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Prime Time for Enzymatic Creatinine Methods in Pediatrics

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BACKGROUND: The literature regarding the nonspecificity of applications of the Jaffe (alkaline picrate) reaction for creatinine is generally outdated. We conducted a specificity study to update the nonspecificity information for current Jaffe and enzymatic creatinine assays.

METHODS: Two serum pools with creatinine concentrations within the pediatric reference interval were spiked with albumin, IgG, unconjugated bilirubin, adult hemoglobin (Hb A), and fetal hemoglobin (Hb F) to produce 1 unspiked and 5 spiked samples per pool. The 35 laboratories participating in the survey used a total of 14 method–analyzer combinations. Measurements were performed in triplicate in a single run in accordance with manufacturer instructions. Absolute differences in creatinine concentration between spiked and unspiked samples were calculated per laboratory. Mixed ANOVA was used to quantify the interferent-related CV component for the Jaffe and enzymatic methods.

RESULTS: The interference by bilirubin and Hb A on serum creatinine measurements was <10% for most of the Jaffe and enzymatic methods. Obvious interference was observed among the Jaffe methods in samples spiked with Hb F, albumin, and IgG, but not among the enzymatic methods. The within-laboratory interferent-related CVs for the Jaffe method–analyzer combinations ranged from 8.0%–27% at a creatinine concentration of 40.4 μmol/L (0.46 mg/dL) and from 5.4%–15% at 73.4 μmol/L (0.83 mg/dL). Enzymatic methods had within-laboratory interferent-related CVs of <4% at both concentrations.

CONCLUSIONS: Albumin, IgG, and Hb F interfered with Jaffe creatinine assays, leading to inaccuracies in estimated glomerular filtration rates that are clinically important, especially in children and neonates. Because protein error and Hb F interference do not occur with any of the enzymatic methods tested, we conclude that enzymatic creatinine methods are preferred for evaluation of kidney function in pediatric cases.

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Proteins and substances with a ketone group are known to interfere in the Jaffe (alkaline picrate) reaction for the measurement of creatinine in serum *(1, 2)*, and many authors have attempted to improve the performance characteristics of the Jaffe reaction *(3–8)*. To prevent the oxidation of bilirubin to biliverdin, investigators have included potassium ferricyanide, bilirubin oxidase, or rate blanking and lithium dodecyl sulfate in the reagents. Some manufacturers have advocated the use of rate blanking to reduce the effects of interfering substances and subtraction of a fixed factor (−27 μmol/L, −0.31 mg/dL) to compensate for the nonspecific reaction of protein in Jaffe methods *(9)*. Although substantial improvements have been made, uncertainties regarding nonspecific effects have remained *(10–14)*. Beginning in the 1970s, enzymatic methods have been developed to improve creatinine specificity *(11, 15)*; however, despite the known limitations, methods based on the Jaffe reaction are still extensively used for measuring serum creatinine.

The serum creatinine concentration and the estimated glomerular filtration rate (GFR)[4] are interrelated; consequently, the performance of the creatinine analysis affects GFR estimates *(11, 16)*. The accuracy of the GFR estimate is important for the early detection of chronic kidney disease in adults and children, as has been recognized in the recent awareness campaigns in the European Union and in the US (http://www.NKDEP.nih.gov). The Laboratory Working Group report of the National Kidney Disease Education Program in the US has reviewed the information on the nonspecificity of routine creatinine methods and recommended that manufacturers of in vitro diagnos-

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[4] Nonstandard abbreviations: GFR, glomerular filtration rate; Hb A, adult hemoglobin; Hb F, fetal hemoglobin.

tics address and reduce the influence of interfering substances in patient samples *(11)*. No specific recommendations were provided, however, because no recent literature has described the specificity characteristics of current routine procedures of creatinine measurement. Krouwer pointed out that the goal for the total error in creatinine assays should take into account not only calibration bias and imprecision but also the nonspecificity component *(17)*.

We report the results of a specificity study we conducted to update such information and to establish the impact of relevant matrix components and frequently encountered interfering substances on serum creatinine results, as measured with several current enzymatic and Jaffe reaction–based methods. We also quantitated the nonspecificity component in Jaffe and enzymatic methods to evaluate its contribution to the total error in creatinine concentrations within the pediatric range.

Materials and Methods

STUDY DESIGN

We created 2 serum pools with different creatinine concentrations, one at approximately 73 μmol/L (0.83 mg/dL), which is the 97.5th percentile of term neonates, and the other at approximately 40 μmol/L (0.45 mg/dL), which is the upper limit of the reference interval for children 5–7 years of age *(18)*. Interfering substances were spiked into these pools so that the matrix was uniform across all of the samples except for the spiked substance. The concentration of creatinine in spiked and unspiked serum samples was measured in selected clinical chemistry laboratories with their routine serum creatinine methods. Within-laboratory within-run differences in serum creatinine between spiked and unspiked samples were calculated at each concentration. In addition, within-laboratory interference-related CV components (which we call "interferential CVs" throughout the remainder of this report) were calculated across the method–analyzer combinations. This design allowed the evaluation of nonspecificity independently of the analytical bias of the participating laboratories.

