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Comparison Between the Enzymatic Vitros Assay for Creatinine Determination and Three Other Methods Adapted on the Olympus Analyzer

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To evaluate the relationship between the enzymatic Vitros assay for creatinine determination and other methods, we determined creatinine concentration in 400 heparin samples. Plasma creatinine level was successively determined on the Vitros 750 analyzer (Johnson & Johnson Co., Rochester, NY) and on the Olympus AU2700 analyzer (Olympus France, Rungis, France), using three reagents in the same assay: Olympus-Jaffé and two enzymatic commercial kits—Crea Plus (Roche Diagnostics, Meylan, France) and Enzymatic Creatinine (Randox, Mauguio, France). Comparison of Jaffé and enzymatic measurements of plasma creatinine levels revealed a high correlation when considering all values ranged from 20–1,000 μmol/l ($r > 0.99$). However, for values <60 μmol/l, enzymatic reagents provided best results. Enzymatic methodology is a better clinical choice for the accurate measurement of creatinine, especially for neonates, pediatrics, and hematology units. Because analytical performance and the costs of Randox creatinine were satisfactory for our laboratory, this method, adapted on the Olympus analyzer, was chosen for routine determination of creatinine levels. According to the Valtec protocol (8), no interferences such as hemolysis, lipemia, or bilirubin were detected for Enzymatic Creatinine Randox. J. Clin. Lab. Anal. 17:235–240, 2003.
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Key words: creatinine; enzymatic; interference; Jaffé

INTRODUCTION

Plasma creatinine concentration is the most widely used and commonly accepted criterion in the medical laboratory for the evaluation of renal function. Knowledge of the methodology used for creatinine measurement is essential since methodological interferences may significantly alter the results and might influence therapeutic decisions.

Plasma creatinine determination is an important activity in our laboratory, with about 10,000 requests per month coming from various clinical departments (nephrology and transplantation, diabetology, cardiology, hematology, neonates, pediatrics, and general surgery intensive care units). Twenty-five percent of patients have mean values of creatinine below 60μmol/l. Indeed, serum creatinine is affected by the level of the glomerular filtration rate (GFR) and by factors independent of GFR, including age, gender, body size, diet, and certain drugs. Having to transfer from solid phase methodology (Vitros 750) to liquid methodology (Olympus AU2700), we recently had the opportunity, and the necessity, to evaluate the respective specificity and costs of three creatinine reagents and to compare them with the previously used enzymatic Vitros method, considered as a reference in this study. The Olympus AU2700 is an open automated clinical chemistry analyzer that allows the simultaneous use of different creatinine reagents. Plasma creatinine concentration was successively determined on the Vitros analyzer and the Olympus AU2700 analyzer, using three reagents in the same assay: Olympus-Jaffé and two enzymatic commercial kits—Crea Plus and Enzymatic Creatinine. The Jaffé kinetic method is known to be subject to several interferences and poor specificity (1–4). In addition, the commonly used Jaffé and modified Jaffé reaction methods systematically overestimated creatinine by 20% compared to enzymatic methods (5). Enzymatic procedure is more expensive than the Jaffé method but

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has the advantage of being unaffected by bilirubin, cephalosporin, and acetoacetate (6,7).

The aim of this study was to determine the best adapted reagent for creatinine determination in our laboratory, recently equipped with Olympus analyzers.

MATERIALS AND METHODS

Study Design

The analytical performance of Olympus-Jaffé, Crea Plus Roche, and Enzymatic Creatinine Randox reagents were tested on the Olympus AU2700 analyzer. The Crea Vitros Plus assay on the Vitros 750 analyzer was used as a reference method. Linear regression analysis was performed to compare data from different methods. The most frequent interferences, such as hemolysis, lipids, and bilirubin, were examined with spiked human sera on the selected method.

Clinical Samples

Heparin plasma samples from 400 patients, admitted to different clinical departments of Lapeyronie Hospital (Montpellier, France), were analyzed in this study.

Methods

The reaction principles of the methods used in this study are described in Table 1.

The Vitros enzymatic method is based on the established determination of sarcosine after conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase. The final reaction uses a leuko dye to generate a blue compound that is quantified at 670 nm.

