Investigating Interferences of a Whole-Blood Point-of-Care Creatinine Analyzer: Comparison to Plasma Enzymatic and Definitive Creatinine Methods in an Acute-Care Setting

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BACKGROUND: Although measurement of whole-blood creatinine at the point of care offers rapid assessment of renal function, agreement of point-of-care (POC) results with central laboratory methods continues to be a concern. We assessed the influence of several potential interferences on POC whole-blood creatinine measurements.

METHODS: We compared POC creatinine (Nova Stat-Sensor) measurements with plasma enzymatic (Roche Modular) and isotope dilution mass spectrometry (IDMS) assays in 119 hospital inpatients. We assessed assay interference by hematocrit, pH, pO2, total and direct bilirubin, creatine, prescribed drugs, diagnosis, red blood cell water fraction, and plasma water fraction.

RESULTS: CVs for POC creatinine were 1.5- to 6-fold greater than those for plasma methods, in part due to meter-to-meter variation. Regression comparison of POC creatinine to IDMS results gave a standard error (Sy/H20841) of 0.61 mg/dL (54 μmol/L), whereas regression of plasma enzymatic creatinine to IDMS was Sy/H20841 0.16 mg/dL (14 μmol/L). By univariate analysis, bilirubin, creatine, drugs, pO2, pH, plasma water fraction, and hematocrit were not found to contribute to method differences. However, multivariate analysis revealed that IDMS creatinine, red blood cell and plasma water fractions, and hematocrit explained 91.8% of variance in POC creatinine results.

CONCLUSIONS: These data suggest that whole-blood POC creatinine measurements should be used with caution. Negative interferences observed with these measurements could erroneously suggest adequate renal function near the decision threshold, particularly if estimated glomerular filtration rate is determined. Disparity between whole-blood and plasma matrices partially explains the discordance between whole-blood and plasma creatinine methods.

The incidence of kidney disease continues to increase worldwide. Creatinine, along with the estimated glomerular filtration rate (eGFR),6 is useful for diagnosing and stratifying kidney disease (1). Patients with limited renal filtration are susceptible to contrast-induced nephropathy (CIN) following contrast computed tomography (CT) or magnetic resonance imaging (MRI) studies. Monitoring whole-blood creatinine at the point of care has gained favor as an immediate assessment of renal impairment, to avoid CIN (2).

Direct biosensors for point-of-care (POC) measuring of analytes such as creatinine, glucose, or lactate detect chemical bioactivity as a function of analyte molarity (amount of analyte per unit of water mass, e.g., mmol/kg). However, most meters report results in units of molarity (amount of analyte per volume of sample, e.g., mmol/L) (3). Conversion between blood creatinine molarity detected and plasma creatinine molarity reported is affected by device calibration that typically assumes healthy mean values for plasma water mass, red blood cell water mass, and hematocrit (4).

The composition of whole blood and plasma varies in many disease states, which may challenge the analytic reliability of whole-blood biosensors in certain patients (5–7).
Recently, several groups have described candidate definitive methods for plasma creatinine. In the past, comparisons of novel whole-blood creatinine methods with plasma enzymatic creatinine reference methods were susceptible to systematic bias, because of the lack of creatinine standardization. To address these concerns and reduce the risk of reference assay interference, we compared POC results to an isotope-dilution mass spectrometry (IDMS) creatinine assay (8).

The goal of this study was to assess the performance of the Nova StatSensor POC whole-blood creatinine analyzer by comparing its performance to the Roche Diagnostics plasma enzymatic creatinine method and an IDMS plasma creatinine method. We investigated possible causes of observed discrepancies, including whole-blood matrix components.

Materials and Methods

Patients and Samples

We selected a random sample of whole-blood specimens collected in lithium heparin Vacutainer Tubes (BD Diagnostics) or heparinized blood syringes with excess blood from 119 intensive care and oncology inpatients (66 men, 53 women, mean age 59 years, 73% non–African American, 27% African American) at Johns Hopkins Medical Institutions (Baltimore, MD). This study conformed to the institutional ethics committee requirements for method evaluation.

