Exogenous Interferences with Jaffe Creatinine Assays: Addition of Sodium Dodecyl Sulfate to Reagent Eliminates Bilirubin and Total Protein Interference with Jaffe Methods

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Background: The study evaluated the impact of interferences on the analytical specificity of three commercial and commonly used creatinine methods (two Jaffe and one enzymatic). **Methods:** Manufacturer creatinine methods plus modified methods were tested with the following interferences: spiking serum with bilirubin, albumin, glucose, hemoglobin and lipid, and patient sera with maximum concentrations of bilirubin, 1,090 $\mu\text{mol/l}$ and protein, 117.8 g/l.

Results: Hemoglobin, 7.5 g/l and lipaemic with triglyceride concentration of 6.27 mmol/l, did not interfere with all assays. Glucose >33.3 mmol/l increased creatinine recovery for Dimension method. Samples spiked with bilirubin imparted a negative bias for Dimension and Architect methods but imparted a positive bias for Vitros assay. However, using patient sera, negative bias with bilirubin was found for all methods, from which Architect method gave the highest effect ($R^2 = 0.861$),

followed by Vitros ($R^2 = 0.239$) and Dimension ($R^2 = 0.163$). Protein provided the positive bias for all creatinine measurements that increased with increasing concentration (R^2 ranging from 0.104 to 0.182, $P < 0.0001$). Addition of sodium dodecyl sulfate (SDS) in alkaline-picrate reagent reduced the effect of bilirubin and protein for kinetic Jaffe method. Although adding potassium ferricyanide was well effective for eliminating negative interference of bilirubin, it was prone to interference from protein.

Conclusions: Endogenous interferences continue to plague creatinine accuracy measurement in both Jaffe and enzymatic methods, and consequentially the estimated glomerular filtration rate. The addition of SDS to the alkaline-picrate reagent was shown to be effective in reducing bilirubin and protein interferences. J. Clin. Lab. Anal. 24:123–133, 2010. © 2009 Wiley-Liss, Inc.

Key words: kinetic Jaffe method; enzymatic method; analytical performance; commercial; glomerular filtration rate

INTRODUCTION

Glomerular filtration rate (GFR) is considered to be the best indicator of kidney function (1). It is widely acceptable that estimations of GFR can be obtained with equations, which are based on serum creatinine along with the readily available demographic variables including age, gender, weight, and race (2–5). International professional organizations have recommended that an estimated GFR based on the Modification of Diet in Renal Disease (MDRD) formula be calculated with every request for measurement of plasma creatinine concentration in adult patients (6–8).

The reliability of estimated GFR depends critically upon the accuracy and precision of the serum creatinine measurement used in the calculation. The impact of bias and imprecision of creatinine measurement are

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significant at creatinine concentrations less than $133\text{ }\mu\text{mol/l}$ or 1.5 mg/dl , which corresponds to GFR values near a cut-off threshold ($60\text{ ml/min/1.73 m}^2$) for identifying kidney disease. To improve the accuracy of MDRD equation, a Laboratory Working Group of National Kidney Disease Education Program has published the recommendations for the measurement of serum creatinine (6). All creatinine methods should be traceable to a reference method based on isotope dilution-mass spectrometry (IDMS) for meeting the minimum total error goal ($<11.4\%$).

The Jaffe method is one of the oldest methods for creatinine determination and was first published in 1886 (9) and has remained in use today with various modifications. Creatinine may be measured enzymatically as well with creatine amidohydrolase/sarcosine oxidase (10), or creatinine iminohydrolase/glutamate dehydrogenase (11). It has been known that the modified Jaffe methods are hampered by interferences as up to 25% by noncreatinine chromogens, especially bilirubin (12), protein (13), and ketones (14). The error from these interferences may compound differences in concordance using estimated GFR (6,15).

Several approaches have been used to minimize nonspecific interferences, such as deproteinization for eliminating the pseudo-chromogen effect of protein (16), early read reaction times (17), addition of some chemicals in reagent systems (18,19), rate-blanking measurement (20), higher temperatures (21), or use of single value compensation for noncreatinine Jaffe reacting chromogens (22). Using the enzymatic creatinine assays, although often recommended as the method of choice, they can have their own interference problems (23).

