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Validation of a point-of-care quantitative immunoassay for total bile acid measurement in dogs and cats

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Abstract

Total bile acid (TBA) concentrations in the blood are measured using an enzymatic method and an automated chemistry analyzer to evaluate liver function in dogs and cats. This study aimed to validate the results of FUJI DRI-CHEM IMMUNO AU in the measurement of TBA concentrations (FDC v-BA; FUJIFILM Corporation, Tokyo, Japan). Serum and plasma samples from dogs and cats were analyzed using FDC v-BA; intra- and interassay precision, accuracy, correlation, and linearity were evaluated. The correlation between FDC v-BA and an automated chemistry analyzer was also statistically assessed. Intra-assay (between 0.8% and 1.34%) and interassay (1.22%) coefficients of variation were determined, and the linearity of canine and feline serum samples showed excellent correlations (correlation coefficient for dogs and cats: R = 1.00 and 1.00). Analysis results of the canine and feline plasma samples by using FDC v-BA were highly correlated with those obtained using the enzymatic method (R = 0.976 for dogs and 0.979 for cats). The cross-reactivity of FDC v-BA with ursodeoxycholic acid was lower than with the enzymatic reagent. FDC v-BA was used to determine the reference intervals. The fasting reference intervals were ≤7.9 μmol/L for dogs and ≤4.7 μmol/L for cats, and the postprandial values were ≤26.2 μmol/L for dogs and ≤9.1 μmol/L for cats. TBA concentrations in dogs with a portosystemic shunt exceeded the reference range when measured with FDC v-BA, demonstrating the validity of our reference ranges. Thus, FDC v-BA can be a useful point-of-care test for clinical diagnoses in dogs and cats.

Key Words: bile acid, immunoassay, plasma, serum, validation

Introduction

Bile acids synthesized from cholesterol in hepatocytes are secreted into bile and stored in the gallbladder[4]. After food consumption, bile acids are secreted into the duodenum and promote the decomposition and absorption of fat[4]. The synthesized bile acids are conjugated in the liver[4]. Primary bile acids, namely, cholic acid and chenodeoxycholic acid, are synthesized in the liver; metabolism of these bile acids by enterobacteria produces deoxycholic acid and lithocholic acid as secondary bile acids[4]. Taurine-conjugated bile acids are predominant in dogs and cats[20]. Approximately 95% of bile acids are absorbed in the terminal ileum and subsequently

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return to the liver via portal circulation[4]. Serum bile acids are often used to assess liver function and indicated for the further evaluation of persistently increased serum liver enzyme activities[7]. Some studies have shown that serum bile acid concentrations are useful for diagnosing hepatobiliary disease in dogs and cats[5,6]. The sensitivity of tests can be increased by submitting a fasting sample and a 2-h postprandial sample[19].

In dogs and cats, bile acid concentrations are useful for diagnosing a portosystemic shunt (PSS)[21]. A PSS is a vascular anomaly that allows the blood from portal circulation to bypass the liver and directly be transported into the systemic circulation[21]. Regardless of shunt type, postprandial bile acid concentrations increase in nearly all (99.3%) patients with an extrahepatic congenital PSS; therefore, this test is the most sensitive biochemical screening test for extrahepatic congenital PSS[12].

At present, bile acids are measured via an enzymatic method that involves the use of an automated chemistry analyzer, such as a Hitachi chemistry analyzer. Bile acids can be commercially measured in an external laboratory, although few systems can be used in-house. For example, a FUJI-DRICHEM IMMUNO AU cartridge v-BA (FDC v-BA), recently released by FUJIFILM Corporation (Tokyo, Japan), is a quantitative bile acid measurement system that can rapidly obtain results in-house for dogs and cats. The total bile acid (TBA) concentration combines the concentrations of individual bile acids; the major bile acids that comprise >98% of the TBA concentration in dogs and cats are taurocholic acid (TCA), taurodeoxycholic acid (TDCA), and taurochenodeoxycholic acid (TCDCA)[20]. The principle of FDC v-BA involves surface plasmon-enhanced fluorescence[2, 22]. In this process, three types of anti-bile acid monoclonal antibodies reacting with TCA, TDCA, and TCDCA are used.

