INTRODUCTION

Pancreatitis is common in dogs. These dogs can present with a wide range of clinical presentations from subclinical disease to mild chronic disease to severe acute disease. This range of clinical presentations makes the diagnosis of pancreatitis challenging. Currently, abdominal ultrasonography and the measurement of serum pancreatic lipase concentrations are considered to be the most useful diagnostic modalities for canine pancreatitis. A variety of ultrasonographic changes have been reported in dogs with pancreatitis. However, these findings can be subjective, and the overall diagnostic efficacy of abdominal ultrasonography for dogs with pancreatitis is dependent on the disease severity, equipment quality, operator experience, and level of suspicion for pancreatitis by the operator.

In contrast to assays for lipase activity, serum pancreatic lipase immunoreactivity (cPLI) measurements are highly specific for the detection of pancreatic lipase. In one study that evaluated serum cPLI concentrations in shelter dogs that had been euthanized for other...
reasons, the specificity of the serum cPLI concentration was 95.7%. Measuring cPLI concentrations is also highly sensitive for the diagnosis of pancreatitis, though reported sensitivities have depended on the clinical presentation and study design. Until recently, the only commercially available assays measuring cPLI were the Spec cPL and SNAP cPL (IDEXX Laboratories). The Spec cPL is a laboratory-based ELISA, using a recombinant antigen (recombinant canine pancreatic lipase) and a monoclonal antibody directed against native canine pancreatic lipase. The analytical validation of this assay has been reported in the peer-reviewed literature. The Spec cPL assay was reported to be linear upon dilution with a working range of 30–2000 µg/L. Intra-assay variability for three samples and 12 repeated measurements were reported to be 7.8%, 9.0%, and 11.2%, and the inter-assay variability for three samples and five repeat measurements were reported to be 3.8%, 7.6%, and 5.6%. The SNAP cPL is a point-of-care semi-quantitative device that is easy to perform and results in either a “normal” read (ie, the associated Spec cPL is in the reference interval [RI] of <200 µg/L) or an “abnormal” read (ie, the associated Spec cPL is either suggestive of pancreatitis or in the questionable range). Abaxis has recently released a point-of-care test for cPLI that is also based on a rapid assay device that is being read by a reader connected to a smartphone. To the authors’ knowledge, no analytical validation data have been provided for this new assay platform either in the peer-reviewed literature or the assay documentation. Thus, the goal of the current study was to perform a partial analytical validation of this new assay platform. This study is especially timely as a recent report suggested that the VetScan cPL correlated well with the Spec cPL and the diagnostic bin of the Spec cPL. However, in that study, the assay was not performed as it would be used in clinical practice, but serum samples were sent to a central research laboratory where no measurements taken on weekend days). Serum sample concentrations of 196, 227, 254, 322, 360, 388, 400, 477, 566, and 1319 mg/dL undiluted, and at dilutions of 1:2 and 1:4.

## 2.1 | Samples

All samples used for the partial validation of the VetScan cPL assay were from leftover serum samples that had been submitted to the Gastrointestinal Laboratory at Texas A&M University. All samples had originally been analyzed at the GI Lab, and then the leftover serum samples were frozen at -20°C for up to 4 months. However, all Spec cPL measurements referred to in this manuscript were performed at the same time as the VetScan cPL measurements. Before analyses, all samples were thawed and brought to room temperature, as suggested by the manufacturer.

## 2.2 | The Vue analyzer and VetScan cPL assay

All VetScan cPL measurements were taken using four different Vue Analyzers obtained from Abaxis (Union City, California). The Vue is a point-of-care analyzer that reads proprietary lateral flow devices, such as the VetScan cPL device. The assay is based on lateral flow technology that uses affinity-purified antibodies directed against canine pancreatic lipase, which is bound to colloidal gold particles. Serum samples are applied to the device, and the pancreatic lipase in the sample binds to antibody-coated gold particles. These complexes are then captured by a secondary antibody on the test strip. The accumulation of the captured gold particle/antibody complex causes the color indicator to become visible on the test line. The signal is further amplified by the use of a competitive antibody calibration scheme employed on the control line. The darkness of the lines is quantified by densitometric analysis in the VetScan VUE (information taken from the product insert of the VetScan cPL). The working range of the assay is 50–60 µg/L to <700 to <800 µg/L. The assay marketing material suggests that the assay is quantitative and leads to results that are within a band of ±60 µg/L of the Spec cPL result. However, while most readers would display and report results as ±60 µg/L, some readers report results as ±50 µg/L.