This study evaluated and compared the analytical specificity from interferences commonly found in serum for the creatinine measurement obtained from three different commercially available assays using the kinetic Jaffe method [Dimension RxL Max (Siemens Medical Solution Diagnostics, Tarrytown, NY), Architect ci8200 (Abbott Diagnosis, Abbott Park, IL)], and the enzymatic method (Vitros 350, Ortho-Clinical Diagnostics, Rochester, NY). Addition of SDS or potassium ferricyanide in the reagent system was also studied for reducing the effect of interferences, particularly bilirubin and protein in creatinine Jaffe method.

MATERIALS AND METHODS

The study for evaluating the susceptibility of the method to interfering substances followed the CLSI protocol EP7-A (24). We used two types of samples to study the effects of potentially interfering substances,

adding the interfering substance to a serum pool or to individual patient specimens.

Assays

All the three methods, the Dimension Max (creatinine Flex #DF33A), the Architect ci8200 (Creatinine #7D64) creatinine assays are kinetic Jaffe reactions and the Vitros 350 sarcosine oxidase enzymatic assay (Creatinine slide #8141947) are traceable to the IDMS reference materials via NIST 914 certified reference material.

Method Comparison

In total, 99 fresh samples with no visible hemolysis, lipaemic, or icteric were analyzed on the three methods. The statistical analysis included the regression equations, correlation coefficients, bias, and the standard deviation of the residuals ($S_{y/x}$) values obtained between each kinetic Jaffe method and the enzymatic method were calculated.

Effect of Potentially Interfering Substances Added to Specimens

We prepared the interference samples by spiking serum with bilirubin, human albumin, glucose, hemoglobin, and lipaemic serum. The stock solutions of each potential interference were prepared as follows:

A stock hemoglobin solution of 15 g/l from lysis of clotted red cells was prepared by centrifugation of 10 ml of clotted blood at $1,500\text{ rpm}$ for 10 min . The serum was separated and red blood cells were washed three times with normal saline (0.9% NaCl). We added an equal volume of distilled water and shock vigorously, centrifuged at $1,500\text{ rpm}$ for 10 min , aspirated the clear erythrocyte hemolysate, and adjusted with distilled water to obtain a hemoglobin concentration of 15 g/l .

The icteric samples were prepared from purified bilirubin (Product #B-4126, Sigma Chemical Company, St. Louis, MO) in bovine serum albumin, 40 g/l to obtain bilirubin concentration ranging from 0 to $1,368\text{ }\mu\text{mol/l}$.

The lipaemic sample pool with triglyceride concentration of 14.64 mmol/l was prepared from milky sera and diluted the pool with NaCl, 0.9% , to obtain the various triglyceride concentrations ranging from 0 to 14.64 mmol/l .

For studying the interfering effects of protein and glucose concentrations, we prepared the set of samples by addition of varying amounts of human albumin (Product #A-1653, Sigma) and glucose [Product #10117, BDH (Chemicals) Ltd., UK] to 0.1 mol/l , phosphate buffered, pH 7.4, to give concentrations ranging from 0 to 240 g/l and 0 to 88.8 mmol/l , respectively.

A 1:2 dilution of each interference sample with pooled serum of three creatinine levels (160, 280, and 560 $\mu\text{mol/l}$) was made to obtain three sets of varying degrees of five interfering substances. All samples were analyzed in triplicate by the three different creatinine methods.

Effect of Potentially Interfering Substances from Patient Sera

To study the interference of bilirubin and protein in human serum, the patient sera with total bilirubin concentrations ranging from 1.0 to 1,090 $\mu\text{mol/l}$ ($n = 174$) and total protein concentrations ranging from 25.0 to 117.8 g/l ($n = 165$) were also tested.

The deproteinization of patient sera was performed using trichloroacetic acid at the final concentration of 0.37 mol/l (16). Apart from reducing the effect of protein interference, this technique also removed protein-bound interfering substances such as bilirubin. Our previous study (16) demonstrated that deproteinization of sample prior to analysis was the best approach to eliminate bilirubin for serum creatinine by the kinetic Jaffe reaction.

