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Comparative repeatability of pancreatic lipase assays in the commercial and in-house laboratory environments

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Abstract

Background: Sensitivity and specificity for commercial and in-house pancreatic lipase immunoreactivity (cPLI) assays have been reported, but repeatability under routine clinical conditions is unknown.

Objectives: To determine:

1 Coefficient of variation (CV) between replicates of a commercial assay (Spec cPL) and 2 in-house assays (VetScan cPL, Vcheck cPL) under routine conditions.
2 Effects of sample condition or personnel on results.
3 Potential directional bias between assays.

Animals: Serum from 12 canine clinical patients.

Methods: Prospective study. Serum Spec cPL, VetScan cPL, and Vcheck cPL (6 aliquots each) were measured, and CVs were calculated, effects of sample condition and personnel were assessed using a linear mixed model, and direction of bias was assessed using least square mean cPLI concentration.

Results: Mean %CVs for Spec cPL, VetScan cPL, and Vcheck cPL were 5.5, 17.0, and 23.7%. Three of 6 VetScan cPL samples and 5/9 Vcheck cPL samples had an unacceptably high %CV (>20%). Transportation (Spec cPL) and sample condition or personnel (VetScan cPL, Vcheck cPL) did not affect repeatability. Least square mean cPL was higher for Spec cPL (807.9 μg/L) than for VetScan cPL (558.5 μg/L) or Vcheck cPL (399.8 μg/L).

Conclusions and Clinical Importance: For clinical use, the commercial Spec cPL has the highest repeatability, and Vcheck cPL has significantly lower repeatability. Both in-house assays evaluated may provide discrepant categorical results ("pancreatitis" versus "equivocal" versus "not pancreatitis") for the same sample. In-house pancreatic lipase concentrations may be lower than those determined by the Spec cPL assay.

KEYWORDS

quality control, Spec cPL, transportation, Vcheck cPL, VetScan cPL

Abbreviations: cPLI, canine pancreatic lipase immunoreactivity; CV, coefficient of variation; ICC, intraclass correlation coefficient; RIA, radio-immunoassay; TAMU, Texas A&M University, Gastrointestinal Laboratory.

1 | INTRODUCTION

Histopathology is the traditional gold standard for the diagnosis of pancreatitis in dogs but its use is limited by the invasiveness of sample collection, potential to miss localized lesions, and potential detection of subclinical pancreatitis.[1-4] Given these limitations, a diagnosis based on clinical criteria has largely replaced histopathology as a surrogate gold standard.[5-10] These clinical gold standards comprise a variety of data, including measurement of canine serum pancreatic lipase immunoreactivity (cPLI).

The Spec cPL (Spec cPL ELISA, Texas A&M Gastrointestinal Laboratory, College Station, TX 77843) is the most commonly utilized assay for quantification of pancreatic lipase in dog serum. It is a send-out test performed under reference laboratory quality control with a turnaround time of approximately 1-2 working days (Guidelines on Utilizing the Feline and Canine Pancreas-Specific Lipase Tests to Assess Pancreatic Health [Internet]. IDEXX Laboratories. 2018 [cited 2019 Jul 7]. Available from: https://www.idexx.com/files/spec_cpl-spec_fpl_dxu_h.pdf). Delays in test results may be detrimental in some patients, and a number of in-house assays have been developed to overcome this limitation. Controversy exists with regard to the influence of testing conditions on these assays.[11,12] Studies evaluating the performance of the Spec cPL in the clinical environment have reported a variety of sensitivities and specificities.[5,13-16] Potential causes for this variation include differences in the gold standard utilized and differences in patient selection, but to the authors' knowledge no studies have evaluated the effect of transportation as a potential contributing factor.

