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Effect of laboratory and sample storage factors on urinary protein:creatinine ratios and clinical decision making in cats

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Abstract

Background: Urinary protein:creatinine ratio (UPC) results affect the diagnosis, prognosis, and therapy of chronic kidney disease in cats.

Objectives: To investigate the interlaboratory and intralaboratory variability and the effect of storage on UPC and International Renal Interest Society (IRIS) proteinuria substaging in cats.

Animals: Healthy and diseased client-owned cats.

Methods: Prospective study. Urine of 60 cats was randomly sent to 4 (of 9) participating laboratories (to assess interlaboratory variability) and per cat, 2 laboratories each received 2 aliquots (to determine intralaboratory variability). Samples of 23 cats were analyzed in the same laboratory the day of collection, after preservation at 22°C for 1 day and at 4°C during 1-7 days (short-term storage) and at −24°C and −80°C for 6-12 months (long-term storage). Storage conditions were compared by equivalence testing.

Results: UPCs showed good interclass correlation (ICC-inter, 0.90) and excellent intraclass correlation (ICC-intra, 0.99). However, in 30/60 (50%) cats at least 1 of 4 laboratories assigned a different IRIS proteinuria substage. Urinary protein:creatinine ratio remained stable with short-term storage, but not after 6 months storage at −24°C and after 12 months storage at −24°C or −80°C. Long-term storage caused a change in IRIS proteinuria substage in 27% of cats, whereas a shift occurred only in 4% of cats during short-term storage.

Conclusions and Clinical Importance: Laboratory choice for UPC measurement can result in different IRIS substaging for the same cat, whereas urine storage at room temperature for 1 day or in the refrigerator for up to 7 days does not clinically affect UPC.

KEYWORDS
analytical variability, feline, kidney disease, preanalytical, proteinuria

Abbreviations: CKD, chronic kidney disease; FIV, feline immunodeficiency virus; IRIS, International Renal Interest Society; SUB, subcutaneous ureteral bypass; UC, urinary creatinine concentration; UP, urinary protein concentration; UPC, urinary protein:creatinine ratio.

1 | INTRODUCTION

Measuring the urinary protein:creatinine ratio (UPC) is important to diagnose and monitor chronic kidney disease (CKD) and determine the need for directed therapy, because proteinuria decreases survival time.[1-3] Whereas moderate to marked proteinuria is more common in humans and dogs with renal disease, cats with CKD mainly exhibit low-level proteinuria (UPC < 1.0).[1,4,5] In cats with azotemic CKD, both overt proteinuria (UPC > 0.4) and borderline proteinuria (UPC 0.2-0.4) decrease survival time compared to a nonproteinuric state (UPC < 0.2).[1] In nonazotemic cats, proteinuria is associated with development of azotemia within 12 months after presentation.[6] Borderline proteinuria also occurs commonly in healthy nonazotemic cats,[7-10] but its clinical relevance is currently unknown.

Despite the importance of UPC measurements, studies on (pre) analytical factors that could affect UPC results in cats are scarce. Automated assays in human medicine show high imprecision and poor accuracy in the reference range of urinary protein (UP) concentrations.[11] In dogs, UPC values can vary depending on the laboratory and that misclassification occurs mainly for values close to the thresholds between the different International Renal Interest Society (IRIS) proteinuria substages.[12,13] In cats, there is good agreement for IRIS proteinuria substages when using 2 colorimetric protein measurement methods in a research laboratory setting, but different colorimetric techniques might affect clinical decision-making.[14] Studies comparing UPC results among commercial laboratories are lacking in cats, as are studies including both colorimetric and turbidimetric methods for UP measurement.

Another complicating factor when determining UPC is that laboratory evaluation within a few hours is not always possible in practice, and that samples can be shipped or stored at different temperatures before analysis. Furthermore, for research purposes, long-term storage of urine for several months is sometimes performed before batch analysis. In dogs, results regarding storage effects on UPC are conflicting (Table 1).[12,15,16] The only currently available study in cats showed stable UPCs in urine stored at 20°C for 6 hours and 4°C for 7 days, but a decrease after storage at −20°C for 2 and 3 months.[17] The latter study however used supernatants after urine centrifugation, whereas in practice, whole urine samples are usually shipped and stored for short-term analysis. Studies on the effect of longer storage at room temperature (>6 hours) or in the freezer (>3 months) on UPC are lacking in cats.

The current prospective study consists of 2 parts. Part A aimed to quantify the interlaboratory and intralaboratory variability of UPC and to additionally assess the agreement between commercial laboratories regarding the IRIS proteinuria substage in cats. The objective of part B was to determine the effect of different storage times and temperatures on UPC in cats and more specifically whether storage leads to clinically relevant differences (ie, a change in the IRIS proteinuria substage).