We mixed a sample with trichloroacetic acid (Product #100807, Merck, E. Merck, Darmstadt, Germany), 0.55 mol/l at the ratio of 2:1 and allowed to stand for 10 min, then centrifuged at 1,200 g for 10 min. The supernatant of each sample was removed and subjected to creatinine analysis and the creatinine value was multiplied with the dilution factor (1.5). All patient sera and their deproteinized samples were analyzed by the three different creatinine methods. The creatinine value in each patient sample was compared separately with the result obtained from the deproteinized sample before the final analysis. Note that the Vitros 350 creatinine assay did not report a quantitative result of creatinine concentration in deproteinized samples, reporting results as less than 4 $\mu\text{mol/l}$ and flagging the error as "OR" (outside range). The acidification might inhibit the enzyme reactions (creatinine amidohydrolase, creatine amidinohydrolase, sarcosine oxidase, and peroxidase) used in this assay. Thus, we selected to use the precipitation method on the Architect ci8200 creatinine assay as the comparative method for the creatinine measurement on Vitros 350 analyzer.

Effect of Potassium Ferricyanide and Sodium Dodecyl Sulfate (SDS) on Jaffe Method

A modified alkaline phosphate buffer solution (R1) following Glick (25) with an absence of SDS was prepared. The two working alkaline buffer reagents were prepared with the addition of potassium ferricyanide

(Art. #4976, Merck), 0.13 mol/l (R2) and SDS (Product #L-4509, Sigma), 20 g/l/detergent (R3) into the reagent R1. Three alkaline picrate reagents were prepared by mixing each alkaline buffer reagent (R1, R2, and R3) with saturated picrate reagent (Art. #623, Merck), 60 mmol/l in proportion 4:1 by volume.

We performed the experimental creatinine user-defined methods by using each in-house alkaline picrate reagent. The parameter of the Dimension Max analyzer was set as follows: Reaction Mode (Rate), Test/Blank wavelength (510/577 nm), Sample/chase volume (30/10 μl), Reagent/chase volume (300/40 μl). The absorbance was read at 20 (P1), 50 (P2), and 80 sec (P3) after the sample addition. Using the Architect ci8200 analyzer, the parameter was set as follow: Reaction Mode (Rate up), Test/Blank wavelength (500/572 nm), Main read (3–6), Last read (15), Sample volume (30 μl), Reagent volume (300 μl).

Calibrators

We used the serum-based calibrators (Multiconstituent Calibrator, Product #1E65-03 E. Abbott Diagnostics) with creatinine concentrations of 92.8 $\mu\text{mol/l}$ and 434.9 $\mu\text{mol/l}$ to calibrate the user-defined creatinine methods on Dimension Max and Architect ci8200.

STATISTIC ANALYSIS

Statistical analysis was carried out using the Statistical Program for Social Sciences (version 12.0 SPSS Inc., Chicago, IL). The relationship between bias and concentration was tested using linear regression analysis. The results were regarded as statistically significant for a P value < 0.05 . Multiple regression analysis of interferences was performed using StatMost for Windows (version 3.5, Dataxiom Software Inc., Los Angeles, CA).

RESULTS

Comparison of Creatinine Methods

We compared the creatinine concentrations measured with the Dimension Max (Fig. 1A) and Architect ci8200 (Fig. 1B) kinetic Jaffe methods with the Vitros 350 enzymatic method by least-square linear regression analysis. The regression statistics obtained between the Dimension Max or Architect ci8200 assays (y) and the Vitros 350 assay (x) are tabulated as shown in Table 1. The mean difference reported from the Architect ci8200 method ($-8.05 \mu\text{mol/l}$) was larger than that from the Dimension Max assay ($0.93 \mu\text{mol/l}$). In addition, paired t -test shows a significant mean difference ($P < 0.0001$)

between the Vitros 350 and Architect ci8200 methods but no significant difference ($P = 0.455$) when compared with the Dimension Max method.

Effect of Potentially Interfering Substances

The interfering effect of bilirubin, hemoglobin, and lipid on the creatinine assays was demonstrated in Table 2. Substances altering results $>11.4\%$ (the minimum total error goal for creatinine determination) (6), higher or lower, than the original creatinine concentration were considered to show significant interference.

