Letter to the Editor

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Creatinine, Jaffe, and glucose: another inconvenient truth

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To the Editor,

The Stichting Kwaliteitsbewaking Medische Laboratoria (SKML, the External Quality Assessment/Proficiency Test (EQA/PT) organizer in the Netherlands uses commutable, targeted samples [1]. Targets are assigned with Joint Committee Traceability Laboratory Medicine (JCTLM)-listed reference measurement procedures (RMP). The commutability of the samples is systematically verified in every annual cycle with a so-called spy-sample. This is a serum collected according to CLSI document C37-A [2]. In the EQA/PT program, regular frozen human pool sera and a spy sample are included. When for a given analyte the bias of all routine assays in the regular EQA/PT sample is similar to the bias in the spy-sample, the commutability of the regular samples is confirmed [1].

In 2014 however we observed a substantial difference for serum creatinine. In Table 1 results are shown for regular human serum sample 1 (S1) and spy serum sample 2 (S2). The creatinine target of the regular sample was 54 μmol/L (0.61 mg/dL). With the Abbott Architect Jaffe test (Abbott Laboratories, IL, USA), the mean of eight laboratories was 77 μmol/L (0.87 mg/dL): a bias of +23 μmol/L (+0.26 mg/dL). In the spy sample the target value was 72 μmol/L (0.81 mg/dL) and the mean of the same eight laboratories was 71 μmol/L (0.80 mg/dL): a bias of –1 μmol/L (–0.01 mg/dL). The difference in bias between both samples was [+23 to (–1)]=+24 μmol/L (0.27 mg/dL)]. Similar differences were calculated for Jaffe tests from other manufacturers. These differences were suspect for either a matrix effect for Jaffe tests in the regular EQA/PT samples due to the manufacture of the samples (spiking), or for analytical interference by endogenous analytes. In respect to this it should be noted that the total protein concentration in S1 was 48 g/L and 66 g/L is S2. In a previous study we demonstrated that a 25 g/L increase in total protein resulted in a 10–15 μmol higher creatinine with Jaffe methods, but no measured increase in measured creatinine with enzymatic methods [3]. The positive bias in S1 due to the high glucose in S1 will be compensated by the negative bias due to the low total protein concentration. Due to these mixed interferences a clear picture of glucose interference cannot be derived from S1 and S2.

This prompted us to a split sample experiment: one aliquot of a serum with a creatinine concentration of 60 μmol/L (0.67 mg/dL) and a total protein concentration of 65 g/L was spiked with 25 mmol/L glucose (450 mg/dL) and the other aliquot was not spiked (S3 and S4 in Table 1). In this way the exact analytical interference of glucose could be estimated. Split samples were assayed by colleagues operating the respective tests. In Table 1 it can be seen that the differences in measured serum creatinine concentration ranged from +10 to +19 μmol/L

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Table 1: Creatinine results in EQA/PT, CLSI C37A-based, and glucose spiked split samples.

Measurement procedure	EQA/PT samples					Split samples				
	n	Creatinine, µmol/L		Bias to RMP, µmol/L		ΔBias, µmol/L	Creatinine, µmol/L		Bias to RMP, µmol/L		ΔBias, µmol/L
		S1[a] Gluc 29.5 Protein 48 g/L	S2[b] Gluc 5.1 Protein 66 g/L	S1	S2	S1–S2	S3[c] Gluc 29.1 Protein 65 g/L	S4[c] Gluc 4.1 Protein 65 g/L	S3	S4	S3–S4
RMP	1	54	72	0	0	0	60	60	0	0	0
Jaffe procedures											
Abbott Architect	8	77	71	+23[d]	−1	+24	73	57	+13[d]	−3	+16[e]
Beckman UniCel DxC	29	59	69	+5[d]	−3	+8	71	61	+11[d]	+1	+10[e]
Roche Cobas 6000	17	56	71	+2	−1	+3	64	54	+4[d]	−6[d]	+10[e]
Siemens VISTA	4	59	70	+5	−2	+7	73	61	+13[d]	+1	+12[e]
Siemens VISTA NR							73	54	+13[d]	−6[d]	+19[e]
Enzymatic procedures											
Abbott Architect	11	56	72	+2	0	+2	60	60	0	0	0
Beckman Unicel DxC	1	51	70	−3	−2	−1					
Roche Cobas 6000	111	55	71	+1	−1	+2	62	62	+2	+2	0
Siemens VISTA	7	53	71	−1	−1	0	59	60	−1	0	−1

[a]Sample S1: Human Pool serum (glucose 29.5 mmol/L; total protein 48 g/L); [b]Sample S2: Single Donation serum collected according to CLSI protocol C37-A (glucose 5.1 mmol/L; total protein 66 g/L); [c]Samples S3 and S4: Split Sample Human Pool serum with (S3; glucose 29.1 mmol/L; total protein 65 g/L) and without spiking with glucose (S4; glucose 4.1 mmol/L; total protein 65 g/L); [d]Significant bias as compared to the RMP (t-test 95% CI); [e]Significant difference in measured creatinine concentration between the split samples with and without glucose spiking (t-test 95% CI). NR, new reagent; RMP, reference measurement procedure.

(0.11–0.21 mg/dL) for the Jaffe assays, whereas enzymatic tests showed no differences. Although the trend was the same as in the EQA/PT samples, results were not exactly the same. This is explained by the fact that EQA/PT S1 and S2 were different samples (with known different concentrations of total protein and unknown other unspecificities) whereas split samples S3 and S4 were identical sera.

In their hallmark investigation of specificity characteristics of seven commercial creatinine assays, Greenberg et al. found a relation between bias and diabetes but concluded that their data did not identify the root cause [4]. Recently, an Australian group published on glucose as a significant interferent with the Abbott Architect Jaffe test [5]. Results in their spiking experiment were in agreement with our findings. These authors also reported a similar mean bias in a group of 132 patients but with a broad variation between individuals. They inferred that in samples from patients with diabetes, there are many other interferents present, both positive and negative in addition to the glucose interference [5].

The split samples S3 and S4 can be regarded as mimics of the same diabetic person in a normo- and hyperglycemic phase. When the estimated glomerular filtration rate (eGFR) is calculated using the CKD-EPI formula for a 55-year-old Caucasian woman, the Abbott Architect Jaffe test would have revealed an eGFR of 101 mL/min/1.73 m^2 in the normoglycemic samples (measured creatinine 57 µmol/L; 0.64 mg/dL) and of 80 mL/min/1.73 m^2 in the hyperglycemic sample (measured creatinine 73 µmol/L; 0.82 mg/dL). This widely exceeds the total error budget and impacts CKD classification, questioning the acceptability of Jaffe methods for routine clinical use.

In our EQA/PT program we observed analytical interference of glucose. However, as this effect derives from normal variation in concentration of an endogenous analyte this can not be considered as non-commutability of the EQA/PT-samples [6]. On the contrary: it discloses non-selectivity of Jaffe methods. In a previous paper of our group we reported on the effect of another endogenous analyte (variation in protein concentration) and made a plea for a change to enzymatic tests [3]. This new evidence of a high interfering effect of common endogenous analytes, such as glucose (along with all other known and unknown unselectivities of the Jaffe tests), strengthens us in our advice as EQA/PT organizer to our participants to abandon (or at least reconsider) Jaffe methods in favor of enzymatic tests.

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