2 | MATERIALS AND METHODS

Urine samples were prospectively collected from healthy and diseased client-owned cats from August 2019 to December 2019 (part A) and in February 2020 (part B). Informed signed consent from the owner was required for participation. This study was approved by the local ethical committee of the Faculty of Veterinary Medicine, Ghent University, Belgium and the deontological committee of the Belgian Federal Agency for the Safety of the Food Chain (EC 2018/54). Healthy cats needed to be "healthy for the owner" (ie, without clinical signs or changes in general behavior and with stable body weight) and free of medication (except preventive medication) for at least 2 months before inclusion. Diseased cats could suffer from renal or other diseases and were presented for routine diagnostic investigations at the Small Animal Department of Ghent University, Belgium. Because of the analytical nature of the study, cats of any age, breed, sex, and

TABLE 1 Overview of the findings of different studies on the effect of storage on canine UPC.

	Short-term storage			Long-term storage		
Reference	Time	Temperature (°C)	Effect on UPC	Time	Temperature (°C)	Effect on UPC
Rossi et al.[12]	4 h	20	No	1 wk	−20	Increase
	12 h	20	Increase	3 mo	−20	No
	12 h	4	Increase			
	3 d	4	No			
	7 d	4	Increase			
Théron et al.[15]	5 d	20	No	1-6 mo	−20	Decrease
	3 d	4	No	1 mo	−80	No
	5 d	4	Decrease	3-6 mo	−80	Decrease
Moyle et al.[16]	4 h	24	No	-	-	-
	12 h	4	No	-	-	-
	3 d	−20	No	-	-	-

Abbreviation: UPC, urinary protein:creatinine ratio.

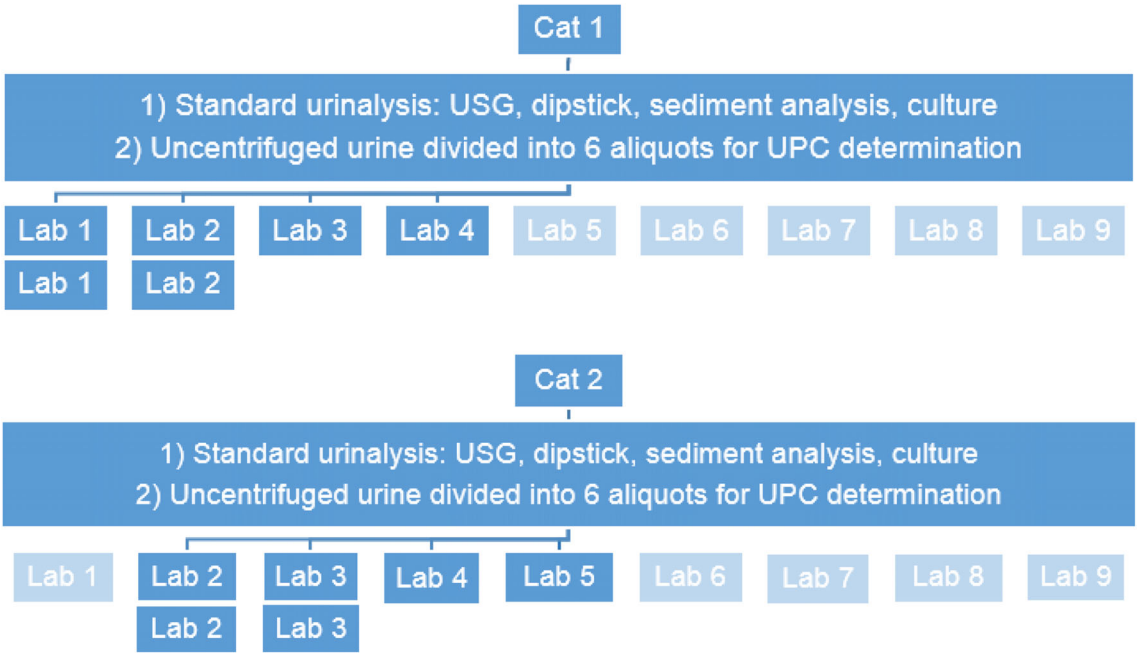

FIGURE 1 Distribution of the different aliquots of 1 urine sample over different laboratories, for the first 2 cats of study part A. UPC, urinary protein:creatinine ratio; USG, urine specific gravity.

neuter status could be included, and all diseases were allowed including those potentially causing renal or postrenal proteinuria.

Urine samples (10 mL) were collected by ultrasound-guided cystocentesis (with a 22G or 23G needle) or via a subcutaneous ureteral bypass device (with a Huber needle), if present. Macroscopic evaluation of urine samples and measurement of the urine specific gravity with a handheld refractometer (MASTER-SUR/NM, Atago) were performed on site. Urine samples were aliquoted and for each cat 2 mL was retained in the original syringe and sent overnight at ambient temperature to IDEXX Laboratories, where dipstick analysis, sediment evaluation, and bacterial culture were performed. Samples with an active urine sediment were not excluded.

