

## Evaluation of SNAP cPL, Spec cPL, VetScan cPL Rapid Test, and Precision PSL Assays for the Diagnosis of Clinical Pancreatitis in Dogs

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**Background:** The sensitivity, specificity, and agreement of 4 diagnostic assays (SNAP canine pancreatic lipase (cPL), specific cPL (Spec cPL), VetScan cPL Rapid Test, and Precision PSL) for pancreatitis in dogs have not been directly compared.

**Hypothesis/Objectives:** To determine the level of agreement among each of the 4 assays and a clinical suspicion score, level of agreement among the assays, and sensitivity and specificity of each assay in a clinically relevant patient group.

**Animals:** Fifty client-owned dogs with clinical signs of gastrointestinal disease.

**Methods:** Prospective study. History, physical examination, complete blood count, serum biochemistry, abdominal ultrasound examination, and the 4 diagnostic assays for pancreatitis were performed. Intraclass correlation coefficients (ICC) were used to determine the level of agreement between each assay and a clinical suspicion score determined by a panel of 5 board-certified veterinary internists.

**Results:** The ICC between the clinical suspicion score and the 4 assays were SNAP cPL, 0.61; Spec cPL, 0.68; VetScan cPL Rapid Test, 0.68; and Precision PSL, 0.60. The sensitivities of the assays ranged from 73.9 to 100.0%, whereas the specificities were SNAP cPL, 71.1–77.8%; Spec cPL, 74.1–81.1%; VetScan cPL Rapid Test, 76.9–83.8%; and Precision PSL, 64.0–74.3%.

**Conclusions and Clinical Importance:** A good to excellent level of agreement was demonstrated among the 4 assays. The previously unreported sensitivity and specificity of the VetScan cPL Rapid Test were 73.9–83.3% and 76.9–83.8%, respectively. Results of any of the 4 diagnostic assays alone, in the absence of supporting clinical findings, are insufficient to establish a diagnosis of clinical pancreatitis in dogs.

**Key words:** Canine; DGGR; Lipase; Pancreas.

Pancreatitis is the most common disorder of the exocrine pancreas in dogs<sup>1</sup> and is a common differential diagnosis for patients with nonspecific gastrointestinal signs such as abdominal pain and vomiting.<sup>2,3</sup> Pancreatic histopathology is considered the most reliable method for diagnosing pancreatitis in dogs,<sup>4</sup> but it is rarely performed because of its invasive nature and a number of limitations, including the potential to miss localized lesions of pancreatitis,<sup>5,6</sup> a lack of standardized criteria for interpretation, and detection of subclinical, potentially clinically irrelevant pancreatitis.<sup>5</sup>

### Abbreviations:

CBC	complete blood count
cPL	canine pancreatic lipase
DGGR	1,2-o-dilauryl-rac-glycero-3-glutaric acid (6'-methyl-resorufin) ester
EPI	exocrine pancreatic insufficiency
ICC	intraclass correlation coefficient
Spec cPL	specific canine pancreatic lipase
TAMU	Texas A&M University, Gastrointestinal Laboratory

Serum amylase and lipase activities historically were used to assess patients for pancreatitis, and serum activities of these enzymes are increased in experimentally induced pancreatitis.<sup>7</sup> However, amylase and lipase have poor sensitivities and specificities for diagnosing naturally occurring pancreatitis in dogs.<sup>8–10</sup>

More recently, several newer pancreatic lipase immunoassays and a 1,2-o-dilauryl-rac-glycero-3-glutaric acid (6'-methyl-resorufin) ester (DGGR)-based assay have been developed. In the absence of a practical and clinically justifiable reason to obtain pancreatic biopsies, these newer diagnostic assays are being used, in conjunction with signalment, history, physical examination, complete blood count (CBC), serum biochemistry, and abdominal ultrasound examination, to establish a clinical diagnosis of pancreatitis.<sup>10–12</sup> Lipases originate from many cells, including pancreatic, hepatic, and gastric cells, and function to hydrolyze triglycerides. Serum lipase activity measures lipase molecules of any origin, whereas canine pancreatic lipase (cPL) assays measure lipase molecules of pancreatic acinar cell origin,<sup>13</sup> and would therefore only be expected to be increased during times of active pancreatic disease. A

