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# Comparison of three types of analyzers for urine protein-to-creatinine ratios in dogs

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## ABSTRACT

**Background:** Quantitation of urine protein is important in dogs with chronic kidney disease. Various analyzers are used to measure urine protein-to-creatinine ratios (UPCR).

**Objectives:** This study aimed to compare the UPCR obtained by three types of analyzers (automated wet chemistry analyzer, in-house dry chemistry analyzer, and dipstick reading device) and investigate whether the differences could affect clinical decision process.

**Methods:** Urine samples were collected from 115 dogs. UPCR values were obtained using three analyzers. Bland-Altman and Passing Bablok tests were used to analyze agreement between the UPCR values. Urine samples were classified as normal or proteinuria based on the UPCR values obtained by each analyzer and concordance in the classification evaluated with Cohen's kappa coefficient.

**Results:** Passing and Bablok regression showed that there were proportional as well as constant difference between UPCR values obtained by a dipstick reading device and those obtained by the other analyzers. The concordance in the classification of proteinuria was very high ( $\kappa = 0.82$ ) between the automated wet chemistry analyzer and in-house dry chemistry analyzer, while the dipstick reading device showed moderate concordance with the automated wet chemistry analyzer ( $\kappa = 0.52$ ) and in-house dry chemistry analyzer ( $\kappa = 0.53$ ).

**Conclusions:** Although the urine dipstick test is simple and a widely used point-of-care test, our results indicate that UPCR values obtained by the dipstick test are not appropriate for clinical use. Inter-instrumental variability may affect clinical decision process based on UPCR values and should be emphasized in veterinary practice.

**Keywords:** Dog; creatinine; point-of-care testing; proteinuria; urinalysis

## INTRODUCTION

Proteinuria is not only an early marker of kidney injury but also a risk factor of uremic crisis and renal disease-related death in dogs with chronic kidney disease (CKD) [1]. Moreover, proteinuria itself may aggravate the kidney disease by promoting inflammation and fibrosis [2]. Therefore, evaluation and early intervention in dogs with renal proteinuria are

**Conflict of Interest**

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**Author Contributions**

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considered important. To evaluate and monitor proteinuria, urine protein quantification is essential. In veterinary medicine, the urine protein-to-creatinine ratio (UPCR) determined from a single urine sample is utilized for the quantification of urine protein [3].

Various instruments are available for determining the UPCR. Automated wet chemistry analyzers are commonly used in referral laboratories, and in-house bench top dry chemistry analyzers are popular in local animal hospitals. Some dipstick reading devices that analyze change in the color of dipstick pads also offer UPCR values based on semiquantitative evaluation of creatinine and protein concentrations in urine [4,5]. Each analyzer uses different analytical method to measure the concentrations of urine creatinine and urine protein. For example, wet chemistry analyzers use Jaffe's method or an enzymatic method to measure urine creatinine concentration and pyrogallol red or benzethonium chloride to measure urine protein concentration. In-house dry chemistry analyzers use an enzymatic method to determine urine creatinine concentration and pyrocatechol violet to measure urine protein concentration. The urine dipstick test detects urine creatinine from the peroxidase activity of copper-creatinine complexes and urine protein from color change of pH indicator, tetrabromophenol blue [6]. Because of the different methodologies, UPCR values obtained from different analyzers likely differ [7-9]. However, so far, no data were available on inter-instrumental variability of UPCR and whether the inter-instrumental variability could affect clinical decision. This study aimed to investigate inter-instrumental variability of UPCR obtained from three types of analyzers and its clinical implications.

## MATERIALS AND METHODS

### Animals and sampling

Urine samples were collected from 115 dogs that were presented at the Veterinary Medical Teaching Hospital of Seoul National University because of health problems or health screening assessment over a five-month period (March – July 2018). All urine samples were collected as a part of the diagnostic workup. Breed, age, sex, method of urine collection (free catch, catheterization or cystocentesis), and underlying clinical conditions were recorded.