The VetScan cPL (VetScan cPL Rapid Test, Abaxis Inc., Union City, CA 94587) is an in-house semiquantitative assay for the detection of canine pancreatic lipase. A recent study found similar diagnostic sensitivity and specificity to the Spec cPL, but in this study the assay was not performed under field conditions.[7] Caution therefore has been advised when using this assay in-house on clinical patients.[11] Additionally, a recent study reported poor linearity, questionable precision, and high interassay variability among VetScan cPL results, but this study also was not performed under field conditions, because the test was performed in a research laboratory, rather than patient-side.[17] The Vcheck cPL (Vcheck cPL, Bionote Co. LTD. 22 Samseong 1-ro 4-gil, Hwaseong-si, Gyeonggi-do, Republic of Korea) is an in-house quantitative pancreatic lipase assay that is advertised to have a similar performance to the Spec cPL, but a recent partial analytic validation study performed on research samples also reported lack of linearity, precision, and reproducibility (Steiner JM, Liu J, Drexel J et al. Partial analytical validation of a new in-clinic cPLI test (Vcheck cPL). 2019 WSAVA Abstracts. Page 681).

Our study had 3 main aims: first, to assess the repeatability of 3 previously described assays under the clinical circumstances in which they are designed to be utilized (the Spec cPL as a send-out test, and the VetScan cPL and Vcheck cPL tests as patient-side tests); second, to assess whether sample condition, transportation batch, or in-house personnel performing the test had an effect on the repeatability of each assay; and third, to determine whether the assays produced similar pancreatic lipase concentration results, and, if not, to determine the direction of bias among the assays. An additional goal was to compare our results to previously reported studies, and thus help determine the impact of factors such as a lack of reference laboratory quality control on in-house pancreatic lipase assays, and the impact of transportation on reference laboratory pancreatic lipase assays.

2 | MATERIALS AND METHODS

2.1 | Study overview

From August to September 2019, 12 client-owned dogs with clinical signs of gastrointestinal disease were prospectively enrolled in the study. Two authors (A. J. M., H. C.) assigned each patient into 1 of the following categories: (1) suspected pancreatitis, (2) equivocal pancreatitis, or (3) unlikely to have pancreatitis based on history, signalment, clinicopathologic data and, when available, the results of a SNAP cPL assay (SNAP cPL Test Kit, Idexx Laboratories Inc., One Idexx Drive, Westbrook, ME 04092) and pancreatic ultrasound examination. The aim of this step was to enroll patients (4 into each of the above categories) that would likely yield a range of pancreatic lipase concentrations, thus allowing assessment of test performance across a variety of concentrations. Approximately 12 mL of whole blood was collected into glass tubes without anticoagulant (Covidien Monoject no additive red top tubes. Medtronic, 710 Medtronic Parkway, Minneapolis, MN 55432) from each dog at the time of enrollment. The sample then was left to clot at room temperature for 20 minutes before centrifugation at 1000*g* for 10 minutes. Serum then was separated from the clot for evaluation of pancreatic lipase concentration using the Spec cPL, VetScan cPL, and the Vcheck cPL assays. Immediately after serum separation, serum from each patient was divided into 18 equal aliquots (approximately 0.3 mL) by 1 of the authors (H. C.). Each aliquot then was assigned a random number using a commercial software package (Microsoft Excel, One Microsoft Way, Redmond, WA 98052). Six aliquots from each patient then were frozen immediately at −20°C for storage and subsequent submission to a reference laboratory for determination of Spec cPL concentration. Samples for Spec cPL measurement were batched for shipment across 3 days (3 shipments of 24 samples each, with each shipment containing 2 samples from each patient) to mimic day-to-day variation in transportation conditions. Samples were shipped under standard shipping conditions recommended by the laboratory for clinical submissions (ie, overnight shipment of frozen samples in a Styrofoam box also containing a cold pack). The reference laboratory was blinded to patient identity, and to which aliquots came from the same patient. The 12 remaining aliquots from each dog were used for in-house determination of pancreatic lipase concentration using both in-house analyzers. Samples were analyzed on the day of collection, immediately after randomization. Each assay was performed in accordance with manufacturer guidelines.[18,19] To allow for adequate blinding of in-house assays, 3 different personnel on-call at the time of patient sample collection (ie,