SAMPLE PREPARATION

We prepared samples to create 6 sets of paired samples at creatinine concentrations of 40 μmol/L (0.45 mg/dL) and 70 μmol/L (0.79 mg/dL) and with exactly the same matrix except for the addition of albumin, IgG, bilirubin, adult hemoglobin (Hb A), and fetal hemoglobin (Hb F) (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue3).

BASE SERUM POOL

The starting point for all sample preparation was a serum pool prepared according to the CLSI C37A protocol. The pool was prepared from serum samples obtained from 3 donors with low concentrations of total protein, albumin, IgG, and creatinine. The pool characteristics were as follows: total protein, 45 g/L; albumin, 21 g/L; IgG, 4.6 g/L; bilirubin, 3 μmol/L; and creatinine, 42 μmol/L (0.48 mg/dL). The pool was hemolysis free and was negative for hepatitis B surface antigen, hepatitis C virus, and HIV.

STOCK SOLUTIONS

Creatinine stock. Creatinine (56.1 mg; NIST SRM914a) was dissolved in 45 mL base serum, transferred to a 50-mL volumetric flask, and made up to 50 mL with base serum (creatinine concentration, 9931 μmol/L or 112 mg/dL).

Bilirubin stock. Bilirubin (194.9 mg; Sigma–Aldrich, $M_r = 584.7$) was dissolved in 8 mL of 0.1 mol/L NaOH and diluted with 0.1 mol/L NaOH to 10 mL in a volumetric flask (bilirubin concentration, 33.3 mmol/L).

Hb A stock. EDTA-treated blood from a Caucasian adult (Hb type AA) was washed 3 times with physiological saline (9 g/L; 1 part cells plus 3 parts saline), and 1 mL of packed cells was lysed with 7 mL water. After centrifugation, the Hb A concentration of the clear supernate was measured (1.88 mmol/L).

Hb F stock. Hb F was prepared from EDTA-treated blood of a newborn in the same way as for the Hb A stock. The Hb F concentration was 2.03 mmol/L.

Low-creatinine serum stock. A 310-mL aliquot was taken from the base serum (stock A).

High-creatinine serum stock. To 300 mL of the base serum was added 1 mL of the creatinine stock (increase in creatinine concentration: $1/301 \times 9931$ μmol/L = 33 μmol/L or 0.37 mg/dL; stock B).

SAMPLES: LOW-CREATININE SERIES

Sample 1. Forty-eight milliliters of stock A was diluted with physiological saline to 50 mL in a volumetric flask.

Sample 2. Forty-eight milliliters of stock A was transferred to a 50-mL volumetric flask, 1.25 g albumin (Sigma–Aldrich) was added and dissolved, and the solution was made up to the mark with physiological saline (increase in albumin of 25 g/L).

Sample 3. Forty-eight milliliters of stock A was transferred to a 50-mL volumetric flask, 1.00 g IgG (Sigma–Aldrich) was added and dissolved, and the volume was

made up to the mark with physiological saline (increase in of 20 g/L).

Sample 4. Forty-eight milliliters of stock A was transferred to a 50-mL volumetric flask, 150 μL of bilirubin stock solution was added and mixed, and 150 μL 0.1 mol/L HCl was added to neutralize the NaOH in the bilirubin stock. The volume was diluted to 50 mL with physiological saline (increase in bilirubin of 100 μmol/L).

Sample 5. Forty-eight milliliters of stock A was transferred to a 50-mL volumetric flask, 1.60 mL Hb A stock was added, and the volume made up to the mark with physiological saline (Hb A increase of 60 μmol/L).

Sample 6. Forty-eight milliliters of stock A was transferred to a 50-mL volumetric flask, 1.48 mL Hb F stock was added, and the volume made up to the mark with physiological saline (Hb F increase of 60 μmol/L).

SAMPLES: HIGH-CREATININE SERIES

Samples 7–12 were prepared exactly as for samples 1–6, but with stock B instead of stock A.

Table 1 in the online Data Supplement summarizes sample preparation.

DISTRIBUTION OF SAMPLES, LOGISTICS, AND ANALYSIS

Aliquots (0.5 mL) of the respective unspiked (native) and spiked pools were dispensed into Cryovials and frozen at −84 °C. Sets of 12 samples were shipped on dry ice to the participating laboratories. On arrival, the samples were stored frozen at −70 °C until analysis and were assayed in triplicate within 2 weeks. Laboratories assayed the samples as unknowns with their routine clinical chemistry analyzers exactly as recommended by the manufacturer. Study samples were all run on a single day in 1 run in June 2008. Data were reported centrally to the Dutch external quality-assessment organization.

