Clinical Chemistry 58:2
391–401 (2012)

Specificity Characteristics of 7 Commercial Creatinine Measurement Procedures by Enzymatic and Jaffe Method Principles

Neil Greenberg,[1*] William L. Roberts,[2] Lorin M. Bachmann,[3] Elizabeth C. Wright,[4] R. Neil Dalton,[5] Jack J. Zakowski,[6] and W. Greg Miller[3]

BACKGROUND: Standardized calibration does not change a creatinine measurement procedure's susceptibility to potentially interfering substances.

METHODS: We obtained individual residual serum or plasma samples (n = 365) from patients with 19 different disease categories associated with potentially interfering substances and from healthy controls. Additional sera at 0.9 mg/dL (80 μmol/L) and 3.8 mg/dL (336 μmol/L) creatinine were supplemented with acetoacetate, acetone, ascorbate, and pyruvate. We measured samples by 4 enzymatic and 3 Jaffe commercially available procedures and by a liquid chromatography/isotope dilution/mass spectrometry measurement procedure against which biases were determined.

RESULTS: The number of instances when 3 or more results in a disease category had biases greater than the limits of acceptability was 28 of 57 (49%) for Jaffe and 14 of 76 (18%) for enzymatic procedures. For the aggregate group of 59 diabetes samples with increased β-hydroxybutyrate, glucose, or glycosylated hemoglobin (Hb A_{1c}), the enzymatic procedures had 10 biased results of 236 (4.2%) compared with 89 of 177 (50.3%) for the Jaffe procedures, and these interferences were highly procedure dependent. For supplemented sera, interferences were observed in 11 of 24 (46%) of groups for Jaffe and 8 of 32 (25%) of groups for enzymatic procedures and were different at low or high creatinine concentrations.

CONCLUSIONS: There were differences in both magnitude and direction of bias among measurement procedures, whether enzymatic or Jaffe. The influence of interfering substances was less frequent with the enzymatic procedures, but no procedure was unaffected. The details of implementation of a method principle influenced its susceptibility to potential interfering substances.

The 2006 report from the National Kidney Disease Education Program (NKDEP)[7] Laboratory Working Group (LWG) *(1)* highlighted the need for improved standardization of routine measurements of serum and plasma creatinine *(2, 3)*. Although calibration was emphasized in the report, interference-related bias was not overlooked. In recommendations for in vitro diagnostics (IVD) manufacturers, the report stated, "IVD manufacturers must address analytical nonspecificity bias in current routine serum creatinine methods."

Standardization of creatinine measurement procedures with calibrations traceable to isotope dilution mass spectrometry (IDMS) reference measurement procedures has largely been accomplished for major manufacturers in North America, as evidenced in external quality assessment schemes using commutable samples. For example, in the February 2010 College of American Pathologists (CAP) Creatinine Accuracy Calibration Verification/Linearity (LN24) Survey, 11 method groups had mean bias of 0% (range −5.8% to 7.7%) vs NIST reference measurement procedure values for creatinine concentrations between 0.77 mg/dL (68 μmol/L) and 4.09 mg/dL (362 μmol/L), and the CV for all participants (n = 372) was between 6.6% and 2.9% *(4)*.

The NKDEP LWG and the International Federation of Clinical Chemistry and Laboratory Medicine Working Group on Glomerular Filtration Rate Assessment designed the present study to obtain contemporary data for

[1] Ortho Clinical Diagnostics, Rochester, NY; [2] University of Utah, Salt Lake City, UT; [3] Virginia Commonwealth University, Richmond, VA; [4] National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Department of Health and Human Services, Bethesda, MD; [5] WellChild Laboratory, King's College London, Evelina Children's Hospital, London, UK; [6] Beckman Coulter, Inc., Brea, CA.
* Address correspondence to this author at: 40 Chipping Ridge, Fairport, NY 14450. E-mail ngreenbe@frontier.com.
Received July 5, 2011; accepted October 11, 2011.
Previously published online at DOI: 10.1373/clinchem.2011.172288

[7] Nonstandard abbreviations: NKDEP, National Kidney Disease Education Program; LWG, Laboratory Working Group; IVD, in vitro diagnostics; IDMS, isotope dilution mass spectrometry; CAP, College of American Pathologists; VCU, Virginia Commonwealth University; ARUP, Associated Regional University Pathologists; DCMP, designated comparison measurement procedure; LC-IDMS, liquid chromatography/IDMS; SRM, Standard Reference Material; Hb A_{1c}, glycosylated hemoglobin; JCTLM, Joint Committee for Traceability in Laboratory Medicine; eGFR, estimated glomerular filtration rate; IFU, instructions for use.

use in establishing specificity performance recommendations for serum creatinine measurement procedures.

Materials and Methods

SAMPLES EXAMINED

Individual patient samples. The study was approved by the respective institutional review boards at both clinical sample collection locations: Virginia Commonwealth University (VCU) and University of Utah Associated Regional University Pathologists (ARUP) laboratories.

Sample handling and storage conditions were consistent with published information on stability of creatinine in human serum or plasma (*5*). Individual patient serum or heparinized plasma samples were collected in accordance with standard laboratory practices. Residual samples submitted for laboratory testing were stored up to 8 h at room temperature then up to 14 days at 2–4 °C before division into aliquots and frozen storage at −70 °C (VCU) or up to 24 h at room temperature then up to 30 days at −20 to −30 °C before thawing, aliquoting, and refreezing at −70 °C (ARUP). Five 0.25-mL aliquots, in 1.2-mL cryovials (VWR International) were prepared from each residual sample and stored at <−70 °C until being shipped on solid CO_2 to participating manufacturers and the designated comparison measurement procedure (DCMP) laboratory. Samples were stored at <−70 °C at each participating laboratory until being measured between January and March 2009.

We selected samples to obtain up to 20 individual serum or plasma samples in each of 19 disease categories established to have either known concentrations of, or a high probability to contain, substances suspected as potential interferents in creatinine measurement procedures (*1*). Samples were not pooled, except those with increased pyruvate were obtained from pooled whole blood that was incubated at room temperature 24–48 h before centrifugation. We identified the selected samples in each category (Table 1) on the basis of previously measured laboratory values, patient location consistent with 1 or more of the selected disease categories, or medical record review. In addition, for a control group, we collected samples from 20 nondiseased individuals (laboratory staff volunteers) who were not using any prescription or over-the-counter pharmaceutical or dietary supplement products.