2.1 | Study design part A: Interlaboratory and intralaboratory variability of UPC in cats

Remaining whole urine was aliquoted into 6 plain tubes (1 mL each). The day of sampling, these aliquots were sent to 4 different laboratories: 2 laboratories received 2 aliquots and 2 laboratories received 1. For each cat, UPC was determined by these 4 laboratories (interlaboratory variability) and in 2 of these laboratories, UPC was analyzed in duplicate (intralaboratory variability). Nine laboratories participated, and urine was sent to these laboratories according to a random scheme (Figure 1).

Samples were stored at ambient temperature until shipping. For each commercial laboratory, routine shipping procedures were applied. Depending on the laboratory, analysis was performed on the day of sampling or the day after. Cooperating laboratories were 7 commercial laboratories (5 located in Belgium and 2 in Germany), 1 clinical laboratory of the Small Animal Department (IDEXX Catalyst Dx Chemistry Analyzer) and 1 research laboratory of the Faculty of Veterinary Medicine in Ghent, Belgium (Department of Veterinary and Biosciences, research group Biochemistry). Participating commercial laboratories were IDEXX Laboratories (Kornwestheim and Leipzig), Laboklin (Bad Kissingen), Medlab Bruyland (Kortrijk), Medvet (Antwerp), Synlab (Heppignies), Velab (Aalter), and Zoolyx (Aalst). Urine centrifugation before analysis was standardly performed in 5 laboratories, not performed in 3 laboratories or performed on turbid samples only in 1 laboratory. Urinary protein determination was performed by turbidimetry based on benzethonium chloride in 5 laboratories or colorimetry based on pyrogallol red molybdate in 4 laboratories. Urinary creatinine (UC) concentration was measured with the modified Jaffe method in all laboratories.

2.2 | Study design part B: Effect of short- and long-term storage on UPC in cats

All UPC measurements for part B of the study were performed by the commercial laboratory Medvet (Antwerp, Belgium). Urine for this study part was divided into 10 aliquots that were stored at the Small Animal Department, Ghent University and shipped to that laboratory on the day the UPC needed to be measured.

On the day of urine sampling, urine was preserved at room temperature (+22°C) and UPC was determined as soon as possible by the commercial laboratory aiming to measure UPC within 8 hours of sampling (D_0). Remaining whole urine aliquots were analyzed by the same

TABLE 2 Distribution of the different aliquots of 1 urine sample over different storage times and temperatures, for cats of study part B.

Temperature (°C)	Storage time before analysis					
+22	≤8 h D_0	1 d D_1				
+4		1 d D_1	1 wk D_7			
−24				6 mo D_{180}	12 mo D_{365}	12 mo + freeze/thaw D_{366}
−80				6 mo D_{180}	12 mo D_{365}	12 mo + freeze/thaw D_{366}

laboratory after storage at room temperature for 24 more hours (D_1) and after being refrigerated (+4°C) during 1 day (D_1) and 1 week (D_7), and values were compared to D_0 to assess short-term storage. The effect of long-term storage was evaluated by comparing UPC ratios in supernatants that were frozen (−24°C and −80°C) for 6 months (D_{180}) and 12 months (D_{365}) with results from D_0. To evaluate the effect of an additional freeze-thaw cycle, samples were defrosted during 1 hour at D_{365} and subsequently refrozen for analysis the next day (D_{366}; Table 2).

For the short-term storage study, whole urine samples were stored and shipped, to mimic practice circumstances. These aliquots were centrifuged (3 minutes, 2200 rpm) by the laboratory immediately before UPC measurement in supernatant, as was the D_0 sample. Urine for long-term (frozen) storage was centrifuged immediately after sampling and supernatant divided into aliquots of 0.5 mL before being placed in the −24°C or −80°C freezer. Samples were shipped frozen at −24°C on D180, D365 or D366 to the laboratory, where UP was measured via turbidimetry using benzethonium chloride (Abbott kit urine/CSF protein 7D79-21). Urinary creatinine concentrations were measured via colorimetry using a modified Jaffe method.

2.3 | Statistical analysis

For part A of the study, statistical analysis was based on a random effects model with cat sample, laboratory and their interaction introduced as random effects. The interclass and intraclass correlation coefficients were obtained from the restricted maximum likelihood estimates of the variance components. Next, protein measurement method and centrifugation were added to the model as fixed effects to assess their importance. Not only the numerical difference between different UPC measurements was assessed, but also the number of cats in which differences in UPC values led to a different classification according to IRIS, that is, the substages nonproteinuric (UPC < 0.2), borderline proteinuric (UPC 0.2-0.4) or proteinuric (UPC > 0.4).

For part B, a fixed effects model was used with cat sample and the storage method as categorical fixed effects factor. The 95% confidence intervals (CI) of the mean differences between storage conditions were derived. If the 95% CI was completely contained in the equivalence region (ie, a region of clinically irrelevant difference, with equivalence bounds for UPC set to −0.03 and +0.03), storage conditions were accepted as being equivalent. First, the effects over time at fixed temperatures were compared. Next, different temperatures and number of thawing cycles were compared at fixed times.