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radioimmunoassay for measurement of cPL immunoreactivity was developed and validated<sup>9</sup> and then was replaced by a quantitative ELISA<sup>14</sup> known as the specific cPL (Spec cPL).<sup>a</sup>

Although the Spec cPL immunoassay was quickly established as a valuable tool in the diagnosis of pancreatitis, results take at least 24 hours to return, an important limitation for some patients. A rapid point-of-care semiquantitative cPL immunoassay (SNAP cPL<sup>b</sup>) therefore was developed to permit more rapid return of results. The SNAP cPL test is used to rapidly rule out pancreatitis, and it is recommended that a positive result be followed by laboratory assessment using a quantitative immunoassay, such as the Spec cPL.<sup>15</sup>

Recently, the VetScan cPL Rapid Test<sup>c</sup> was developed, with the aim of combining the benefits of a quantitative assay with the point-of-care benefits of the SNAP cPL. The VetScan cPL is a semiquantitative immunoassay for the detection of cPL that gives rapid point-of-care results. Unlike the SNAP cPL, the results of this point-of-care assay are numerical rather than binary, and can be used to distinguish among patients without pancreatitis, those with equivocal results, and those with cPL results consistent with pancreatitis.

Non-immunologic colorimetric lipase assays are also available. The Precision PSL<sup>d</sup> is a colorimetric diagnostic assay that recently has become available. It utilizes the substrate DGGR, which has been validated for the diagnosis of acute pancreatitis in dogs.<sup>11</sup> The DGGR-based assay is not specific for pancreatic lipase.<sup>16</sup>

To date, no studies have directly compared the sensitivity and specificity of all 4 assays when a clinical diagnosis of pancreatitis is suspected. The level of agreement among these 4 assays and a clinical diagnosis of pancreatitis also has not been evaluated.

## Materials and Methods

### Study Overview

From January 2017 to June 2017, 50 client-owned dogs with gastrointestinal clinical signs presented to an emergency clinic were prospectively enrolled into the study. Potential clinical signs of gastrointestinal disease included  $\geq 1$  of the following clinical signs: anorexia, lethargy, vomiting, diarrhea, or abdominal pain. Signalment, history, physical examination, CBC, serum biochemistry, and abdominal ultrasound examination were required for each patient to be enrolled in the study, with additional diagnostic tests performed at the discretion of the attending clinician. Each patient had additional blood collected at the time of presentation for performance of the 4 diagnostic assays evaluated in the study. A SNAP cPL was performed in-house, and the remaining serum then was submitted to 3 different commercial laboratories for determination of Spec cPL, VetScan cPL Rapid Test, and Precision PSL.

All abdominal ultrasound examinations were performed by either an emergency clinician or a board-certified veterinary radiologist. Most abdominal ultrasound examinations were performed and reported by a single radiologist (A.G. MacLeod). When the ultrasound examination was performed by another individual, the still images and video clips were stored, and for the purposes of our study, they were later evaluated and reported by the same board-certified radiologist.

After collection of data obtained from physical examination, diagnostic imaging, and laboratory evaluation, each case was retrospectively and

independently reviewed by a panel of 5 board-certified small animal veterinary internists (A.J. Mackin, A.M. Sullivant, K.V. Lunsford, J.M. Thomson, and T.M. Archer). A clinical suspicion score of "0" was assigned when the internist believed that the patient almost certainly did not have pancreatitis. A score of "1" was assigned when the internist believed that a diagnosis of pancreatitis could not be ruled in or out based on the available information (diagnostically equivocal). A score of "2" was assigned when the internist believed that the patient almost certainly had pancreatitis. When all scores were within 1 category of each other, the score provided by the majority of internists (3, 4, or 5 individuals) was assigned to the case ("consensus score"). No cases were excluded from the study because of failure to reach a consensus score. Internists were blinded to the results of the SNAP cPL, Spec cPL, VetScan cPL Rapid Test, and Precision PSL assays at the time of assigning scores. The clinical suspicion score for pancreatitis assigned by the panel of internists then was used as the standard against which the tests were evaluated. The results of the 4 diagnostic assays were compared to the clinical suspicion score, and to each other to determine the level of agreement. The level of agreement was determined by calculation of intraclass correlation coefficients (ICCs). Sensitivity and specificity then were calculated. Our study was approved by the Animal Care and Use Committee of the Blue Pearl Science Clinical Trial Review Board. All owners of the patients enrolled in the study signed an informed consent agreement.