In the Olympus-Jaffé method (Olympus France, Rungis, France), creatinine forms a yellow-orange colored compound with picric acid in the presence of alkaline solution. The concentration of the dye formed is a measure of creatinine concentration. The reaction is measured kinetically in order to minimize interference from endogenous substances.

The first enzymatic method (Crea Plus, Roche Diagnostics, Meylan, France), uses a reaction scheme to convert creatinine and release hydrogen peroxide, similar to the Vitros enzymatic method. The liberated hydrogen peroxide is measured via a modified Trinder reaction (10).

In the second enzymatic method (Randox, Mauguio, France), creatinine is converted by creatinine deiminase to ammonia and N-methylhydantoin. The ammonia is subsequently assayed by use of alpha-oxoglutarate and glutamate dehydrogenase. The reduction in absorbance at 340 nm, caused by oxidation of NADPH, is proportional to the ammonia concentration released by creatinine.

Reagents

Vitros Crea Slides: Reactive ingredients are creatinine amidohydrolase (creatininase, EC 3.5.2.10), creatine amidinohydrolase (creatinase, E.C.3.5.3.3), sarcosine

TABLE 1. Reaction principles used for creatinine determinations in our study

Reagents	Enzymes	Reaction principle
Vitros Crea slides	Creatininase	Creatinine + H_2O → creatine
	Creatinase	Creatine + H_2O → sarcosine + urea
	Sarcosine oxidase	Sarcosine + H_2O + O_2 → glycine + HCHO + H_2O_2
	Peroxidase	H_2O_2 + leucodye → dye
		The final reaction involves the peroxidase-catalyzed oxidation of a leucodye to produce a coloured product. Reflectance measurements are made at 3.85 and 5mn. The change in reflectance between two consecutive readings is proportional to the creatinine concentration in the sample
Olympus		Creatinine + picric acid (in the presence of alkaline solution) → creatinine picrate complex
Roche	Creatininase	Creatinine + H_2O → creatine
	Creatinase	Creatine + H_2O → sarcosine + urea
	Sarcosine oxidase	Sarcosine + H_2O + O_2 → glycine + HCHO + H_2O_2
	Peroxidase	H_2O_2 + amino-4 phénazone + HTIB → colored compound (benzoquinone-mono-imine) + $2H_2O$ + HI
		The color intensity is directly proportional to the concentration of creatinine present and measured photometrically HTIB acide hydoxy-3 triodo-2,4,6 benzoique
Randox	Creatinine deiminase	Creatinine + H_2O → 1-Methylhydantoin + NH_3
	Glutamate dehydrogenase	NH_3 + α oxoglutarate + NADPH + H^+ → L-Glutamate + NADP+ + H_2O
		Any endogenous ammonia present in the sample is removed by the presence of GLDH before the addition of creatinine deiminase. The reduction in absorbance at 340 nm caused by the oxidation of NADPH is proportional to the ammonia concentration released by creatinine.

oxidase (E.C.1.5.3.1), peroxidase (E.C.1.11.1.7), and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(4-dimethyl-aminophenyl) imidazole (leuko dye).

Olympus Creatinine Jaffé Method: Reagent 1 contains lithium hydroxyde and Reagent 2 is lithium picrate.

Crea Plus Roche: Reagent 1 (R1: buffer/enzymes/HTIB) ingredients are TAPS buffer (3-([tris(hydroxy-metyl)methyl]amino)propanosulfonic acid), creatinase, sarcosine oxidase, ascorbate oxidase, and HTIB (2,4,6-triiodo-3-hydroxybenzoic acid). Reagent 2 (R2: buffer/enzymes/4-aminophenazone) are TAPS buffer, creatininase, peroxidase, 4-aminophenazone, and potassium hexacyanoferrate.

Enzymatic Creatinine Randox: Reagent 1 is a substrate constituted by NADPH, ADP, alpha-oxoglutarate, and GLDH and Tris buffer. Reagent 2 contains, in addition, creatinine deiminase.