## 2.3 | Linearity

The linearity of the assay was assessed by measuring dilutional parallelism of six canine serum samples of high-quality undiluted and at dilutions of 1:2, 1:4, and 1:8 with a pooled nonlipemic serum sample with an undetectable serum Spec cPL concentration.

## 2.4 | Effect of lipemia

For this experiment, we evaluated 3 naturally hypertriglyceridemic serum samples with serum triglyceride concentrations of 525, 580, and 1319 mg/dL undiluted, and at dilutions of 1:2 and 1:4.

## 2.5 | Reproducibility

Intra-assay variability was tested with three high-quality serum samples, designated Samples 1, 2, and 3, evaluated 10 times on four different Vue analyzers. Measurements were performed on the same analyzer during a single session on a single day. For this study, an intra-assay variability of ±10% was considered acceptable, an intra-assay variability 10% < %CV ≤ 20% was considered poor but acceptable, and an intra-assay variability of >20% was considered unacceptable.

## 2.6 | Repeatability

For the assessment of inter-assay variability, 10 high-quality serum samples were measured eight times on the same analyzer on different days (measurements were performed on consecutive days with no measurements taken on weekend days). Serum sample concentrations spanned the lower third of the Spec cPL assay working range since our previous findings showed that serum samples with a serum Spec cPL concentration >700–800 µg/L would often read outside the VetScan cPL assay working range. The 10 samples had Spec cPL concentrations of 196, 227, 254, 322, 360, 388, 400, 477, 566, and...
3.2 | Adherence to the manufacturer’s specifications

To determine whether the new VetScan cPL adhered to the manufacturer’s specification that the results would be within ±60 µg/L of the Spec cPL assay, each nonlipemic measurement was assessed. It should be noted that one VUE analyzer read the result as ±50 µg/L of the Spec cPL assay, but the more conservative criterion of ±60 µg/L was used for these assessments. Spec cPL concentrations were determined and used as the target values. However, only VetScan Vue measurements were used to evaluate this criterion and were measured close to the same time as that of the Spec cPL concentrations. Thus, for the inter-assay variability determinations where samples were run multiple times on consecutive days, only the initial Spec cPL concentration was used.

2.7 | Correlations

While we did not run a correlation study per se, our experiments created a set of data pairs that were then used to assess the correlation. The values for undiluted samples from the dilutional parallelism and lipemia experiments were used. Also, the first data point, each from the intra- and inter-assay variability experiments was used for this analysis, which generated a total of 34 data pairs. After excluding data pairs with results that were outside the working range for either assay (five data pairs had results that were outside the working range of the VetScan assay, one data pair from the intra-assay variability study had a VetScan cPL of <60 µg/L and a Spec cPL of 120 µg/L, one data pair from the inter-assay variability study had a VetScan cPL of <50 µg/L and a Spec cPL of 254 µg/L, one data pair from the inter-assay variability study had a VetScan cPL of <60 µg/L and a Spec cPL of 196 µg/L, and two data pairs from the dilutional parallelism study had a VetScan cPL of >800 µg/L and a Spec cPL of 1265 and 1287 µg/L, respectively), a total of 29 data pairs were selected for analysis.

2.9 | The statistical methods

For statistical analyses, single results outside the assay working range (eg, <60 µg/L) were transcribed as one unit above or below the working range limit (eg, 59 µg/L). However, sample sets that had results mostly outside of the working range (eg, all 10 samples <60 µg/L) were not used for the statistical analyses. Repeated measure ANOVA was used to assess differences among the different analyzers.