This study aimed to validate FDC v-BA analytically by evaluating its intra- and interassay precision, accuracy, recovery, linearity, and correlation. Cross-reactivity with substances with a bile acid-like structure was also examined. The reference ranges of FDC v-BA for dogs and cats were established, and their clinical availability was assessed by measuring the TBA concentration of dogs and cats with PSS.

Materials and Methods

Animals and samples: For the intra-assay, interassay, linearity, and cross-reactivity tests, 50 serum samples from each healthy dog and cat were pooled and used. The method for adjustment of bile acid concentration is described in the method validation.

For the correlation and reference interval test, plasma samples were collected from dogs and cats in the Veterinary Medical Centre at the University of Tokyo, Japan. For the reference interval test, samples with liver enzyme activities outside the normal range (shown in brackets) were considered unhealthy and were excluded: blood alkaline phosphatase ([dogs] (≥ 1 year) 47–254 U/L, (< 1 year) 69–333 U/L), [cats] (≥ 1 year) 38–165 U/L), (< 1 year) 77-358 U/L), alanine aminotransferase ([dogs] 17–78 U/L, [cats] 22–84 U/L), γ-glutamyl transpeptidase ([dogs] ≤ 14 U/L, [cats] ≤ 10 U/L), and aspartate aminotransferase ([dogs] 17–44 U/L, [cats] 18–51 U/L). These items were measured with a FUJI DRI-CHEM 7000.

For the measurement of PSS samples, plasma from the blood samples of dogs and cats with a PSS was used if the PSS was confirmed through a contrast computed tomography examination. Of 32 dogs (21 measured preprandially only, three measured postprandially only, and eight measured both preprandially and postprandially), 21 were males (neutered, seven) and 11 were females (neutered, three). Their ages ranged between 0.3 and 11 years (median, 3.5 years). Of eight cats (six preprandial only and two measured both preprandially and postprandially), three were males (neutered, 0) and five were females (neutered, three); their age range was 0.4–12 years (median, 4.5 years).

After blood collection, the serum and plasma were separated and stored at −20°C until measurement. All the procedures were approved by the Animal Welfare Committee of the Pharmaceutical and Healthcare Research Laboratories in FUJIFILM Corporation (Number: I27I004NPI27010 and I27I003NPI27006) before the study commenced.

Measurement of TBA: Fasted specimens were obtained in a state in which an animal received no food for more than 12 h. Postprandial specimens were obtained 2 h after a meal. Blood bile acid concentrations were measured using FDC v-BA with a FUJI DRI-CHEM IMMUNO AU10V analyzer (FUJIFILM Corporation, Tokyo, Japan). An Aqua-auto Kainos TBA (Kainos TBA) test kit (Kainos Laboratories, Inc., Tokyo, Japan) run on a Hitachi 7180 chemistry analyzer (Hitachi, Ltd., Tokyo, Japan) served as the reference method.

Method validation: Intra- and interassay coefficients of variation, accuracy, linearity, and correlation were evaluated through analytical validation. Intra-assay validation shows within-run precision, whereas interassay validation presents between-run precision. Accuracy is defined as the closeness of a measured value to the true value. Linearity indicates the properness of a measurement range. Correlation is described as the relationship between FDC v-BA and the Hitachi 7180 chemistry analyzer.