The Crea Vitros slide cartridge must be equilibrated at the room temperature. This requires 60 min after removal from the freezer or 30 min after removal from the refrigerator. The Olympus and Roche reagents are ready for use and can be placed directly in the instrument. Randox creatinine reagents require reconstitution before use.

Calibrators

As indicated by the manufacturers, the analyzers were calibrated once a month and once a day for Vitros and Olympus, respectively. The Crea Vitros slide required Vitros chemistry calibrator kit 1. Olympus multicalibrator for serum application was used for calibration of the Jaffé and Roche reagents. A Randox calibrator was used for Randox reagents.

Quality Control

Quality control was performed once a day for each method. Commercially available calibrators and control materials are specified in Table 2. All characteristics of the methods are detailed in Tables 1 and 2.

Assay Procedures

Imprecision: Within-day imprecision for the Olympus, Roche, and Randox methods were determined through 10 replicated analyses of samples with low, medium, and high values.

Linearity: Plasma samples with creatinine concentrations of 613, 631, and 717 μmol/l were used to test the linearity of the Olympus, Roche, and Randox methods, respectively. Samples were diluted at the following final percentages: 100, 75, 50, 25, 10, 5, and 2.5%.

Interferences: Interferences from hemolysis, lipemia, and hyperbilirubinemia were carried out according to the protocol of the Société Française de Biologie Clinique (SFBC) (8). The plasma was spiked with different amounts of interfering substances. Hemoglobin was obtained from Sigma (ref: H-7379, St. Quentin

TABLE 2. Conditions for creatinine determination

	Vitros Crea	Jaffé method	Roche	Randox
Apparatus	Vitros 750 XRC	Olympus AU2700	Olympus AU2700	Olympus AU2700
Methods	Enzymatic single slide	Kinetic Jaffé method	Enzymatic	Enzymatic
Volumes (μl)				
Samples	10	25	6	20
Reagent 1		15 (+ 90 μl of diluent)	250	125
Reagent 2		15 (+ 90 μl of diluent)	125	25
Wavelength (nm)	670	520–660	540–700	340–410
Stability of reagents on board (days)	30	7	28	30
Calibration				
Calibrators	Kit calibrage n°1	Olympus system calibrators 66300	Olympus system calibrators 66300	Randox OCR 08A
Days	At a change in reagent lot number or when required by critical situation (maintenance, or when indicated by QC)	Every day	Every day	Every day
Quality controls				
Controls	Johnson and Johnson performance verifiers I and II	Olympus system reagent level 1 and level 2	Olympus system reagent level 1 and level 2	Olympus system reagent level 1 and level 2
Linearity SI units (μmol/l)	4–1238	Up to a range of 2650	3–2652	10–880

TABLE 3. Imprecision and linearity of Jaffé and enzymatic methods for creatinine measurement

	Jaffé method			Roche creatinine			Randox creatinine		
Intraassay imprecision									
Creatinine levels (μmol/l)	52	109	726	35	98	771	42	107	603
CVs (%)	0.61	1.04	0.77	1.35	0.49	0.37	2.18	1.31	0.28
Linearity									
Creatinine levels (μmol/l)		613			631			717	
Determination coefficient:r		0.997			0.999			0.999	

Fallavier, France), Endolipide® 20% from Braun Médical (Boulogne, France), and Bilirubin from Fluka (ref: 14370, Sigma Aldrich Chimie, St. Quentin Fallavier, France). The maximal values of added hemoglobin, triglyceride (TG) equivalent, and bilirubin were 9g/l, 25 mmol/l, and 600 μmol/l, respectively. Interference effect was automatically detected by the analyzer with a @ alarm.

RESULTS AND DISCUSSION

Analytical Performance

Total intraassay variation coefficients (CVs) for the different methods tested on the Olympus AU2700 analyzer ranged from 0.49–2.18% (Table 3). The coefficient of linearity obtained with the three assays yielded r = 0.997 using Olympus-Jaffé, r = 0.999 using Roche, and r = 0.999 using Randox (Table 3). Analytically, all three of the methods showed satisfactory performance.