ASSIGNMENT OF CREATININE VALUES

Samples 1 and 7 were assigned creatinine values with reference material endorsed by the Joint Committee for Traceability in Laboratory Medicine and with the Deutsche Gesellschaft für Klinische Chemie (Bonn, Germany) definitive method for serum creatinine (http://www.bipm.org). Final creatinine concentrations were as follows: 40.4 μmol/L in sample 1 (expanded uncertainty = 0.4 μmol/L, with coverage factor $k = 2.4$ and 7 degrees of freedom) and 73.4 μmol/L in sample 7 (expanded uncertainty = 0.73 μmol/L, with coverage factor $k = 2.4$ and 7 degrees of freedom). The expanded-uncertainty value is obtained by multiplying the standard uncertainty by the coverage factor (2.4) *(19)*. At a coverage factor derived from the *t* distribution with 7 effective degrees of freedom, the value of the measurand lies within the assigned range of values with a probability of 95%. Although the exact bias data are not addressed in this publication, we wanted to keep track of the traceability and accuracy of current serum creatinine measurements during the nonspecificity study to obtain accurate, absolute estimates of the nonspecificity components for the absolute creatinine concentrations.

PARTICIPATING CLINICAL CHEMISTRY LABORATORIES AND CREATININE METHODS

Thirty-five clinical chemistry laboratories (24 laboratories from The Netherlands and 11 laboratories from other Western European countries) were invited to participate in this specificity study. The invitation to laboratories was selective and made so that enzymatic combinations as well as uncompensated and compensated Jaffe combinations were included. The selection initially produced 15 method–analyzer combinations from 35 laboratories: 1, enzymatic method with the Abbott Diagnostics ARCHITECT analyzer (4 laboratories); 2, enzymatic method with the Ortho Clinical Diagnostics VITROS analyzer (2 laboratories); 3, enzymatic method with the Roche Diagnostics Cobas analyzer (7 laboratories); 4, enzymatic method with the Roche Diagnostics Modular Analytics analyzer (3 laboratories); 5, compensated Jaffe method with the Roche Diagnostics Cobas analyzer (2 laboratories); 6, compensated Jaffe method with the Roche Diagnostics Integra 400 analyzer (2 laboratories); 7, compensated Jaffe method with the Roche Diagnostics Modular Analytics analyzer (2 laboratories); 8, compensated Jaffe method with the Siemens ADVIA 1650 and 2400 analyzers (2 laboratories); 9, compensated Jaffe method with the Siemens Dimension RxL analyzer (1 laboratory); 10, uncompensated Jaffe method with the Abbott Diagnostics ARCHITECT analyzer (2 laboratories); 11, new uncompensated Jaffe method with the Abbott Diagnostics ARCHITECT analyzer (1 laboratory); 12, uncompensated Jaffe method with the Beckman Coulter LX20 analyzer (3 laboratories); 13, uncompensated Jaffe method with the Siemens Dimension RxL analyzer (2 laboratories); 14, uncompensated Jaffe method with the Siemens ADVIA 1650 analyzer (1 laboratory); and 15, uncompensated Jaffe method with the Siemens Dimension VISTA analyzer (1 laboratory). According to Abbott Diagnostics, the new uncompensated Jaffe method is a modified version that is less susceptible to bilirubin interference. Compensated methods subtract a fixed concentration from all results in an attempt to adjust for the nonspecific protein influence. To simplify the visual inspection of the results presented in the figures, we present the data from enzymatic method–analyzer combinations in green, compensated Jaffe method–analyzer combinations in red, and uncompensated Jaffe method–analyzer combinations in orange.

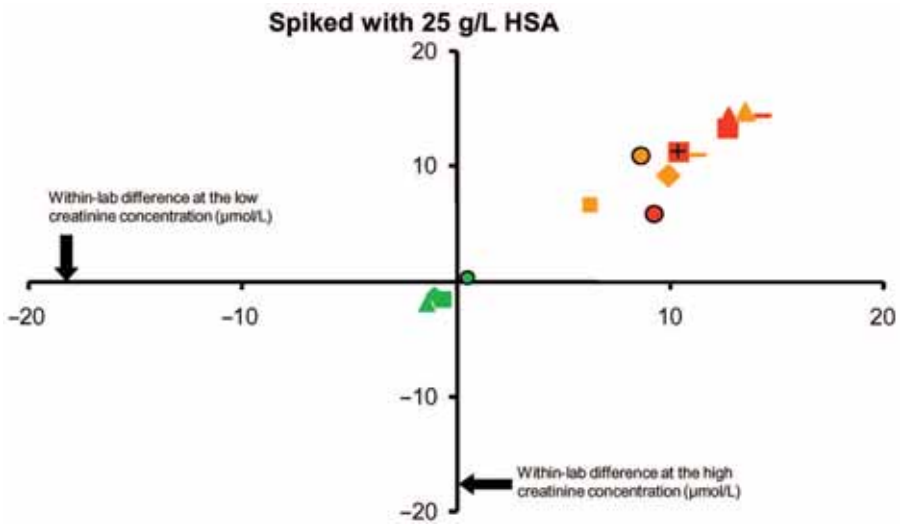

Fig. 1. Scattergram of differences in within-laboratory serum creatinine concentration between human serum albumin (HSA)-spiked and unspiked samples at low creatinine (*x* axis) and high creatinine (*y* axis) concentrations.

