Inaccuracy of routine creatinine measurement in canine urine

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Background: Urine creatinine concentration often is used in ratios such as urine protein:creatinine to compensate for dilution or concentration of spot urine samples. **Objective:** The purpose of this study was to compare the accuracy of different techniques of urine creatinine measurement currently available for veterinary practitioners. **Methods:** In 104 samples of canine urine diluted 1:20 with distilled water, creatinine concentration was measured using a kinetic Jaffé reaction assay, and an enzymatic technique on an automatic analyzer (Elimat) and 3 benchtop analyzers (Reflovet, Scil; Vitros DT2, Ortho-Clinical Diagnostics; Vettest 8008, IDEXX) used in veterinary practice. **Results:** The Jaffé and enzymatic techniques on the Elimat were not significantly different, and their inaccuracy tested with human control urines was <5%. The benchtop analyzers underestimated creatinine concentration, especially at concentrations >2000 mg/L. Inaccuracy was higher with multilayer slide technology systems (Vitros and Vettest) than with the Reflovet system. Results were approximately 25% and 2% lower, respectively, than with the Elimat at urine creatinine concentrations about 2000 mg/L. **Conclusion:** Inaccuracy in urine creatinine measurements using benchtop analyzers should be taken into account when defining decision thresholds, which should be corrected according to the method used to avoid misinterpretations. (*Vet Clin Pathol.* 2004;33:128–132)

Key Words: Creatinine, dog, laboratory method, quality control, urine

The concentration of creatinine in canine urine (U-creatinine) is often used as a criterion to compensate for dilution or concentration of urine by expressing the elimination of analytes such as proteins, cortisol, or enzymes as their ratio to U-creatinine. This ratio is based on the assumption that the total mass excretion of creatinine is constant over time in a given dog so that U-creatinine is an inverse function of the dilution of urine. Thus, the urine concentration of any analyte can be compensated for urine dilution or concentration by expressing its excretion as a ratio to creatinine concentration. Only moderate changes in creatinine excretion over 24 hours have been reported in dogs[1] and humans,[2] and there is good correlation between 24-hour urine protein concentrations and protein:creatinine ratios in spot urine samples from dogs.[3–5]

When using a ratio, there is a possibility of analytical errors for both measurements, thus increasing imprecision and making diagnostic interpretation more difficult. In our laboratory, the routine measurement of urine creatinine was analytically unsatisfactory. Comparison of our results and results issued from neighboring laboratories showed large differences. These differences probably resulted partly from the inaccuracy of creatinine measurement, previously reported in human serum.[6,7]

To the best of our knowledge, inaccuracy has not been reported for the measurement of urine creatinine concentration. The aim of this study was to test the accuracy of urine creatinine measurements performed in the same specimens of canine urine, using standard enzymatic or Jaffé methods and 3 benchtop analyzers frequently used in veterinary practice.

Materials and Methods

After routine urinalysis, 104 centrifuged urine samples were randomly taken among samples submitted in the morning to the laboratory of the Veterinary School of Toulouse, independently of breed, age, sex, disease status, conditions of collection, and storage. Samples were frozen at –20°C until analysis 2–7 weeks later. Analyses were performed on groups of 7–15 samples that were thawed at room temperature (~20°C) for approximately 1 hour and homogenized before analysis.

All samples were diluted 1:20 (vol:vol) with distilled water, and dilutions were then analyzed for

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Table 1. Comparison of the 5 methods tested for canine urine creatinine measurement (manufacturers' information).