Samples spiked with bilirubin greater than 171 and 340 $\mu\text{mol/l}$ imparted a negative bias for Architect ci8200

and Dimension RxL Max methods, respectively, but imparted a positive bias for Vitros 350 assay when bilirubin was more than 171 $\mu\text{mol/l}$. Hemoglobin concentration, as high as 7.5 g/l, did not interfere with all assays. Lipaemic sample with triglyceride concentrations greater than 6.27 and 7.32 mmol/l gave a positive bias for Dimension RxL Max and Architect ci8200 methods, respectively. Triglyceride concentrations up to 7.32 mmol/l did not interfere with Vitros assay. Glucose more than 33.3 mmol/l increased creatinine recovery only for the Dimension Max method as shown in Table 3. For protein interference, the spiked samples greater than 90, 90, and 120 g/l gave a significant positive bias for Dimension Max, Architect ci8200, and Vitros 350 methods, respectively.

To minimize the effect of different forms of human serum bilirubin and protein, we also performed creatinine analysis in patient sera with maximum concentrations of bilirubin, (1,090 $\mu\text{mol/l}$) and protein (117.8 g/l). The bias plots, difference in creatinine concentration obtained between the nonprotein precipitate (NPPT) sample, and the same patient sera with protein precipitate (PPT) vs. bilirubin concentration are shown in Figure 2. All experimental assays showed a significant effect of bilirubin concentration on their creatinine measurements ($P < 0.0001$). It was found that the Architect ci8200 method (Fig. 2B) provided the highest effect ($R^2 = 0.861$), followed by the Vitros 350 assays ($R^2 = 0.239$, Fig. 2C) and the Dimension Max ($R^2 = 0.163$, Fig. 2A). When we divided the data based on bilirubin concentration less than 700 $\mu\text{mol/l}$, a weak but significant concentration bias effect were apparent for the Dimension Max ($R^2 = 0.038$, $P = 0.012$) and Vitros 350 assays ($R^2 = 0.041$, $P = 0.008$). In addition, we also observed a constant positive bias between NPPT and PPT when using Dimension Max assay (Fig. 2A).

The bias plots, vs. serum protein concentration, are illustrated in Figure 3. All experimental creatinine assays provided the positive bias that increased with increasing protein concentration (R^2 ranging from 0.104 to 0.182, $P < 0.0001$). Dimension Max assay gave the highest mean bias (23.54 $\mu\text{mol/l}$, Fig. 3A), followed by the Vitros 350 (7.47 $\mu\text{mol/l}$, Fig. 3C) and the Architect ci8200 methods (−1.01 $\mu\text{mol/l}$, Fig. 3B). Although Architect ci8200 method used the kinetic Jaffe reaction

Fig. 1. Correlation of serum creatinine obtained from the Dimension Max (A) and Architect ci8200 (B) kinetic Jaffe methods with the Vitros 350 enzymatic method.

TABLE 1. Regression Analyses Between the Dimension RxL Max, the Architect ci8200 Assays (y), and the Vitros 350 method (x)

Method	R^2	$S_{y/x}$	Bias ($\mu\text{mol/l}$)	Coefficients		P -value
				Slope (95% CI)	Intercept (95% CI)	
Dimension RxL	0.994	12.19	0.93	1.015 (0.999 to 1.032)	−1.043 (−4.266 to 2.180)	<0.0001
Architect ci8200	0.996	9.18	−8.05	0.936 (0.924 to 0.949)	0.206 (−2.22 to 2.63)	<0.0001

TABLE 2. Interfering Effect of Sera with Abnormal Appearance on Creatinine Determination