Creatinine Methods

The StatSensor (Nova Biomedical) handheld creatinine device uses capillary flow of whole blood into a test strip impregnated with creatinine amidohydrolase, creatine aminohydrolase, sarcosine oxidase, and peroxidase which oxidize ferric-cyanide to ferrous-cyanide, producing a current (e−). The current is measured amperometrically to determine creatinine concentrations.

The Creatinine Plus assay (Roche Hitachi Modular, Roche Diagnostics) is an enzymatic creatinase reaction approved for use in plasma or serum, with calibrators assigned by IDMS. The IDMS creatinine method has been described (8) and is traceable to serum-based NIST calibrators (Standard Reference Material 967a, levels 1 and 2).

Study Design

We manually applied whole-blood samples to StatSensor handheld devices. A subset of samples (n = 50) were tested across 4 different StatSensor meters. Whole-blood samples were centrifuged (4500g, 5 min) and plasma creatinine was measured with the Roche Hitachi Modular automated chemistry analyzer (mean time between measurements, approximately 1 h). Plasma was stored at −20 °C for IDMS creatinine analysis. We calculated eGFR using the Modification of Diet in Renal Disease 4 parameter equation (9, 10). Patient variables were determined by electronic medical record review.

Imprecision and Linearity

We determined within-day imprecision by repeated measures (n = 10) of 3 levels of quality control material (Nova Biomedical), and between-day imprecision, by daily testing of these materials (n = 17). Two levels of control were used for the Roche Modular creatinine assay (Liquichek Unassayed Chemistry Control, Bio-Rad Laboratories), and linearity was verified to be 0.1–30 mg/dL. We determined between-meter imprecision by measuring 3 control levels (Nova Biomedical) on 4 handheld StatSensor devices (n = 8 days). The analytical measurement range was verified with 5 concentrations of linearity material (Nova Biomedical).

Additional Measurements

We measured hematocrit and mean corpuscular hemoglobin concentration (MCHC) using Sysmex automated hematology analyzers (Sysmex Corporation). Spun hematocrits were performed on a subset of samples, in duplicate. Radiometer ABL blood gas analyzers (Radiometer A/S) were used to measure whole-blood pH and oxygen concentrations (pO2), and total protein was determined with the Roche Modular analyzer. If analyte results were not available from the initial sample, we used record review of results within 24 h of the initial collection. Direct and total bilirubin plasma concentrations were determined with the Hitachi 917 automated analyzers (Roche Diagnostics). Creatine concentrations were measured with ELISA (K635–100, Biovision Research Products).

We derived plasma water fraction (PW) from the plasma total protein using a partial specific volume for plasma protein of 0.725 (PW = 0.95 at total protein of 71.5 g/L) (11). We derived red blood cell water fraction (RW) from the MCHC in g/L using the equation RW = 1.050 − (MCHC × 10−3) (12), with the specific gravity of blood reduced from 1.090 to 1.050 to account for membrane components of red blood cells (RW = 0.715 at MCHC 335 g/L) (12, 13).

Statistics

We evaluated method comparison data using Deming regression, Pearson correlation coefficients, and Bland–Altman plots (EP Evaluator 8.0 software, David G. Rhoads Associates). We compared creatinine and eGFR values for each method using Wilcoxon and Mann–Whitney tests using Analyse-it® software (Analyse-it Software). Linear regression, ANOVA, 2-tailed, 2-sample t-tests were performed with Microsoft Excel. The statisti-
cal significance of differences in diagnoses and racial distributions was determined with tests of proportions (MedCalc Software). Multiple linear regression analyses were performed using Stata 11 statistical software (StataCorp LP). The Fisher method of pooled probability was calculated manually. A probability of $P < 0.05$ was considered statistically significant.