Correlations

Samples used for the between-method correlations ranged from 20–1,000 μmol/l. All regression equations are shown in Table 4. Comparison graphs are presented in Fig. 1. The comparative study between Crea Vitros (x) and other method values (y) led to correlation coefficients >0.99, with no significant difference between the Jaffé, Roche, or Randox methods when all samples (20–1,000 μmol/l) were included. However, when considering low creatinine levels (<60 μmol/l), the methods performed differently. When linear regression was performed with intersection to zero, the two enzymatic procedures scored better correlation coefficients than the Jaffé method (Table 4). It is generally assumed that the Jaffé method leads to a 20% overestimation of creatinine determination when compared to enzymatic methods (5). A 26% overestimation was observed with Olympus-Jaffé results only for low creatinine values (<60 μmol/l). The value of the slope was nearest to one using the Randox reagent. The equation of linear regression between the two enzymatic methods (Roche and Randox) is shown in Table 5.

TABLE 4. Regression equation of all comparisons

X: Vitros 750, n = 400 Range: 20–1000 μmol/l	Y = ax + b	r	SD	95% CI
Y: Jaffé method	0.83x + 20.6	0.998	9.5	0.821–0.832
Y: Roche	0.87x –3.0	0.998	8.7	0.863–0.873
Y: Randox	0.94x + 5.7	0.998	11.6	0.930–0.944
X: Vitros 750, n = 400 Range: 20–1000 μmol/l	**Y = a'x**	**r**	**SD**	**95% CI**
Y: Jaffé method	0.88x	0.991	18.7	0.874–0.891
Y: Roche	0.86x	0.998	9.0	0.856–0.864
Y: Randox	0.95x	0.997	12.4	0.947–0.958
X: Vitros 750, n = 95 Range: 20–60 μmol/l	**Y = ax + b**	**r**	**SD**	**95% CI**
Y: Jaffé method	0.82x + 21.3	0.916	3.7	0.743–0.890
Y: Roche	0.77x + 2.2	0.886	4.2	0.687–0.852
Y: Randox	0.94x + 6.4	0.858	5.8	0.832–1.063
X: Vitros 750, n = 95 Range: 20–60 μmol/l	**Y = a'x**	**r**	**SD**	**95% CI**
Y: Jaffé method	1.26x	0.764	5.9	1.230–1.280
Y: Roche	0.81x	0.885	4.2	0.797–0.832
Y: Randox	1.07x	0.850	6.0	1.050–1.100

r, determination coefficient; SD, standard error of the slope; 95% CI, confidence intervall of the slope.

Because analytical performance and the cost of Randox creatinine (the relative cost is 30% lower than