TABLE 3 Group characteristics for study part A.

	Healthy (n = 30)	Diseased (n = 30)
Median (range) age	6 (1-16) y	11 (1-18) y
Sex	10 male neutered 20 female spayed	19 male neutered 11 female (10 spayed)
Breed (n > 1)	DSH and DLH (n = 25) Birman (n = 2)	DSH and DLH (n = 24)
Diseases (n > 1)	-	Hyperthyroidism (n = 15) CKD (n = 6) Cardiac disease (n = 4) FIV (n = 3)
Sampling method	Cystocentesis (n = 30)	Cystocentesis (n = 27) SUB device (n = 3)
Median (range) UPC	0.10 (0.05-1.47)	0.29 (0.08-4.53)
Nonproteinuric cats	26	11
Borderline proteinuric cats	2	13
Proteinuric cats	2	6

Abbreviations: CKD, chronic kidney disease; DLH, domestic longhair; DSH, domestic shorthair; FIV, feline immunodeficiency virus; SUB, subcutaneous ureteral bypass; UPC, urinary protein:creatinine ratio.

3 | RESULTS

3.1 | Interlaboratory and intralaboratory variability

Sixty cats, 30 healthy and 30 diseased, were included in part A of the study (Table 3). Their UPC values, calculated as the median value of the different laboratory results, ranged from 0.05 to 4.53 (median 0.15). When applying the IRIS proteinuria substages, 37/60 (62%) cats

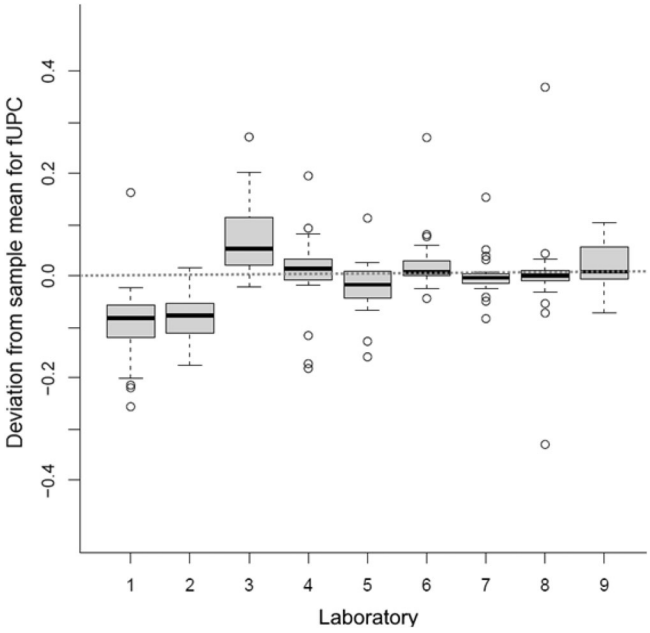

FIGURE 2 Box plots of the deviation of a sample from the mean of that sample by lab. The value 0 for urinary protein:creatinine ratio (UPC) on the y-axis reflects the mean value of the UPC ratios obtained by different laboratories for the same urine sample. The numbers on the x-axis depict the different laboratories. The box plots reflect the deviation of UPC ratios from that mean value for each laboratory separately.

were nonproteinuric, 15/60 (25%) were borderline proteinuric, and 8/60 (13%) cats had proteinuria.

Each laboratory examined urine samples from 25 to 27 cats. When comparing the results obtained by different laboratories for the same sample, absolute UPC values showed good interclass correlation (ICC-inter = 0.90) and excellent intraclass correlation (ICC-intra = 0.99). In Figure 2, the box plots of UPC ratios measured by each laboratory are shown. Laboratories 1 and 2 measured lower UPC ratios than the mean value measured by all laboratories together, whereas laboratory 3 on average reported higher UPC ratios than the sample mean.

The UPC values obtained with turbidimetry vs colorimetry did not significantly differ, nor did centrifugation of urine samples before analysis affect UPC results significantly.

Agreement on IRIS substages between laboratories was moderate (Fleiss' κ coefficient = 0.55). When 2 laboratories evaluating the same urine sample were compared, 253/360 (70%) of UPC comparisons showed a classification within the same IRIS proteinuria substage by different laboratories. In 30/60 cats (50%), at least 1 of the 4 laboratories reported a UPC value within a different substage than the other laboratories that analyzed the same urine sample. In these 30 cats, the disagreement concerned nonproteinuric vs borderline proteinuric substage in 18 cases, and borderline vs overt proteinuria in 9 cases. In the 3 remaining cases, the 4 laboratories reported values in the complete range of substages, from nonproteinuric over borderline proteinuric to proteinuric. The difference in UPC measured between laboratories assigning a different substage to the same cat was ≤0.1 in 5/30 (17%) cats, 0.11-0.2 in 11/30 (37%) cats, 0.21-0.3 in 9/30 (30%) cats, 0.31-0.4 in 3 (10%) cats, 0.41-0.5 in 1 (3%) cat, and >0.5 in 1 (3%) cat.