veterinary technicians, small animal rotating interns, or residents), all trained in the use of the in-house assays, performed the VetScan cPL on 2 randomly assigned aliquots each, and performed the Vcheck cPL, also on 2 randomly assigned aliquots of patient serum. Each of the personnel who performed the in-house assays was blinded to the results of the other 2 personnel performing the same assay from the same patient. In total, each assay was tested for repeatability across 6 samples from 12 patients (72 samples). Sample condition (ie, normal, hemolyzed, lipemic) also was recorded. The study protocol was approved by the IACUC committee of Mississippi State University (protocol # IACUC-18-249).

2.2 | Spec cPL Assay

The Spec cPL assay is a second-generation ELISA for the measurement of cPLI concentration and offered as a send-out test by the Gastrointestinal Laboratory at Texas A&M University and Idexx Laboratories. As is standard practice on most ELISAs, samples are run in duplicate fashion. The lower limit of detection is 30 μg/L, and the upper limit of detection is 2000 μg/L. A Spec cPL concentration ≥400 μg/L is considered by the laboratory as consistent with a diagnosis of pancreatitis, whereas a concentration ≤200 μg/L considered to be within the reference interval. Concentrations between 201 μg/L and 399 μg/L are considered equivocal for the diagnosis of pancreatitis in dogs.

2.3 | VetScan cPL Rapid Test assay

The VetScan cPL Rapid Test is an in-house immunoassay that utilizes lateral-flow technology for the semiquantitative assessment of canine pancreatic lipase. The assay utilizes purified polyclonal antibodies that bind to canine pancreatic lipase to assess its concentration.[19] The sample size required is 1 drop of serum or plasma, and results are available within 10 minutes. The analyzer utilized had a lower limit of detection of 40 μg/L, and an upper limit of detection of 600 μg/L. The reference intervals provided by the manufacturer for diagnostic categories of pancreatitis, within the reference interval, or equivocal, are the same as used for the Spec cPL assay, as reported earlier.

2.4 | Vcheck cPL assay

The Vcheck cPL assay is an in-house fluorescent immunoassay for the quantitative measurement of canine pancreatic lipase.[18] The sample size required is 25 μL, and results are available within 5 minutes. The lower limit of detection is 50 ng/mL, and the upper limit of detection is 2000 ng/mL. The reference interval provided by the manufacturer as well as the cutoff values for pancreatitis or equivocal are the same as used for the Spec cPL assay, as described above.

2.5 | Statistical analysis

For assessment of repeatability, the coefficients of variation were calculated for each dog for each of the assays (ie, Spec cPL, VetScan cPL, and Vcheck cPL) using a statistical software package (Microsoft Excel, One Microsoft Way, Redmond, WA 98052). For this part of the study, analysis was performed only on samples for which at least 4 of 6 measurements fell within the working range of the respective assay. If a patient had at least 4 of 6 measurements within the working range of the assay, but another measurement was outside of the working range, the measurement outside of the range was adjusted by 0.1 from the lower or upper limits of the range to give a quantitative value. The mean, SD, and range for each of the test results subsequently were calculated for the coefficients of variation. The effect of test type and sample condition on coefficient of variation was evaluated by linear mixed modeling using PROC MIXED in a statistical software package (SAS for Windows 9.4, SAS Institute Inc, Cary, North Carolina). The coefficient of variation, calculated from quantitative values, was the dependent variable in the model. Test type, sample condition, and their interaction were included as fixed effects, and dog identity was included as a random effect.

For the following analyses, a data set excluding individual values above and below the working limits of each assay was used for analysis. The effect of test type on test results was assessed by linear mixed modeling using PROC MIXED with test value as the dependent variable, test type as the fixed effect, and dog identity as a random effect. The effect of shipping day on Spec cPL results was assessed using a similar linear mixed model. The effects of the identity of the in-house personnel performing the assays on VetScan cPL and V-check results were assessed using similar linear mixed models. In the case of a significant effect in the linear mixed models, differences in least squares means with Tukey adjustment for multiple comparisons were determined. The distribution of the conditional residuals was evaluated for all of the linear mixed models to ensure the assumptions of normality and homoscedasticity for the statistical models had been met. An alpha level of .05 was used to determine statistical significance for all methods.