**Results**

**IMPRECISION**  
StatSensor within-day ($n = 10$ measurements) and between-day ($n = 17$ days) imprecision results using QC materials are presented in Table 1 and show greater variation than the plasma enzymatic method. Mean between-day imprecision of the Roche Modular enzymatic assay is 4.6% CV, mean 0.82 mg/dL (72 μmol/L), and 1.9% CV, mean 5.78 mg/dL (511 μmol/L). To determine if different StatSensor devices generated equivalent results, we also measured control levels on 4 different StatSensor meters for 8 days (Table 1). CV was calculated between the 4 meters each day, with pooled daily results ranging from 3.0% [level 3, mean 5.24 mg/dL (463 μmol/L)] to 24.7% CV [level 1, mean 0.73 mg/dL (65 μmol/L)]. The results generated by the 4 meters were significantly different from each other ($P = 0.021$ at level 1 and $P = 0.0035$ overall). Similarly, whole-blood patient samples ($n = 50$) were measured for creatinine using 4 different meters. Between-device imprecision for these 4 meters ranged from 3.1% [mean creatinine 6.99 mg/dL (618 μmol/L)] to 26.5% [mean creatinine 2.54 mg/dL (225 μmol/L)], with a mean CV of 11.4% (see Supplementary Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue11). Using supplemented whole blood samples, the StatSensor manufacturer reports imprecision of 8.7% at level 1, 3.0% at level 2, and 4.4% at level 3.

**METHOD COMPARISON**  
We analyzed 119 whole-blood samples with the StatSensor meter and corresponding plasma samples with the Roche Modular enzymatic creatinine method (Fig. 1A). Increased discordance was observed at higher creatinine concentrations, with better agreement at lower creatinine values (Fig. 1B). Using linearity material supplied by the manufacturer, we found the StatSensor to be linear over the measured range of 1.01 mg/dL (89

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**Table 1. Imprecision of StatSensor single meter (within-day) and multiple meter (between-day) creatinine readings.**

<table>
<thead>
<tr>
<th>Quality control level</th>
<th>Within-day, single meter ($n = 10$)</th>
<th>Between-day, single meter ($n = 17$ days)</th>
<th>Between-day, multiple meters ($n = 8$ days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dL μmol/L</td>
<td>mg/dL μmol/L</td>
<td>mg/dL μmol/L</td>
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<tr>
<td>Level 1</td>
<td></td>
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<tr>
<td>Meter 1</td>
<td>0.82 (0.03), 3.9 73 (3)</td>
<td>0.76 (0.10), 12.9 67 (9)</td>
<td>0.82 (0.06), 7.1 73 (5)</td>
</tr>
<tr>
<td>Meter 2</td>
<td>0.68 (0.08), 12.0 60 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meter 3</td>
<td>0.68 (0.10), 15.1 60 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meter 4</td>
<td>0.73 (0.11), 14.8 65 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group, level 1</td>
<td>0.73 (0.10), 13.4 65 (9)</td>
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<tr>
<td>Level 2</td>
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<tr>
<td>Meter 1</td>
<td>1.70 (0.09), 5.3 150 (8)</td>
<td>1.64 (0.13), 8.1 145 (12)</td>
<td>1.76 (0.15), 8.3 156 (13)</td>
</tr>
<tr>
<td>Meter 2</td>
<td>1.54 (0.15), 9.6 136 (13)</td>
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<tr>
<td>Meter 3</td>
<td>1.67 (0.15), 8.8 148 (13)</td>
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<tr>
<td>Meter 4</td>
<td>1.63 (0.12), 7.2 144 (11)</td>
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<tr>
<td>Group, level 2</td>
<td>1.65 (0.15), 9.3 146 (13)</td>
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<tr>
<td>Level 3</td>
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</tr>
<tr>
<td>Meter 1</td>
<td>5.01 (0.22), 4.3 443 (19)</td>
<td>5.09 (0.41), 8.0 450 (36)</td>
<td>5.43 (0.20), 3.7 480 (18)</td>
</tr>
<tr>
<td>Meter 2</td>
<td>5.16 (0.23), 4.4 456 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meter 3</td>
<td>5.27 (0.25), 4.7 466 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meter 4</td>
<td>5.10 (0.31), 6.1 451 (27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group, level 3</td>
<td>5.24 (0.26), 4.9 463 (23)</td>
<td></td>
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</tbody>
</table>

* Data are means (SD), % CV.
from 7.96 mg/dL (704 μmol/L) to 7.96 mg/dL (704 μmol/L) (y = 0.779x + 0.266), with a mean recovery of 91% (range 81%–106%).