### Data Collection

The following results from each patient were required for inclusion in the study: signalment, history, physical examination, CBC, serum biochemistry, abdominal ultrasound examination, and 4 results of the diagnostic assays investigated in the study. The CBC and serum biochemistry results could be supplied by a primary veterinarian at the time of referral or performed in-house at the time of presentation. Each abdominal ultrasound examination was performed by an emergency clinician or a board-certified veterinary radiologist, and representative still images and video clips were stored.

### Data Interpretation

The panel of internists could use the CBC and serum biochemistry results from either in-house or commercial laboratories, or a combination of both, provided reference intervals were available.

Although the abdominal ultrasound examination could be performed by either an emergency clinician or a board-certified veterinary radiologist, the interpretation of still images and video clips of the abdominal ultrasound examination were all performed by a single board-certified radiologist (A.G. MacLeod), and the interpretation results were available to the panel of internists when determining the clinical score for pancreatitis. Abdominal ultrasound examination reports were evaluated by the panel of internists and a combination of the following findings were considered to be suggestive of pancreatitis: hypoechoic areas within the pancreas, increased echogenicity of the mesentery surrounding the pancreas, and enlargement or irregularity of the pancreas.

The SNAP cPL was recorded as either visually normal or visually abnormal. A normal SNAP cPL was noted when the test spot had a lighter color than the reference spot. An abnormal SNAP cPL was noted when the test spot was darker or equal in color to the reference spot. An abnormal result is reported to correspond to a cPL  $\geq 200$   $\mu\text{g/L}$ .<sup>17</sup>

Serum was submitted to a commercial laboratory for assessment of Spec cPL.<sup>a</sup> The Spec cPL is an ELISA immunoassay offered through commercial laboratories.<sup>a,b</sup> A Spec cPL result  $\leq 200$   $\mu\text{g/L}$  is considered to be not consistent with pancreatitis, whereas a result  $\geq 400$   $\mu\text{g/L}$  is consistent with pancreatitis. A Spec cPL result of 201–399  $\mu\text{g/L}$  is considered to be equivocal for the diagnosis of pancreatitis, and retesting is recommended in 2–3 weeks.

Serum was submitted to a commercial laboratory,<sup>c</sup> where a VetScan cPL Rapid Test immunoassay was performed. A VetScan cPL Rapid

Test result of  $\leq 200$   $\mu\text{g/L}$  is considered to be not consistent with pancreatitis, whereas a result of  $\geq 400$   $\mu\text{g/L}$  is consistent with pancreatitis. A VetScan Rapid cPL result of 201–399  $\mu\text{g/L}$  is considered to be equivocal for the diagnosis of pancreatitis.

Serum also was submitted to another commercial laboratory,<sup>f</sup> where a Precision PSL non-immunologic colorimetric assay was performed. A Precision PSL result of  $\leq 140$  U/L is reported as within reference limits, whereas a result  $> 216$  U/L is considered to be supportive of a diagnosis of pancreatitis. A Precision PSL result of 141–216 U/L is considered to be equivocal for the diagnosis of pancreatitis.

### Statistical Analysis

Statistical analysis for agreement among assays and among the internists was assessed by ICC<sup>18,19</sup> using PROC MIXED in a statistical program.<sup>g</sup> The ICC is a measure of rating reliability from 0 to 1 that compares the variability of different scorings of the same dog to the total variation across all internists, or pairs of assays, and all dogs.<sup>20</sup> To calculate the ICC to assess the agreement among pairs of assays and between the consensus and each assay, a generalized linear mixed model was fit with score as the outcome, assay identity as a fixed effect and dog identity as a random effect. To calculate the ICC to assess the agreement among the 5 internists, an intercept-only generalized linear mixed model was used with score as the outcome and internist identity and dog identity as random effects. The restricted maximum likelihood estimation method was utilized for all models. Covariance variables from the models then were utilized to calculate the ICC using PROC SQL<sup>21</sup> in the same statistical program. It was calculated by dividing the between-dog variance by the total variance, which is the sum of the between-dog variance and the pooled variance within dogs. Thus, a higher ICC indicated greater agreement between 2 assays or among internists. Sensitivity and specificity calculations were performed under 2 different assumptions: assuming that equivocal clinical suspicion score cases did not have pancreatitis and that equivocal assay results also did not suggest pancreatitis, and assuming that equivocal clinical suspicion score cases had pancreatitis and that equivocal assay results also suggested pancreatitis, resulting in a range for both sensitivity and specificity. Standard sensitivity and specificity formulas were used. The number of true positives, false positives, true negatives, and false negative results for each diagnostic assay were determined by comparing each result with the clinical suspicion score.