When 2 aliquots of a single sample were examined by the same laboratory, 116/120 (97%) of UPC comparisons resulted in the same IRIS proteinuria substage. The discrepancies in these 4 (3%) cats were between the non- and borderline proteinuric substage in 1 case, and between borderline and overt proteinuria in the 3 remaining cases. In these 4 cases, the difference between both UPC values reported by the same laboratory for the same urine sample ranged from 0.05 to 0.2.

When investigating UP and UC measurements separately, UP values also showed good interclass correlation (ICC-inter = 0.87) and excellent intraclass correlation (ICC-intra = 0.99). Results for UC showed both excellent interclass (ICC-inter = 0.93) and intraclass correlation (ICC-intra = 0.99).

In Figure 3, the mean UPC, UP, and UC of a sample are plotted against the SD of all measurements of that sample.

3.2 | Effect of sample storage

Twenty-three cats were included in part B of the study (Table 4). At D_0, median (range) UPC was 0.19 (0.07-1.67) with 13 cats being nonproteinuric (UPC < 0.2), 6 borderline proteinuric (UPC 0.2-0.4) and 4 proteinuric (UPC > 0.4).

The amount of urine obtained from 1 proteinuric cat sufficed for aliquots for short-term storage only. With short-term storage (D_1 and D_7), UPC results were equivalent to D_0 (95% CI −0.006 to 0.025 for D_1 at 22°C, −0.001 to 0.029 for D_1 at 4°C, and −0.005 to 0.026 for D_7 at 22°C). After long-term storage, UPC remained equivalent to D_0 after storage for 180 days at −80°C (95% CI −0.004 to 0.027), but a clinically unacceptable decrease in UPC compared to D_0 was seen at D_{180} and D_{365} with storage at −24°C (95% CI 0.015-0.047 for D_{180} at −24°C and 0.024-0.056 for D_{365} at −24°C), and at D_{365} for urine stored at −80°C (95% CI 0.008-0.039; Figure 4A). When comparing samples stored long-term at different temperatures, UPC in samples stored at −24°C for 180 days (95% CI 0.003-0.035) or 365 days (95% CI 0.001-0.032) were not equivalent to UPC after storage at −80°C for the same time period. (Figure 4B), with a larger decrease in UPC occurring at −24°C compared to −80°C. After an extra freeze-thaw cycle, UPC values at D_{366} were still equivalent to D_{365} for samples stored at both −24°C (95% CI −0.012 to 0.020) and − 80°C (95% CI −0.009 to 0.023; Figure 4C).

After long-term storage of urine, a change in IRIS substage for UPC occurred at ≥1 time point in 6/22 (27%) cats, whereas only in 1/23 cats (4%) a shift took place during short-term storage. The cat that had a change in IRIS substage during short-term storage had a UPC of 0.19 at D_0 and a value of 0.20-0.21 at D_1 (+4 and +22°C) and D_7 (+4°C). In 5/6 cats where IRIS proteinuria substage changed after long-term storage, UPC shifted (in both directions) between the nonproteinuric and borderline proteinuric substage as well, with the arisen difference in UPC ranging from 0.01 to 0.1. In 1 cat the UPC