Instrument	Method	Temperature	Analytical Range (mg/L)
Elimat (Jaffé)	Kinetic (10–120 s) Calibration by 20 mg/L solution	37°C	1.7–150
Elimat (Enzymatic)	Creatininase Calibration by 20 mg/L solution	37°C	1.4–200
Reflovet	Creatininase Reagent strip Calibration by magnetic strip	37°C	5–100
Vettest	Creatininase Multilayer slide Calibration by bar code	37°C	0.5–140
Vitros	Creatininase Multilayer slide Calibration by 5.2, 15.6, and 125.6 mg/L solutions	37°C	0.5–140

creatinine using 5 techniques and 4 analyzers (Table 1). An automatic transfer analyzer (Elimat, Elitech, Labarthe Inard, France) was used with 2 different commercial reagents based on the Jaffé reaction and on creatininase (Creatinine Jaffé and Creatinine Enzymatique, Elitech). Each technique was used according to the manufacturer's recommendation; calibration was done with a solution containing 20 mg creatinine/L (Elical, Elitech); accuracy was verified with the calibrator and a commercial urine control (Sigma Diagnostics, L'Isle d'Abeau, France; targets = 850 and 860 mg/L for the Jaffé reaction and enzymatic technique, respectively). When results were above the upper limit of linearity, samples were rediluted 1:2 (vol:vol) in distilled water and run again. Three different benchtop analyzers used in veterinary practice also were used, the Vitros DT2 (Ortho-Clinical Diagnostics, Illkirch, France), Reflovet Plus (Scil, Holzheim, France), and Vettest 8008 (IDEXX, Cergy Pontoise, France). Measurements were based on enzymatic techniques. When results were above the upper limit of linearity, samples were rediluted 1:2 (vol:vol) in distilled water and run again (14 cases for the Reflovet, 0 for the Vettest, and 1 for the Vitros). Results were expressed as mean ± SD. U-creatinine concentration was expressed as mg/L. For correspondence with SI units, 1 mg/L = 8.84 μmol/L and 1 mmol/L = 113.12 mg/L; for conversion to conventional units, 1 mg/L = 0.1 mg/dL.

Table 2. Comparison of the results of urine (U)-creatinine measurements by 5 different techniques in 104 canine urine samples.

	U-Creatinine (mg/L)				
	Elimat		Reflovet	Vettest	Vitros
	Jaffé	Enzymatic			
Mean	1309	1321	1204	991	1029
Median	1157	1203	1078	858	922
SD	861	898	776	602	626
Minimum	142	121	129	118	131
Maximum	4170	4344	3384	2717	2857

Comparison of results was performed according to standard recommendations for comparing analytical techniques, based on Student's paired *t*-test, Deming's regression, and difference plots.[8,9] Calculations were performed using Method Validator freeware (http://perso.easynet.fr/~philimar)[9] and a Microsoft Excel (Microsoft Corp, Redmond, WA, USA) spreadsheet with the Analyse-it set of macroinstructions (Analyse-it Software, Leeds, England).

Results

Urine creatinine concentrations ranged from ~100 to ~4000 mg/L (Table 2). Accuracy of the Jaffé and creatininase techniques with the Elimat analyzer was good. The Elimat moderately underestimated the concentration of the human urine control, with mean values (n = 7) of 831 and 819 mg/L with the Jaffé and enzymatic techniques, respectively, so that respective inaccuracies were 2.2% and 4.8%. Between-series imprecision (n = 7) at 400 mg/L and 850 mg/L was 7% and 6%, respectively, for the Jaffé technique and 10% and 6% for the enzymatic technique.

There was no significant difference between results obtained by the Jaffé reaction and the enzymatic technique with the Elimat (Student's paired *t*-test, $P > .05$). The correlation between the 2 series of results was excellent: r = 0.997 (Figure 1), Deming's regression (95% confidence interval between brackets) gave: $\text{U-Creatinine}_{(\text{Enzyme})} = 1.043\ (1.022/1.063) \times \text{U-creatinine}_{(\text{Jaffé})} - 43.7\ (-65.6/-21.9)$. Although it was not significant (ANOVA, $P > .05$), the difference (Jaffé – enzymatic) was moderately proportional because its mean value decreased from 24 to –67 mg/L in the 25% less concentrated and 25% most concentrated samples. In the following comparisons, the mean of the results obtained by the 2 above techniques were used as reference.