Samples spiked with potential interfering substances. Serum with a creatinine concentration of 0.90 mg/dL (80 μmol/L) was obtained from a healthy male volunteer, age 48 years. Half was supplemented with a solution of 150 mg/dL (13.26 mmol/L) creatinine hydrochloride (Sigma Aldrich) dissolved in water to a final creatinine concentration of 3.80 mg/dL (336 μmol/L). Candidate interfering substances were added as described in the Supplemental Data, which accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue2.

INSTRUMENTS AND MEASUREMENT PROCEDURES

Designated comparison measurement procedure. We measured serum or plasma creatinine by liquid chromatography/IDMS (LC-IDMS) based on the method described by Preiss et al. (*6*). Procedural details are provided in the online Supplemental Data.

Commercial clinical laboratory measurement procedures. Participating manufacturers and respective measurement procedures were as follows: Beckman Coulter Synchron Unicel enzymatic (E1) and Jaffe (J1), Ortho Clinical Diagnostics Vitros 5,1 FS Chemistry System enzymatic (E2), Roche Diagnostics Integra enzymatic (E3) and Jaffe (J2), and Siemens Dimension RxL enzymatic (E4) and Jaffe (J3). Characteristics of the commercial creatinine procedures, and details of the protocol for preanalytical sample handling and creatinine measurement at the participating manufacturers' laboratories, are provided in online Supplemental Table S1.

Characterization of additional analytes. Procedures for measurement of concentrations of additional analytes used for selection and characterization of patient samples were as described in Table 1, footnote a.

STATISTICAL PROCEDURES

For each commercial procedure and the DCMP, we used the mean of quadruplicate creatinine measurements on each sample for all statistical analyses and comparisons.

For samples supplemented with interfering substances, we calculated percentage recovery as the creatinine concentration at each concentration of the interferent divided by the creatinine concentration of the unspiked sample, multiplied by 100. Percentage bias was the percentage recovery minus 100. We used standard linear or polynomial regression to estimate the relationship between percentage recovery of creatinine and the concentration of an interferent.

For individual patient samples, we calculated bias as the difference from the DCMP creatinine concentration. On the basis of the distribution of biases observed for the samples from healthy controls (Fig. 1, A and B), the criteria for presence of a nonspecificity bias was defined as the larger of 0.10 mg/dL (8.8 μmol/L) or 10%. For the rationale for these criteria, see online Supplemental Data: Additional Statistical Considerations.

Table 1. Patient sample selection criteria for potential interfering substances.[a]

Category	Selection criteria	Patient samples in the category	Median creatinine, mg/dL (range)[b]
Albumin, low	Serum/plasma concentrations 1.4–4.0 mg/dL (median 2.0 mg/dL)	20 samples from 14 patients, 4 of whom had 3, 3, 2, and 2 different samples each	0.82 (0.30–5.27)
β-Hydroxybutyrate	Serum/plasma concentrations 33–103 mg/dL (median 64 mg/dL)	19 samples from 19 patients	0.89 (0.22–10.11)
Bilirubin, high	Siemens Advia index 4; serum/plasma concentrations 9.3 mg/dL for 1 sample and 15.6–37.1 mg/dL (median 32.5) for the remaining samples	20 samples from 5 patients, 2 of whom had 10 and 7 different samples each	2.23 (0.47–3.01)
Cardiovascular disease	Outpatient cardiac care clinics; with hypertension and taking 3 or more HTN/CVD medications[c]	20 samples from 20 patients	1.35 (0.56–4.48)
Cephalosporins	Patients receiving cefepime, ceftriaxone, cefazolin, cefoxitin, or cefpodoxime[d]	20 samples from 19 patients, 1 of whom had 2 different samples	0.76 (0.47–9.04)
Dialysis	Blood collected immediately before hemodialysis procedure	20 samples from 20 patients	5.38 (1.50–11.33)
Dobutamine[e]	Patients receiving dobutamine[d]	18 samples from 11 patients, 6 of whom had 3, 2, 2, 2, 2, and 2 different samples each	1.96 (0.66–2.96)
Dopamine[e]	Patients receiving dopamine[d]	11 samples from 6 patients, 3 of whom had 3, 3, and 2 different samples each	2.19 (0.55–2.61)
eGFR, low	eGFR 15–30 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$	19 samples from 18 patients, 1 of whom had 2 different samples	3.02 (2.15–4.24)
Glucose, high	Serum/plasma concentrations 388–816 mg/dL (median 455)	20 samples from 18 patients, 2 of whom had 2 different samples	1.10 (0.34–2.83)
Hb A_{1c}, high	Plasma Hb A_{1c} concentrations 8.1%–13.2% (median 8.9%); glucose concentrations 77–461 mg/dL (median 261)	20 samples from 20 patients	0.83 (0.64–2.03)
Hemolyzed	Siemens Advia index 4; approximate serum/plasma hemoglobin concentrations 350–>1000 mg/dL (median 450)	20 samples from 18 patients, 1 of whom had 2 different samples, and 1 of whom had a second sample in the high urine protein group	0.88 (0.46–8.35)
Kidney transplant	Patient status posttransplant, taking 1 or more immunosuppressant drugs (tacrolimus, sirolimus, cyclosporine, mycophenolic acid)	20 samples from 20 patients	1.31 (1.00–2.94)
Lidocaine	Serum/plasma concentrations 0.3–10.8 mg/L (median 4.3)	20 samples from 20 patients	0.79 (0.49–6.10)
Lipemic	Siemens Advia index 2–3, approximate serum/plasma triglycerides 500-1000 mg/dL based on intralipid equivalents	20 samples from 15 patients, 2 of whom had 4 and 3 different samples each	1.39 (0.21–4.99)
Protein, high	Serum/plasma concentrations 6.9–17.9 mg/dL (median 10.4)	20 samples from 20 patients	0.99 (0.51–1.88)
Protein, low	Serum/plasma concentrations 3.1–6.2 mg/dL (median 4.2)	20 samples from 20 patients	1.02 (0.34–4.72)
Protein, urine, high	Urine albumin 226 and 547 mg/L (n = 2), albumin-creatinine ratio 2983 mg/g (n = 1), urine protein between 3.1 and 21.8 g/L (n = 15)	18 samples from 15 patients, 2 of whom had 2 different samples, and 1 of whom had a second sample in the hemolyzed group	2.94 (0.97–7.95)
Pyruvate	Pooled heparinized blood at room temperature for 24–48 h, plasma concentrations 0.19–0.34 mmol/L (median 0.29)	20 different pooled samples	1.16 (0.77–2.28)

