected patient samples, using different analytical methods (some with offsets).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Target</th>
<th>LCMSMS</th>
<th>H917 Enzymatic</th>
<th>H917 Jaffe R/B with offset</th>
<th>Olympus 5400 with offset</th>
<th>Advia 2400 with offset</th>
<th>Vitros 950</th>
<th>Integra 700 with offset</th>
<th>Dimension RxL</th>
<th>Beckman LX20</th>
<th>Abbott 8200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Blank</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-26</td>
<td>-16</td>
<td>-33</td>
<td>9</td>
<td>&lt;18</td>
<td>N/A</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>Albumin (HSA) 43 g/L</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>-7</td>
<td>8</td>
<td>&lt;18</td>
<td>0</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Beckman CX3 aqueous std</td>
<td>442</td>
<td>442</td>
<td>441</td>
<td>416</td>
<td>352</td>
<td>402</td>
<td>496</td>
<td>428</td>
<td>370</td>
<td>443</td>
<td>396</td>
</tr>
<tr>
<td>BioRad Liquichek QC1 Unassayed</td>
<td>69</td>
<td>67</td>
<td>65</td>
<td>63</td>
<td>58</td>
<td>68</td>
<td>58</td>
<td>66</td>
<td>70</td>
<td>73</td>
<td></td>
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<tr>
<td>BioRad Liquichek QC2 Unassayed</td>
<td>524</td>
<td>518</td>
<td>524</td>
<td>497</td>
<td>501</td>
<td>557</td>
<td>449</td>
<td>521</td>
<td>512</td>
<td>473</td>
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<tr>
<td>SRM 967 Lo</td>
<td>66.5</td>
<td>67</td>
<td>67</td>
<td>68</td>
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<td>60</td>
<td>74</td>
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<tr>
<td>SRM 967 Hi</td>
<td>346.2</td>
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<td>Selected Patient Sample Results</td>
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Manufacturer Offset
-26.5 -18 -35.4 -18

N/A = not available
Beckman LX20 [Beckman Coulter]
- The STAT (cup) assay has an excellent formulation to minimise protein, bilirubin and glucose interference. Other than the LCMS and Roche enzymatic assays, it is the only Jaffe assay in this group that recovered the weighed-in concentration (442 µmol/L) of an aqueous creatinine standard (Table 3).

Olympus 5400 [Olympus Diagnostic Systems]
- An offset of -18 µmol/L is recommended for the Olympus method in Australia. Contains unstated additives (?) to reduce bilirubin interference.
- The package insert correctly indicates that absorption of CO₂ can alter the calibration of the assay and this is an important consideration for other assays because of the high pH of all Jaffe assays.
- Calibration is traceable to SRM 909-b level 2.

Dimension RxL [Dade Behring]
- The only assay of this group using ferricyanide to eliminate bilirubin interference.
- Calibration materials containing at least 30 g/L of protein are recommended, mainly to partially compensate for protein interference.
- No offsets are used.

Vitros 950 [Ortho Clinical Diagnostics]
- A dry chemistry system using an enzymatic reagent sequence discussed previously.
- Calibration currently traceable to purified SRM 914a creatinine via HPLC.
- Bilirubin and haemoglobin interference are minimised by retention in the spreading layer.

The measured creatinine concentrations were converted to eGFR values using the MDRD equation for results traceable to an IDMS method. These data are shown in Figure 3.

Examination of these data shows an increasing spread in eGFR values as the true value increases. At values below 50 mL/min/1.73m², all instrument/method groups are in reasonable agreement. At the current reporting point of 60 mL/min/1.73m², measured values ranged from 52 to 67, or 13% low to 12% high which is outside the goal of ± 10%. Removal of the three instrument/method groups with the highest bias (points shown as – in Figure 3) improves the estimated GFR range to 57–63 mL/min/1.73m² or ± 5%.

A series of control samples were assayed with patient samples (Table 3). For most methods, saline and albumin solutions produced noticeably different creatinine results, indicating the extent of protein interference in Jaffe assays. This is the main reason why offsets need to be used (note difference in saline and albumin solution results in Table 3).

For SRM 967 (target 66.5 µmol/L), six of the nine methods recovered results within 3 µmol/L (4.5%) of the target value. For SRM 967 (target 346.2 µmol/L), seven of nine methods recovered results within 17 µmol/L (4.9%). The overall recoveries for SRM 967 were better than expected as most methods have not yet been IDMS-recalibrated using SRM 967.