Within-laboratory differences between HSA-spiked and unspiked samples are grouped by method–analyzer combination. Data are presented as mean differences and are calculated from the individual laboratory data. Green, red, and orange symbols indicate enzymatic, compensated Jaffe, and uncompensated Jaffe method–analyzer combinations, respectively. Specific combinations: ◆, enzymatic method with the Abbott Diagnostics ARCHITECT analyzer; ●, enzymatic method with the Ortho Clinical Diagnostics VITROS analyzer; ▲, enzymatic method with the Roche Diagnostics Cobas analyzer; ■, enzymatic method with the Roche Diagnostics Modular ANALYTICS analyzer; ▲, compensated Jaffe method with the Roche Diagnostics Cobas analyzer; ●, compensated Jaffe method with the Roche Diagnostics Integra 400 analyzer; ■, compensated Jaffe method with the Roche Diagnostics Modular ANALYTICS analyzer; –, compensated Jaffe method with the Siemens Advia 1650 and 2400 analyzers; +, compensated Jaffe method with the Siemens Dimension RxL analyzer; ◆, uncompensated Jaffe method with the Abbott Diagnostics ARCHITECT analyzer; –, new uncompensated Jaffe method with the Abbott Diagnostics ARCHITECT analyzer; ■, uncompensated Jaffe method with the Beckman Coulter LX20 analyzer; ●, uncompensated Jaffe method with the Siemens Dimension RxL analyzer; ▲, uncompensated Jaffe method with the Siemens Advia 1650 analyzer.

DATA REVIEW

The within-run CV was calculated for each sample at each concentration. Results from one laboratory (Siemens Dimension VISTA) were excluded because of high within-run CVs (up to 15%) in multiple samples. Both the direction and magnitude of the interference were inspected visually from the scattergrams (see Statistical Analysis below) for consistency at the 2 creatinine concentrations. Reanalysis was necessary in the one laboratory that reported inconsistent results for samples spiked with bilirubin. After reanalysis by this laboratory, all interference data were consistent.

STATISTICAL ANALYSIS

Calculation of the interference effect. The effect of an interfering substance is defined as the difference in creatinine results between the spiked and unspiked samples in a series, irrespective of the calibration status of the laboratory. For example, if a laboratory obtained a creatinine concentration of 53 μmol/L (0.60 mg/dL) in the albumin-enriched sample of the low-creatinine series (sample 2) and 41 μmol/L (0.46 mg/dL) in the unspiked sample of the low-creatinine series (sample 1), the interfering effect of 25 g/L albumin in that laboratory was 12 μmol/L (i.e., 53 μmol/L − 41 μmol/L), or 0.14 mg/dL. The effect of 25 g/L albumin in the high-creatinine series can similarly be derived from the measurements for samples 7 and 8.

Differences between creatinine concentrations measured in spiked and unspiked samples were calculated per laboratory per creatinine concentration. Absolute differences were averaged per analyzer–method combination and plotted in scattergrams for the low creatinine concentration (*x* axis) and