		Creatinine concentration (μmol/l) kinetic Jaffe		
Degree interferent ^a		Dimension Max	Architect ci8200	Enzymatic Vitros 350
Icteric	Bilirubin (μmol/l)			
Baseline	0	87	82	88
±	85.5	84	70 ^b	92
1+	171.0	76	61	97
2+	343.0	65 ^b	42	104 ^b
3+	514.0	58	24	109
4+	686.0	44	14	108
Baseline	0	145	137	145
±	85.5	142	127	154
1+	171.0	136	114 ^b	164 ^b
2+	343.0	124 ^b	94	177
3+	514.0	114	75	180
4+	686.0	99	61	184
Baseline	0	284	272	278
±	85.5	287	259	302
1+	171.0	280	249	328 ^b
2+	343.0	279	232 ^b	352
3+	514.0	272	207	364
4+	686.0	247 ^b	191	369
Hemolysis	Hemoglobin (g/l)			
Baseline	0	73	80	83
±	0.1	72	79	85
1+	0.9	68	80	83
2+	1.9	66	78	82
3+	3.8	69	79	80
4+	7.5	79	81	82
Baseline	0	143	137	136
±	0.1	140	135	135
1+	0.9	137	135	137
2+	1.9	136	136	137
3+	3.8	138	136	134
4+	7.5	144	139	134
Baseline	0	284	277	268
±	0.1	278	275	263
1+	0.9	280	273	262
2+	1.9	286	273	257
3+	3.8	283	274	260
4+	7.5	295	279	262
Lipaemic	Triglyceride (mmol/l)			
Baseline	0.67	84	80	92
±	3.10	82	85	97
1+	4.14	90	93	100
2+	5.14	101 ^b	96 ^b	102
3+	6.27	107	99	106
4+	7.32	117	104	111
Baseline	0.67	144	138	148
±	3.10	144	139	147
1+	4.14	153	143	150
2+	5.14	159	149	153
3+	6.27	164 ^b	155 ^b	158
4+	7.32	171	158	160
Baseline	0.67	289	280	273
±	3.10	298	283	270
1+	4.14	297	290	270
2+	5.14	309	294	273
3+	6.27	306	295	275
4+	7.32	322 ^b	300	278

^aHemoglobin values are given in System International (SI) units: to convert to conventional units (g/dL), divide by 10; bilirubin values given in SI units: to convert to conventional units (mg/dL), multiply by 0.058.

^bStarting significant negative interference (>11.4% decreasing from baseline level) (6).

TABLE 3. Interfering Effect of Protein and Glucose Concentrations on Creatinine Determination

		Creatinine concentration (μmol/l) kinetic Jaffe		
Substance ^a		Dimension Max	Architect ci8200	Enzymatic Vitros 350
<i>Protein (g/l)</i>				
30		83	77	81
60		97	89	84
90		115 ^b	101 ^b	90
120		126	115	94 ^b
150		143	127	99
30		143	133	135
60		158	144	139
90		178 ^b	161 ^b	146
120		190	172	156 ^b
150		200	186	162
30		281	268	267
60		296	278	280
90		315 ^b	289 ^b	290
120		331	302	310 ^b
150		346	318	329
<i>Glucose (mmol/l)</i>				
0		83	77	82
11.1		87	81	83
22.2		90	85	82
33.3		93 ^b	86	82
44.4		99	90	81
0		141	133	140
11.1		143	135	138
22.2		149	140	139
33.3		153	144	140
44.4		161 ^b	147	139
0		285	267	270
11.1		293	268	273
22.2		292	276	272
33.3		302	281	272
44.4		310 ^b	285	274

^aProtein values are given in SI units: to convert to conventional units (g/dl), divide by 10; glucose values given in SI units: to convert to conventional units (mg/dl), multiply by 5.55.

^bStarting significant positive interference (>11.4% increasing from baseline level) (6).

as similar as the Dimension Max method, the protein precipitation technique did not appear to affect creatinine measurement.

Analyte-Dependent Interference

Table 4 tabulates the multiple regression analysis with each commercial creatinine concentration as the dependent variable and with the bilirubin or protein concentrations as independent variable. We found analyte-dependent interference by bilirubin and protein with the Vitros method for creatinine. The general formula is $\text{Vitros creatinine} = 0.5 - 0.015[\text{Protein}] + 0.93[\text{Creatinine}] + 0.002[\text{Protein}][\text{Creatinine}]$, $R^2 = 0.99$.

Fig. 2. Bias plot of serum creatinine concentration between the nonprotein precipitate sample (NPPT) and the deproteinized with trichloroacetic acid sample (PPT) using Dimension Max (A), Architect ci8200 (B), and Vitros 350 method (C) (Y-axis) vs. serum bilirubin concentration (X-axis). Because PPT sample was not appropriate for analyzing by Vitros 350 method, the PPT creatinine value was obtained from the Architect ci8200 method.

The second general formula is Vitros creatinine = $-4.1 - 0.017[\text{Bilirubin}] + 1.07[\text{Creatinine}] + 0.0005[\text{Bilirubin}][\text{Creatinine}]$, $R^2 = 0.99$.

We did not observe analyte-dependent interference by bilirubin or protein with the Dimension and Architect methodologies.