All statistical comparisons were performed using a statistical software package (GraphPad Prism 6.07). The level of statistical significance was set at .05 for all statistical comparisons. Spearman’s correlation coefficient (r) was calculated using a statistical software package (GraphPad Prism).

3 | RESULTS

3.1 | Linearity

The average observed-to-expected (O/E) ratio for a total of 13 observations was 119.3% (±SD: 28.7%). Of those O/E ratios, two were between 90% and 110% (ideal range), four were between 80% and 120% (acceptable range), and seven were outside the acceptable range (77.4, 123.4, 130.2, 140.0, 150.6, 159.1, and 162.9%) (Table 1).

3.2 | The effects of lipemia

For this experiment, we evaluated serum samples from three hypertriglyceridemic dogs with serum triglyceride concentrations of 525, 580, and 1319 mg/dL. For two samples, the VetScan cPL rapid test neat measurement was outside the analyzer working range of 700 µg/L, while the third sample had a concentration of 320 µg/L. Concentrations for the 1:2 and 1:4 dilutions were 292 µg/L with an O/E ratio of 182.5% and 65 µg/L with an O/E ratio of 81.3%, respectively. Both measurements were outside the preferred range of ±10%, and one was outside the acceptable range of ±20%. For the other two samples that had undiluted cPLI concentrations above the upper limit of the working range (eg, all 10 samples >800 µg/L), one was outside the acceptable range of ±20%. For the other two samples, the results mostly outside of the working range (eg, all 10 samples <60 µg/L) were not used for the statistical analyses. Repeated measure ANOVA was used to assess differences among the different analyzers.

One out of four analyzers had %CVs that were acceptable but poor (10% > %CV ≤ 20%). Sample 1 had values that ranged from 54 to 188 µg/L with an overall mean of 106 µg/L. Also, 33 Sample 1 measurements (82.5%) met the manufacturer’s specifications of being in the range of Spec cPL ± 60 µg/L while seven did not, and all results fell into the RI. The four analyzers produced significantly different

594 µg/L. Four different analyzers were used for this experiment, and eight serum samples were analyzed eight times on a single analyzer on eight different days, while two serum samples were analyzed eight times on eight different days on each of the four analyzers. Leftover serum sample concentrations were chosen throughout the working range of the VetScan cPL assay. They were then aliquoted, and each aliquot was frozen in a separate sample tube at −20°C. Samples were removed from the freezer and thawed immediately prior to analysis. For this study, an inter-assay variability of ≤10% was considered acceptable, an inter-assay variability 10% ≤ %CV ≤ 20% was considered poor but acceptable, and an inter-assay variability of > 20% was considered unacceptable.
results from one another with sample means of 66, 105, 122, and 133 µg/L; \( P = .0037 \) (Table 3).

Sample 2 had %CVs of 22.3, 26.0, 30.3, and 34.7% for the four different analyzers, which would be considered unacceptable for all four analyzers. For this sample, the values ranged from 56 to 423 µg/L, with an overall mean of 232 µg/L. Twenty-one measurements (52.5%) met the manufacturer's specifications of being in the range of the Spec cPL ± 60 µg/L, while 19 (47.5%) did not. For Sample 6 were also outside the working range of the analyzer.

