Letter to the Editor

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Glucose and total protein: unacceptable interference on Jaffe creatinine assays in patients

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To the Editor,

Accurate measurement of serum creatinine is essential for the correct estimation of glomerular filtration rate (eGFR) and, consequently, adequate classification of the presence of chronic kidney disease (CKD). The variability in serum creatinine test results among medical laboratories and manufacturers has greatly been reduced since the development and availability of NIST SRM 967 and NIST SRM 967a and the rapid adoption of these matrix-based reference materials for standardizing creatinine tests to SI units [1]. However, standardization of calibration does not eradicate analytical interferences by non-creatinine chromogens such as ketones, glucose and proteins [1–4]. Using data from the Dutch external quality assessment (EQA) organization SKML, we demonstrated that Jaffe techniques overestimate serum creatinine values, leading to substantial misclassification of patients into a lower CKD category [2]. In addition, when, more recently, frozen commutable samples were circulated to 89 laboratories in four European countries, Jaffe methods still showed unsatisfactory performance in terms of bias, imprecision and specificity, particularly when the samples were spiked with glucose [1]. These problems were not encountered with specific enzymatic methods [1–4].

The results of the previous study were criticized because modified (non-native) patient samples were used. Therefore, we here illustrate the degree of interference by glucose and total protein on serum creatinine measurements and eGFR (CKD-EPI) calculations using the Jaffe and enzymatic techniques in fresh patient samples. For this purpose, 78 patient samples with total protein concentrations <65 g/L or >75 g/L and glucose concentrations <7 mmol/L or >15 mmol/L were centrally collected at the Department of Clinical Chemistry of the Queen Beatrix Hospital in Winterswijk. Split samples were stored at –70 °C and shipped on dry ice to participating clinical laboratories. Creatinine concentrations were measured in duplicate with the Jaffe and enzymatic methods in four laboratories, each representing one of the four major platforms in the Netherlands (LangeLand Ziekenhuis Zoetermeer: Abbott [Architect], Maasziekenhuis Pantein, Beugen: Beckman-Coulter [UniCel DxC 860i], Queen Beatrix Hospital, Winterswijk: Roche [Cobas 6000], Bernhoven, Uden: Siemens [Vista]).

The measurements were performed according to the manufacturers' instructions. In all laboratories, the samples were measured in a standardized order in one

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batch. In addition, two IDMS-RMP targeted calibrators were measured eight times for each method. The means of these calibrator measurements were plotted against the target calibrator values. The estimated deviations from the target calibrator values were used to adjust the patient results, generating IDMS traceable creatinine measurements and eliminating differences in calibration between methods. Because enzymatic methods have been shown to be insensitive to interfering substances [4], the target values of the patient samples were defined as the mean of the IDMS traceable patient results of the four enzymatic methods. This approach was supported by a verification experiment in which all laboratories measured 10 IDMS targeted EQA samples, showing (a) exchangeable results for the four enzymatic methods and (b) no substantial bias compared to the target values (data not shown).

The results are summarized in Tables 1 and 2. When creatinine was measured with enzymatic methods, no interference was observed in samples with low or high total protein concentrations, or in samples with normal or high glucose concentrations (Table 1). By contrast, when, for example, using the Jaffe method on the Abbott platform, a negative bias of −6 μmol/L was found in samples with a low total protein concentration (50 g/L), and a positive bias of +4 μmol/L was found in samples with a high total protein concentration (80 g/L). In addition, a positive bias of +33 μmol/L was observed for samples with a high glucose concentration (30 mmol/L). Similar results were obtained when samples were measured using the Jaffe method on the Beckman-Coulter, Roche and Siemens platforms. As is illustrated in Table 2, these biases in creatinine outcomes using Jaffe methods lead to incorrect eGFR calculations on all platforms, ranging from severe overestimations (>20% above the actual eGFR) in case of low total protein (30 g/L) and low glucose (0–5 mmol/L) concentrations or underestimations (>30% below the actual eGFR) in case of high protein (100 g/L) and high glucose (30 mmol/L) concentrations.

Because CKD staging directly relies on eGFR calculations [2], the observed systematic errors in creatinine measurements using the Jaffe method and subsequent incorrect eGFR calculations have an unacceptable impact on CKD patient care with respect to diagnosis, prognosis, follow-up and treatment. Despite earlier efforts to abandon Jaffe methods, the majority of European laboratories are still using these methods [1]. It is of crucial importance that Jaffe methods are replaced by enzymatic assays, to further prevent patient harm. We therefore hereby repeat our former recommendation to laboratory specialists and manufacturers to take their responsibility and to implement exclusively creatinine tests that are *fit-for-clinical-purpose* [1, 4].