Intra-assay validation was conducted as follows: a mixture consisting of TCA sodium salt hydrate (Sigma-Aldrich, St. Louis, MO, USA), sodium taurochenodeoxycholate (Sigma-Aldrich), and sodium taurodeoxycholate hydrate (Sigma-Aldrich) at a ratio of 62:11:27 was added to the canine pooled serum. Control liquids were prepared with high (≥40 μmol/L), medium (≥20 μmol/L to <40 μmol/L), and low (<20 μmol/L) concentrations of bile acids. The control liquids were measured 10 times each and evaluated in terms of standard deviation (SD) and coefficient of variation (CV). Interassay variation was assessed to measure the control liquid containing the medium concentration of bile acids (≥20 μmol/L to <40 μmol/L) twice daily for 10 days. The interassay was conducted by using the difference between the mean of 10 measurements of the control liquid (composed of each different assay) and the mean of the control material measured with the Hitachi 7180 chemistry analyzer. Linearity was assessed by diluting the canine serum pools with high concentrations of bile acids (approximately 180 μmol/L) containing TCA, TCDCA, and TDCA at a ratio of 62:11:27 with the pooled serum from 50 healthy dogs treated with activated carbon[14]. The linearity of the serum was evaluated from the mean of three measurements for each serum sample (undiluted high-concentration serum at ratios of 4:1, 3:2, 2:3, and 1:4 from the undiluted pooled serum of dogs treated with activated carbon). The pooled serum of cats was similarly evaluated. Correlation was examined by measuring and comparing dog and cat plasma examined with the Hitachi 7180 chemistry analyzer and FDC v-BA.

Cross-reactivity with substances with a bile acid-like structure: Cross-reactivity with substances with a bile acid-like structure[11] and ursodeoxycholic acid (UDCA), which is a hydrophilic bile acid with poorly defined hepatocyte-protective properties[16], was evaluated. Cortisol (276 μmol/L; National Institute of Standards and Technology, MD, USA), cholesterol (259 μmol/L; Sigma-Aldrich), testosterone (35 μmol/L; Sigma-Aldrich), and UDCA (100 μmol/L; Sigma-Aldrich) were added to the pooled serum from 50 healthy dogs, and they were measured three times each by using FDC v-BA and the Kainos TBA test kit.

Cross-reactivity is the ratio of the reacted cross-reactive substance, which can be calculated using the following formula:

[(bile acid concentration measured after adding the cross-reactive substance − bile acid value measured before adding the cross-

Table 1. Intra-assay repeatability of assays for the detection of high (≥40 μmol/L), medium (≥20 μmol/L to <40 μmol/L), and low (<20 μmol/L) concentrations of bile acids

Test	High concentration (≥40 μmol/L)	Medium concentration (≥20 μmol/L to <40 μmol/L)	Low concentration (<20 μmol/L)
Mean ± SD	98.72 ± 0.79	34.91 ± 0.40	10.04 ± 0.13
CV (%)	0.80	1.14	1.34

Mean, standard deviation (SD), and coefficient of variation (CV) are calculated from 10 replicate measurements for each concentration.

reactive substance)/concentration of the added cross-reactive substances] × 100.

Establishment of the reference interval of TBA: The dog and cat plasma samples in which four biochemical items were within the normal range (as described in the "Animals and samples" section above) were measured to set the reference range of FDC v-BA. The lower and upper limits of the reference range were calculated as follows: (1) all measured values were logarithmically converted, and outliers were excluded; (2) mean and SD were derived from logarithms; (3) logarithms in the 95% interval were determined with mean ± 1.96 × SD; and (4) logarithms were exponentiated to obtain the lower and upper limits[18].

Statistical analysis: The CV of the measurements was calculated by dividing the SD by the means of the measured values and multiplying them 100 times. The linearity of serum was calculated using Excel 2007 (Microsoft Corp., Redmond, WA). Bland–Altman difference plot and Deming regression analyses were conducted with a comparison method using XLSTAT (Addinsoft, Okayama, Japan).

Results

Method validation

As shown in Table 1, the intra-assay precision ranged from 0.80% (minimum) to 1.34% (maximum). As a result of analyzing the control liquid with the medium bile acid concentration for 7 days, the mean ± the SD interassay precision was 34.61 ± 0.42 μmol/L, and the CV was 1.22%.