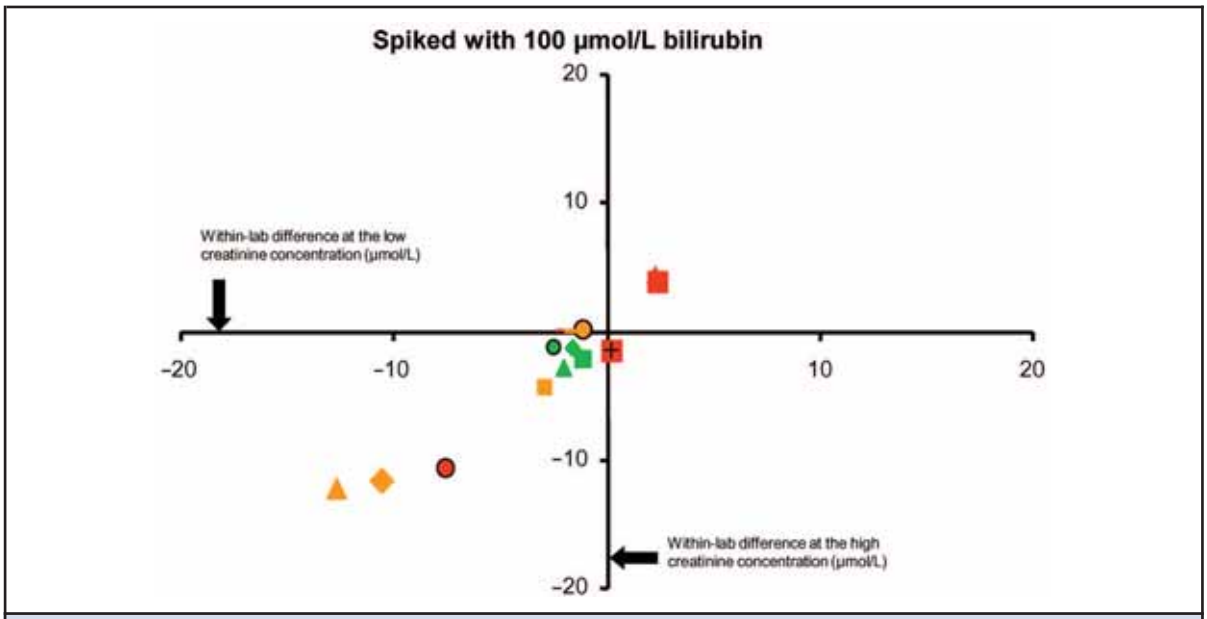

Fig. 2. Scattergram of differences in within-laboratory serum creatinine concentration between bilirubin-spiked and unspiked samples at low creatinine (*x* axis) and high creatinine (*y* axis) concentrations.

Within-laboratory differences between bilirubin-spiked and unspiked samples are grouped by method–analyzer combination (see legend to Fig. 1 for symbol definitions; green, red, and orange symbols indicate enzymatic, compensated Jaffe, and uncompensated Jaffe method–analyzer combinations, respectively). Data are presented as mean differences and are calculated from the individual laboratory data.

the high creatinine concentration (*y* axis) for each interfering substance.

Quantification of the interferential CV. The Statistical Package for the Social Sciences (version 11.0; SPSS) was used for statistical analyses. Data were analyzed per method–analyzer combination (14 combinations) and per concentration (2 concentrations). A mixed-model ANOVA [performed with the PROC MIXED module in the SAS System for Windows, Release 8.2, 2004; SAS Institute *(20)*] was used for analyzing the outcome variable (creatinine) with the following random explanatory factors: laboratory (34 laboratories), spike (5 spiked samples per creatinine concentration), and replication (3 replicates). The resulting variance components were estimated with a restricted maximum-likelihood method. The random laboratory factor represents the between-laboratory component. The sum of the main spike component and the spike component nested within laboratories was considered the interferential component. The sum of all other components containing replication error and the residual error was considered the analytical component. The CVs were calculated by taking the square root of the variance components and expressing them as a percentage of the relevant reference concentration.

Results

The absolute differences in serum creatinine concentration between spiked and unspiked samples for the low and high creatinine concentrations and averaged for each method–analyzer combination are presented in the figures. Fig. 1 shows positive interference by a 25-g/L albumin increment for all Jaffe methods but not for the enzymatic methods. Fig. 2 illustrates small and comparable negative interferences by bilirubin in the enzymatic and most Jaffe methods. Three Jaffe methods (compensated Jaffe method on the Roche Diagnostics Integra 400 analyzer, uncompensated Jaffe method on the Abbott Diagnostics ARCHITECT analyzer, and uncompensated Jaffe method on the Siemens ADVIA 1650 analyzer) showed more pronounced interference by bilirubin. Fig. 3 shows positive interference by IgG in most of the Jaffe methods but negligible interference in the enzymatic methods. Fig. 4 shows negligible interference by Hb A for the Roche enzymatic methods and for most Jaffe methods, small negative interferences for the enzymatic methods with the Ortho Clinical Diagnostics and Abbott Diagnostics analyzers, and more pronounced negative interference with compensated and uncompensated Jaffe methods that used the