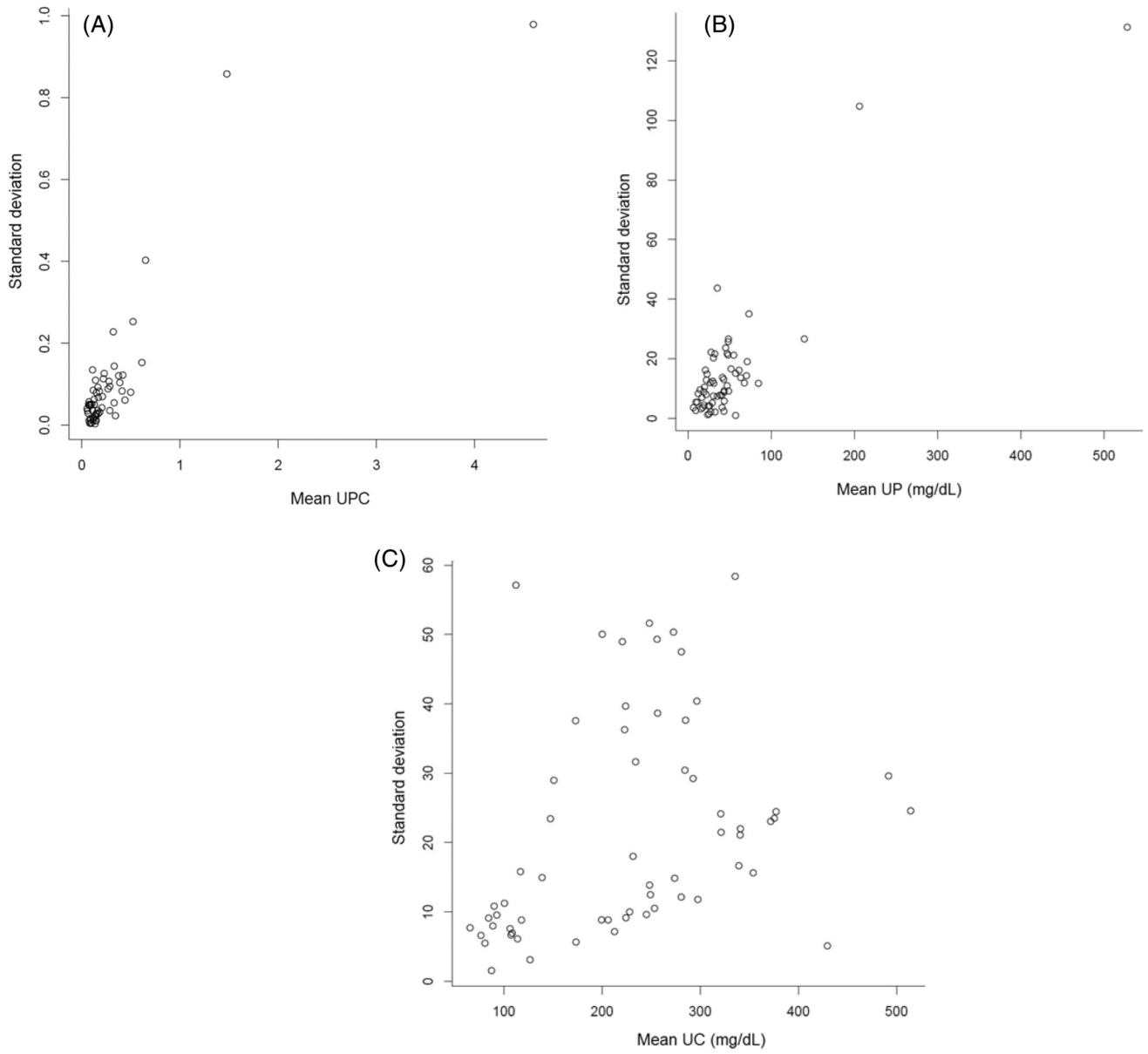

FIGURE 3 SD of all measurements of a sample as a function of the mean of that sample, for urinary protein: creatinine ratio (UPC, A), urinary protein concentration (UP, B), and urinary creatinine concentration (UC, C).

dropped from the proteinuric substage at D_0 (UPC 0.47) to the borderline proteinuric substage at D_{180} (UPC 0.33) and D_{365} (UPC 0.32) upon storage at −24°C, but not with storage at −80°C.

When looking at UP and UC separately, UP significantly decreased after long-term storage for 180 days (at −24°C and −80°C) and 365 days (at −24°C), whereas UC decreased only after storage for 180 days (at −24°C and −80°C), after which it increased again.

4 | DISCUSSION

This study compares UPC values measured by different commercial laboratories and by colorimetric and turbidimetric techniques for UP determination in cats. Our results show that the choice of a particular (commercial) laboratory to assess urine samples of cats does not significantly affect absolute UPC values. This is reflected by the good interclass correlation (ICC-inter = 0.90), similar to reported values in dogs (ICC-inter = 0.86-0.90).[13] When looking at the clinically relevant IRIS substages for proteinuria, however, agreement is only moderate (Fleiss' κ coefficient = 0.55), which is somewhat lower than what was reported in a study comparing 2 different colorimetric methods for UP determination in cats (Cohen's κ coefficient = 0.62).[14]

A different IRIS proteinuria substage holds clinical consequences with regard to starting (or increasing) therapy for proteinuria, which IRIS recommends for all cats with a UPC persistently >0.4.[18] For cats with CKD, it is known that a UPC ≥ 0.2 already leads to a decreased survival time compared to a UPC < 0.2.[1] In the current study, lack of

TABLE 4 Group characteristics for study part B.

	Healthy (n = 11)	Diseased (n = 12)
Median (range) age	5 (0-14) y	10 (4-15) y
Sex	6 male neutered 5 female spayed	5 male neutered 7 female spayed
Breed (n > 1)	DSH and DLH (n = 7)	DSH and DLH (n = 11)
Diseases (n > 1)	-	CKD (n = 6) Hyperthyroidism (n = 4)
Sampling method	Cystocentesis (n = 11)	Cystocentesis (n = 9) SUB device (n = 3)
Median (range) UPC at D_0	0.15 (0.07-0.29)	0.24 (0.11-1.67)
Nonproteinuric cats	8	5
Borderline proteinuric cats	3	3
Proteinuric cats	0	4

Abbreviations: CKD, chronic kidney disease; DLH, domestic longhair; DSH, domestic shorthair; SUB, subcutaneous ureteral bypass; UPC, urinary protein:creatinine ratio.

substantial agreement between laboratories led to differences in classification of proteinuria according to IRIS guidelines.[19] When a sample was sent to 4 laboratories, at least 1 of these reported a different substage than the other laboratories in half of the cats in the current study. This implicates that careful interpretation of UPC values near the IRIS thresholds for the different substages is warranted, as a different laboratory could place the same cat in a different therapeutic and prognostic category. Therefore, clinicians need to be aware that such misclassifications are possible. Furthermore, IRIS guidelines state that proteinuria substage is ideally based on at least 2 subsequent UPC measurements.[19] Based on the results of study part B, it is then important to always send the urine sample to the same laboratory when monitoring the same cat for the development or evolution of proteinuria, or when monitoring the response to antiproteinuric treatment.