After classification of quantitative test results, using a data set that included individual results above and below the working limits of each assay, into categories of "normal, pancreatic lipase concentration ≤200 μg/L" (NP), "equivocal, pancreatic lipase concentration 201-399 μg/L" (E), and "suggestive of pancreatitis, pancreatic lipase concentration ≥ 400μg/L" (P), the replicates from each dog for each test then were evaluated for agreement in diagnostic category determination.

3 | RESULTS

3.1 | Animals

Seven neutered male and 5 spayed female dogs were enrolled in the study. The median age of dogs enrolled was 10 years and 6 months (range, 5 to 16 years). The median weight of patients enrolled was

6.9 kg (range, 5.1-21.3 kg). There were 2 mixed breed dogs. The remaining 10 dogs represented a variety of breeds including Miniature Schnauzer (n = 2), Pomeranian (n = 2), and 1 each of the following breeds: Chihuahua, Dachshund, Shetland Sheepdog, Shih Tzu, Standard Poodle, and Yorkshire Terrier.

3.2 | Repeatability of the Spec cPL, VetScan cPL, and Vcheck cPL assays

Results of all 3 assays for pancreatic lipase concentration are presented in Tables S1-S3. For calculation of repeatability, sample sets from 6 dogs were used for the VetScan cPL assay, and sample sets from 9 dogs were used for the Spec cPL and Vcheck cPL assays. When evaluating those sample sets, the mean %CV for the Spec cPL was 5.5% (range, 2.9%-8.2%), for the VetScan cPL 17.0% (range, 4.7%-32.6%), and for the Vcheck cPL 23.7% (range, 4.6%-40.8%). The standard deviations of the %CV for the Spec cPL, VetScan cPL, and Vcheck cPL were 1.6, 11.2, and 11.1%, respectively. Similar to previous studies, an intraassay variability of ≤10% was considered acceptable, an intraassay variability of 10% < %CV ≤ 20% was considered suboptimal but acceptable, and a value >20% was considered unacceptable.[17] The mean %CV and individual values of repeatability from all 9 dogs for the Spec cPL met acceptable performance criteria. For the VetScan cPL, the mean %CV was in the suboptimal but acceptable range, and 2 %CVs from samples from individual dogs were in the acceptable range (4.7 and 6.2%), 1 more was in the suboptimal but acceptable range (12.1%), and the remainder were in the unacceptable range (20.1, 26.0, and 32.6%). For the Vcheck cPL, the mean %CV was in the unacceptable range. One %CV from a sample from an individual dog was in the acceptable range (4.6%), 3 more were in the suboptimal but acceptable range (17.0, 17.2, and 19.8%), and the remainder were in the unacceptable range (22.1, 23.5, 32.3, 36.1, and 40.8).

3.3 | Agreement of diagnostic category determination by the Spec cPL, VetScan cPL, and Vcheck cPL assays