The differences in the measured values of the control liquid with high (97.9 μmol/L), medium (35.2 μmol/L), and low (10.0 μmol/L) concentrations of bile acids from the values measured using the Kainos TBA test kit were 0.9%, 1.9%, and 5.0%, respectively.

The results of the two linearity experiments of the canine and feline lithium heparin plasma samples are shown in Figs. 1A and 1B. The expected and measured results were highly correlated. The correlation coefficient for the dogs and cats was 1.00. For FDC v-BA, linearity was presented up to the highest bile acid concentrations assessed with a mean of 180.3 μmol/L (for dogs) and 179.6 μmol/L (for cats).

Furthermore, canine and feline plasma samples analyzed with FDC v-BA were highly correlated with the enzymatic method (i.e., the Kainos TBA test kit; Figs. 2A and 2B). The correlation coefficients for dogs (n=55) and cats (n=45) were 0.976 and 0.979, respectively.

The Bland–Altman plot revealed a positive bias for dogs (1.2 μmol/L; Fig. 3A) and a negative bias for cats (0.4 μmol/L; Fig. 3B). The correlation between the Kainos TBA test kit and FDC v-BA was described using the following equation:

For dogs: FDC v-BA value = 1.043 (95% confidential interval [CI], 0.851 to 1.234) × Kainos TBA + 0.082 (95% CI, −3.547 to 3.711; Fig. 3A)

For cats: FDC v-BA value = 0.930 (95% CI, 0.739 to 1.122) × Kainos TBA + 0.084 (95% CI, −0.975 to 1.143; Fig. 3B)

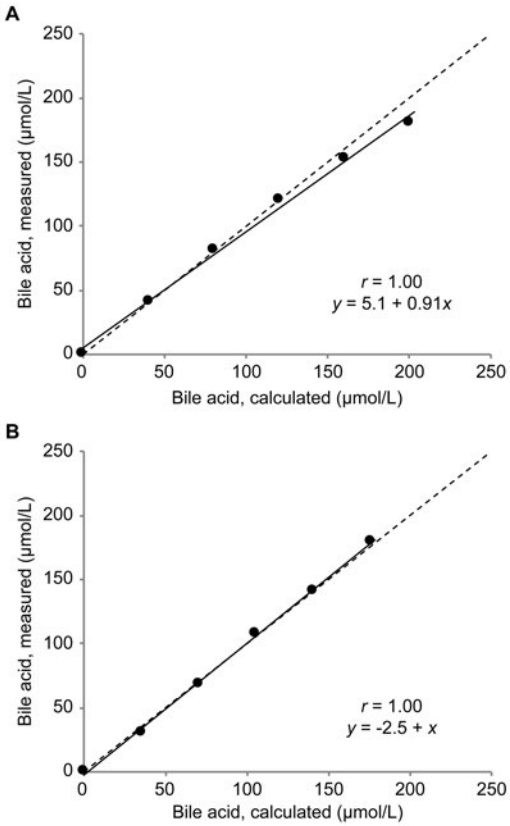

Fig. 1. Linearity of canine and feline serum samples with high bile acid concentrations.
(A) Linearity under dilution for measuring canine pool serum samples containing 200.6 μmol/L bile acids. (B) Linearity under dilution for examining feline pool serum samples containing 176.1 μmol/L. A serial dilution was performed for both groups to achieve six bile acid concentrations from the original concentration as follows: 1.0, 0.8, 0.6, 0.4, 0.2, and 0 parts.