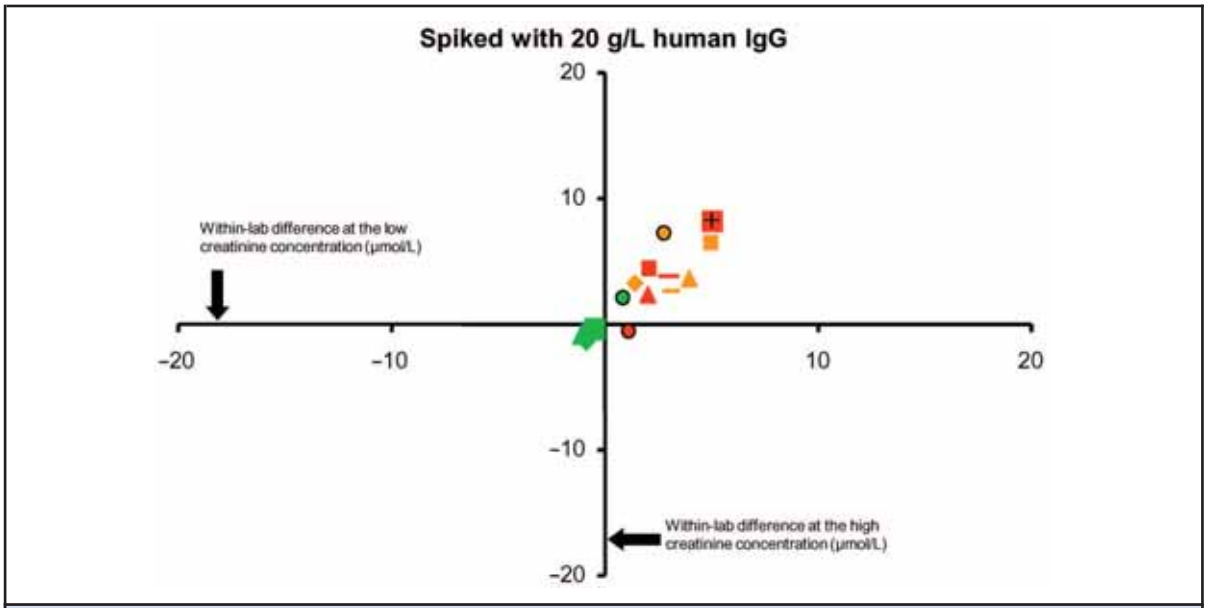

Fig. 3. Scattergram of differences in within-laboratory serum creatinine concentration between human IgG-spiked and unspiked samples at low creatinine (*x* axis) and high creatinine (*y* axis) concentrations.

Within-laboratory differences between human IgG-spiked and unspiked samples are grouped by method–analyzer combination (see legend to Fig. 1 for symbol definitions; green, red, and orange symbols indicate enzymatic, compensated Jaffe, and uncompensated Jaffe method–analyzer combinations, respectively). Data are presented as mean differences and are calculated from the individual laboratory data.

Siemens Dimension RxL analyzer. Fig. 5 shows negligible interference by Hb F in all of the enzymatic methods and in the uncompensated Jaffe methods with the Beckman Coulter, Abbott Diagnostics, and Siemens ADVIA 1650 analyzers, but substantial and generally negative interference was observed for the other Jaffe methods. The Jaffe method with Roche Diagnostics Integra 400 analyzer showed positive interference by Hb F.

The results of the mixed-model ANOVA (Table 1) indicate that Jaffe methods had a larger within-laboratory interferential CV component than the enzymatic methods, demonstrating the appreciably greater nonspecificity of the Jaffe methods. The range of within-laboratory interferential CVs of the Jaffe method–analyzer combinations was 8.0%–27% at a creatinine concentration of 40.4 μmol/L (0.46 mg/dL) and 5.4%–15% at 73.4 μmol/L (0.83 mg/dL). This range of interferential CVs corresponds to errors in serum creatinine concentration of −18 μmol/L to +14 μmol/L (−0.20 mg/dL to +0.16 mg/dL) at 40.4 μmol/L (0.46 mg/dL) and −20 μmol/L to +16 μmol/L (−0.23 mg/dL to +0.18 mg/dL) at 73.4 μmol/L (0.83 mg/dL). Enzymatic methods have small within-laboratory interferential CVs (0.5%–3.9% at the low creatinine concentration and 1.0%–3.1% at the high concentration). These values correspond to errors in serum creatinine concentration of −4 μmol/L to 0 μmol/L at a creatinine concentration of 40.4 μmol/L (0.46 mg/dL) and −6 μmol/L to 0 μmol/L at 73.4 μmol/L (0.83 mg/dL). Table 1 also shows that within-laboratory analytical CVs are generally smaller for the enzymatic methods than for the Jaffe methods.

Discussion

The scattergrams in Figs. 1–5 reveal no or negligible interference by the interfering substances tested for the enzymatic methods from Roche Diagnostics, Abbott Diagnostics, and Ortho Clinical Diagnostics. In contrast, most Jaffe methods showed substantial interference by these substances. The small within-laboratory interferential CV components obtained for the enzymatic methods (Table 1) support the recent statement from the IFCC Scientific Division recommending that more specific creatinine methods be adopted *(21)*. Figs. 2 and 4 show that manufacturers have been successful in minimizing bilirubin and Hb A interference in most Jaffe and enzymatic methods, as has been reported in the literature *(8–11, 13–14)*. In the case of Abbott Diagnostics, the newest uncompensated Jaffe version has negligible bilirubin