[a] Albumin, glucose, and total protein in serum/plasma samples were measured by use of a Vitros 5,1 FS Chemistry System (Ortho Clinical Diagnostics). β-Hydroxybutyrate was measured by use of Liquicolor reagent (Stanbio Laboratory) on a Cobas c501 analyzer (Roche Diagnostics). Bilirubin, hemolysis, and lipemia indexes were estimated by use of a Siemens Advia 1650 analyzer (Siemens Medical Solutions Diagnostics). Bilirubin concentrations were measured by use of the Siemens Advia procedure. Approximate hemoglobin concentrations were based on index values from Ortho Vitros and approximate triglyceride values on index values from Siemens Advia. eGFR was calculated with the IDMS traceable MDRD Study equation with creatinine measured by use of Roche enzymatic Creatinine Plus reagents and Calibrator for Automated Systems adapted to a Siemens Advia 1650 analyzer. Hb A_{1c} was measured by use of phenylboronic acid affinity HPLC procedure (Trinity Biotech), lidocaine by use of an Abbott TDx analyzer (Abbott Laboratories), urine albumin and protein by use of a Siemens Advia 1650 analyzer, and pyruvate by use of a spectrophotometric procedure with lactate dehydrogenase and NADH *(19)*.

[b] Measured by LC-ID-MS/MS.

[c] Hypertension (HTN)/cardiovascular disease (CVD) medications included diuretics, vasodilators, calcium channel blockers, β-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, 3-hydroxy-3-methylglutaryl-coenzyme A–reductase inhibitors (statins), or other cholesterol-reducing agents and platelet aggregation inhibitors.

[d] Samples were obtained during the drug-dosing interval when patients were expected to be at steady state.

[e] Two patients received both dopamine and dobutamine.

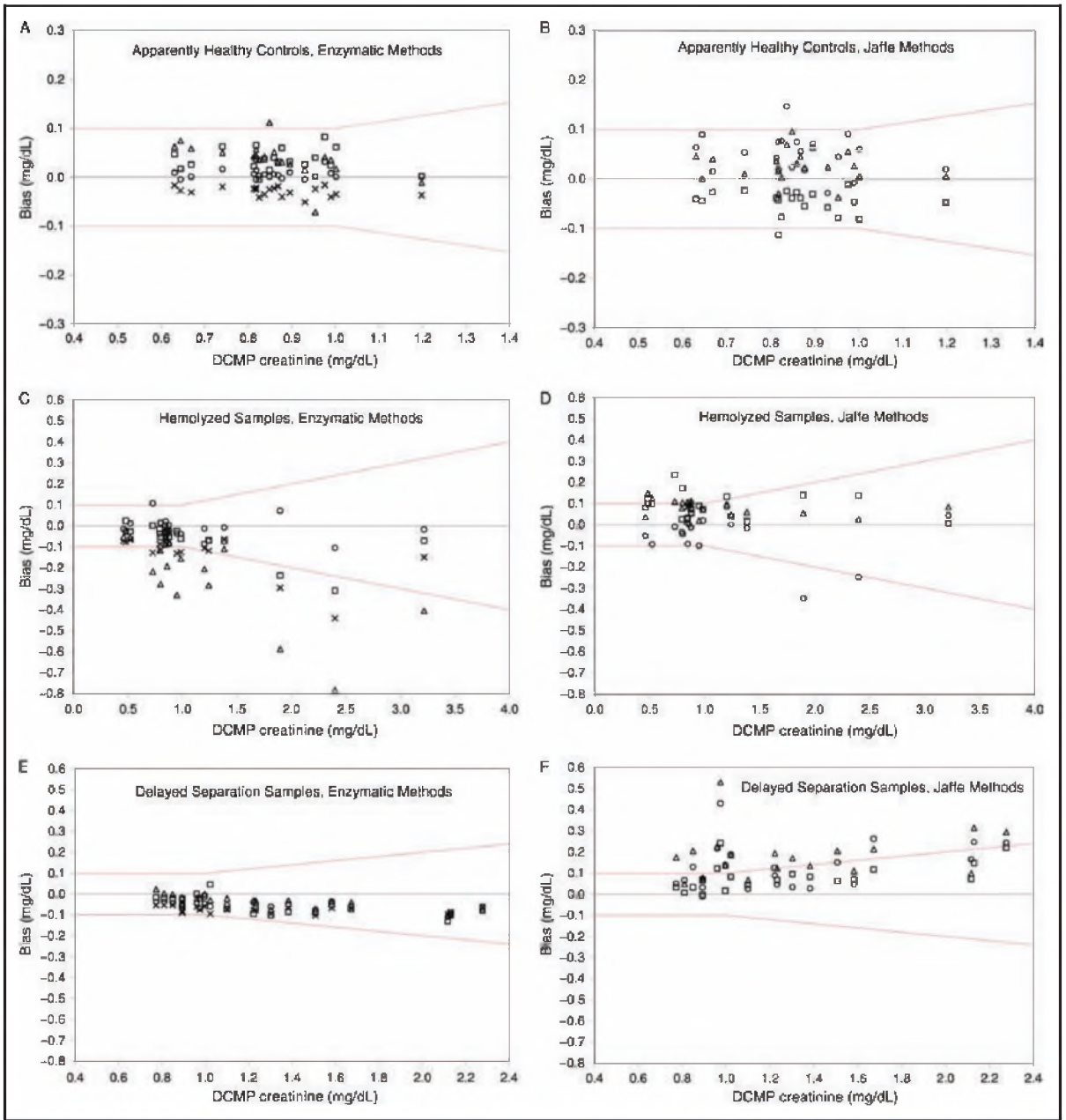

Fig. 1. Creatinine measurement bias vs DCMP for individual serum or plasma samples from the apparently healthy control group (A and B), the hemolyzed group (C and D), and the delayed separation group (E and F).

Note that 1 sample with creatinine 8.35 mg/dL is not shown in C but had biases of 0.10, −0.02, −0.28, and −0.56 for procedures E3, E4, E2, and E1, respectively, nor in panel D but had biases of 0.20, 0.20, and 0.14 mg/dL for procedures J1, J2, and J3. Symbols: △, procedure E1 or J1; □, procedure E2 or J2; ○, procedure E3 or J3; ×, procedure E4. The lines indicate the criteria used for a meaningful nonspecificity bias. Multiply by 88.4 to convert creatinine to μmol/L.