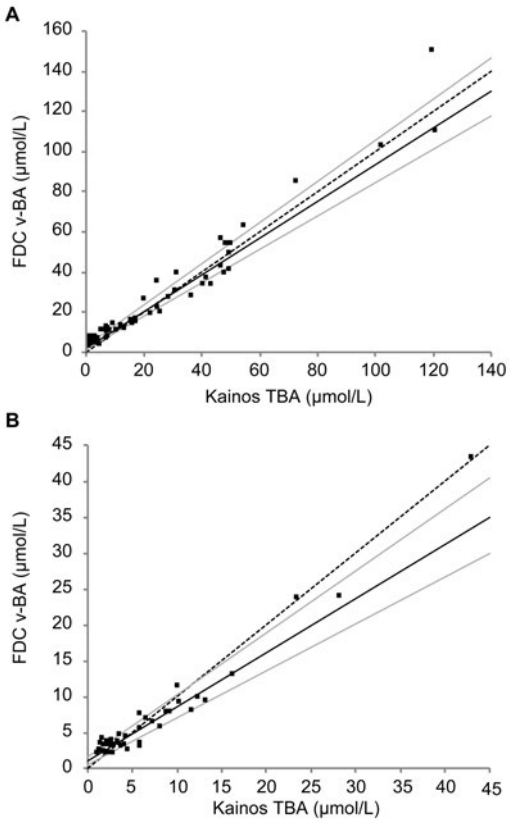

Fig. 2. Deming regression analysis for bile acid as determined using the FDC v-BA versus the Kainos TBA test kit.
Samples were obtained from (A) dogs and (B) cats. The solid black line illustrates the regression equation with its 95% confidence interval (solid gray line). The black dotted line represents the identity line, which is consistent with a perfect correlation of the two methods.
FDC v-BA, FUJI DRI-CHEM IMMUNO AU cartridge v-BA; Kainos TBA, Aqua-auto Kainos TBA.
Note: FUJI DRI-CHEM IMMUNO AU cartridge v-BA is produced by FUJIFILM Corporation (Tokyo, Japan), and Aqua-auto Kainos TBA test kit is produced by Kainos Laboratories, Inc. (Tokyo, Japan).

Cross-reactivity with substances with a bile acid-like structure

No cross-reactivity with cortisol, cholesterol, or testosterone was detected with FDC v-BA or the Kainos TBA test kit. The cross-reactivities with UDCA were 31% with FDC v-BA and 84% with the Kainos TBA test kit (Table 3).

Establishment of the reference interval of TBA

The specimens for the reference interval were 90 dogs (22 males [7 neutered]; 68 females [7 neutered]) and 219 cats (89 males [42 neutered]; 130 females [39 neutered]; Supplemental Tables S1 and S2). They were collected from veterinary hospitals in Japan. For the preprandial test, 90 dogs and 144 cats were used. For the postprandial tests, 88 dogs and 144 cats were used (Table 2, Supplemental Table S1 and S2).

The mean ± SD concentrations were 3.2 ± 2.3, 2.0 ± 1.6, 10.0 ± 5.0, and 3.9 ± 1.8 μmol/L for the fasted dogs, fasted cats, postprandial dogs, and postprandial cats, respectively. The

Table 2. Specimen characteristics for the reference interval

	Dogs		Cats	
Characteristics	Preprandial	Postprandial	Preprandial	Postprandial
Sex, n [%]				
Neutered Male	7 [8%]	7 [8%]	11 [8%]	36 [25%]
Neutered Female	7 [8%]	7 [8%]	15 [10%]	32 [22%]
Intact Male	15 [17%]	15 [17%]	39 [27%]	21 [15%]
Intact Female	61 [68%]	59 [67%]	79 [55%]	55 [38%]
Age, year, median (range)	2.6 (0.5–13.8)	2.5 (0.5–13.8)	2.8 (0.4–18.3)	3.7 (0.1–17.0)
Alanine aminotransferase, U/L, median (range)	45 (27–78)	45 (27–78)	46 (22–84)	50 (24–84)
Aspartate aminotransferase, U/L, median (range)	27 (18–44)	27 (18–44)	23 (18–47)	26 (18–51)
γ-Glutamyl transpeptidase, U/L, median (range)	4 (2–9)	4 (2–9)	1 (0.5–3)	1 (1–10)
Alkaline phosphatase, U/L, median (range) (≥1 year)	149 (74–254)	149 (74–254)	93 (43–165)	97 (49–165)
(<1 year)	175 (124–321)	175 (124–321)	205 (70–358)	217 (111–358)

*Percentages may not total 100 due to rounding.