### Urinalysis

Complete urinalysis including specific gravity by refractometer (Reichert VET 360, Reichert Technologies Inc., USA), dipstick tests (Combur Test, Roche Diagnostics Ltd., Switzerland), and sediment examination, was carried out within 2 h of sample collection. To perform sediment analysis, 2 mL of each urine sample was centrifuged at 400 g for 5 min. Then, 1.8 mL of the supernatant was transferred into separate tubes for determining the UPCR. In case immediate analysis of UPCR was not possible, the supernatant was stored at  $-20^{\circ}\text{C}$  for a maximum of 3 months. The urine pellet was resuspended and microscopically examined. Urine sediment was classified as active when there were more than 5 red blood cells or white blood cells at high power field ( $40\times$  objective) or bacteriuria was detected [10]. When stored supernatants were subjected to determine the UPCR, frozen samples were gently thawed at room temperature for 1 h, further centrifuged so that the crystals or particles were settled, and then analyzed.

### UPCR

Urine concentrations of protein and creatinine were determined by an automated wet chemistry analyzer (Hitachi 7180, Hitachi Ltd., Japan), an in-house dry chemistry analyzer

(Catalyst One, Idexx Laboratories Inc., USA), and a dipstick reading device (BS201, Bionics Ltd., Korea). These instruments were selected because they are commonly used in referral laboratories and local animal hospitals. All analyzers were operated according to the manufacturers' instructions.

Using the automated wet chemistry analyzer, urine protein concentration was measured by the pyrogallol red method and urine creatinine concentration by the Jaffe method. The measurement range using the pyrogallol red method was 2.58 to 400 mg/dL; samples were diluted at a ratio of 1:20 and reanalyzed when the values were higher than the upper limit [11]. The results were recorded as a range with an inequality sign if the values were below the lower limit. If the urine creatinine value was higher than 80 mg/dL, the upper limit of linearity of the Jaffe method, the values were obtained using a linear equation calculated from serial dilutions of the sample at 1:4, 1:20, and 1:100 with deionized water.

The in-house dry chemistry analyzer used pyrocatechol violet dye to measure the concentration of urine protein and an enzymatic method to determine urine creatinine concentration. The measurement ranges of urine protein and urine creatinine were 5 to 400 mg/dL and 6 to 350 mg/dL, respectively. The values outside of these ranges were recorded with inequality sign.

For evaluating urine protein and urine creatinine by the dipstick reading device, the urine sample was dropped on the urine strip pad (Bionics Vet 12, DFI Ltd., Korea), excessive urine was tapped off, and then, the strip was placed in the reading device's tray. The reading was done at 60 seconds from urine application. The urine dipstick reading device evaluated color changes of urine dipsticks by reflectance photometry.

UPCR was calculated by dividing the urine protein concentration (mg/dL) by urine creatinine concentration (mg/dL) on the wet and dry chemistry analyzers. By the urine dipstick reading device, the automatically calculated UPCR was shown.

### **Precision assay, repeatability, and linearity**

For precision assay, two levels of commercial urine chemistry control (Liquichek Urine chemistry Control Level 1 and 2, Bio-Rad Inc., USA) were used. These urine chemistry controls are human urine based and have urine substances including protein and creatinine. Control materials were measured three times per day over 5 days, totaling 15 runs for each level. The mean, standard deviation (SD), and coefficient of variation (CV) of urine protein concentration, urine creatinine concentration, and UPCR were calculated [12].

To evaluate repeatability, the concentrations of urine protein and urine creatinine were measured 5 times in succession from three samples. The mean, SD, and CV were calculated.

Urine samples were mixed in serial proportions (0:100, 20:80, 40:60, 60:40, 80:20, and 100:0) to evaluate the linearity. The concentrations of urine protein and urine creatinine were measured and compared to the calculated expected concentrations.