After assessment of the quantitative data, the results were categorized as follows: "within the reference interval" (pancreatic lipase concentration ≤200 μg/L), "equivocal for pancreatitis" (pancreatic lipase concentration 201-399 μg/L), or "suggestive of pancreatitis" (pancreatic lipase concentration ≥400 μg/L), according to the manufacturer's guidelines. All Spec cPL replicates yielded the same diagnostic category for all 12 dogs, with 4 of those samples being interpreted as being within the reference interval and the remaining 8 being suggestive of pancreatitis. For the VetScan cPL, replicates from 9 dogs yielded the same diagnostic category, with 4 of those samples being interpreted as being within the reference interval and the remaining 5 being suggestive of pancreatitis. Replicates from 3 dogs had discordant results. Patient H had 2 results suggestive of pancreatitis and 4 results equivocal for the diagnosis of pancreatitis, whereas patient I had 5 results suggestive of pancreatitis and 1 result equivocal for the diagnosis of pancreatitis. Patient J, however, had replicates in each of the 3 diagnostic categories, with 2 replicates suggestive of pancreatitis, 3 replicates equivocal for pancreatitis, and 1 replicate within the reference interval. For the Vcheck cPL, replicates from 8 dogs yielded the same diagnostic category, with 4 of those samples being interpreted as within the reference interval and results of the remaining 4 dogs being suggestive of pancreatitis. Replicates from 4 dogs had discordant results. Patients H, I, and J had 5 replicates suggestive of pancreatitis and 1 replicate equivocal for the diagnosis of pancreatitis. Patient K had 3 replicates equivocal for the diagnosis of pancreatitis and 3 replicates suggestive of pancreatitis. No patient had replicates in all 3 diagnostic categories using the Vcheck cPL.

3.4 | Comparison of the repeatability of the Spec cPL assay among different transportation batches

Serum samples for Spec cPL assessment were stored at −20°C before shipment and were shipped as 3 batches across 3 consecutive days to a commercial laboratory. Day of shipment had no significant effect on the Spec cPL results ($P = .4$).

3.5 | Effect of assay and sample condition on repeatability

No significant effect on %CV was detected for sample condition ($P = .20$) or the sample condition and test interaction ($P = .10$), but test did have a significant effect ($P = .001$). The Vcheck cPL had significantly higher %CV than the Spec cPL ($P = .001$) and the VetScan cPL ($P = .04$). A significant difference was not detected between the Spec cPL and VetScan cPL ($P = .10$).

3.6 | Effect of laboratory personnel on pancreatic lipase concentration as measured by the VetScan cPL and Vcheck cPL assays

A significant effect, because of the laboratory personnel utilized, on pancreatic lipase concentration was not detected for either the VetScan cPL ($P = .30$) or the Vcheck cPL ($P = .07$) assay.

3.7 | Comparative assessment and direction of bias between pancreatic lipase assay results

Test type had a statistically significant effect on pancreatic lipase concentration ($P < .001$). The least squares means of pancreatic lipase concentration produced by each of the tests (Spec cPL, VetScan cPL, and Vcheck cPL) was significantly different (adjusted $P < .001$). The least squares means of pancreatic lipase concentrations were

807.9 μg/L for the Spec cPL, 558.5 μg/L for the VetScan cPL, and 399.8 μg/L for the Vcheck cPL.

4 | DISCUSSION

Pancreatic lipase assays are being used with increasing frequency in the clinical diagnosis of pancreatitis in dogs. The Spec cPL frequently is utilized because it has been reported to have the highest sensitivity and specificity for detection of histopathologic pancreatitis in dogs but the turnaround time of at least 1-2 business days may be limiting in some cases, which has led to development of in-house quantitative pancreatic lipase assays.[14] Before our study, the repeatability of these in-house assays had not been directly compared in the same patient group, and controversy existed regarding testing conditions in prior studies.[11,12] Our study indicates that the evaluated send-out pancreatic lipase assay (Spec cPL) had less variation between replicates than did the evaluated in-house assays (VetScan cPL and Vcheck cPL). Our results suggest that a lack of formal commercial quality control in some in-house assays may have more effect on repeatability of test results than the impact of transportation variables on the send-out assay.