## Results

### Animals

Fifty client-owned dogs, 27 males (23 neutered males and 4 intact males) and 23 females (22 spayed females and 1 intact female) were included. The mean age of dogs enrolled was 7 years and 10 months (range, 5 months to 14 years and 11 months). The median weight of patients enrolled was 20.05 kg (range, 4.4–57.3 kg). There were 15 mixed-breed dogs. The remaining 35 patients represented a variety of breeds including Labrador Retriever ( $n = 4$ ), Pitbull Terrier ( $n = 4$ ), English Mastiff Dog ( $n = 2$ ), Siberian Husky Dog ( $n = 2$ ), and 1 of each of the following breeds: American Eskimo Dog, Bichon Frise, Boxer, Brittany Spaniel, Cairn Terrier, Chihuahua, Dachshund, English Setter Dog, French Bulldog, German Shepherd Dog, Great Dane, Greyhound, Husky Dog, Jack Russell Terrier, Maltese, Miniature Schnauzer, Pembroke Welsh Corgi, Pug, Rottweiler, Shetland Sheepdog, Standard Poodle, Welsh Terrier, and Yorkshire Terrier.

**Table 1.** Intraclass correlation coefficients (ICCs) between each of the four tests evaluated in our study.

	ICC (0 to 1)
SNAP cPL—Spec cPL	0.92
SNAP cPL—Precision PSL	0.86
SNAP cPL—VetScan cPL Rapid Test	0.93
Spec cPL—Precision PSL	0.89
Spec cPL—VetScan cPL Rapid Test	0.96
Precision PSL—VetScan cPL Rapid Test	0.91

### Abdominal Ultrasound Examination Findings

All 50 abdominal ultrasound examinations were evaluated for signs consistent with pancreatitis. The most common abnormalities consistent with pancreatitis included enlargement or irregularity of the pancreas (28% of cases), hypoechoic areas within the pancreas (26% of cases), hyper-echoic mesentery surrounding the pancreas (24% of cases), or some combination of these (22% of cases).

### Combined Internist Scores

Records from all 50 dogs enrolled in the study were evaluated by each of the 5 internists (total of 250 individual scores) before the consensus clinical suspicion score was determined. After review of the cases, 56.4% (141/250) of the individual scores were classified as 0 (not pancreatitis), whereas 19.2% (48/250) of the scores were classified as 1 (equivocal for pancreatitis), and 24.4% (61/250) of the scores were classified as 2 (consistent with pancreatitis). Fifty-four percent (27/50) had a consensus score of 0 (not pancreatitis), 22% (11/50) had a consensus score of 1 (equivocal), and 24% (12/50) had a consensus score of 2 (consistent with pancreatitis). An ICC was determined for the level of agreement among the internists' individual scores. The ICC was 0.87, indicating a good level of agreement among the internists. An ICC value  $< 0.5$  is considered poor agreement, a value of 0.5–0.75 indicates moderate agreement, a score of 0.75–0.90 indicates good agreement, and a score of 0.90 indicates excellent agreement.<sup>22</sup>

### Agreement between the Consensus Score and the 4 Diagnostic Assays

The ICC was calculated between the internist consensus scores and each of the pancreatic lipase assays evaluated in the study. The results of the pancreatic lipase assays were classified as "0" if the assay result was in the range not consistent with pancreatitis, as "1" if the assay result was within the equivocal range, and as "2" if the assay result was within the range considered by each manufacturer or laboratory to be consistent with pancreatitis. Overall, a moderate level of agreement was found between each of the diagnostic assays and the consensus internist scores, with the lowest ICC being 0.60. The greatest level of agreement between the consensus score and the diagnostic assays evaluated was with the Spec cPL and the VetScan Rapid cPL, each with an ICC of 0.68. The next highest level of agreement was between the consensus score and the SNAP cPL, with an

ICC of 0.61. The lowest level of agreement was between the consensus score and the Precision PSL, with an ICC of 0.60.