The poor result concordance observed prompted further investigation of the agreement between StatSensor and enzymatic methods. The patient population was divided into 2 parts: a discrepant group and a control group (Fig. 2). The discrepant group consisted of 22 patients with creatinine results that differed by ≥0.50 mg/dL (44 μmol/L) between the StatSensor and Roche Modular methods [StatSensor creatinine range 0.69–8.61 mg/dL, median 3.09 mg/dL (61–761 μmol/L, median 273 μmol/L); Roche Modular range 0.84–12.90 mg/dL; median 3.96 mg/dL (74–1140 μmol/L, median 350 μmol/L)] (P = 0.017). Four patients had >1 discrepant sample (total samples n = 27); these values were included in the study to investigate whether discrepant results were method or patient specific. An age- and sex-matched group of control patients was identified from the initial sample population (both groups, mean age 57 years, 12 men and 10 women). Racial composition of the control (68% non–African American, 32% African American) and discrepant (45% non–African American, 55% African American) patient groups was also similar (P = 0.568).

Median creatinine values for the control group were statistically different from those of the discrepant patient group using either the StatSensor or Roche Modular methods (both P < 0.0001). A summary of demographics and renal function for each of the patient groups, including comparisons with the entire patient population, are provided in Table 2.

Nineteen of 22 discrepant patients (86%) had eGFR values <30 mL/min/1.73 m², compared to 5% of control patients (1 of 22, using Roche creatinine values). The majority of discrepant patient results were
underestimated by the StatSensor, with a negative bias that exceeded that of the whole patient population. Thirty-three percent of the discrepant results were from patients with StatSensor creatinine values >5.01 mg/dL (443 μmol/L), with 52% of results corresponding to creatinine values between 1.31 and 5.00 mg/dL (116 and 442 μmol/L).

INVESTIGATION OF INTERFERENCE

We investigated potential causes for the discrepancies observed between the StatSensor meter and the enzymatic method. When available, whole-blood hematocrit, pH, \( \text{pO}_2 \), and total and direct bilirubin values were compared between the discrepant patient population and controls. No statistically significant differences between groups were found, and linear regression revealed no correlations with creatinine results in either population (mean slope 0.987, mean \( R^2 \) 0.062).

Creatine concentrations were measured in a subset of discrepant (n = 12) and control (n = 14) patients. Although there was a significant difference in StatSensor creatinine concentrations between these 2 groups [discrepant patients, mean 3.36 (2.59) mg/dL [297 (229) μmol/L], control patients, mean 1.24 (0.68) mg/dL [110 (60) μmol/L]; \( P = 0.022 \)], creatine concentrations were identical [discrepant patients, mean 0.421 (0.490) mg/dL [32 (37) μmol/L], control patients, mean 0.462 (0.614) [35 (47) μmol/L]; \( P = 0.851 \)] (SDs in parentheses).

To determine if specific drugs interfered with StatSensor creatinine measurements, we reviewed prescribed medications in the health records of a subset of discrepant and control patients (n = 10 each). No association was found between drugs and discrepant creatinine results. However, further review of the diagnosis carried by each patient revealed that 77% (17 of 22) of discrepant samples were from individuals with renal insufficiency or renal-related diagnoses (e.g., acute or chronic renal failure, end-stage renal disease, renal transplantation). Twenty-three percent of control patients (5 of 22) were similarly categorized with some type of renal dysfunction (\( P = 0.0011 \)). The 4 patients who had multiple discrepant samples were all diagnosed with end-stage renal disease or renal failure. The contribution of interfering substances or differences in the whole-blood matrix of these renal disease patients was not resolved.