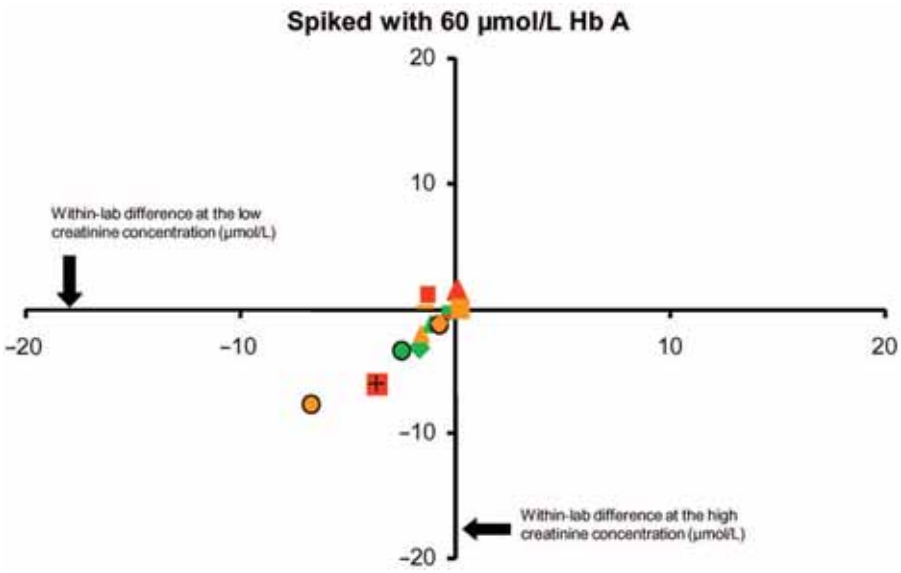

Fig. 4. Scattergram of differences in within-laboratory serum creatinine concentration between human Hb A–spiked and unspiked samples at low creatinine (*x* axis) and high creatinine (*y* axis) concentrations.

Within-laboratory differences between human Hb A–spiked and unspiked samples are grouped by method–analyzer combination (see legend to Fig. 1 for symbol definitions; green, red, and orange symbols indicate enzymatic, compensated Jaffe, and uncompensated Jaffe method–analyzer combinations, respectively). Data are presented as mean differences and are calculated from the individual laboratory data.

interference, compared with the previous version of the assay.

On the other hand, positive interferences due to albumin and/or IgG (Figs. 1 and 3) and Hb F (Fig. 5) are still substantial for most Jaffe methods. Although manufacturers try to correct for the protein error through the use of a fixed compensation factor for the protein content in adults (i.e., −27 μmol/L, or −0.31 mg/dL) *(9)*, this procedure overcorrects with pediatric samples because of children's low serum protein concentrations. This overcorrection produces inaccurate GFR estimates, especially in neonates and children *(22)*. Concerns about the impact of variation in normally occurring serum matrix components, such as albumin and IgG, on serum creatinine concentration and the GFR estimate have also been described by Parry *(23)*.

The nonspecificity error component in most Jaffe methods is appreciable (see Table 1), with a range of 8.0%–27% at a creatinine concentration of 40.4 μmol/L (0.46 mg/dL) and 5.4%–15% at 73.4 μmol/L (0.83 mg/dL), and this component by itself can cause errors that exceed the desirable total error for creatinine of 7.6% *(11)*. In this study, the nonspecificity component in enzymatic methods contributed a maximum of 3.9% to total error.

In this study, special attention was given to representing the composition of the serum matrix in children (i.e., low concentrations of creatinine, total protein, and IgG) because the impact of method nonspecificity on serum creatinine and GFR estimation is most pronounced in these samples. Common interferences and/or matrix components, which generally vary among patient samples, were tested across 14 current method–analyzer combinations. The purpose was not to estimate an effect for each interfering substance but to obtain a global estimate of the contribution of these commonly encountered interferences. We expressed that contribution as the interferential CV, which is the aggregate of variance components that might occur in a serum sample. The interferential CV is consistent with Krouwer's recommendation *(17)* to consider random sample-specific interferences as a component of imprecision in the formula for total allowable error.

EFFECT OF NONSPECIFICITY ON THE GFR ESTIMATE

As an example, GFR was calculated with the Schwartz equation (http://www.NKDEP.nih.gov/professionals/gfr_calculators/gfr_children.htm), assuming a term neonate ($k = 0.45$, where k is the constant in the Schwartz equation) with a height of