Results obtained with benchtop analyzers were lower than the reference results (Student's paired *t*-test, Bonferroni's correction for multiple comparisons, $P < .001$) (Table 2). Results with the Reflovet (Figures 2a and 2b) gave a moderate negative bias up to 2000 mg/L; then the bias increased significantly, reaching a mean of –261 mg/L in the 25% most concentrated samples. Correlation with the Elimat results was high: $r = 0.982$. The Deming's regression equation gave the following: U-creatinine$_{(Reflovet)}$ = 0.916 (0.893/0.940) × U-creatinine$_{(Elimat)}$ + 4.2 (–5.2/13.7).

Results with the Vettest and Vitros systems (Figures 3a, 3b, 4a , and 4b) were lower over the whole range of measurements, and the difference from Elimat results increased with creatinine concentration, reaching 718 ± 85 mg/L and 643 ± 379 mg/L (mean ± SD) in the 25% most concentrated samples.

Correlations with the Elimat results were high: 0.959 and 0.977, respectively. Deming's regression equations gave the following: creatinine$_{(Vettest)}$ = 0.714 (0.672/0.762) × creatinine$_{(Elimat)}$ + 56.2 (25.7/86.8); and creatinine$_{(Vitros)}$ = 0.706 (0.640/0.773) × creatinine$_{(Elimat)}$ + 100.6 (36.0/165.1). The results obtained with these 2 analyzers based on the same technology were highly correlated (r = 0.996) and moderately but significantly different (Student's paired *t*-test, $P < 0.05$). Moreover, the dispersion of results was higher with benchtop analyzers than with the Elimat analyzer. For instance, when results obtained by the latter were in the range of 900–1100 mg/L by the Elimat (n = 13 samples), ranges of values measured using the Reflovet, Vitros, and Vettest systems were 832–1128, 499–1051, and 703–956 mg/L, respectively. Mean bias around 2000 mg/L was –2.4%, –25.1%, and –24.2%, with the Reflovet, Vettest, and Vitros, respectively.

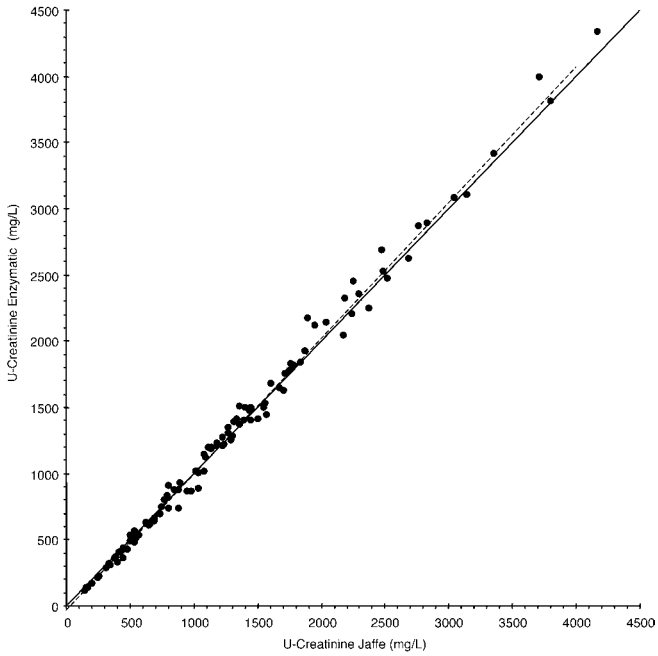

Figure 1. Scatterplot of results of U-creatinine measurements in 104 canine urine samples using the Jaffé reaction assay and an enzymatic technique on the Elimat analyzer (solid line = equivalence; dotted line = regression).

Discussion

To the best of our knowledge, there is no internationally or nationally recommended procedure for the measurement of creatinine in the urine of humans or any animal species. Usually manufacturers recommend using techniques for plasma creatinine measurement after prior dilution of samples, which helps ensure values fall within the limits of linearity of the method. As we had previously observed that the dilution of canine urine could introduce a bias in the accuracy of results (unpublished, sigmoid relationship), it was decided that all urines would be diluted 1:20 prior to analysis, whatever the sample. In most cases, this dilution was adequate to ensure results fell within the limits of measurement indicated by the manufacturers. In cases in which another dilution was needed, it was likely that this caused an additional bias. In human biochemistry, the accuracy of the different techniques of plasma creatinine measurement, which are the same as used in urine, has long been a topic of debate and is considered unsatisfactory.[6,10]