**Agreement between the Different Diagnostic Assays**

The levels of agreement between the results of each of the 4 diagnostic assays are summarized in Table 1. The overall levels of agreement for the 4 diagnostic assays evaluated were good to excellent. The highest level of agreement between tests was between the Spec cPL and the VetScan Rapid cPL, with an ICC of 0.96 (excellent agreement). Overall, the Precision PSL had the lowest level of agreement with the other tests.

**Sensitivity and Specificity**

The sensitivity and specificity of each of the 4 tests were calculated under 2 assumptions: assuming that equivocal clinical suspicion score cases did not have pancreatitis and that equivocal assay results also did not suggest pancreatitis, and assuming that equivocal clinical suspicion score cases had pancreatitis and that equivocal assay results also suggested pancreatitis. These 2 calculations resulted in a range in both sensitivity and specificity for each test. The results are listed and compared to other studies in Table 2.

When classifying equivocal clinical suspicion score cases and assay results as not having pancreatitis, the sensitivities of the assays were SNAP cPL, 100.0%; Spec cPL, 90.9%; VetScan cPL Rapid Test, 83.3%; and Precision PSL 90.9%. The specificities of the assays under the same assumptions were SNAP cPL, 71.1%; Spec cPL, 81.1%; VetScan cPL Rapid Test, 83.8%; and Precision PSL, 74.3%.

In contrast, when classifying equivocal clinical suspicion score cases and assay results as having pancreatitis, the sensitivities of the assays were SNAP cPL, 73.9%; Spec cPL, 81.0%; VetScan cPL Rapid Test, 73.9%; and Precision PSL, 85.7%. The specificities of the assays under the same assumptions were SNAP cPL, 77.8%; Spec cPL, 74.1%; VetScan cPL Rapid Test, 76.9%; and Precision PSL, 64.0%.

**Discussion**

The nonspecific clinical signs associated with pancreatitis in dogs, in combination with the poor sensitivities and specificities of traditional lipase and amylase enzyme assays, make the definitive diagnosis of pancreatitis in dogs challenging. In recent years, this clinical dilemma has been addressed by the development of a number of newer, lipase-based diagnostic assays for pancreatitis in dogs. The full range of newer lipase-based assays available in the United States, however, has not previously been directly evaluated for agreement, sensitivity, or specificity. Our study indicates a good to excellent level of agreement among the 4 diagnostic assays, and a good level of agreement between each test and a clinical suspicion score. Our study also evaluates, in a group of dogs with gastrointestinal clinical signs due to a variety of different causes, the diagnostic sensitivity and specificity of the VetScan cPL Rapid Test immunoassay, which have not previously been reported, along with the sensitivities and specificities of the other 3 assays.

**Table 2.** Comparison between the sensitivities and specificities reported in our study to previous studies.

	Gold Standard	Spec cPL ( $\geq 400$ $\mu\text{g/L}$ )		SNAP cPL		Precision PSL		VetScan cPL Rapid Test		Total Serum Lipase	
		Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Cridge et al	Clinical Criteria	81.0–90.9	74.1–81.1	73.9–100	71.1–77.8	85.7–90.9	64.0–74.3	73.9–83.3	76.9–83.8	–	–
Haworth et al <sup>12</sup>	Clinical Criteria	70.0 <sup>a</sup>	77.0 <sup>a</sup>	82.0	59.0	–	–	–	–	–	–
Mansfield et al <sup>23</sup>	Histopathology	33.0	90.0	–	–	–	–	–	–	–	–
McCord et al <sup>10</sup>	Clinical Criteria	71.7–77.8	80.5–88.0	91.5–94.1	71.1–77.5	–	–	–	–	43.4–53.6	89.3–92.5
Neilson-Carley et al <sup>24</sup>	Histopathology	–	97.5	–	–	–	–	–	–	–	–
Trivedi et al <sup>8</sup>	Histopathology	21.0 (mild)	100.0 (mild)	–	–	–	–	–	–	54.0	43.0
Graca et al <sup>11</sup>	Clinical Criteria	71.0 (severe)	100.0 (mild)	–	–	93.0	53.0	–	–	–	–

<sup>a</sup>Spec cPL.