### **Statistical analyses**

Statistical analyses were performed using Excel 2016 (Microsoft Corporation Inc., USA) and XLSTAT software (Addinsoft Inc., USA). The  $p$  value  $< 0.05$  was considered statistically significant. Differences in UPCR values between sexes or sampling methods were assessed

by Kruskal-Wallis test. The correlation between each UPCR value obtained from the three analyzers was evaluated using the Pearson correlation test. The agreement between UPCR values were analyzed using the Bland-Altman and Passing Bablok tests. Cohen's kappa coefficient was calculated to evaluate the concordance in classification of proteinuria between analyzers. The results of Cohen's kappa concordance tests represented as very good ( $\kappa = 0.81-1.00$ ), good ( $\kappa = 0.61-0.80$ ), moderate ( $\kappa = 0.41-0.60$ ), fair ( $\kappa = 0.21-0.40$ ), and poor ( $\kappa = 0.00-0.20$ ) [13].

## RESULTS

A total of 115 dogs consisted of 11 male, 50 castrated male, 9 female, and 45 spayed female. Median age was 11 years and ranged from 3 months to 18 years. There were 24 breeds of dogs; Maltese ( $n = 27$ ) and Shih-tzu ( $n = 17$ ) were the most common breeds. Twenty-one urine samples were collected by free catch, 15 were catheterization, and 79 were cystocentesis. There was no significant difference in the UPCR values between sexes ( $p = 0.242$ ) or sampling methods ( $p = 0.954$ ). From urine sediment tests, 15 samples were evaluated as active urine sediments.

### Precision assay, repeatability, and linearity

For precision assay, the automated wet chemistry analyzer had higher CV values of urine protein, urine creatinine, and UPCR for low-level control material than for high-level control; nevertheless, all CV values were lower than 10%. All CV values of urine protein, urine creatinine, and UPCR in the in-house dry chemistry analyzer were lower than 5%. The dipstick reading device had the highest CV values of UPCR among three analyzers, which were slightly higher than 10% (Table 1).

The two chemistry analyzers showed good repeatability, and those of CV values were less than 5%. The dipstick reading device had higher CV values of UPCR than did the other two chemistry analyzers (Table 2).

In the linearity study, the regression analysis of the measured value and the expected value showed a very good linear relationship for both urine creatinine ( $R^2 = 0.9999$ ) and urine protein concentration ( $R^2 = 0.9991$ ) obtained by the automated wet chemistry analyzer. The in-house

**Table 1.** Mean, SD and CV of urine protein concentration, urine creatinine concentration, and UPCR measured by three analyzers using urine chemistry control materials (15 runs; 3 replications per day for 5 days)

Analyzer	Control level 1			Control level 2		
	UP	UC	UPCR	UP	UC	UPCR
<b>Analyzer 1</b>						
Mean	15.9	59.2	0.27	55.7	118.6	0.47
SD	1.5	2.1	0.02	1.3	3.5	0.01
CV (%)	9.42	3.61	9.23	2.28	2.96	2.89
<b>Analyzer 2</b>						
Mean	32.0	38.3	0.84	72.9	71.7	1.02
SD	1.0	1.1	0.02	2.1	1.9	0.03
CV (%)	3.11	2.90	2.76	2.89	2.73	2.60
<b>Analyzer 3</b>						
Mean	27.3	121.8	0.23	66.0	192.1	0.35
SD	0.9	12.9	0.02	4.8	17.0	0.04
CV (%)	3.32	10.58	10.03	7.30	8.87	11.32

The unit of mean and SD of urine protein and urine creatinine concentration is mg/dL.

SD, standard deviation; CV, coefficient of variation; UPCR, urine protein-to-creatinine ratio; UP, urine protein; UC, urine creatinine; Analyzer 1, automated wet chemistry analyzer; Analyzer 2, in-house dry chemistry analyzer; Analyzer 3, dipstick reading device.