Difficulties in the measurement of creatinine in canine urine have already been reported in the determination of endogenous creatinine clearance and were attributed to the poor specificity of the Jaffé reaction used in that study.[11] The first useful information from the present comparison is that the Jaffé and enzymatic techniques gave the same results in canine urine using the Elimat. This could have been expected because the higher specificity of enzymatic procedures is valuable in eliminating possible interferences by such compounds as glucose, bilirubin, and ketone bodies, which have relatively high plasma but low urine concentrations, except in rare situations.

We also demonstrated that urine creatinine can easily be measured in urine by the relatively inexpensive Jaffé technique with the same precision and accuracy as an expensive enzymatic technique. The main object of controls in both cases was to ensure the accuracy of the results and their transferability from one laboratory to another. At this point, the use of commercially available human urine controls should

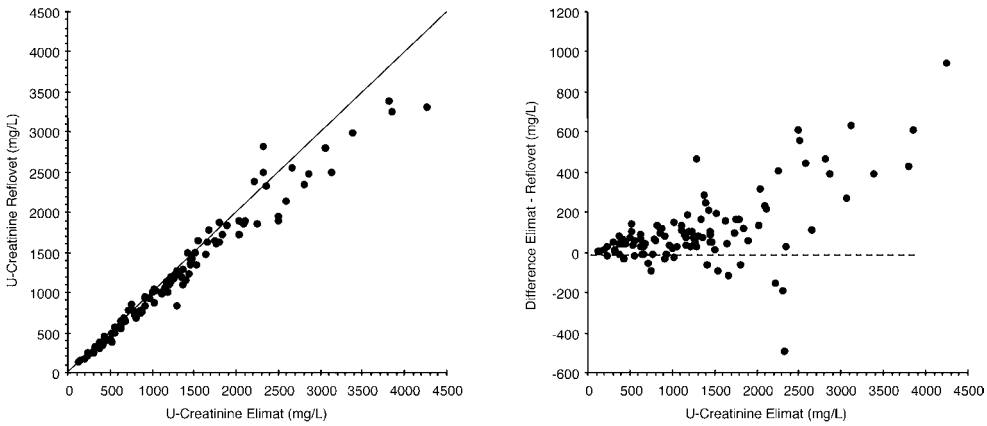

Figure 2. Scatterplot (left) of results of U-creatinine measurements of 104 canine urine samples using the Reflovet Plus and the mean of results obtained using the Jaffé reaction and an enzymatic technique with the Elimat (black line = equivalence). Difference plot (right) of results according to the mean of results using the Elimat analyzer.

be recommended to evaluate inaccuracy; in our hands, it was <5%. It is likely that better accuracy might be achieved by use of a calibrator with a higher concentration than the 20 mg/L solution provided, which is adequate for plasma creatinine measurement. Even with this low-value calibrator, inaccuracy was lower than imprecision, yielding a maximal total error in the range of 15%, as recommended for plasma creatinine measurement in human medicine.[12] Possible errors due to redilution of concentrated samples could be avoided by diluting all samples 1:40 or 1:50 instead of 1:20. A higher dilution would help avoid the need for a second dilution, and permit measurements even at the lowest concentrations because the quantification limits are lower than 2 mg/L.

The benchtop analyzers available in many veterinary practices are not well suited for accurate measurement of U-creatinine at higher concentrations because they underestimated urine creatinine concentration, especially at higher levels. Even at lower U-creatinine concentrations, which are often observed in dogs with polyuria, inaccuracy was notable with the analyzers using multilayer slide technology (Vitros and Vettest), whereas it was acceptable with the analyzer using test strips (Reflovet). This inaccuracy and the dispersion of results could lead to erroneous interpretations. For instance, a dog having a urine protein concentration of 0.3 g/L and a urine creatinine concentration ranging from 900 to 1100 mg/L would have a urine protein: creatinine ratio ranging from 0.25 to 0.33 using the Elimat and would thus be considered normal. With the benchtop analyzers the ranges would be 0.27–0.36, 0.38–0.80, and 0.42–0.57 with the Reflovet, Vitros, and Vettest systems, respectively, and thus would be considered questionable in some cases, according to the commonly accepted thresholds of 0.3, 0.3–1, and >1 for normal, questionable and abnormal.[13]