Our study confirms that there is at least good agreement among all of the 4 evaluated diagnostic assays, and that there is excellent agreement ( $ICC = 0.96$ ) between the Spec cPL and the VetScan cPL Rapid Test immunoassay. Although kappa analysis and ICC cannot be directly compared, the level of agreement between the SNAP cPL and the Spec cPL immunoassays in our study ( $ICC = 0.92$ ), is subjectively greater than previously reported ( $\kappa = 0.78$ ).<sup>12</sup>

The weakest level of agreement in our study was between the numerical pancreatic-lipase specific immunoassays (Spec cPL and VetScan cPL Rapid Test) and the colorimetric DGGR non-immunologic assay (Precision PSL), although agreement was still good (ICCs between the Precision PSL and the Spec cPL, Precision PSL and VetScan Rapid cPL, and the Precision PSL and SNAP cPL were 0.89, 0.91, and 0.86, respectively). The DGGR-based assay is not specific for pancreatic lipase, and results therefore may be increased in conditions other than pancreatitis.<sup>16</sup> Patients with exocrine pancreatic insufficiency (EPI) would be expected to have poor pancreatic secretory capacity but 33/48 (69%) dogs with EPI had serum lipase activities, as measured by DGGR, within the reference interval.<sup>16</sup> This finding supports the lack of specificity of DGGR-based assays for pancreatic lipase. We found similar agreement between the DGGR-based assay and the Spec cPL ( $ICC = 0.89$ ) as previously reported ( $\kappa = 0.80$ ).<sup>25</sup> A lack of strong agreement between 2 tests does not directly provide information regarding the diagnostic accuracy of each test and, with 2 tests that utilize very different methodologies, some lack of agreement between test results is to be expected.

Similar to previously published studies,<sup>10–12</sup> we used a clinical diagnosis of pancreatitis based on clinical suspicion scores derived from an integrated and expert analysis of a comprehensive body of information (including signalment, history, physical examination findings such as cranial abdominal pain, CBC, serum biochemistry, and abdominal ultrasound examination). A high level of agreement among board-certified internists is required to use a clinical diagnosis of pancreatitis as the “gold standard” when calculating sensitivity and specificity, and consequently an agreement study among the individual internist scores was performed as part of our study. In our study, an ICC of 0.87 indicated strong agreement among the internists when assessing clinical patients. The agreement reported in our study is similar to previously reported where kappa analysis was used to determine the level of agreement among 4 board-certified veterinary internists in establishing a clinical diagnosis of pancreatitis in 84 dogs ( $\kappa = 0.87$ ).<sup>10</sup>

Overall, the agreement among assays was greater than between each individual assay and the combined internist score, indicating that assay results do not necessarily correspond to a diagnosis of clinical pancreatitis. This was not an unexpected finding especially because subclinical pancreatitis is thought to be common in dogs. In 1 study, 64% of dogs presented for necropsy for various reasons had histopathologic evidence of pancreatitis,<sup>5</sup> which is much higher than the reported prevalence of clinical pancreatitis in dogs. In another study, 34% of necropsied dogs had histopathological evidence of pancreatitis,<sup>26</sup> once again suggesting that subclinical pancreatitis is common. In yet another study, 78% of dogs with upper gastrointestinal obstruction had at least mildly increased Spec cPL concentrations ( $\geq 200 \mu\text{g/L}$ ),<sup>27</sup> suggesting that diseases

outside of the pancreas can lead to a secondary pancreatopathy, or that there is increased leakage of immunoassay-positive lipase into the circulation in non-pancreatic disease.<sup>27</sup> Interestingly, 6/50 (12%) dogs in our study were diagnosed with foreign bodies, 4 of which had no false-positive results, and 2 of which had at least 1 false-positive result. One of the foreign body cases had a false-positive Precision PSL and equivocal results for the Spec cPL and VetScan cPL Rapid Test diagnostic assays. The second foreign body case had a false-positive Precision PSL diagnostic assay and an equivocal result for the Spec cPL diagnostic assay. Increases in Spec cPL concentration and increased numbers of SNAP cPL positive results were reported in dogs with naturally occurring hyperadrenocorticism, even when there was no evidence of clinical pancreatitis.<sup>28</sup> Similarly, hyperadrenocorticism in some of the dogs in our study could potentially explain some cases with increased lipase immunoassay concentrations or positive SNAP cPL results in the absence of a diagnosis of clinical pancreatitis (based on the consensus internist scores). Three patients (6%) in our study had suspected hyperadrenocorticism. One of these patients had clinical pancreatitis, and the other 2 patients had no evidence of clinical pancreatitis (based on the consensus internist scores). One of the patients with suspected hyperadrenocorticism without clinical pancreatitis had an equivocal Precision PSL result. It has been suggested that increased Spec cPL concentrations in dogs with hyperadrenocorticism potentially may be due a consequence of undetected subclinical pancreatitis,<sup>29</sup> but this is as yet unproven.