Results

CALIBRATION TRACEABILITY

We assessed trueness for each creatinine measurement procedure by recovery of the certified concentration with or without expanded uncertainty for NIST Standard Reference Material (SRM) 967 Creatinine in Frozen Human Serum *(7)* (online Supplemental Fig. S3). The DCMP method results (mean and expanded uncertainty) were 0.751 (0.029) mg/dL [66.4 (2.6) μmol/L] and 3.850

Table 2. Maximum percentage bias observed for serum supplemented with potential interfering substances.

	Creatinine measurement procedure, maximum bias, %						
Substance	E1	E2	E3	E4	J1	J2	J3
Acetoacetate 174 mg/dL							
Creatinine 0.9 mg/dL	1.9	2.1	−0.5	−0.2	122	−43.9	27.2
Creatinine 3.8 mg/dL	30.9	−0.1	−11.3	5.5	4.7	−0.6	−0.7
Acetone 100 mg/dL							
Creatinine 0.9 mg/dL	−1.9	−0.1	−0.8	−0.8	28.3	20.5	30.3
Creatinine 3.8 mg/dL	7.5	−0.8	6	8.3	2.2	0.1	−0.6
Ascorbate 20 mg/dL							
Creatinine 0.9 mg/dL	−12.4	0	−11.6	−9.6	7.7	86.1	177.4
Creatinine 3.8 mg/dL	2.5	−5.7	13.3	42.3	−5.4	−6.8	0
Pyruvate 1.2 mmol/L							
Creatinine 0.9 mg/dL	−1	0.2	0.5	−2	54.7	40.2	50.2
Creatinine 3.8 mg/dL	14.1	−0.4	7.3	11.5	0.8	−1.7	0.4

(0.081) mg/dL [340.4 (7.2) μmol/L] for the 2 concentrations, and were within the SRM uncertainty intervals. In addition, Community Bureau of Reference Certified Reference Materials 573, 574, and 575, Human Serum, were included in all runs for the DCMP. See online Supplemental Tables S2 and S3 for additional data on the calibration traceability of the DCMP.

For the commercial procedures, bias was ≤5.9% for SRM 967-1 and ≤3.8% for SRM 967-2. Four of 7 means were within the uncertainty interval of SRM 967-1 (see online Supplemental Fig. S3A). The ±2 SD ranges for 2 means overlapped the uncertainty interval, and the ±2 SD range for 1 mean (procedure E4) was just outside the uncertainty interval. For SRM 967-2 (see online Supplemental Fig. S3B), 3 of 7 means were within the uncertainty interval, the ±2 SD ranges for 3 means overlapped the uncertainty interval, and the ±2 SD range for 1 mean (procedure E1) was just outside the uncertainty interval.

For all commercial procedures, mean biases for 137 of 140 results (98%) in the healthy individuals were within 0.10 mg/dL (8.8 μmol/L) of the DCMP (Fig. 1, A and B), and the mean biases for each individual procedure ranged from −5.5% to 5.8%, further supporting calibration traceability to IDMS reference measurement procedures *(8)*. With either the mean bias with SRM 967-1 or the mean bias for healthy individuals, in conjunction with CV estimates based on either the within-procedure imprecision for SRM 967-1 obtained in this study or with the within-procedure/among-laboratories CV from the CAP Comprehensive Chemistry Survey C-B (2011), the total allowable error of all commercial procedures was within the recommendations of the NKDEP LWG *(1)* (see online Supplemental Fig. S4). Therefore, we used no corrections for calibration biases in any of the commercial procedures in the data analysis.

INTERFERING SUBSTANCES SUPPLEMENTED INTO SERUM

Owing to concerns about their stability, acetoacetate, acetone, ascorbate, and pyruvate were supplemented into serum with within–reference interval and increased creatinine concentrations. The maximum percent biases observed for each supplemented interferent at each concentration of creatinine are summarized in Table 2, and interferographs are shown in online Supplemental Data Figs. S5–S8.

INDIVIDUAL SAMPLES FROM CLINICAL CATEGORIES

Difference plots for the biases observed between routine creatinine measurement procedures and the DCMP are shown for apparently healthy controls and hemolyzed and delayed sample processing (Fig. 1); increased glucose, increased glycosylated hemoglobin (Hb A_{1c}), and increased β-hydroxybutyrate (Fig. 2); and low albumin, low total protein, and high total protein (Fig. 3) clinical sample categories. The biases observed for the other clinical sample categories are shown in online Supplemental Figs. S9–S19.

Table 3 summarizes results for all clinical sample categories by measurement procedure. For 1 or more of the Jaffe procedures, ≥3 biased creatinine values per clinical sample group were observed for the increased β-hydroxybutyrate, increased glucose, increased Hb A_{1c}, cardiovascular disease, cephalosporin, dobutamine, lidocaine, increased bilirubin, delayed sample processing, hemolyzed, lipemic, low albumin, high total protein, and post–kidney transplant groups. For 1 or more of the enzymatic procedures, ≥3 biased creat-