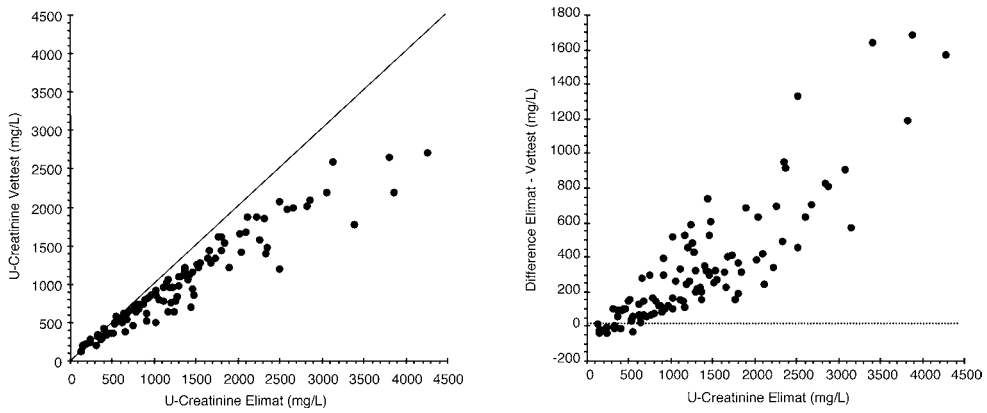

Figure 3. Scatterplot (left) of results of U-creatinine measurements in 104 canine urine samples using the Vettest and the mean of results obtained using the Jaffé reaction and an enzymatic technique with the Elimat (black line = equivalence); Difference plot (right) of results according to the mean of results using the Elimat analyzer.

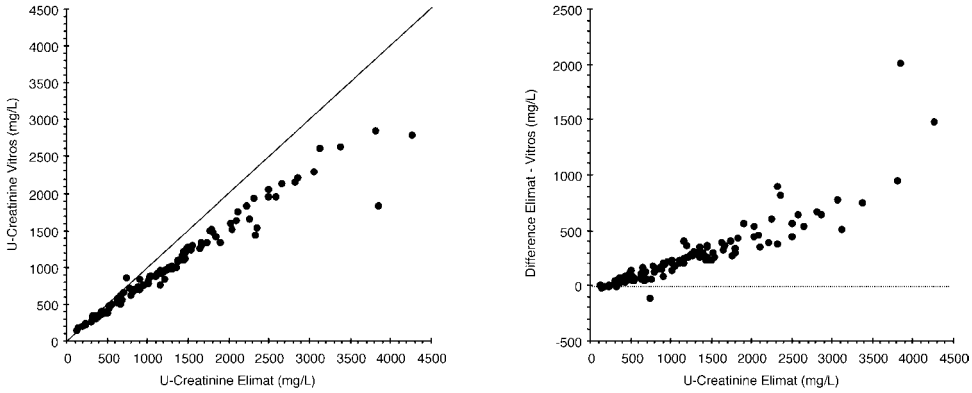

Figure 4. Scatterplot (left) of results of U-creatinine measurements in 104 canine urine samples using the Vitros and the mean of results obtained using the Jaffé reaction and an enzymatic technique with the Elimat (black line = equivalence); Difference plot (right) of results according to the mean of results using the Elimat analyzer.

The inaccuracy of U-creatinine measurements found in this study emphasizes the need for proper assessment of the accuracy of methods used in each laboratory and the use of appropriate reference intervals for urine creatinine and for ratios of any analyte to urine creatinine. Otherwise, underestimation of creatinine may lead to overestimation of the ratios and possible false positive diagnoses. Moreover, because the imprecision of creatinine measurement is relatively high, triplicate measurements would yield more accurate results.

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