Effect of Potassium Ferricyanide and SDS on Kinetic Jaffe Method

Figure 4 illustrates the bias plots vs. bilirubin concentration up to $795 \mu\text{mol/L}$ ($n = 129$) by using three modified alkaline picrate reagents (with and without the addition of potassium ferricyanide or SDS) on the Dimension Max and the Architect ci8200 analyzers. Without the addition of potassium ferricyanide and SDS, a greater bilirubin concentration effect was seen with the assays on both Dimension Max (Fig. 4A) and Architect ci8200 (Fig. 4B) analyzers. Bilirubin imparted

Fig. 3. Bias plot of serum creatinine concentration between the nonprotein precipitate sample (NPPT) and the deproteinized with trichloroacetic acid sample (PPT) using Dimension Max (A), Architect ci8200 (B), and Vitros 350 method (C) (Y-axis) vs. serum protein concentration (X-axis).

a significant negative bias for these creatinine determinations (mean bias of -11.30 and $-13.37 \mu\text{mol/L}$, respectively), which significantly increased with increasing concentrations ($R^2 = 0.765$, $P < 0.0001$ and $R^2 = 0.80$, $P < 0.0001$, respectively). These interfering patterns were similar to the Architect ci8200 commercial assay (Fig. 2B). Using the reagent with the addition of potassium ferricyanide (Fig. 4C, D), the interfering effect of bilirubin concentration was significant ($P < 0.001$ and $R^2 = 0.081$, 0.1032 for Dimension Max and Architect ci8200 methods, respectively), but less of an effect than using the reagent without ferricyanide (Fig. 4A, B); however, they showed greater mean biases, 14.43 and $15.92 \mu\text{mol/L}$, respectively. For the assay using the reagent with the addition of SDS (Fig. 4E, F), the interfering effect was not significant ($P = 0.864$, $R^2 = 0.0002$) for Dimension Max but was slightly significant ($P = 0.001$, $R^2 = 0.079$) for Architect ci8200 methods. The mean biases obtained from the Dimension Max and the Architect ci8200 analyzers were -4.02 and $-0.34 \mu\text{mol/L}$, respectively.

TABLE 4. Multiple Regression Parameters for Bilirubin or Protein Interferences in Three Commercial Creatinine Assays^a

Variable	Regression parameters kinetic Jaffe								
	Dimension Max			Architect ci8200			Enzymatic Vitros 350		
	β^b	<i>t</i> -value	<i>P</i> -value	β	<i>t</i> -value	<i>P</i> -value	β	<i>t</i> -value	<i>P</i> -value
<i>Independent: Bilirubin</i>									
β_0 , Constant	-1.705	-0.435	0.67	-10.191	-0.514	0.62	-4.136	-0.558	0.586
β_1 , [Bilirubin]	-0.073	-7.152	<0.001	-0.104	-2.014	0.06	-0.017	-0.874	0.397
β_2 , [Creatinine]	1.026	49.940	<0.001	1.073	9.856	<0.001	1.07	27.14	<0.001
β_3 , [Bilirubin]*[Creatinine]	7.718	1.447	0.17	-1.752	-0.062	0.95	0.0005	5.099	<0.001
R_{adj}^2 ^c	0.998			0.940			0.990		
<i>Independent: Protein</i>									
β_0 , Constant	-12.809	-4.364	0.001	-12.873	-5.064	<0.001	0.500	0.147	0.886
β_1 , [Protein]	0.46	15.593	<0.001	0.439	17.160	<0.001	-0.015	-0.451	0.661
β_2 , [Creatinine]	0.988	63.348	<0.001	0.995	69.781	<0.001	0.931	49.24	<0.001
β_3 , [Protein]*[Creatinine]	0.0003	1.929	0.080	-0.0001	-0.517	0.616	0.002	10.35	<0.001
R_{adj}^2	0.999			0.999			0.990		

^aThe multiple regression formula is commercial creatinine = $\beta_0 + \beta_1[\text{Interferant}] + \beta_2[\text{Creatinine}] + \beta_3[\text{Interferant}][\text{Creatinine}]$.

^bCoefficient of variable.

^cAdjusted R^2 .