The diagnostic sensitivity and specificity of the SNAP cPL assay based on our results (73.9–100% and 71.1–77.8%, respectively) are similar to those reported in a previous study that used clinical criteria as the gold standard, and calculated a sensitivity of 91.5–94.1% and a specificity of 71.1–77.5% for the SNAP cPL.<sup>10</sup> The sensitivity of the Spec cPL assay based on our results (81.0–90.9%) was higher than that previously reported (71.7–77.8%).<sup>10</sup> The specificity of the Spec cPL assay in our study (74.1–81.1%) is lower than previously reported (80.5–88.0%).<sup>10</sup> Our study, however, is not directly comparable to other studies using clinical scoring systems because our scoring internists included an equivocal score for cases in which the diagnosis of pancreatitis was considered to be possible but not conclusive, rather than simply assigning a dichotomous score of either “pancreatitis” or “not pancreatitis”. Sensitivities and specificities then were calculated under 2 different assumptions (either that equivocal cases were pancreatitis, or that equivocal cases were not pancreatitis) to enable some degree of comparison with previous studies that used a simple dichotomous scoring system. The sensitivities and specificities of the Spec cPL reported in our study and 1 previous study<sup>10</sup> differ significantly from those reported by another study (sensitivity of 21% for mild pancreatitis and 71% for moderate to severe pancreatitis, and a specificity of 100.0% for both mild and moderate to severe pancreatitis).<sup>8</sup> The differences in the reported sensitivities and specificities in these studies likely originate from the different gold standards used in each study. In our study and another study,<sup>10</sup> clinical criteria were interpreted as the gold standard, whereas in yet another study,<sup>8</sup> histopathology was used as the gold standard. Therefore, it is likely that a larger number of subclinical pancreatitis cases were diagnosed in that study.<sup>8</sup>

when compared to our study and the other study.<sup>10</sup> The clinical relevance of subclinical pancreatitis that is only detectable by pancreatic biopsy is currently unknown. In addition, our study did not seek to quantify the severity of clinical pancreatitis, unlike the other study.<sup>8</sup>

To our knowledge, our study is the first to evaluate the diagnostic sensitivity and specificity of the VetScan cPL Rapid Test assay. The diagnostic sensitivity of the VetScan cPL Rapid Test assay based on our results (73.9–83.3%) was moderately lower than the sensitivity of the other assays evaluated. The diagnostic specificity however is higher than that of the other assays evaluated (76.9–83.3%), including the Spec cPL. With the appropriate point-of-care analyzer, the VetScan cPL Rapid Test can be performed in practice, and this assay has the combined benefits of fast turn-around time and diagnostic accuracy associated with lipase immunoassays currently being used by commercial laboratories.

Although the diagnostic sensitivity of the Precision PSL assay based on our results was similar, if not higher than, the other 3 diagnostic assays evaluated (85.7–90.9%), the specificity of the Precision PSL was lower (64.0–74.3%). The difference in diagnostic specificity potentially can be explained by the fact that the Precision PSL is a DGGR-based assay, and DGGR is likely not specific for pancreatic lipase.<sup>16</sup> The lower specificity of the Precision PSL assay suggests that it may be more suited as a screening assay, rather than a confirmatory assay.