Table 3. Cross-reactivity with compounds with a bile acid-like structure and ursodeoxycholic acid

Bile acid-like compound	Bile acid concentration	Bile acid-like structure concentration	FDC v-BA		Kainos TBA test kit	
			Bile acid concentration	Cross-reactivity	Bile acid concentration	Cross-reactivity
	(μmol/L)	(μmol/L)	(μmol/L)		(μmol/L)	
Cortisol	9.8	276	10.1	0%	9.7	0%
Cholesterol	9.8	259	9.4	0%	9.7	0%
Testosterone	9.8	35	9.9	0%	9.7	0%
UDCA	9.8	100	40.8	31%	93.4	84%

FDC v-BA, FUJI DRI-CHEM IMMUNO AU cartridge v-BA (by FUJIFILM Corp. Tokyo, Japan); UDCA, ursodeoxycholic acid.
Note: FUJI DRI-CHEM IMMUNO AU cartridge v-BA is produced by FUJIFILM Corporation (Tokyo, Japan); Kainos TBA test kit is produced by Kainos Laboratories, Inc. (Tokyo, Japan).

concentration ranges were 0.7–13.9, 0.5–10.2, 1.1–22.6, and 0.3–9.8 μmol/L for the fasted dogs, fasted cats, postprandial dogs, and postprandial cats, respectively. The measured values of the fasted and postprandial dogs and cats were logarithmically converted. After the abnormal values were excluded, the following results (average ± SD) were obtained: 0.430 ± 0.238, 0.227 ± 0.227, 0.938 ± 0.245, and 0.538 ± 0.215 for the fasted dogs, fasted cats, postprandial dogs, and postprandial cats, respectively. Based on these values, the results of calculating with a 95% CI were as follows: −0.036 to 0.897 for the fasted dogs, −0.21 to 0.672 for the fasted cats, 0.459 to 1.418 for the postprandial dogs, and 0.116 to 0.960 for the postprandial cats. These values were then converted to real numbers. The values for the fasted dogs, fasted cats, postprandial dogs, and postprandial cats with their corresponding 95% CIs were 0.9–7.9, 0.6–4.7, 2.9–26.2, and 1.3–9.1 μmol/L, respectively. The upper limit of each CI was set as the reference range. The fasting reference range was ≤7.9 μmol/L for dogs and ≤4.7 μmol/L for cats, and the postprandial values were ≤26.2 μmol/L for dogs and ≤9.1 μmol/L for cats.

TBA concentrations in canine and feline patients with PSS

The TBA concentrations of all the plasma samples of dogs with PSS in the fasting and postprandial states exceeded the reference range set in this study (Fig. 4). The median (range)