## Comparison of UPCR analyzers

**Table 2.** Mean, SD, CV of five repeated measurements of urine protein concentration, urine creatinine concentration, and UPCR in canine urine samples

Analyzer	Sample 1			Sample 2			Sample 3		
	UP	UC	UPCR	UP	UC	UPCR	UP	UC	UPCR
Analyzer 1									
Mean	223.8	34.14	6.6	24.8	25.61	1.0	55.2	28.55	1.9
SD	4.6	0.4	0.1	0.8	0.1	0.0	1.3	0.3	0.0
CV (%)	2.1	1.0	1.6	3.4	0.6	3.8	2.4	1.0	1.7
Analyzer 2									
Mean	356.8	49.4	7.2	36.2	32.6	1.1	69.0	32.4	2.1
SD	5.8	1.1	0.2	1.1	0.5	0.0	1.2	0.5	0.0
CV (%)	1.6	2.3	2.4	3.0	1.7	2.4	1.8	1.7	1.3
Analyzer 3									
Mean	127.2	98.6	1.31	63.0	52.6	1.22	74.6	85.2	0.88
SD	7.3	12.4	0.2	2.9	7.3	0.2	5.6	8.9	0.1
CV (%)	5.8	12.6	15.0	4.6	13.8	16.3	7.5	10.4	8.2

The unit of mean and SD of urine protein and urine creatinine is mg/dL.

SD, standard deviation; CV, coefficient of variation; UPCR, urine protein-to-creatinine ratio; UP, urine protein; UC, urine creatinine; Analyzer 1, automated wet chemistry analyzer; Analyzer 2, in-house dry chemistry analyzer; Analyzer 3, dipstick reading device.

dry chemistry analyzer also showed a good linear relationship for urine creatinine ( $R^2 = 0.9113$ ) and urine protein concentration ( $R^2 = 0.9494$ ). However, the dipstick reading device showed a poor linear relation (urine creatinine  $R^2 = 0.4548$ ; urine protein  $R^2 = 0.528$ ) (**Fig. 1**).

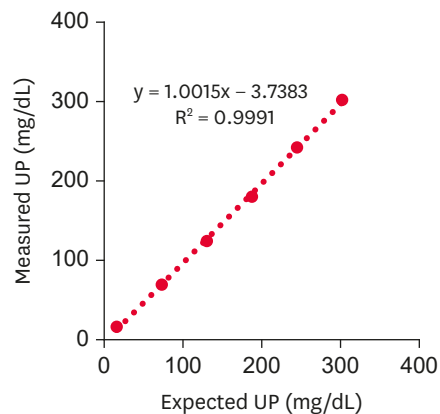
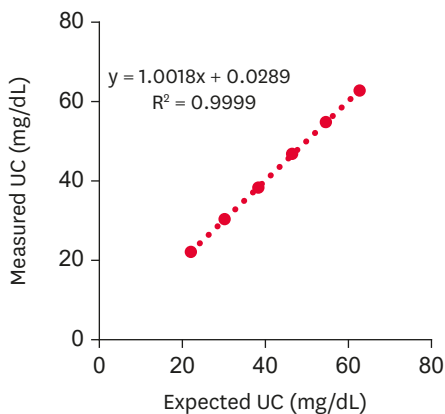
### Comparison of UPCR results between three analyzers

Urine creatinine or urine protein concentration values that were below or above the measurement range of each analyzer were recorded as ranges with an inequality sign. Thus, UPCR values of those cases were excluded from estimation of the correlation coefficient or plotting due to the difficulty in determining the numerical value. Of the 115 UPCR values measured from each analyzer, 18 from the automated wet chemistry analyzer and 24 from the in-house dry chemistry analyzer were excluded. UPCR values of 81, 97, and 91 dogs were used for the comparison analysis. The UPCR values obtained using the automated wet chemistry analyzer and in-house dry chemistry analyzer were highly correlated ( $r = 0.911$ ,  $p < 0.001$ ). The correlation was moderate ( $r = 0.54$ ,  $p < 0.001$ ) between the dipstick reading device and automated wet chemistry analyzer and poor ( $r = 0.14$ ,  $p < 0.001$ ) between the dipstick reading device and in-house dry chemistry analyzer.