Figure 5 showed the bias plots vs. protein concentration up to 112.8 g/l ($n = 125$) by using three alkaline picrate reagents on the Dimension Max and the Architect ci82000 analyzers. Using the reagent with and without the addition of potassium ferricyanide on both Dimension Max (Fig. 5A, C) and Architect ci8200 analyzers (Fig. 5B, D), a positive bias for creatinine determination of 1.93 and 14.56 $\mu\text{mol/l}$ for Dimension Max and 1.75 and 15.58 $\mu\text{mol/l}$ for Architect ci8200, respectively, was seen. Moreover, the biases were slightly increased with increasing serum protein concentrations ($R^2 = 0.081$, $P = 0.001$ and $R^2 = 0.076$, $P = 0.002$ for Dimension Max and $R^2 = 0.0452$, $P = 0.017$ and $R^2 = 0.0396$, $P = 0.026$ for Architect ci8200, respectively). We observed that the interfering patterns obtained from the assay using the reagent with potassium ferricyanide were similar to that of the Dimension Max commercial assay (Fig. 3A), which showed the constant positive bias. On the other hand, with the assay using the reagent with the addition of SDS, the effect of serum protein concentration was not significant on both analyzers ($R^2 = 0.008$, $P = 0.327$ and $R^2 = 0.002$, $P = 0.627$ for Dimension Max and Architect ci8200 methods, respectively). The mean biases were reported as -3.78 and -2.56 $\mu\text{mol/l}$, respectively.

DISCUSSION

Although most current commercial creatinine methods established the calibration traceable to the reference method, varying estimates of GFR have been reported (26,27). Our data clearly demonstrated that the Architect ci8200 unlike the Dimension Max assay has

clinically significant negative bias compared with the Vitros enzymatic method. It seems to show that the inter-assay variable of creatinine determination may occur even though the assays use similar methodological principle and calibration reference material (NIST 914) as seen between Dimension Max and Architect ci8200 assays. NIST 914 is not considered a commutable reference material; it is based on crystalline creatinine in aqueous buffer.

The influence of serum matrix components such as nonspecific proteins in Jaffe methods, which form a protein-picric acid complex as an absorbing species resembled to picric acid-creatinine complex, is quite different between the patient serum and the non-commutable reference materials. In addition, nonspecific bias varies depending on Jaffe methodology applications. To date, NIST has developed a new serum reference material (SRM), designated SRM 967 made from off-the-clot frozen serum pools. It will be critical for effective implementation of creatinine standardization, which will reduce the inter-assay bias in result.

Endogenous and exogenous interfering substances contribute to the lack of analytical specificity of creatinine methods (12–14). Most interference studies for creatinine assays, especially in Jaffe reaction, date back to 10–30 years ago. Our results have indicated that the present popular commercial creatinine methods including both a kinetic Jaffe reaction and an enzymatic method still demonstrate clinically significant interferences from many substances tested, especially bilirubin and protein (Tables 2 and 3 and Figs. 2 and 3).

Increasing serum protein concentrations provided the positive bias for the creatinine measurement (Fig. 3).

Fig. 4. Bias plot of serum creatinine concentration between the nonprotein precipitate sample (NPPT) and the deproteinized with trichloroacetic acid sample (PPT) on the Dimension Max (A, C, E), Architect ci8200 analyzers (B, D, F) by using three alkaline picrate reagents without (A, B) and with addition of potassium ferricyanide (C, D) or SDS (E, F) vs. bilirubin concentration.

Fig. 5. Bias plot of serum creatinine concentration between the nonprotein precipitate sample (NPPT) and the deproteinized with trichloroacetic acid sample (PPT) on the Dimension Max (A, C, E), Architect ci8200 analyzers (B, D, F) by using three alkaline picrate reagents without (A, B) and with addition of potassium ferricyanide (C, D) or SDS (E, F) vs. protein concentration.

This may lead to wrong interpretation. To account for the sensitivity of creatinine kinetic Jaffe method, some manufacturers have used a single value correction factor to adjust the offset of calibration curve to minimize nonspecific protein interference. This approach does not represent an accurate correction since it assumes an average protein concentration for all samples, but serum protein concentrations vary among patients (28). Moreover, as creatinine concentrations are normally quite low, such as found with children, elderly, pregnant women, or cancer patients, any protein interference can have a very significant effect on the correct value. In addition, as there is no noncreatinine chromogens, especially protein present in urine, the protein error will not affect creatinine determinations in urine. Thus, if serum and urine samples are measured on the same instrument channel, which is influenced by protein concentration, the results for urine will show a basic negative bias. Therefore, creatinine clearance is underestimated when creatinine methods affected by the protein error are used. If an accurate measurement of urinary creatinine is needed, e.g., in the estimation of the albumin/creatinine ratio or for creatinine clearance determination, a method that minimizes protein interference has to be suggested for using.