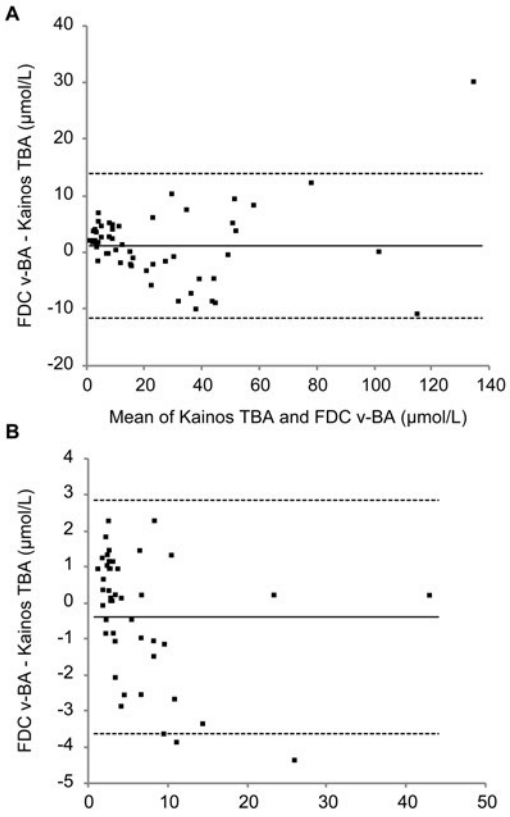

Fig. 3. Bland–Altman difference plot for bile acid concentrations.
The concentrations were measured in the canine plasma samples with Kainos TBA and FDC v-BA for (A) dogs and (B) cats. The black line indicates the mean bias and its 95% confidence interval (black dotted line). The black dotted line is consistent with the 1.96-fold standard deviation (SD) line of the mean absolute bias, which indicates the limits of agreement. FDC v-BA, FUJI DRI-CHEM IMMUNO AU cartridge v-BA; Kainos TBA, Aqua-auto Kainos TBA.

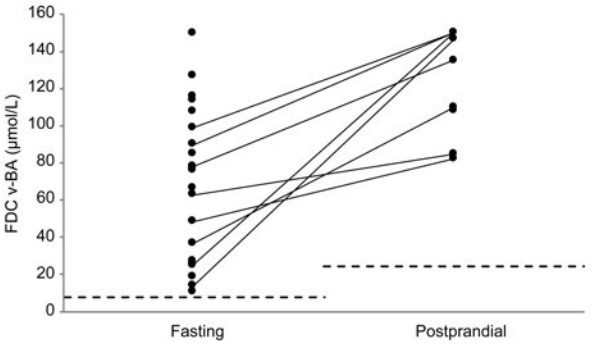

Fig. 4. Bile acid concentrations in fasting (29) and postprandial (11) dogs with a portosystemic shunt.
The dotted line indicates the reference values. It is connected by a straight line between the fasting and postprandial values from the same dog (five dogs).

of bile acid concentrations in the preprandial specimens from dogs with PSS was 76.1 μmol/L (10.6 μmol/L to >150 μmol/L), and the median (range) of the bile acid concentrations in the postprandial samples was 146.3 μmol/L (82.3 μmol/L to >150 μmol/L). In eight dogs measured pre- and postprandially, all postprandial TBA concentrations increased from their preprandial levels.

The TBA concentrations of all the plasma samples from cats with PSS in the fasting and postprandial states also exceeded the reference range set in this study. The median (range) of bile acid concentrations in the preprandial specimens from cats with PSS was 69.7 μmol/L (10.8–119.9 μmol/L), and the median (range) of bile acid concentrations in the postprandial specimens was 54.6 μmol/L (37.6–71.5 μmol/L). All the measured specimens of PSS from animals in the pre- and postprandial states deviated from the reference range. In the two cats measured pre- and postprandially, all the postprandial TBA concentrations increased compared to their preprandial levels.

Discussion

This study aimed to confirm the validity of FDC v-BA by evaluating its intra- and interassay precision, accuracy, recovery, linearity, and correlation. Based on these evaluations, we confirmed the validity of FDC v-BA as a quantitative immunoassay of TBA. FDC v-BA was confirmed to have an adequately satisfactory CV within 20%, which is the acceptable range of intra-assay precision for quantitative measurement[17]. The accuracy test using the mixtures with pure bile acid yielded accuracy errors within 15%, which confirmed that FDC v-BA can accurately measure bile acids[17]. Standard substances were originally used in the accuracy test. However,