ENZYMATIC METHOD CONFIRMATION

Although a link between diminished renal function and discordant creatinine results was suggested, the cause of interference in this patient population was not clear. To confirm the performance of the core laboratory method (Roche Modular enzymatic assay), frozen aliquots of the initial patient samples were assayed using an IDMS creatinine method (8), and results were compared with both enzymatic (Fig. 3A) and StatSensor (Fig. 3B) values. Regression analysis indicated that the StatSensor analysis of whole-blood creatinine had greater variation than plasma creatinine values determined with the Roche Modular method. This was evident by the SD about the regression lines (\( S_{yy} \)) and the Pearson correlation coefficient and was further supported by a Fisher F-test (\( P = 0.048 \)). Using the value of \( R^2 \) to represent explained variance, IDMS plasma creatinine measurements explained 88% of variance in StatSensor whole-blood values and 99% of variance in plasma enzymatic values.

MULTIVARIATE ANALYSIS

We established a multiple linear regression model to test the influence of whole-blood matrix components on StatSensor creatinine measurements. Hematocrit, red blood cell water fraction (RW), plasma water fraction (PW), and their joint interaction terms were assessed in the model because of the known association of

| Table 2. Demographic information for entire patient population, the discrepant patient group, and the control group, including creatinine concentrations and diagnoses. |
|-----------------|------------|------------|---|
|                  | Discrepent | Control    | All |
| No. of patients  | 22         | 22         | 119 |
| No. of samples   | 27         | 22         | 119 |
| Mean age, years (range) | 57 (15–83) | 57 (16–87) | 59 (6–97) |
| Men, n (%)       | 12 (55)    | 12 (55)    | 66 (55) |
| Women, n (%)     | 10 (45)    | 10 (45)    | 53 (45) |
| African American, n (%) | 12 (55) | 7 (32) | 32 (27) |
| Non–African American, n (%) | 10 (45) | 15 (68) | 87 (73) |
| eGFR <30 mL/min/1.73m², n (%) | 19 (86) | 1 (5) | 38 (22) |
| Renal diagnosis, n (%) | 17 (77) | 5 (23) | 29 (24) |
these factors with conversion of whole blood to plasma, as shown with direct biosensor POC glucose meters (5, 7). Terms were retained within the model when the Wald test $P < 0.1$ and the coefficient was different from zero; model coefficients are shown in online Supplementary Table 2, and the final model is shown in Eq. 1:

$$E(\ln\text{Creatinine}_{\text{StatSensor}}) = \beta_0 + \beta_1(\ln\text{Creatinine}_{\text{IDMS}}) + \beta_2\text{Hematocrit} + \beta_3\text{RW} + \beta_4(\text{RW})(\ln\text{Creatinine}_{\text{IDMS}}) + \beta_5(\ln\text{Creatinine}_{\text{IDMS}})^2.$$ (1)

It is apparent that the whole-blood matrix contributes a small but statistically significant influence on StatSensor creatinine results. StatSensor creatinine concentrations were predicted by a polynomial equation that was modified by the RW and PW and confounded by hematocrit. The $R^2$ value indicated that Eq. 1 explained 91.8% of variance of the StatSensor creatinine results, 3.8% more than the 88.0% variance explained in the univariate analysis of IDMS creatinine in Fig. 3B. This small influence of RW on StatSensor whole-blood creatinine is depicted in Fig. 4. This suggests that variance between the StatSensor and the IDMS method is only partly explained by this whole-blood matrix variable, and the variance largely remains unexplained.