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Table 1: Interference of total protein and glucose expressed as bias in creatinine measurement in μmol/L creatinine.

Method	Total protein		Glucose	
	50 g/L	80 g/L	5 mmol/L	30 mmol/L
Jaffe methods				
Abbott	−6	+4	0	+33
Beckman	−2	+1	0	+16
Roche	−4	+4	0	+15
Siemens	−5	+4	−1	+26
Enzymatic methods				
Abbott	0	0	0	0
Beckman	0	0	0	0
Roche	0	0	0	0
Jaffe	0	0	0	0

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Table 2: Calculated eGFR[a] (in mL/min/1.72 m²) and deviations in eGFR (%, colored background) based on creatinine measurements using Jaffe and enzymatic methods of Abbott, Beckman-Coulter, Roche and Siemens at glucose concentrations ranging from 0 to 30 mmol/L and total protein concentrations ranging from 30 to 100 g/L.

		Jaffe methods								Enzymatic methods						
Abbott	Total protein, g/L	100	58	53	49	46	43	38	Total protein, g/L	100	60	60	59	59	59	59
		80	63	57	53	49	46	40		80	60	60	59	59	59	59
		70	66	60	55	51	47	41		70	60	60	59	59	59	59
		60	68	62	57	53	49	42		60	60	60	59	59	59	59
		50	72	65	59	55	50	44		50	60	60	59	59	59	59
		30	79	70	64	59	54	47		30	60	60	59	59	59	59
			0–5	5–10	10–15	15–20	20–30	30			0–5	5–10	10–15	15–20	20–30	30
			Glucose, mmol/L								Glucose, mmol/L					
Beckman	Total protein, g/L	100	60	57	55	53	51	47	Total protein, g/L	100	60	60	59	59	59	59
		80	61	59	57	54	52	48		80	60	60	60	60	60	59
		70	62	59	57	55	53	49		70	60	60	60	60	60	60
		60	63	60	58	55	53	49		60	60	60	60	60	60	60
		50	64	61	59	56	54	50		50	60	60	60	60	60	60
		30	66	63	60	57	55	52		30	60	60	60	60	60	60
			0–5	5–10	10–15	15–20	20–30	30			0–5	5–10	10–15	15–20	20–30	30
			Glucose, mmol/L								Glucose, mmol/L					
Roche	Total protein, g/L	100	57	53	52	50	48	45	Total protein, g/L	100	59	59	59	59	59	59
		80	59	57	55	53	51	47		80	59	59	59	59	59	59
		70	60	59	57	55	53	49		70	60	60	60	60	60	60
		60	63	60	58	56	54	50		60	60	60	60	60	60	60
		50	66	63	60	58	56	52		50	60	60	60	60	60	60
		30	70	67	65	62	60	55		30	60	60	60	60	60	60
			0–5	5–10	10–15	15–20	20–30	30			0–5	5–10	10–15	15–20	20–30	30
			Glucose, mmol/L								Glucose, mmol/L					
Siemens	Total protein, g/L	100	56	53	50	47	44	40	Total protein, g/L	100	58	58	59	59	59	59
		80	60	57	53	50	48	42		80	59	59	59	59	59	59
		70	63	59	55	52	49	44		70	59	59	59	59	59	59
		60	66	61	57	54	51	45		60	59	59	59	59	59	59
		50	69	65	60	56	53	47		50	59	59	59	59	59	59
		30	76	70	66	61	57	50		30	59	59	59	59	59	60
			0–5	5–10	10–15	15–20	20–30	30			0–5	5–10	10–15	15–20	20–30	30
			Glucose, mmol/L								Glucose, mmol/L					

[a]eGFR (CKD-EPI) of a Caucasian female aged 55 years with a creatinine concentration of 93 µmol/L

Legend:
Deviations in eGFR
>20% higher than actual eGFR
10–20% higher than actual eGFR
3–10% higher than actual eGFR
<3% difference with actual eGFR
3–10% lower than actual eGFR
10–20% lower than actual eGFR
20–30% lower than actual eGFR
>30% lower than actual eGFR