In our study, coefficients of variation were calculated using quantitative data, provided at least 4 replicates were within the working range of each assay. We also evaluated for agreement between replicates in diagnostic category determination. When evaluating quantitative data, the repeatability of results was highest (lowest CV) with the Spec cPL assay (mean CV, 5.5%), followed by the VetScan cPL assay (mean CV, 17.0%), whereas the Vcheck cPL had the lowest repeatability (mean CV, 23.7%). No dogs had an unacceptable CV with the Spec cPL assay but 3 dogs had unacceptable CV (> 20%) with the VetScan cPL and 5 dogs had unacceptable CV (>20%) with the Vcheck cPL assay. The median CV of the VetScan cPL reported in our study (17.0%) was lower than the median CV (25.1%) reported in a prior study of research samples performed by the Texas A&M GI Laboratory.[18] This result may be a consequence of the broader range of pancreatic lipase concentrations evaluated in the clinical patients in our study, and because of improved performance of the VetScan cPL under the in-house conditions used in our study. The median CV of the Vcheck cPL reported in our study (23.7%) is similar to that reported in a prior partial validation study using research samples (23%-36%), and published in abstract form (Steiner JM, Liu J, Drexel J, Chandrashekar C. Partial analytical validation of a new in-clinic cPLI test [Vcheck cPL]. 2018 WSAVA Abstracts. Page 681).

When evaluating agreement between replicates for diagnostic category determination (ie, within the reference interval, equivocal for the diagnosis of pancreatitis, or suggestive of pancreatitis), the Spec cPL had the highest agreement, with all 6 replicates for the 12 patients yielding the same diagnostic category. In contrast, 3 dogs for the VetScan cPL and 4 dogs for the Vcheck cPL had replicates classified in different diagnostic categories. Of particular concern, 1 dog had replicates in each of the 3 different diagnostic categories when utilizing the VetScan cPL assay. It therefore is recommended that, when VetScan cPL or Vcheck cPL results are obtained that are unexpected based on a combination of history, physical examination findings, clinicopathologic data and pancreatic ultrasound examination, in-house tests be repeated to reach a consensus, a Spec cPL assay be performed because of its lower CV and 100% agreement in categoric determination or both. The fact that a higher proportion of the CVs for the Vcheck cPL and the VetScan cPL assays were in the acceptable but suboptimal range, or in the unacceptable range, suggests that performance improvement of these assays may be necessary before routine clinical use. The higher number of patients with unacceptable CVs in the VetScan cPL and the Vcheck cPL groups suggests that these assays are more suitable for use as semiquantitative assays. The performance of the Spec cPL assay in our study, with low coefficients of variation and 100% agreement in diagnostic categorization, suggests that it currently is the most precise biochemical marker of pancreatic lipase in dogs.

Some previous studies have suggested measurement of Spec cPL to monitor disease progression and response to treatment (Lim SY, Nakamura K, Morishita K et al). Serial evaluation of specific canine pancreatic lipase immunoreactivity and C-reactive protein in dogs with cerulein-induced acute pancreatitis. 2015 WSAVA Abstracts. Page 122). In addition, a prior study also has documented that a markedly increased Spec cPL was associated with nonsurvival in acute pancreatitis.[20] The VetScan cPL assay, with an upper limit of detection of 600 μg/L compared to 2000 μg/L for the other 2 assays, may have less value for assessing temporal changes in pancreatic lipase concentration in some patients, and for assessing prognosis based on the magnitude of the increased concentrations.

The effect of transportation variables on the repeatability of send-out pancreatic lipase assays previously has been questioned, because repeatability testing of a single sample multiple times in a commercial laboratory environment will not account for the effects of transportation.[12] In our study, we did not detect a statistically significant effect of day of transportation or transportation batch on Spec cPL concentration, suggesting that transportation variables across 3 consecutive days had minimal effect on Spec cPL measurement. However, future studies would be required to determine the effects of longer transportation times or a greater variability in weather conditions during shipment.