**Discussion**

Rapid determination of creatinine concentration and eGFR provides beneficial information in several clinical settings. Most notably, near-patient testing of renal function may decrease the risk of CIN in patients with renal impairment, as exposure to contrast dye should be limited in these individuals (2, 14). Numerous published reports have focused on agreement between POC creatinine and centralized laboratory measurements in a variety of disease states and patient settings (15–25). Although rapidly obtained creatinine values...
do offer certain benefits, discrepancies with centralized methods may cause confusion for clinicians and laboratorians and subsequently compromise patient care. This study compared 119 sample results between the Nova StatSensor handheld device, a centralized Roche Modular enzymatic method, and an IDMS method and further investigated causes for the discrepancies observed in a subset of those patients.

Similar to other reports (22–25), we observed a small overall negative bias between the StatSensor and the centralized enzymatic method. Bias measurements for these types of data do not reveal the whole story, however. Although bias was relatively small for the entire 119-sample population, further investigation revealed clinically significant discrepancies in a subset of patients. Almost a quarter of the samples in this study had creatinine values that differed by 0.50 mg/dL (44 μmol/L) or more between the Nova StatSensor and the enzymatic Roche Modular method (27 of 119), with increased discordance observed at creatinine values >2.00 mg/dL (177 μmol/L). Whole-blood analysis at the point of care is not expected to perform with the same precision as serum-based measurements, thus the discrepancy cutoff used in this study was more lenient than the CLIA total allowable error of 0.3 mg/dL (26 μmol/L) or 15% (no error margins exist for whole-blood creatinine at this time). The Roche Modular enzymatic results correlated well with the IDMS creatinine results; however, StatSensor whole-blood creatinine results exhibited much larger discrepancies with both the IDMS and enzymatic plasma creatinine methods. These results suggest that the enzymatic plasma method was not prone to interference and the discrepancy was due to a negative interference with the whole-blood method.

The discrepant patient population had renal impairment compared with a control group of similar age, sex, and race, as indicated by the statistically significant increases in creatinine values, the majority (86%) having eGFR values <30 mL/min/1.73 m², and 77% of patients having a diagnosis associated with renal disease. Schnabl et al. (25) similarly found an increased bias between StatSensor and plasma creatinine measurements in renally impaired patients, with discordance noted in samples from predialysis patients that were not present in samples taken after dialysis was completed. Dialysis patients also had StatSensor creatinine values that were significantly lower than Roche enzymatic assay results in a study by Shepard et al. (24). These findings suggest that a parameter associated with renal disease promoted a negative discrepancy with the whole-blood creatinine method. Possible interferences include renally cleared metabolites that accumulate in plasma or changes to the matrix, such as low protein concentrations associated with renal disease or alterations related to the cellular components of blood.

The discrepant patient group was investigated for possible interfering factors, and no evidence was found that drugs, pH, total and direct bilirubin, creatine, hematocrit, or PO₂ were related to discrepant creatinine results. A multiple linear regression evaluation of hematocrit, PW, and RW revealed that all 3 factors affected StatSensor creatinine concentrations. The RW fraction had greater predictive influence than PW and hematocrit, and an explanation for this observation will require additional experimentation. The RW fraction represents intracellular water volume not occupied by hemoglobin, and it is possible that RW is associated with the volume of intracellular fluid containing metabolites that interfere with the creatinine reagent strips. Alternatively, the reagent strip could osmotically promote movement of water out of red blood cells, leading to dilution of plasma creatinine because zwitterionic creatinine diffuses more slowly than water across red blood cell membranes (26). Through either mechanism, a negative interference with the StatSensor creatinine results was observed as the RW fraction increased (see Fig. 4).

The data presented here suggest that whole-blood POC creatinine measurements should be used with caution. Negative interference was observed in these measurements, which could lead to an erroneous determination of adequate renal function near the decision threshold, particularly when eGFR is calculated. Whole-blood creatinine measurement with the StatSensor POC meter was closely related to plasma creatinine concentrations and is useful for detecting loss of renal function. However, the StatSensor was less precise than the plasma enzymatic method with increased variability, partly due to the whole-blood matrix.

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