It might be advised to not only consider the IRIS substages but also laboratory-specific reference intervals for UPC, when making clinical decisions regarding proteinuria in cats. Another option would be to introduce standardization of UPC measurement in veterinary medicine, in order to minimize interlaboratory variation. Based on the present study, different measurement techniques for UP determination (ie, colorimetric vs turbidimetric) and whether or not urine was centrifuged before analysis did not have a statistically significant effect on UPC in cats. Nonetheless, the difference in UPC was mostly explained by the difference in UP measurements and less by differences in UC measurement, which might indicate that a more robust or standardized UP technique could be advantageous.

It could be argued that results between laboratories might have differed because of differences in storage times, since samples were processed by some laboratories the same day and by others the next day. Additionally, samples could be stored either at ambient temperature or in the refrigerator by the participating laboratories. Considering the results of part B of this study, however, transportation times and storage temperatures did not play a significant role in the observed interlaboratory variability.

Repeating a UPC measurement in the same laboratory led to a different IRIS classification in only 3% of measurements. Considering the excellent intraclass correlation, it does not seem necessary or useful to let the same sample be analyzed by the same laboratory multiple times and have the mean value reported, but a single measurement suffices.

Finally, it is noteworthy to mention that 3/9 laboratories rounded their UPC values, which means that a reported UPC value of 0.4 for example could mean either borderline proteinuria (if the fUPC is 0.35-0.40) or overt proteinuria (if the exact UPC value is 0.41-0.44). For the current study, exact UPC values were calculated based on UP and UC results, but in practice, veterinarians might often work with the rounded UPC values reported by the laboratory. To facilitate comparison between laboratories, it would be best to use a uniform way to report UPC values (eg, using 2 decimals, as is done for IRIS substages).

The present study also investigates both the effect of long-term storage (>3 months) on UPC in cats and the effect of short-term storage on whole urine, the sample type typically transported in practice. The only previous study in cats examined urine stored at ambient temperature for a maximum of 6 hours, whereas practical circumstances can lead to overnight storage or transportation, with analysis taking place the next day. In the current study, short-term storage of urine only led to a change in proteinuria substage in 1/23 cats with a UPC on the border between the non- and borderline proteinuric substage (0.19 at D_0 and 0.20 at D_1 and D_7). Therefore, it is acceptable to send urine samples of cats to external laboratories the day after collection, even upon storage at room temperature. When samples are refrigerated in the clinic or at the laboratory, UPC can even be determined up to 7 days after sampling without influencing clinical decisions regarding therapy, monitoring, or prognosis.

This study mainly showed an effect of long-term storage on UPC, with a shift in substage occurring in 6/22 cats. This shift occurred mostly between the nonproteinuric and borderline proteinuric substage. In 1 cat, the UPC dropped from the proteinuric substage (at D_0, D_1, and D_7) to the borderline proteinuria substage after storage for 180 and 365 days at −24°C (but not −80°C). It is important to take this into account when measuring UPC in frozen urine samples for research purposes. Storage at −80°C has less effect on UPC than storage at −24°C for 180 or 365 days. Therefore, urine samples of cats are best stored long-term at −80°C rather than −24°C, in accordance with recommendations in human medicine.[20]

The most likely cause for changes in UP and UPC over time is degradation of albumin and other proteins during sample storage,[15,20] although shifts in substages may also be attributable to intra-assay variability. Part A of the study however showed excellent intraclass correlation for UP and UPC measurement, making the latter a less likely explanation.