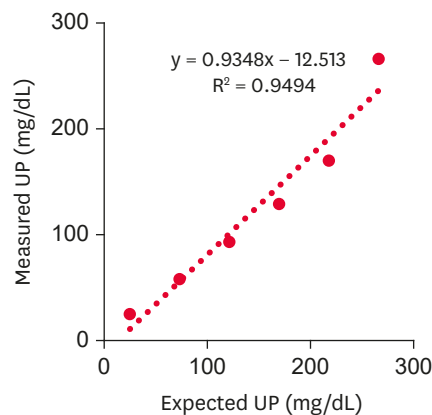
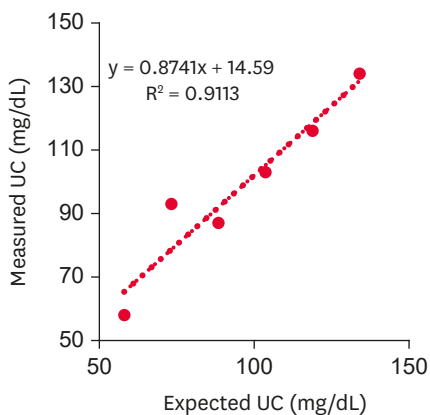
The 95% confidence interval (CI) for intercept and the 95% CI for slope of Passing Bablok regression on UPCR values obtained by automated wet chemistry analyzer and in-house dry chemistry analyzer included value zero and one, respectively, which means there was no constant difference nor proportional difference between two methods [14]. In addition, Bland-Altman analysis showed that the bias between two analyzers was not significant. However, proportional difference and constant difference existed between dipstick reading device and the other analyzers, though the biases were not significant (**Figs. 2 and 3**).

Excluding the 15 samples with active urine sediments, 100 samples were used to evaluate concordance in the classification of proteinuria between analyzers. The UPCR values  $\geq 0.5$  were considered to indicate proteinuria [15]. The automated wet chemistry analyzer and in-house dry chemistry analyzer showed very good concordance ( $\kappa = 0.82$ ), whereas the dipstick reading device showed moderate concordance ( $\kappa = 0.52$  or  $0.51$ ) with the other two chemistry analyzers (**Tables 3-5**).

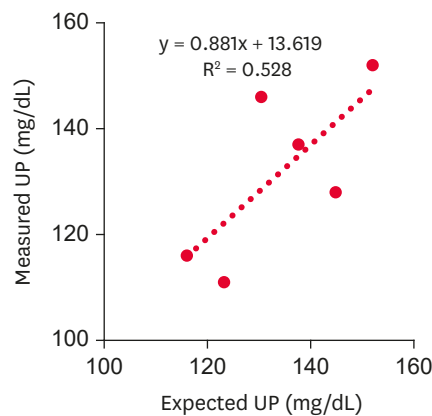
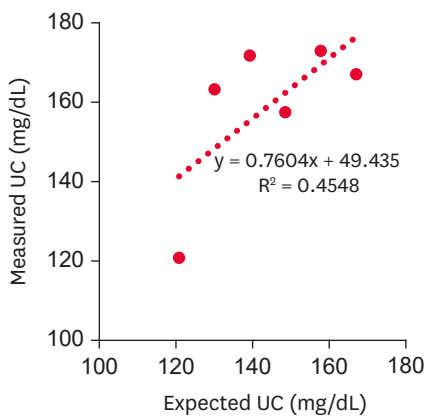
**Analyzer 1**