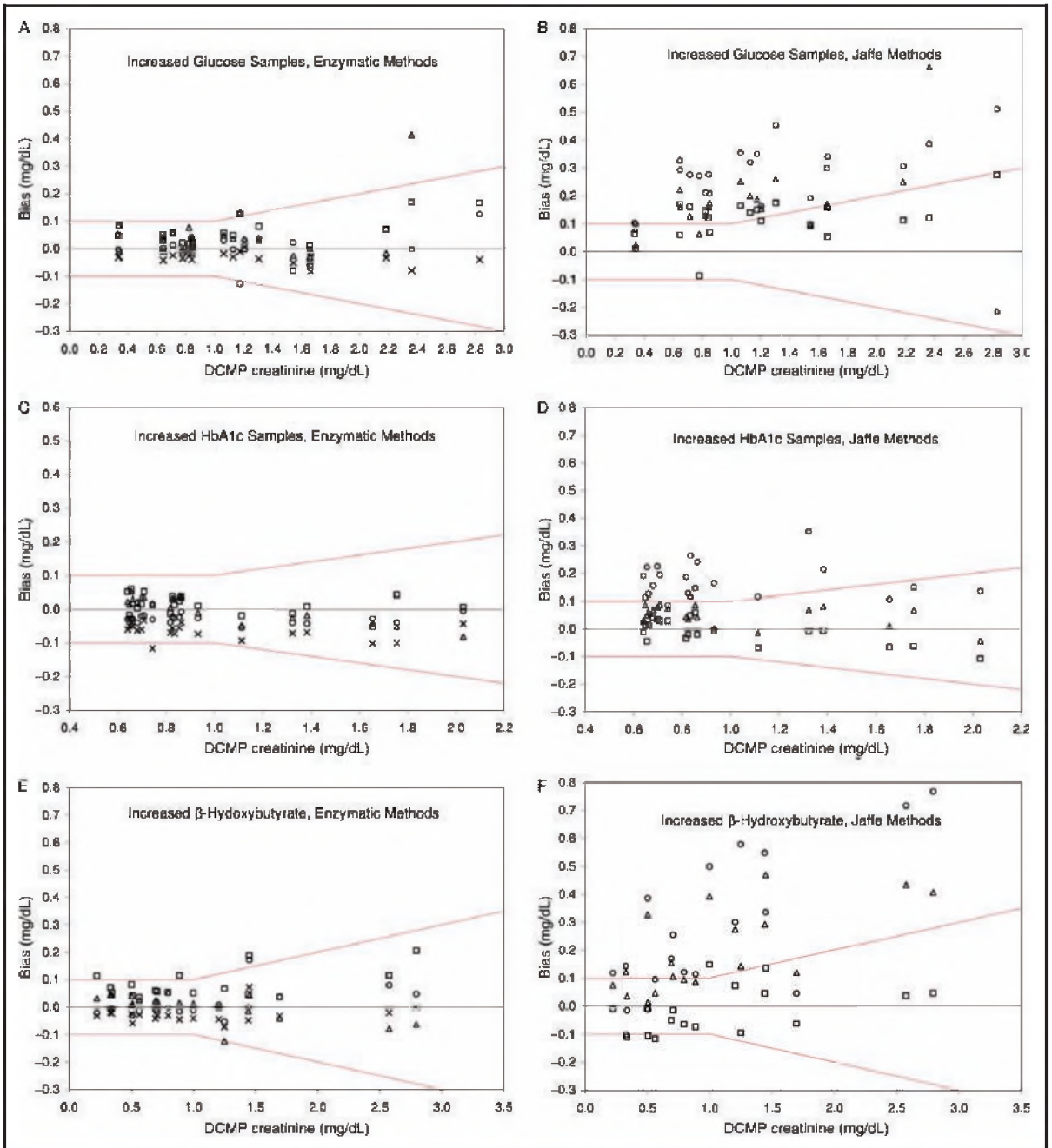

Fig. 2. Creatinine measurement bias vs DCMP for individual serum or plasma samples from the groups with increased glucose (A and B), increased Hb A_{1c} (C and D), and increased β-hydroxybutyrate (E and F).

Note that 1 sample with creatinine 10.11 mg/dL is not shown in panel E but had biases of 0.39, −0.31, −0.48, and −0.96 for procedures E2, E3, E4, and E1, respectively, nor in panel F but had biases of 0.33, 0.02, and −1.27 mg/dL for procedures J3, J1, and J2. △, procedure E1 or J1; □, procedure E2 or J2; ○, procedure E3 or J3; ×, procedure E4. The lines indicate the criteria used for a meaningful nonspecificity bias. Multiply by 88.4 to convert creatinine to μmol/L.

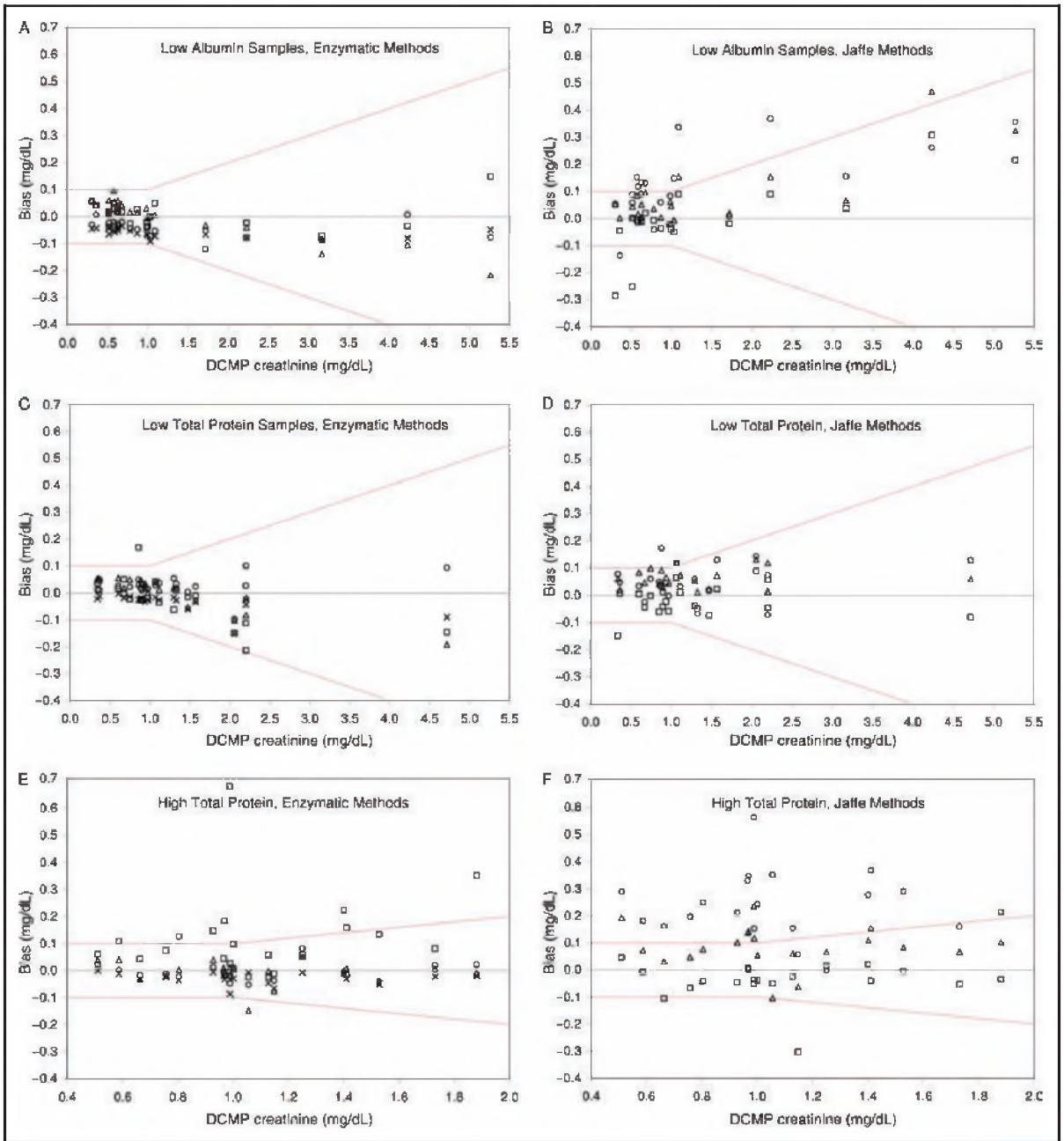

Fig. 3. Creatinine measurement bias vs DCMP for individual serum or plasma samples from the low albumin group (A and B), the low total protein group (C and D), and the high total protein group (E and F).