| Sample# | Dilution | Spec cPL | Observed | Expected | O/E ratio |
|---------|----------|----------|----------|----------|-----------|
| 1       | Neat     | 1287     | >800     | N/A      | N/A       |
|         | 1:2      | >800     | 617      | N/A      | N/A       |
|         | 1:4      | 328      | 309      | 106.3    |           |
| 2       | Neat     | 1265     | >800     | 757      | N/A       |
|         | 1:2      | 320      | 379      | 84.5     |           |
|         | 1:4      | 204      | 189      | 107.8    |           |
| 3       | Neat     | 621      | 496      | 323      | 130.2     |
|         | 1:2      | 96       | 124      | 77.4     |           |
|         | 1:4      | 101      | 62       | 162.9    |           |
| 4       | Neat     | 548      | 548      | 318      | 116.1     |
|         | 1:2      | 169      | 137      | 123.4    |           |
|         | 1:4      | 109      | 69       | 159.1    |           |
| 5       | Neat     | 367      | 542      | 218      | 80.4      |
|         | 1:2      | 152      | 136      | 112.2    |           |
|         | 1:4      | 102      | 68       | 150.6    |           |
| 6       | Neat     | 182      | 110      | 77       | 140.0     |
|         | 1:2      | <50      | 28       | N/A      |           |
|         | 1:4      | <50      | 14       | N/A      |           |

Mean 119.3
SD 28.7

| Sample# | Dilution | Triglyceride | Spec cPL | VetScan cPL | O/E ratio |
|---------|----------|--------------|----------|-------------|-----------|
| 1       | Neat     | 525          | 337      | 320         | 182.5     |
|         | 1:2      | 580          | 737      | >700        | N/A       |
|         | 1:4      | 1319         | 1368     | >700        | N/A       |
| 2       | Neat     | 580          | 737      | >700        | N/A       |
|         | 1:2      | 334          | 449      | 268.9       |           |
| 3       | Neat     | 621          | 496      | 323         | 130.2     |
|         | 1:2      | 96           | 124      | 77.4        |           |
|         | 1:4      | 101          | 62       | 162.9       |           |

Mean 149.2
SD 95.4
Sample 2, with a Spec cPL in the questionable range, the VetScan cPL rapid test results had 25 measurements within the RI, 14 measurements in the questionable range, and one measurement in the range suggestive for pancreatitis. The four analyzers produced significantly different results from one another with sample means of 142, 248, 255, and 284 µg/L, \( P = .0005 \).

Sample 3 had %CVs of 16.9, 18.7, 21.0, and 34.6% for the four different analyzers, which would be considered unacceptable for two analyzers and acceptable but poor for the other two. For this sample, values ranged from 175 to >700 µg/L, with an overall mean of 536 µg/L. Only 11 of the measurements (27.5%) met the manufacturer’s specifications of being in the range of the Spec cPL ±60 µg/L, while 29 (72.5%) did not. For this sample, with a Spec cPL in the range of being suggestive for pancreatitis, the VetScan cPL rapid test results had one measurement within the RI, three measurements in the questionable range, and 36 measurements in the range suggestive for pancreatitis. The four analyzers produced significantly different results from one another with sample means of 441, 557, 568, and 577 µg/L; \( P = .014 \).

### 3.4 Reproducibility

For inter-assay variability, one of 10 samples (Spec cPL = 196 µg/L) read <60 µg/L for every measurement. The %CV for this sample could not be assessed (Table 4; Figure 1). For another sample (Spec cPL = 254 µg/L), five measurements were <50 µg/L, and only three other measurements resulted in values within the working range of the assay. Many of the samples spanned all three diagnostic bins of the assay. For example, one sample, with a Spec cPL of 566 µg/L, had two results within the RI (109 and 152 µg/L), four in the questionable range (247, 270, 297, and 392 µg/L), and two in the range suggestive for pancreatitis (467 and 477 µg/L). Only four of the samples had measurements in a single diagnostic bin, seven samples spanned two diagnostic bins, and five samples spanned all three diagnostic bins. To illustrate these findings further, the actual measurements for the three samples are provided in Figure 1. Overall, inter-assay variability was high, ranging from 14.1% to 51.2% (mean %CV for all samples 31.8%; with a total of 14 data points).

### 3.5 Adherence to the manufacturer’s specifications

A total of 252 measurements were assessed for adherence to manufacturer’s specifications, which would require the results of the VetScan cPL to be within a range of the Spec cPL result ±60 µg/L. Adherence to this specification was only achieved for 99 measurements (39%) but was not achieved for the majority of the measurements (153 samples or 61%).