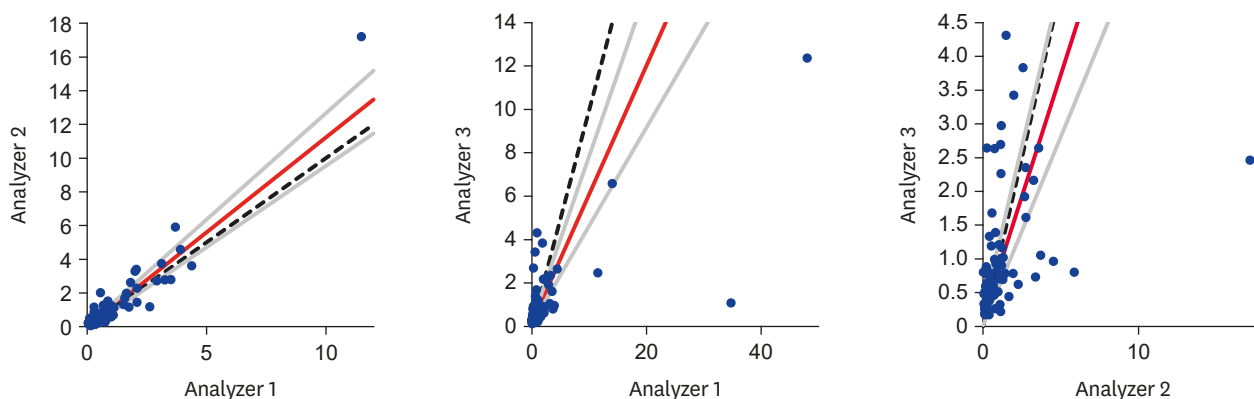
**Analyzer 2**



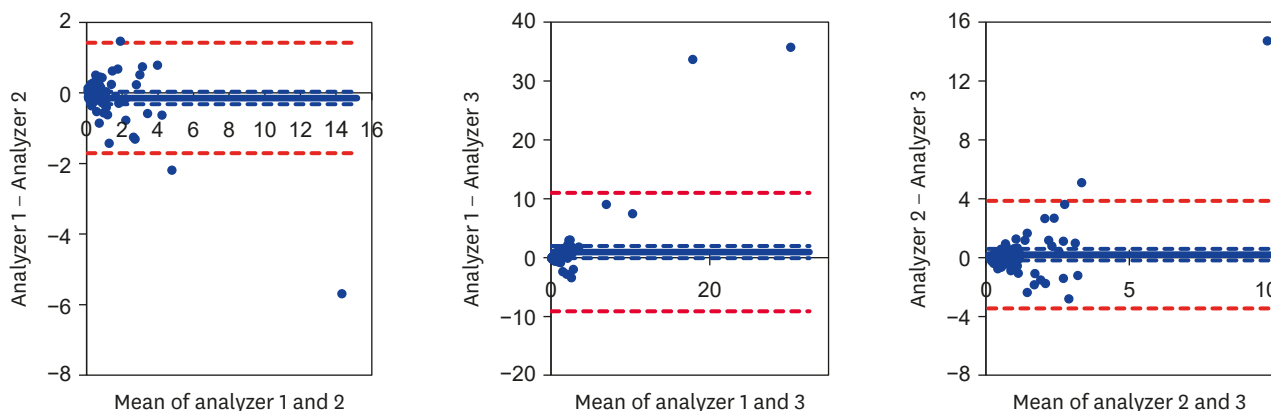
**Analyzer 3**



**Fig. 1.** Scatter plot of expected UC or UP concentrations against measured UC or UP concentrations. Patient samples and serially mixed samples (20:80, 40:60, 60:40, 80:20) were used. Dotted lines are regression lines. Analyzer 1, automated wet chemistry analyzer; Analyzer 2, in-house dry chemistry analyzer; Analyzer 3, dipstick reading device. UC, urine creatinine; UP, urine protein.



**Fig. 2.** Passing Bablok regression regarding the comparison of UPCR results between analyzers. The plot shows the UPCR values with the regression line (red line), the confidence interval for regression line (grey line), and identity line ( $x = y$ , dotted line). Analyzer 1, automated wet chemistry analyzer; Analyzer 2, in-house dry chemistry analyzer; Analyzer 3, dipstick reading device. UPCR, urine protein-to-creatinine ratio.