Two commercial quality control samples (BioRad Liquichek) were also assayed and results for the low control (IDMS 69 µmol/L) agreed within 4 µmol/L (5.8%) for six assays. This was unexpected and suggests that it may be possible to produce a commercial product with a creatinine concentration around 80–90 µmol/L to assist with inter-laboratory control of creatinine results. Agreement was not as good for the high control, which may reflect calibration and interference problems.

Future Goals and Recommendations
The recently published report of the Laboratory Working Group of the NKDEP provides a well-developed plan for the standardisation of serum creatinine measurements to IDMS. Although most routine creatinine methods have not yet been aligned, IVD manufacturers are currently working towards this goal using SRM 967 from NIST as a commutable calibration material and recalibration of methods is at an advanced stage. Companies in Australia have been asked to supply information to clients indicating when this task will be completed and how it will change creatinine results and reference intervals.

All methods for measuring serum creatinine should have their calibration traceable to an IDMS reference measurement.
procedure. With low combinations of bias and imprecision, routine methods have the potential to meet the goal of <10% total error recommended by NKDEP. In our survey of nine instrument/method groups using patient samples, it would appear that the performances of three methods with bias outside acceptable ranges require further investigation to assess whether the results found in our study are consistent and applicable to the same instruments in other laboratories. For some methods, it is difficult to assay manufacturer supplied calibrators as “unknowns” to validate the accuracy of the low calibration point, as results below a defined limit are not easily accessible (eg Roche Integra assay results <18 µmol/L are unavailable), and IVD manufacturers should address this problem (see Table 3). In absolute terms, the goal should be to reduce bias to <5 µmol/L in the low serum creatinine concentration range.

For methods exceeding the required bias in this study, further comparisons in these laboratories have indicated that methods can be mathematically aligned with either IDMS or the Roche enzymatic assay by careful selection of calibration values and offsets. Prior to official method recalibration, each laboratory can consider this option, especially if they are concerned about the accuracy of their creatinine results. Selection of the IDMS version of the eGFR equation will then become important. Variations in creatinine results and their influence on eGFR have been discussed in many papers, and an excellent review of options for measuring and estimating GFR has recently been published. Different calibration materials and offsets are used for certain instrument/method groups in different parts of the world, making extrapolations from the literature and quality assurance data difficult. As quality assurance samples
may not have the same properties as patient specimens, they
cannot be recommended as a simplistic means of correcting
or calibrating methods. Because of the complexities involved
in accurately calibrating serum creatinine assays, it is
recommended that any “unofficial” regional recalibration
procedures should have the support of the Australasian
Creatinine Working Group.

We have found that reasonably good comparisons can be
obtained between carefully calibrated enzymatic and Jaffe
creatinine methods (see Figure 1 and Table 3), although
the lack of specificity of the chemical assay means that
an alternative method may be needed in certain clinical
situations. Care should be taken when considering the use of
the Jaffe assay for neonatal samples and samples containing
cephalosporins. In addition, creatinine results can vary
depending on the protein concentration of the sample, which
explains why bias may still be evident in samples with unusual
protein concentrations, even after IDMS recalibration. For
enzymatic creatinine assays based on the generation of
hydrogen peroxide, dopamine and dobutamine interferences
have been reported. Thus although enzymatic methods
have improved, they still do not show complete specificity
for creatinine.

Once instrument manufacturers have established the
relationship between their previous conventionally calibrated
method and their re-standardised IDMS-traceable method,
clinical laboratories must notify clients of the impact on their
reported creatinine and eGFR values. The availability of
commutable reference materials and laboratories capable of
performing an IDMS reference method for serum creatinine
will provide a valuable resource for other laboratories to
independently check the accuracy of their methods. The end
result of these standardisation activities should be to remove
calibration bias between laboratories, as is being achieved
for other analytes such as haemoglobin A\textsubscript{1c}, and to reach
the goal of minimising variability in the reporting of eGFR
for assessing renal function. This assumes, however, that
laboratories are prepared to carefully monitor the calibration
accuracy of their methods. Failure to adequately perform
this task is one reason for the variable results seen for any
individual method in quality assurance programmes. Access
to a commutable routine quality control material (IDMS
creatinine concentration approximately 80-90 µmol/L) could
help improve agreement between laboratories in an important
concentration range.

In conclusion, despite the concerns of Behre and Benedict
which preface this commentary, much better alignment of
serum creatinine methods should soon be achieved, despite the
specificity limitations of most routine methods. In addition,
more detailed evidence for the reliability of the eGFR equation
for IDMS - traceable methods has now become available.

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Competing Interests: None declared.

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