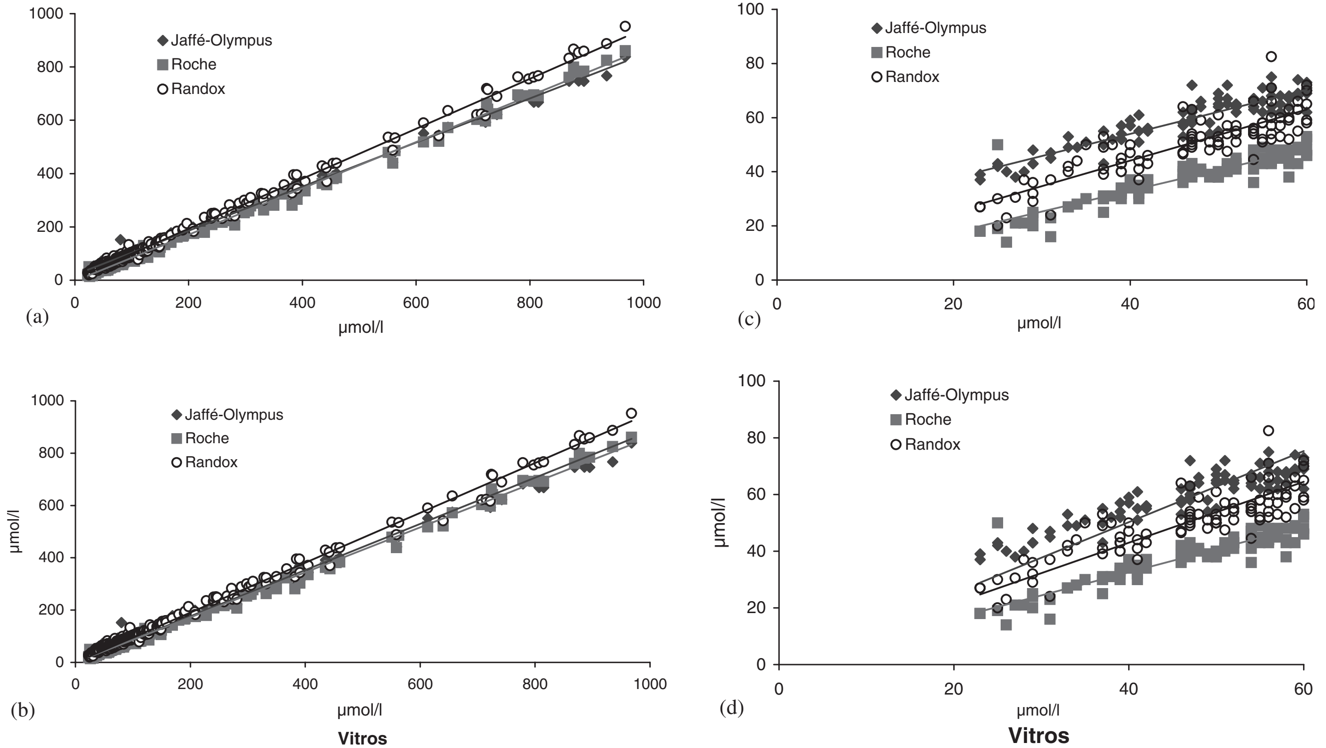

Fig. 1. Correlation between creatinine determination on a Vitros analyzer (x) and an Olympus analyzer using Olympus, Roche, or Randox reagents. **a:** correlation is y = ax + b, range is 20–1,000 µmol/l; **b:** correlation is y = ax, range is 20–1,000 µ mol/l; **c:** correlation is y = ax + b, range is 20–60 µmol/l; **d:** correlation is y = ax, range is 20–60 µmol/l. Regression equation of comparisons are listed in Table 4.

TABLE 5. Regression equation between Roche (X) and Randox (Y) creatinine determination.

Range (µmol/l)	n	Y = ax + b	r	SD	95% CI
20–1000	400	1.08x + 9.1	0.997	8.9	1.073–1.085
20–60	100	1.06x + 10.1	0.889	5.1	0.954–1.178

the Roche enzymatic test) were satisfactory for our laboratory, this method, adapted on the Olympus analyzer, was chosen for routine determination of creatinine levels.

Bilirubin, Hemolysis, and Interference by Lipids

Three "visible" interferences—hemolysis, lipemia, and bilirubin—were studied on Randox creatinine determination (Fig. 2). According to the Valtec protocol, we performed graphs with the concentration of the interfering component on the x-axis (concentration of hemoglobin, TG equivalent, and bilirubin), and the difference between the results of the pool and the spiked samples was expressed in percentage (%) of the pool concentration on the y-axis (8). Each data point represented a mean of three values obtained with spiked plasma and the graphs showed the deviation from the control value. The interferences were tested by adding solutions to sera with low (95 µmol/l), medium (220 µmol/l), or elevated (425 µmol/l) creatinine concentrations.

When a 10% variation was retained as interference cutoff, the addition of interfering substances to the concentration defined by the protocol of SFBC (4.4 g/l of hemoglobin, 7 mmol/l of TG equivalent, and 550 µmol/l of bilirubin) produced no interference with Randox creatinine measurement (irregardless of the creatinine levels). This was in agreement with other enzymatic methods such as Crea Plus on Vitros (9).