**Fig. 3.** Bland-Altman plot regarding the comparison of UPCR results between analyzers. The plot shows the difference of UPCR values (blue dot), the bias (blue line), the 95% confidence interval for bias (blue dotted line), and the 95% confidence interval for difference (red dotted line). Analyzer 1, automated wet chemistry analyzer; Analyzer 2, in-house dry chemistry analyzer; Analyzer 3, dipstick reading device. UPCR, urine protein-to-creatinine ratio.

**Table 3.** Concordance in the classification of normal or proteinuria between automated wet chemistry analyzer and in-house dry chemistry analyzer

Analyzer 2	Analyzer 1		Total
	N	P	
N	50	5	55
P	4	41	45
Total	54	46	100

Analyzer 1, automated wet chemistry analyzer; Analyzer 2, in-house dry chemistry analyzer. N, normal (UPCR < 0.5); P, proteinuria (UPCR ≥ 0.5).

**Table 4.** Concordance in the classification of normal or proteinuria between automated wet chemistry analyzer and dipstick reading device

Analyzer 3	Analyzer 1		Total
	N	P	
N	37	7	44
P	17	39	56
Total	54	46	100

Analyzer 1, automated wet chemistry analyzer; Analyzer 3, dipstick reading device. N, normal (UPCR < 0.5); P, proteinuria (UPCR ≥ 0.5).

**Table 5.** Concordance in the classification of normal or proteinuria between in-house dry chemistry analyzer and dipstick reading device

Analyzer 3	Analyzer 2		Total
	N	P	
N	37	7	44
P	18	38	56
Total	55	45	100

Analyzer 2, in-house dry chemistry analyzer; Analyzer 3, dipstick reading device.  
N, normal (UPCR < 0.5); P, proteinuria (UPCR ≥ 0.5).

## DISCUSSION

This study demonstrated the differences in UPCR measured by three types of analyzers. Although no significant bias, constant or proportional difference was observed between the UPCR values measured by the automated wet chemistry analyzer and in-house chemistry analyzer; the 95% CI of differences between UPCR results was -1.70 to 1.42. The residual standard deviation (RSD) that represents random differences between the two methods was 0.695. These differences cannot be ignored clinically and the two measurements should be considered non-interchangeable.

The difference in UPCR values could be attributable to the difference in the measurement methods used by the two analyzers. The automated wet chemistry analyzer used the Jaffe method for measuring urine creatinine and pyrogallol red method for urine protein. The in-house dry chemistry analyzer used an enzymatic method for measuring urine creatinine and pyrocatechol violet method for urine protein. The Jaffe method can present falsely increased results owing to the presence of non-creatinine chromogens such as bilirubin, proteins, glucose, and ketone bodies, whereas the enzymatic method does not affected by these materials [7].

According to Lynch and colleagues, the bench-top dry chemistry analyzer using the pyrocatechol violet method retains a positive bias in measurement of total protein concentration compared with the wet chemistry analyzer using the pyrogallol red method, especially at high level [16]. Our results also indicated that the higher are the urine creatinine or protein concentrations, the greater are the differences between results obtained by the automated wet chemistry analyzer and the in-house dry chemistry analyzer. The absolute differences in UPCR also tended to increase in proportion to the average UPCR values of two analyzers. Therefore, the reason for the high consistency ( $r=0.81$ ) of clinical classification of proteinuria between the automated wet chemistry analyzer and in-house dry chemistry analyzer could be explained by relatively small differences in UPCR values near 0.5, the discrimination point of proteinuria.

Therefore, in cases where proteinuria is suspected and needs to be confirmed, the differences between the values obtained by the automated biochemistry analyzer and in-house dry chemistry analyzer would not affect the clinical judgement. However, in cases where serial measurements of UPCR are performed to monitor the clinical courses, it would be better to use the same analyzer to minimize the influence of variability between analyzers.