△, procedure E1 or J1; □, procedure E2 or J2; ○, procedure E3 or J3; ×, procedure E4. The lines indicate the criteria used for a meaningful nonspecificity bias. Multiply by 88.4 to convert creatinine to μmol/L.

inine values per clinical sample group were observed for the increased β-hydroxybutyrate, dobutamine, lidocaine, increased bilirubin, hemolyzed, lipemic, and high total protein groups. Of the 365 clinical samples, the overall proportion of biased results was 11.7%, 9.0%, 8.8%, and 11.2% for the 4 enzymatic methods and 19.5%, 15.6%, and 35.9% for the 3 Jaffe methods. Overall, more biases were observed for the Jaffe procedures, but findings were inconsistent in terms of occurrence rate, direction, and magnitude of bias (Table 3, Figs. 1–3, and online Supplemental Figs. S9–S19), within a method principle (Jaffe or enzymatic).

Table 3. Number of samples in each sample category with a negative (–) or positive (+) bias >0.10 mg/dL (>8.8 μmol/L) or >10%, whichever was greater.

		Creatinine measurement procedure													
		E1		E2		E3		E4		J1		J2		J3	
Sample category	n	–	+	–	+	–	+	–	+	–	+	–	+	–	+
Apparently healthy	20	0	1	0	0	0	0	0	0	0	0	1	0	0	1
Clinical categories	365	31	12	6	27	21	11	33	8	1	70	39	18	3	128
Diabetes mellitus															
β-Hydroxybutyrate 33–103 mg/dL	19	0	0	0	3	0	1	0	0	0	11	5	1	0	14
Glucose 388–816 mg/dL	20	1	2	0	1	1	0	0	0	0	14	0	8	0	19
Hb A_{1c} 8.1%–13.2%	20	0	0	0	0	0	0	1	0	0	1	0	0	0	16
Cardiovascular disease with hypertension	20	0	0	0	0	0	0	0	0	0	0	1	0	0	6
Drugs															
Cephalosporins	20	0	0	0	0	0	0	1	0	0	5	1	0	0	10
Dobutamine	18	3	0	1	0	2	0	2	0	0	3	0	0	0	7
Dopamine	11	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lidocaine	20	0	10	0	11	0	9	0	8	0	4	4	0	0	5
Endogenous substances															
Bilirubin 9–38 mg/dL	20	13	0	1	0	10	0	18	0	0	0	19	0	0	3
Delayed separation 24–48 h	20	0	0	0	0	0	0	0	0	0	13	0	3	0	8
Hemolysis, hemoglobin >350 mg/dL	20c	11	0	2	0	0	1	6	0	0	6	0	5	2	0
Lipemia	20	2	0	1	1	8	0	5	0	1	2	4	1	0	8
Protein abnormalities															
Albumin 1.4–4.0 g/dL	20	0	0	0	0	0	0	0	0	0	2	2	0	1	7
Protein 7–18 g/dL	20	1	0	0	8	0	0	0	0	0	7	2	0	0	17
Protein 3.1–6.2 g/dL	20	0	0	0	1	0	0	0	0	0	1	1	0	0	2
Kidney disease															
Predialysis	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
eGFR 15–30 mL · min^{-1} · $(1.73\ m^2)^{-1}$	19	0	0	1	1	0	0	0	0	0	1	0	0	0	1
Post–kidney transplant	20	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Urine protein 3–22 g/L (n = 15)	18	0	0	0	1	0	0	0	0	0	0	0	0	0	1

Discussion

Calibrations of the LC-IDMS DCMP and the commercial measurement procedures were verified to be traceable to Joint Committee for Traceability in Laboratory Medicine (JCTLM) listed reference measurement procedures and reference materials. Consequently, calibration bias of the commercial creatinine measurement procedures was not a factor in evaluating the influence of potential interfering substances.

The serum creatinine concentration influenced the magnitude of specific interferences in our supplementation studies. The interferographs, particularly for the Jaffe procedures, demonstrated substantial interferences at low creatinine concentrations but no interference at high creatinine concentrations. Nearly all of the interferences observed for the Jaffe procedures were positive, with the exception of acetoacetate for procedure J2. The magnitude of the interference observed for the Jaffe procedures was similar for acetone and pyruvate but differed markedly for acetoacetate and ascorbate.

For enzymatic procedures, the magnitude of the interference observed in the supplementation studies was generally smaller than for the Jaffe procedures, but the pattern of interference across procedures was more complicated. For acetoacetate, the interference was positive for 1 enzymatic procedure, negative for 1, and absent for the other 2. No interference was observed for

acetone with any of the enzymatic procedures. For ascorbate, 2 enzymatic procedures showed negative interference at low creatinine concentrations and 2 showed positive interference at high creatinine concentrations. Procedure E3 showed negative interference at low creatinine concentrations and positive interference at high creatinine concentrations. For pyruvate, 2 procedures showed positive interferences at high creatinine concentrations.

Cobbaert et al. *(9)* examined the effects of albumin, hemoglobin A, IgG, and unconjugated bilirubin on enzymatic and Jaffe creatinine procedures by supplementing these substances into serum pools. Those authors found positive creatinine biases from albumin and IgG for Jaffe procedures but not for enzymatic procedures. An important difference in our experimental design was that we selected individual clinical samples containing the potentially interfering substances. However, biases may have been due to presence of the substance used to select the samples or to unknown substances associated with samples in that disease category. We found that 2 of 3 Jaffe procedures exhibited a positive bias with high protein samples and a few samples had a negative bias with 1 Jaffe procedure; we also observed a positive bias with 1 enzymatic procedure. Our findings for patient samples selected for low albumin concentrations showed that 1 Jaffe procedure had a positive bias with 2 samples, 1 Jaffe procedure had a negative bias with 2 samples, and 1 Jaffe procedure had a negative bias with 1 sample and a positive bias with 7 samples. For the high total protein sample category, 1 enzymatic and 2 Jaffe procedures showed positively biased creatinine values. Although we were not able to specifically identify the interfering substances in the clinical samples, our results for samples with protein concentrations not within reference intervals demonstrated that both enzymatic and Jaffe methods were affected and that the influence of interfering substances was complex and dependent on the technical implementation of a measurement procedure.