According to Kroll et al. (29), it has been purposed that if the interference is established, the type of interference should be assessed in order to engender a clear understanding of its chemical nature. We found bilirubin and protein interfere with the Vitros method for creatinine; these interferences are positive (results increase with increasing concentration of interference) and are analyte-dependent. Neither bilirubin nor protein contributes to the overall effect independent of the creatinine concentration. Such results suggest that these interferences do not independently interact with the method sensing system, nor react singly with the reagents along each step of the reaction process. The positivity of the interferences suggests that the interferences enhance or accelerate one or more of the steps in the reaction. Actual elucidation of a mechanism would require detailed study of each reaction step.

The presence of analyte-dependent interference complicates the evaluation and assessment of interference for a method, because the concentration of interference that exceeds an acceptable limit varies with the product of the creatinine with bilirubin or with protein concentrations. Also, the analyte-dependence complicates any assessments of the impact of reagent modifications to eliminate the interferences. One should establish limits of reporting based on the knowledge that the interference is analyte-dependent. Also, one should investigate whether other methodologies on the Vitros

analyzer may experience analyte-dependent interference, because they may use similar or analogous detection systems. Moreover, the analyte-dependent interference may occur with other enzymatic creatinine assays, because the chemical interaction or competition between the analyte and the interferent for reagents is always possible.

The analytical specific error from bilirubin interference can lead to differences in clinical concordance using estimated GFR. Moreover, it will affect the assessment in patients with liver disease. Model for end-stage liver disease (MELD) scoring to incorporate serum creatinine concentration is used in rating the liver severity and to prioritize patients for liver transplantation waiting list in USA (30). Because serum with high bilirubin concentration occurs frequently in patients with complications of liver disease, MELD scores may greatly vary by different methods of creatinine measurement, which will affect prioritization for liver transplantation.

Although Dimension Max and Architect ci8200 assays used similar kinetic Jaffe method, the effects of interferences were different. The effect of bilirubin varied from slight (Dimension Max) to marked interference (Architect ci8200 assay). On the other hand, the effect of serum proteins on Jaffe reaction with Dimension Max assay was more than that with Architect ci8200 assay. Considering the reagent composition, the Dimension Max reagent contains potassium ferricyanide, while the Architect ci8200 reagent does not. Although potassium ferricyanide rapidly converts oxidized bilirubin to biliverdin, thereby removing interference in the Jaffe reaction, it might accentuate protein interference. Moreover, we observed that it was unstable in the working reagent.

Different laboratory methods are used to overcome the problem of bilirubin interference with creatinine measurement (16–22). Apart from the addition of potassium ferricyanide, SDS was incorporated in our modified reagent and showed improvement in the specificity of Jaffe reaction. We proved that the addition of SDS to the reaction mixture reduced the interferences caused by both protein and bilirubin with the Dimension and Architect methodologies for creatinine. It can be combined with proteins to form stable complexes with a large number of negative charged sulfate groups. Because both conjugated and unconjugated bilirubins are bound with proteins in blood circulation, SDS can prevent the oxidation of bilirubin in the strong alkaline solution.

Our results demonstrated that the addition of SDS to the reagent produced an effective medium to minimize the important interfering substances in creatinine methods based on the Jaffe reaction. Because of its analytical advantage and low cost, this reagent may be applied to automated analyzers worldwide.

CONCLUSION

The sample specific interferences still represent important problems for accuracy of creatinine measurement by the current kinetic Jaffe and enzymatic methods and for estimated GFR. The enzymatic creatinine method was prone to interfere from bilirubin, protein, and glucose substances. The interference caused by bilirubin and protein was analyte-dependent, which created a difficulty in predicting the degree of interference. Its effect from interfering substances was not absolutely less than the kinetic Jaffe method. We found SDS in alkaline-picrate reagent to be effective in reducing the effect of sample-specific interferences, particularly bilirubin, and protein, for the creatinine kinetic Jaffe method.

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