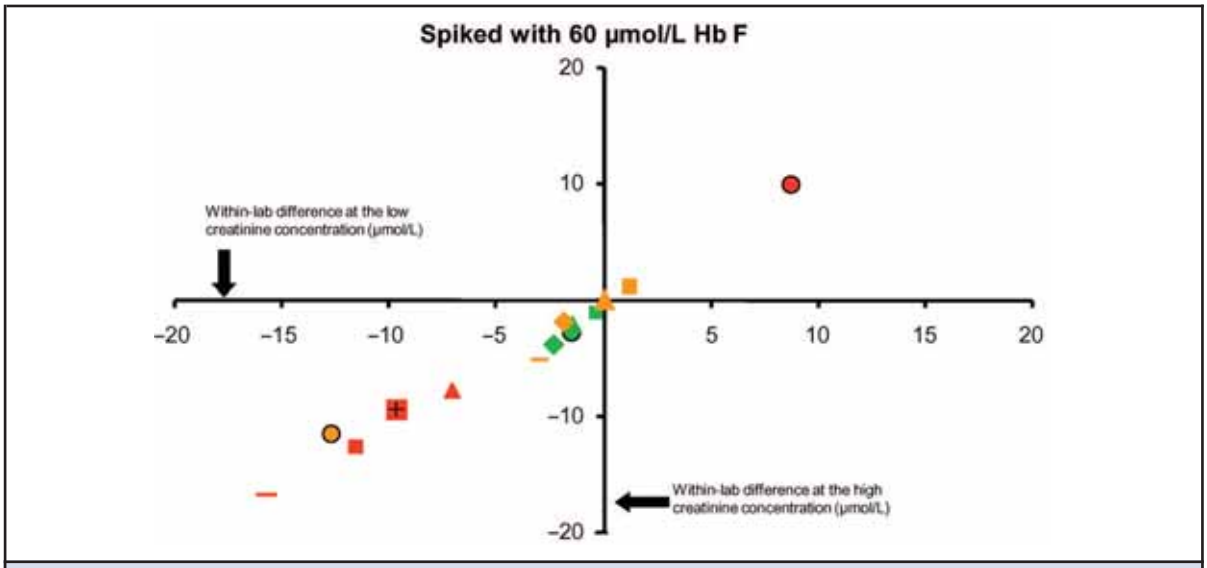

Fig. 5. Scattergram of differences in within-laboratory serum creatinine concentration between human Hb F–spiked and unspiked samples at low creatinine (*x* axis) and high creatinine (*y* axis) concentrations.

Within-laboratory differences between human Hb F–spiked and unspiked samples are grouped by method–analyzer combination (see legend to Fig. 1 for symbol definitions; green, red, and orange symbols indicate enzymatic, compensated Jaffe, and uncompensated Jaffe method–analyzer combinations, respectively). Data are presented as mean differences and are calculated from the individual laboratory data.

50 cm and the results for base serum (38 μmol/L or 0.43 mg/dL) and albumin-spiked serum (52 μmol/L or 0.59 mg/dL) from the compensated Jaffe method with the Siemens ADVIA 1650 analyzer. The corresponding GFR values were 52 and 38 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ for the base and spiked sera, respectively. In the albumin-spiked sample, the GFR was underestimated by 27%. At the higher creatinine concentration, the results were 69 μmol/L (0.78 mg/dL) in the base serum and 83 μmol/L (0.94 mg/dL) in the albumin-spiked sample. The corresponding GFR values were 29 and 24 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ for the base and spiked sera, respectively. In the albumin-spiked sample, the

Table 1. Within-laboratory interferential and analytical CV components calculated from 2 creatinine concentrations in the pediatric concentration interval, as measured in 2 unspiked and 10 spiked samples by 34 clinical chemistry laboratories across 14 method–analyzer combinations.

	Method–analyzer combination (no. of laboratories)[a]													
	Enzymatic methods				Jaffe methods									
	1 (4)	2 (2)	3 (7)	4 (3)	5 (2)	6 (2)	7 (2)	8 (8)	9 (1)	10 (2)	11 (1)	12 (3)	13 (2)	14 (1)
At the low creatinine concentration (40.4 μmol/L)														
Within-lab interferential CV, %	2.1	3.9	0.8	0.5	18	18	22	27	19	19	14	8.0	21	24
Within-lab analytical CV, %	0.8	1.8	2.7	1.7	6.8	3.2	5.8	3.3	1.8	2.1	2.2	2.3	2.4	3.7
At the high creatinine concentration (73.4 μmol/L)														
Within-lab interferential CV, %	1.9	3.1	1.2	1.0	11	11	14	15	12	11	8.1	5.4	12	13
Within-lab analytical CV, %	0.5	1.3	1.6	1.3	4.5	2.5	3.1	2.0	1.9	1.1	0.9	1.6	1.4	0.8

[a] See Materials and Methods for descriptions of method–analyzer combinations.

GFR was underestimated by 17%. Similarly, 60 μmol/L Hb F in this term neonate example caused a reduction of 14 μmol/L (0.16 mg/dL) at both creatinine concentrations: 38 μmol/L (0.43 mg/dL) in the base serum and 24 μmol/L (0.27 mg/dL) in the Hb F–spiked low-creatinine sample and 69 μmol/L (0.78 mg/dL) in the base serum and 55 μmol/L (0.62 mg/dL) in the spiked high-creatinine sample. The corresponding GFR values were overestimated by 60% and 24% at the low and high creatinine concentrations, respectively.

The limitations of this study are related to the fact that only 2 normally occurring serum matrix components and 3 common interfering substances were tested. Moreover, nonspecificity was evaluated at the 97.5th percentile upper reference values of neonates and children *(18)*, and the degree of interference observed cannot be completely generalized or extrapolated to creatinine concentrations in adults. Other interfering substances, such as ketone bodies and cephalosporines, were not investigated.

PRIME TIME FOR ENZYMATIC CREATININE METHODS?

Notwithstanding the ongoing progress made in the current formulations of Jaffe methods for minimizing interferences by bilirubin and Hb A, clinically significant interferences remain for Hb F, albumin, and/or IgG concentrations. These interferences lead to large nonspecificity variances and to inaccurate GFR estimates, especially in children and neonates. Because protein error and Hb F interference do not occur with any of the enzymatic methods tested, we conclude that enzymatic methods should become the methods of choice for evaluating kidney function with samples from pediatric patients.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures of Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:*

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Consultant or Advisory Role: None declared.
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