Previously reported effects of bilirubin on enzymatic and Jaffe procedures for serum creatinine have been contradictory. Cobbaert et al. *(9)* found that enzymatic and some Jaffe procedures demonstrated similar slight positive or negative interferences with unconjugated bilirubin, whereas other Jaffe procedures exhibited >10% negative interference. Owen et al. *(10)* measured creatinine results for 73 patient samples with bilirubin concentrations of 5.8–56 mg/dL (99–958 μmol/L) using Roche enzymatic and Jaffe procedures and an LC-IDMS procedure. They found that 49% of creatinine results had −10% to −35% bias for the enzymatic procedure and, for the Jaffe procedure, 1 result had >10% bias and 2 results had <−10% bias. For the high bilirubin patient group in our study, all but 1 sample had bilirubin concentration >19 mg/dL (>325 μmol/L), and most of these samples were collected from only 3 patients. For 3 of 4 enzymatic procedures and 1 of 3 Jaffe procedures, a large portion of the samples were negatively biased. Interestingly, procedure E2 had only 1 biased result and procedure J1 had no biased results with the increased bilirubin samples. As for protein, our results demonstrated that the influence of bilirubin was complex and dependent on the technical implementation of a measurement procedure.

Delayed separation of serum from cellular components following specimen collection has been reported to cause increased creatinine results with some Jaffe creatinine procedures, likely owing to accumulation of pyruvate *(11)*. For the delayed sample separation category, all 3 Jaffe procedures showed a positive bias with variable numbers of samples. When serum pools were supplemented with pyruvate, all 3 Jaffe procedures demonstrated positive biases at a creatinine concentration of 0.9 mg/dL (80 μmol/L) but almost no bias at creatinine 3.8 mg/dL (336 μmol/L); 2 of 4 enzymatic procedures had positive biases only at the higher creatinine concentration.

Highly procedure-dependent interferences were observed for the aggregate of 3 sample categories with 59 diabetes patient samples known to have increased β-hydroxybutyrate (n = 19), glucose (n = 20), and Hb A_{1c} (n = 20). All 3 Jaffe procedures had a large number of positively biased results for the glucose category. Procedures J1 and J3 had a large number of positively biased results for the β-hydroxybutyrate category, whereas procedure J2 had a smaller number of predominantly negative biases. Procedure J3 had a large number of positive biases for the increased Hb A_{1c} category, and the other Jaffe procedures had essentially no biases. For the enzymatic procedures, there were 0–3 samples with biases in each of the diabetes sample categories, suggesting minimal or no specificity issues for these categories. Our data do not identify the root cause for a given bias (e.g., Hb A_{1c} is likely not the root cause of the bias in the increased Hb A_{1c} patient group), but these disease categories likely included additional substances that influenced some creatinine procedures. For patients with diabetes, enzymatic procedures appeared to be more suitable than Jaffe procedures.

In the cardiovascular disease group, only the J3 procedure showed positive biases. For the kidney disease patient category, none of the procedures had biased results for the sample categories predialysis, low estimated glomerular filtration rate (eGFR), and high urine protein where creatinine concentrations were higher. For the post–kidney transplant group with lower creatinine concentrations, only procedure J3 had

a few positively biased results. For the patient groups for which creatinine is an important biomarker, it appears that properly implemented enzymatic or Jaffe procedures gave satisfactory results.

Lidocaine showed large positive biases in a large number of samples for all enzymatic methods, whereas Jaffe methods had modest biases in a few samples. There was no influence from dopamine. Dobutamine showed positive biases with 2 of 3 Jaffe procedures and negative biases with all enzymatic procedures. Cephalosporin showed positive biases for 2 of 3 Jaffe procedures, with no influence on the enzymatic procedures.

REVIEW OF FINDINGS VS MANUFACTURERS' LABELING

We compared the biases for samples containing known amounts of supplemented interfering substances to interference claims obtained from each manufacturer's instructions for use (IFU). In general, the IFUs did not contain adequate information regarding the effects of interfering substances on the procedures. Acetoacetate, acetone, ascorbate, and pyruvate are well-known interferents in creatinine measurements *(12)*. Nonetheless, only 4 of 7 IFUs had interference claims for acetoacetate, none had claims for acetone, 5 had claims for ascorbate, and 2 had claims for pyruvate. Of those that did have claims for these interferences, most did not have adequate information to interpret the claims. In many cases, the IFUs did not state the concentrations of interfering substances and/or creatinine concentrations tested. In some cases in which the concentration of interferent was stated, it was substantially lower than that expected to be encountered in diseased patient populations *(12–17)*. In addition, the criteria used for evaluation of the effects of interferences were not uniform among manufacturers.

STRENGTHS AND LIMITATIONS

Strengths of this investigation were inclusion of a substantial number of individual patient samples representing diverse clinical conditions selected to have a high probability to contain potentially interfering substances and a control group of samples from healthy individuals. This approach eliminated any noncommutability artifacts from influencing results and included a range of both exogenous pharmaceutical and endogenous metabolic substances. Several labile metabolic substances were examined by supplementing a single donor serum to ensure the substances were present in the samples tested. The LC-IDMS DCMP and the commercial measurement procedures were validated to have calibration traceable to JCTLM listed reference measurement procedures. IDMS technology is considered the best available to be free from the influence of interfering substances. All measurements were made in quadruplicate to minimize the influence of measurement imprecision.

Limitations of this study were that we were unable to include all manufacturers because of limited volumes available (as residual samples from clinical laboratories). The clinical samples were selected to have a high probability to contain various interfering substances; however, the identity and concentrations of the substances responsible for a given interference were either unknown or only partially known based on the selection parameters. In addition, the number of samples included in each clinical category was relatively small (approximately 20), and in some cases, different samples from the same individual were included more than once in a clinical category. The clinical samples were not handled uniformly before aliquoting and freezing, with variable time spent at room temperature, refrigerated, or frozen, with possible metabolic changes or loss of labile components (e.g., dopamine and dobutamine). In addition, samples collected at ARUP were thawed, aliquoted, and refrozen. Given previously published findings that creatinine in serum or plasma is a stable measurand under common sample storage conditions *(5)*, it is unlikely that sample handling or storage contributed to underestimation of creatinine in this study. However, the consequence of sample handling and storage variations on substances that may interfere with creatinine measurements is unknown, and may have led to underestimation of bias in certain disease categories. Finally, the serum creatinine concentration interval examined in the patient samples selected for this study was inadequate to address measurement specificity issues at creatinine concentrations typically found in pediatric patients between the ages of 2 months and 10 years, in whom values are usually $<$0.6 mg/dL (50 μmol/L) *(18)*.