considering the absence of a standard substance for dog and cat bile acids, we used TCA, TDCA, and TCDCA (i.e., the main bile acids in dogs and cats) as substitutes[20]. Recovery was confirmed to be between 80% and 120%, which were within the acceptable range of recovery rates[17]. Correlation analysis revealed an acceptable correlation coefficient[15], and the Bland–Altman analysis demonstrated an acceptable bias[3]. The 95% CI of the Deming analysis equation included "0" in the intercept and "1" in the slope, indicating that the two methods provide the same analytical results[9]. These results suggest that FDC v-BA can accurately measure the bile acid concentrations of dogs and cats in either serum or plasma. Linearity and correlation analyses showed that the different bile acid concentrations of the samples collected from dogs and cats can be accurately determined. Moreover, because it has been confirmed that the addition of bilirubin, chyle, or cholesterol to canine pooled serum has little effect on FDC v-BA, it is considered that any effect of jaundice and hyperlipidemia would be small (Table 3 and Supplemental Table S3). UDCA is a hydrophilic bile acid that is usually in a relatively low concentration in dogs and cats. It has the emission function of bile acids and the replacement function of a hydrophobic bile acid. UDCA affects biliary stasis and is used to treat a variety of hepatobiliary diseases in dogs and cats[1,16]. No cross-reactivities with substances with a bile acid-like structure except UDCA were observed. The cross-reactivity was 31% for FDC v-BA and 84% for the Kainos TBA test kit, but the cross-reactivity of the former was lower than that of the latter. The Kainos TBA test kit is a general bile acid measurement method that measures 3α-hydroxy bile acid concentrations by using an enzymatic method[4]. Therefore, if UDCA exists in the blood, the Kainos TBA test kit measures it as a bile acid. By contrast, FDC v-BA is based on an immunoassay method that uses antibodies reacting to TCA, TDCA, and TCDCA; thus, the reactivity with UDCA is expected to be low[8]. FDC v-BA has an advantage over the Kainos TBA test kit in terms of UDCA interference. This feature may be important because UDCA is commonly used in veterinary medicine. When UDCA is used at a typical dosage for dogs (10 mg/kg), it has been reported that the serum UDCA concentration reaches a maximum concentration of approximately 5 μmol/L (1.98 μg/mL) 1 h after oral administration and then decreases[4,13]. When 5 μmol/L UDCA is measured using the Kainos TBA kit, a value of approximately 4 μmol/L bile acid is expected based on the results of cross-reactivity experiments. As a result, even if normal before UDCA administration, the total bile acid concentration in the fasted state may exceed the reference range. On the other hand, even if 5 μmol/L UDCA is measured using FDC v-BA, the measured value is estimated to be only approximately 1.5 μmol/L. Therefore, it is unlikely that the reference range will be exceeded.

The reference range of the FDC v-BA set in this study corroborates the data reported in previously published work[10]. We confirmed that all PSS samples from the animals in the fasting and postprandial states exceeded the reference interval that was set in this study. We could not evaluate liver diseases other than PSS in dogs and cats. Thus, further studies should be conducted to confirm the clinical usefulness of FDC v-BA for these diseases.

In this study, FDC v-BA, an automated fluorescence immunoassay analyzer, was evaluated to measure TBA concentrations in dogs and cats. It is an easy-to-use point-of-care test instrument that can quickly and precisely measure the concentrations of bile acids in serum and plasma samples. Although multiple measurements cannot be performed simultaneously using this point-of-care test, it correlates well with the enzyme measurement method involving the use of an automated chemistry analyzer at a testing center. Its linearity is good within the assessed range, and its clinical usability is confirmed in the diagnosis of PSS. With FDC v-BA it is possible to quickly obtain the test results in the clinic, meaning

treatment can be started earlier. As a result, since early treatment initiation reduces the period of suffering for dogs and cats, FDC v-BA may help improve the quality of life of dogs and cats.

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Supplemental data

Supplemental data associated with this article can be found, in the online version, at http://dx.doi.org/10.14943/jjvr.69.2.89

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