Some discrepant results were noted during data analysis. Six patients who were considered to not have pancreatitis had positive SNAP cPL assay results. Similar discrepancies were also reported in another study.<sup>12</sup> There are a number of potential explanations for these discrepancies. First the single diagnostic cut-point on the SNAP cPL may be targeted to have increased diagnostic sensitivity at the expense of a decreased specificity. In a highly sensitive assay, pancreatic inflammation associated with diffuse abdominal inflammation (eg, septic peritonitis) may be sufficient to provide a positive result, as may pancreatic hypoperfusion, because studies in humans have shown that the pancreas is highly sensitive to changes in its microcirculation.<sup>30</sup> Two patients also had normal SNAP cPL assay results, but Spec cPL results  $\geq 200$   $\mu\text{g/L}$ . The first dog had a Spec cPL of 237  $\mu\text{g/L}$ , and the second patient had a Spec cPL of 292  $\mu\text{g/L}$ . One possible explanation for this discrepancy would be operator error, however, this is unlikely because all tests were performed by trained individuals who performed the SNAP cPL test in accordance with the manufacturer's guidelines. A second, and more probable, explanation is that the results were within expected variation for these assays. The manufacturer reports 88–92% agreement between SNAP cPL and Spec cPL in abnormal samples, and these 2 discrepant results equate to only 4% of the test population. In 1 study, a comparable level of disparity was noted, in that 6% (5/84) of cases had normal SNAP cPL results but Spec cPL  $\geq 200$   $\mu\text{g/L}$ .<sup>10</sup> Although hemolysis was present in some of the samples in our study, previous studies have shown that hemolysis does not interfere with the results of the SNAP cPL<sup>17</sup> and Spec cPL assays.<sup>31</sup>

One potential limitation of our study was that ultrasound examinations were performed by different individuals, including both emergency clinicians and board-certified veterinary radiologists. The diagnostic sensitivity of abdominal

ultrasound examination for pancreatitis is reported to be approximately 68%,<sup>2</sup> although this is highly dependent on the skill of the ultrasonographer in obtaining and interpreting the images.<sup>15</sup> Steps were taken to improve on limitations reported in previous studies by having all ultrasound examinations recorded as both still images and video clips, and then by having all studies reviewed and reported by a single board-certified radiologist (A.G. MacLeod) if that radiologist had not conducted the original study. Twenty-eight (56%) of patients had the initial ultrasound examination and subsequent interpretation performed by A.G. MacLeod, whereas the other 22 (44%) patients had the initial ultrasound examination by 1 of 4 other clinicians, and stored images were subsequently interpreted by A.G. MacLeod. One patient in our study had an abdominal ultrasound examination in which only the right limb of the pancreas could be visualized in the stored video clips interpreted by the board-certified radiologist. Consequently, ultrasonographic evidence of focal pancreatitis may have been missed in this particular case. A consensus internist score of "0" was given to this patient. A further potential limitation of our study was that enrolled patients were dogs presented to an emergency clinic, and this design feature may have introduced a selection bias toward more severe cases. A further potential limitation was the lack of histopathological follow-up data, which still is considered as the gold standard by some individuals, despite its practical limitations.

In conclusion, we found that the level of agreement between individual diagnostic tests for pancreatitis and the clinical suspicion score was good. The level of agreement among 4 currently available diagnostic assays for pancreatitis however was greater than the level of agreement between an individual diagnostic test and the clinical suspicion score. No single assay had high enough diagnostic specificity to conclusively diagnose pancreatitis based on a single test result, suggesting that a combination of signalment, physical examination, blood tests, a pancreatic lipase test, and ideally, abdominal ultrasound examination when available, may be the most practical means of establishing a definitive diagnosis of clinical pancreatitis in dogs.

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## Footnotes

- <sup>a</sup> Spec cPL ELISA, Texas A&M University, Gastrointestinal Laboratory, College Station, TX
  - <sup>b</sup> SNAP cPL Test Kit, Idexx Laboratories Inc, Westbrook, ME; VetScan cPL Rapid Test, Abaxis Inc, Union City, CA
  - <sup>c</sup> VetScan cPL Rapid Test, Abaxis Inc, Union City, CA
  - <sup>d</sup> Precision PSL, Antech Diagnostics, Irvine, CA
  - <sup>e</sup> Abaxis Laboratories Ltd, Union City, CA
  - <sup>f</sup> Antech Diagnostics, Irvine, CA
  - <sup>g</sup> SAS for Windows 9.4, SAS Institute Inc, Cary, NC
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**Off-label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.

**Institutional Animal Care and Use Committee (IACUC) or Other Approval Declaration:** Our study was approved by the Animal Care and Use Committee of the Blue Pearl Science Clinical Trial Review Board. All owners of the patients enrolled in the study signed an informed consent agreement.

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