Conclusions

Overall, the influence of interfering substances was less frequent with enzymatic procedures than with Jaffe procedures, but no procedure was unaffected by the interfering substances or disease categories examined. There were differences in both magnitude and direction of bias among measurement procedures within a given method principle, enzymatic or Jaffe, indicating that influence of interfering substances depended on details of implementation of the method principle. With the exception of the diabetic disease category, which showed substantially more frequent influence of interfering substances with Jaffe procedures than with enzymatic procedures, no general conclusions regarding Jaffe or enzymatic technologies can be drawn. Supplemented interferents had greater influence at creatinine concentrations within reference intervals than at

higher concentrations, highlighting the importance of evaluating interference at more than 1 concentration of the measurand. The results emphasize the need to evaluate interference characteristics in detail with each particular measurement procedure with consideration of the patient populations served.

Manufacturers' labeling and claims for interferences with commercial creatinine measurement procedures had shortcomings in the information provided. It is recommended that manufacturers' claims be based on testing at clinically relevant concentrations of a broad range of potential interferents as well as at 2 or more concentrations of the measurand.

On the basis of the magnitude of biases observed in the healthy controls category, it is recommended that specifications for bias from interfering substances in creatinine measurement procedures should not exceed the larger of 0.1 mg/dL or 10% at a given concentration of creatinine.

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

Employment or Leadership: N. Greenberg, Ortho Clinical Diagnostics, Inc., a Johnson & Johnson Company; R.N. Dalton, SpOtOn Clinical Diagnostics Ltd.; J.J. Zakowski, CLSI; W.G. Miller, *Clinical Chemistry*, AACC, and CLSI.
Consultant or Advisory Role: None declared.
Stock Ownership: N. Greenberg, Johnson & Johnson; R.N. Dalton, SpOtOn Clinical Diagnostics Ltd.; J.J. Zakowski, Beckman Coulter, Inc.
Honoraria: R.N. Dalton, AstraZeneca.
Research Funding: W.L. Roberts, ARUP Labs (not related to this report); W.G. Miller (Principal Investigator), subcontract from the University of Pennsylvania (2-U01-DK-060990-08) funded by the NIDDK, NIH, and coordinated by the National Kidney Disease Education Program.
Expert Testimony: None declared.
Other Remuneration: R.N. Dalton, Quintiles Ltd.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: We thank NIST for providing SRM 967 Creatinine in Frozen Human Serum, used as a control in all assay runs. We thank the participating manufacturers for their support by performing the measurements in their laboratories, and we especially thank A. Hawayek and S. Wolf at Beckman Coulter; R. Brookes, S. Nealon-Merulla, and A. Tweedie at Ortho Clinical Diagnostics; M. Swartzentruber, B. Wiler, and F. Stubbe at Roche Diagnostics; and S.T. Salyer at Siemens Healthcare Diagnostics for their efforts in managing the analytical studies performed at their respective company laboratories. We also thank P. Vo at VCU and J. Hunsaker at ARUP Laboratories for their assistance with collecting residual patient samples.

References

1. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for improving serum creatinine measurement: a report of the National Kidney Disease Education Program Lab Working Group. Clin Chem 2006;52:5–18.
2. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461–70.
3. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F. Expressing the Modification of Diet in Renal Disease study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007;53:766–72.
4. College of American Pathologists creatinine accuracy calibration verification/linearity survey, 2010 LN24-A participant summary. Northfield (IL): College of American Pathologists.
5. Donnelly JG, Soldin SJ, Nealon DA, Hicks JM. Stability of twenty-five analytes in human serum at 22 degrees C, 4 degrees C, and −20 degrees C. Pediatr Pathol Lab Med 1995;15:869–74.
6. Preiss DJ, Godber IM, Lamb EJ, Dalton RN, Gunn IR. The influence of a cooked-meat meal on estimated glomerular filtration rate. Ann Clin Biochem 2007;44:35–42.
7. Dodder NG, Tai SS-C, Sniegoski LT, Zhang NF, Welch MJ. Certification of creatinine in a human serum reference material by GC-MS and LC-MS. Clin Chem 2007;53:1694–9.
8. Joint Committee for Traceability in Laboratory Medicine (JCTLM) Database of Higher-order Reference Materials, Measurements methods/procedures and services. http://www.bipm.org/jctlm/ (Accessed 13 Dec 2010).
9. Cobbaert CM, Baadenjuijsen H, Weykamp CW. Prime time for enzymatic creatinine methods in pediatrics. Clin Chem 2009;55:549–58.
10. Owen LJ, Keevil BG. Does bilirubin cause interference in Roche creatinine methods? Clin Chem 2007;53:370–1.
11. Shepherd J, Warner MH, Kilpatrick ES. Stability of creatinine with delayed separation of whole blood and implications for eGFR. Ann Clin Biochem 2007;44:384–7.
12. Gerard SK, Khayam-Bashi H. Characterization of creatinine error in ketotic patients. AJCP 1985; 84:659–64.
13. Weber JA, Van Zanten AP. Interferences in current methods for measurements of creatinine. Clin Chem 1991;37:695–700.
14. Bowers LD. Kinetic serum creatinine assays I. The role of various factors in determining specificity. Clin Chem 1980;26:551–4.
15. Bowers LD, Wong ET. Kinetic serum creatinine assays II. A critical evaluation and review. Clin Chem 1980;26:555–61.
16. CLSI. Interference testing in clinical chemistry: approved guideline—second edition. CLSI document EP7-A2. Wayne (PA): CLSI; 2005.
17. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: WB Saunders; 1999. p 790.
18. Ceriotti F, Boyd JC, Klein G, Henny J, Queralto J, Kairisto V, Panteghini M, on behalf of the IFCC Committee on Reference Intervals and Decision Limits (C-RIDL). Reference intervals for serum creatinine concentrations: assessment of available data for global application. Clin Chem 2008;54: 559–66.