Previous studies have documented that hemolysis and lipemia have no effect on measurement of Spec cPL, but to our knowledge, no studies have evaluated the effects of sample condition on the VetScan cPL and Vcheck cPL assays.[21] The manufacturer of the VetScan cPL reports that icterus and lipemia do not affect results, and the manufacturer of the Vcheck cPL reports that results are not affected by bilirubin or triglycerides.[18,19] Our results are in agreement with the manufacturers' reports, and we did not identify sample condition (ie, normal, hemolysis, lipemia) as having an effect on the repeatability of either the VetScan cPL or Vcheck cPL assays. We also evaluated whether different in-house personnel performing the test, as would be expected in typical clinical scenarios, impacted variability in VetScan cPL or Vcheck cPL results. Our results indicated that using differing personnel did not affect the repeatability of either the

Vcheck cPL or VetScan cPL assays, despite the relative complexity of the Vcheck cPL assay when compared to the VetScan cPL. The VetScan cPL is a relatively simple test in which 1 drop of serum is added to 3 drops of chase buffer followed by a timed scan and interpretation of results using the application software. The Vcheck cPL, however, requires more steps before interpretation, thus increasing its complexity.

We documented that each assay resulted in significantly different pancreatic lipase concentrations when measured on the same clinical samples. This observation differs from a previous study that documented a high level of agreement (intraclass correlation coefficient [ICC] = 0.96) between the results of the Spec cPL and VetScan cPL assays.[7] Our results indicate that the assays cannot be directly compared or used interchangeably. Notably, the least square mean Spec cPL pancreatic lipase concentration (807.9 μg/L) was higher than the pancreatic lipase concentration measured by both the VetScan cPL (558.5 μg/L) and Vcheck cPL assay (399.8 μg/L). Our results are similar to those of a previous study that found that 81% of samples had lower pancreatic lipase concentrations when measured by the Vcheck cPL compared to the Spec cPL assay (Steiner JM, Liu J, Drexel J et al. Partial analytical validation of a new in-clinic cPLI test (Vcheck cPL). 2018 WSAVA Abstracts. Page 681).

One potential limitation of our study was the small sample size (72 samples for 12 dogs). At the time of study design, however, an estimation of variability among repeated samples for these particular assays in a clinical environment was unavailable. Future studies could utilize the CVs reported here as part of *a priori* power calculations. Future studies also should evaluate the clinical consequences of the decreased repeatability of the in-house pancreatic lipase assays assessed, compared to the commercial send-out test evaluated, and the subsequent impact on assignment of a clinical diagnosis of pancreatitis.

5 | CONCLUSION

In conclusion, we found that the level of repeatability was highest (lowest CV) with the Spec cPL assay, suggesting that the lack of formal laboratory-based quality control on in-house assays may have more impact on test repeatability than transportation variables associated with send-out pancreatic lipase assays. The Spec cPL had the lowest CV and had a 100% agreement in diagnostic category assignment, suggesting that it is currently the most precise biochemical marker of pancreatic lipase in dogs. The VetScan cPL and the Vcheck cPL had a higher proportion of results with unacceptably high CV, and had disagreements of interpretation with repeat measurements, suggesting that these evaluated assays may require additional improvements before clinical utilization. Sample condition or personnel factors had no effect on repeatability. In-house assays (VetScan cPL and Vcheck cPL) resulted in lower mean pancreatic lipase concentrations than did the Spec cPL assay.

CONFLICT OF INTEREST DECLARATION

J. M. S, J. A. L, and J. S. S. are affiliated with the Texas A&M Gastrointestinal Laboratory, which offers measurement of Spec cPL concentration on a fee-for-service basis. J. M. S. is also a paid consultant and speaker for IDEXX Laboratories, the manufacturer of the Spec cPL assay. Conflict of interest was managed by ensuring that enrollment of patients and randomization of samples was performed at a separate institution (Mississippi State University) by 2 authors, H. C. and A. J. M., who have no association with the Texas A&M Gastrointestinal Laboratory or with IDEXX, and who have no conflict of interests. Additionally, all assays were performed by individuals who were blinded to sample aliquot, patient and other results from that patient. Furthermore, all results have been included in Tables S1-S3 to allow the reader to independently interpret the data.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials

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

Approval was granted by the Mississippi State University IACUC. Protocol ID: IACUC-18-249.

HUMAN ETHICS APPROVAL DECLARATION

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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