In conclusion, evaluation of the Olympus-Jaffé, Roche, and Randox creatinine methods on the Olympus analyzer yielded good imprecision results and satisfactory linearity. Particular attention to low creatinine values must be taken into account when choosing a method. Indeed, correlation between the Olympus-Jaffé and enzymatic methods was altered for low creatinine levels. In order to compromise between the economic aspect and better specificity, we adapted the Randox

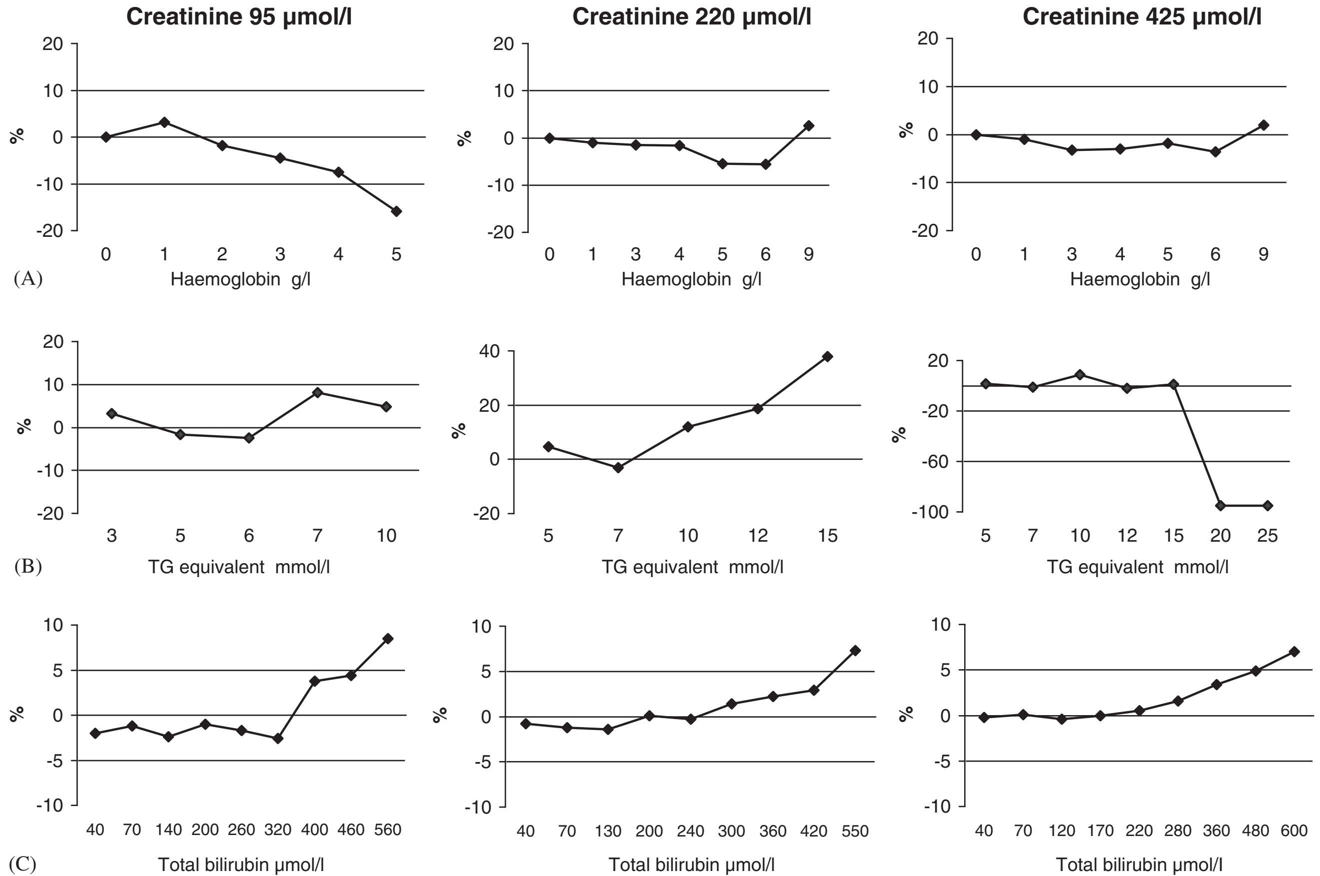

Fig. 2. Interference study on Randox creatinine assay, expressed in percentage of the recovery value. **A:** interference by haemoglobin; **B:** interference by lipid (triglyceride or TG equivalent); **C:** interference by bilirubin.

reagents on the AU2700, AU640, and AU400 Olympus analyzers.

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