Urine dipstick is an easy and inexpensive test to screen proteinuria [17], and there have been some attempts to calculate the UPCR using dipstick strip and dipstick reading devices [4,5,18]. One study reported a good correlation between results of semiquantitative urine protein analysis and measurement of creatinine with the dipstick reading device and the



quantitative measurements [18], whereas other studies concluded that the dipstick test or dipstick reading device cannot be recommended for determination of the UPCR [4,5]. Although different analyzers and statistical tests were used, our study also indicated the poor concordance of dipstick-based test with other methodologies.

In the present study, on comparing UPCR values measured with the dipstick reading device and those measured with the other two chemistry analyzers by Bland-Altman and Passing Bablok tests, proportional difference and consistent difference were found. Additionally, the 95% CI of differences and the  $\pm 1.96$  RSD interval was too large to consider the methods to be in agreement (**Figs. 2 and 3**). In other words, the UPCR values measured by the dipstick reading device cannot be used interchangeably with those of the other two analyzers. Concordance in classifying proteinuria was moderate ( $\kappa = 0.52$  or  $0.51$ ) when comparing UPCR values measured with the dipstick reading device and those measured with the automated wet chemistry analyzer or in-house dry chemistry analyzer.

Our data indicated that there was limitation to get accurate results from dipstick reading device. Two possibilities could be considered as the cause of the differences in UPCR data between dipstick reading device and other analyzers. The first possibility was that the dipstick reading device used in this study produced data that were inconsistent with the true value. This was indicated by the observation that the dipstick reading device showed poor linearity in both urine protein and urine creatinine concentrations (**Fig. 1**). The second possibility was that the dipstick strips were designed for semi-quantitative measurement of protein and creatinine concentration, and thus an accurate UPCR value could not be obtained. Previous study comparing the semi-quantitative dipstick measurement with quantitative measurements showed good agreement of protein concentrations but creatinine concentrations showed only moderate agreement [4]. On the other study, the complete coincidence rate of test results between semi-quantitative dipstick and quantitative method was 69.44% in protein and 52.16% in creatinine tests, respectively [19]. On the other hand, there are some studies reported high correlation between results of quantitative urine strip test using reflectance reading analysis and results of quantitative chemistry analyzer [20,21]. However, correlation and regression studies are not recommended method for assessing agreement because those represent the relationship between variables, not the differences [22]. Therefore, the agreement of data between urine dipstick and laboratory chemistry analyzer is still not addressed.

Based on our data, the dipstick test and dipstick reading device should be cautiously used to estimate the UPCR for classifying proteinuric dogs or to monitor the clinical effect of medication used for reducing urine protein.

A limitation of the present study was that not all the analyzers were operated on the same day. Some urine samples were analyzed by all three analyzers within a short time after the collection, but the other urine samples were analyzed by just one or two analyzers immediately after the collection, with the remaining urine samples being frozen at  $-20^{\circ}\text{C}$  and then analyzed by the other analyzer within 3 months. However, according to previous studies, the UPCR did not significantly change for 3 months when stored at  $-20^{\circ}\text{C}$  [11,23]. Therefore, the storage effect was considered to minimally influence the UPCR in the present study.

Another limitation of this study was that it was not possible to evaluate the accuracy of the analyzers or compare the UPCR obtained by the analyzers with that obtained by a gold

standard method. It was because that there was no definitive method or standardized measurement to determine urine protein or creatinine concentrations. Moreover, quality control material could not provide target values for the three analyzers to assess the accuracy. The present study was not designed to assess which method or analyzer was more accurate but to evaluate whether the difference between various analyzers affected clinical judgement. Further studies are warranted to evaluate which analyzer or analytic method can assess UPCR more accurately and can reflect disease status or efficacy of the medication more precisely.

In conclusion, our study demonstrated differences in UPCR values obtained by different types of analyzers, and the dipstick reading device might not be appropriate for clinical use. Although identification of proteinuric dogs was highly consistent between the automated wet chemistry analyzer and in-house dry chemistry analyzer, a dog requiring sequential measurement of UPCR when monitoring the status of disease or the effect of medication should be done by the same analyzer to minimize the impact of inter-instrumental variance.

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