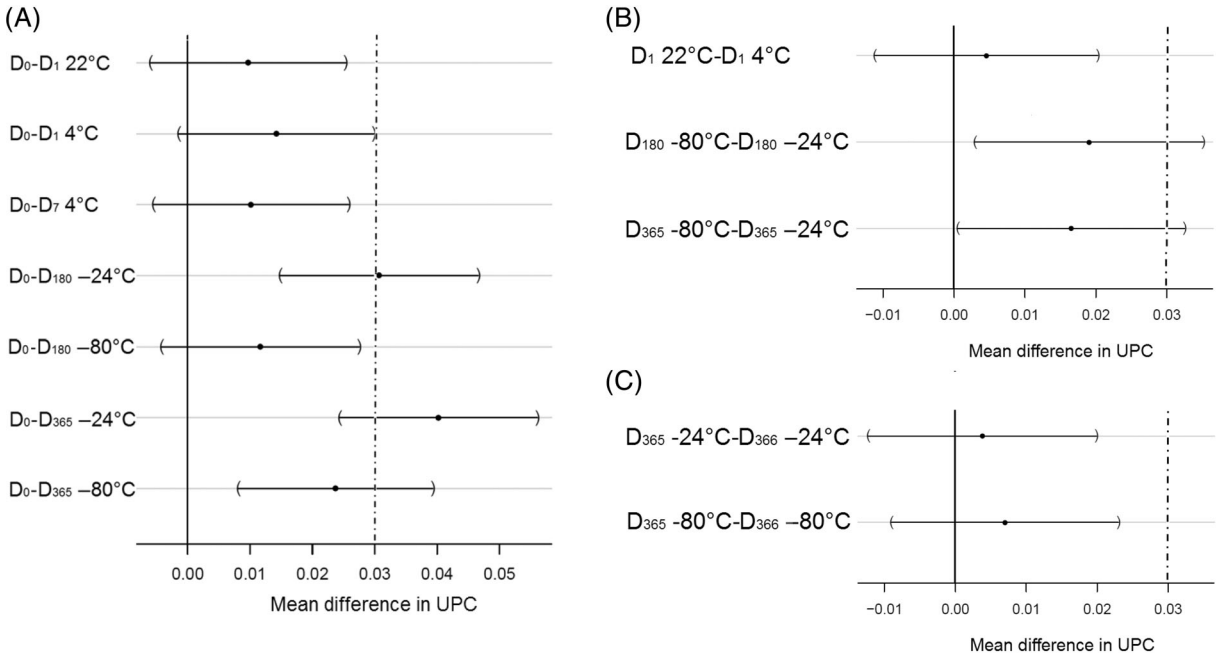

FIGURE 4 95% confidence intervals for mean differences in urinary protein:creatinine ratio (UPC). If the 95% CI was completely contained in the equivalence region (ie, a region of clinically irrelevant difference, with equivalence bounds for UPC set to −0.03 and +0.03), storage conditions were accepted as being equivalent. The dashed vertical line indicates the upper equivalence limit (+0.03), which must not be exceeded in order to claim equivalence. (A) Effect of different storage times and temperatures (each compared to D_0). (B) Effect of storage temperature for storage during 1 day (4°C vs 22°C), 180 or 365 days (−24°C vs −80°C). (C) Effect of an additional freeze-thaw cycle after storage at −24 or −80°C (D_{366} vs D_{365}).

A limitation for study part A is that there is no gold standard assay for UP and therefore UPC determination in cats, to compare other measurement methods and laboratories with. This makes it impossible to determine assay sensitivity and specificity, or assess which laboratory is most accurate and reports the most reliable UPC value. A limitation for both study parts was that urine protein content analysis was not performed and the contributing types of urinary proteins to the total UP may have varied between cats. Since cats with different types of disease were included, it is possible that proteinuria origin differed between cats. For study part A, this implies that differences in UP measurements between laboratories were potentially due to differences in the detection of certain protein types, that could be either renal or postrenal in origin. For study part B, this prohibits the investigation of whether certain proteins may have degraded over time and been responsible for the decline in UP and UPC. A final limitation was the limited number of borderline and overtly proteinuric cats, with a relatively low median UPC of 0.15 and 0.19 in parts A and B, respectively. Yet, it should be emphasized that this is consistent with earlier findings in cats that proteinuria is rare in healthy cats and that even a large proportion of CKD cats does not have proteinuria.[7,9,10] Nonetheless, even borderline proteinuria is of prognostic importance in cats with CKD, and further studies are needed to determine its importance in cats not (yet) diagnosed with CKD.

In addition to analytical factors, it is also important to consider the biological variability of proteinuria in cats when making clinical decisions. Since the biological variability of UPC in cats is currently unknown, studies are needed to further aid in making clinical decisions in case of a clinically relevant change in UPC (ie, a shift in IRIS proteinuria substage) in a single cat.

In conclusion, choosing a particular laboratory to assess urine samples of cats does not significantly affect absolute UPC values. However, since a different IRIS proteinuria substage was diagnosed in 30% of cases where UPC was measured by 2 different laboratories, this choice can affect clinical decisions. Therefore, it is advisable to choose the same laboratory when monitoring the evolution of proteinuria or response to antiproteinuric treatment in the same cat. Short-term storage of urine in the conditions used in practice (for 1 day at room temperature or up to 7 days in the refrigerator) does not cause clinically relevant changes in UPC in cats and is therefore acceptable. Long-term storage (6-12 months) for research purposes more often results in statistically and clinically relevant changes in UPC, and is preferably done at −80°C rather than at −24°C.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the local ethical committee of the Faculty of Veterinary Medicine, Ghent University, Belgium and the deontological committee of the Belgian Federal Agency for the Safety of the Food Chain (EC 2018/54).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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