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**TABLE 3** Repeatability (intra-assay variability) of three serum samples from dogs analyzed on four different analyzers performed 10 times in one run. None of the variabilities was less than 10%, which is a general target value for repeatability, and only three of the 12 variabilities were within the “poor but acceptable” range of 10%-20%.

| Sample# | Spec cPL | Analyzer | Measurements | %CV |
|---------|----------|----------|--------------|-----|
| 1       | 120      | 1        | 10           | 22.9 |
| 2       | 233      | 1        | 10           | 26.0 |
| 3       | 588      | 1        | 10           | 18.7 |
| Mean    | 81       | 25.1     |              |     |
| SD      | 23.8     | 6.2      |              |     |

**TABLE 4** Reproducibility (inter-assay variability) of 10 samples analyzed on four different analyzers (two samples were analyzed on all four analyzers, and eight samples were analyzed on one analyzer). None of the %CVs were less than 10%, which is a general target value for reproducibility. Only three of the 14 variabilities were within the “poor but acceptable” range of 10%-20%. The samples that are shown in red are also depicted in Figure 1.

| Sample# | Spec cPL | Analyzer | Measurements | %CV |
|---------|----------|----------|--------------|-----|
| 1       | 227      | 1        | 8            | 48.3 |
| 2       | 388      | 1        | 8            | 20.4 |
| 3       | 196      | 1        | 8            | N/A  |
| 4       | 254      | 2        | 8            | N/A  |
| 5       | 322      | 1        | 8            | 27.0 |
| 6       | 360      | 3        | 8            | 18.9 |
| 7       | 400      | 4        | 8            | 28.0 |
| 8       | 477      | 1        | 8            | 14.1 |
| 9       | 566      | 2        | 8            | 45.2 |
| 10      | 594      | 2        | 8            | 40.8 |
| Mean    | 369      | 31.8     |              |     |
| SD      | 16.2     | 12.0     |              |     |

Abbreviations: SD, standard deviation; %CV, percent coefficient of variation.
Correlations

Our studies created several data pairs that could be used for rudimentary correlation assessments. The Spearman r for 29 data sets was 0.722 (P < .0001) (Figure 2). The Bland-Altman plot (Figure 3) showed a bias of 40.7 ± 138.3 µg/L (95% limits of agreement: -230.4 to 311.7 µg/L). No correlation between the difference (Spec cPL - VUE) and average of the two measurements (P = .9428) was found on the linear regression analysis, which suggested that proportional bias did not exist among the assay results.

DISCUSSION

In this study, we found that the Abaxis VetScan cPL assay, for measurement of serum cPLI concentrations, showed poor linearity, repeatability, and reproducibility when tested on a single occasion as is usually performed by veterinarians in general practice.

A variety of protocols for analytical validation of newly developed assays exist. Many assays, such as radioimmunoassays and enzyme-linked immunosorbent assays (ELISAs), intrinsically use a duplicate or even triplicate approach to evaluate standards and assess unknown samples. The VetScan cPL assay evaluated in this study only uses a single measurement to determine each sample result. Thus, to mirror conditions, where a veterinarian would assess each sample only once with this assay, we chose to only analyze each sample once in this study. Analyzing samples in duplicate or triplicate fashion would not have provided meaningful results for the analytical performance of the assay in relation to general veterinary practice.
confirm these results. This is important because serum samples from dogs with acute pancreatitis are often lipemic. Thus, the impact of lipemia on assay performance might also affect clinical decisions. Therefore, the VetScan cPL rapid test should be used with caution in lipemic dogs until further studies on the impact of lipemia with this assay have been performed.

We designated acceptable and unacceptable ranges for the inter-assay variability measurements because no universally accepted performance goal standards for diagnostic assays exist. We chose these goals based on the fact that most commercial assay manufacturers achieve and report intra-assay variabilities of <10%. Overall, repeatability of the VetScan cPL assay was poor. For the first sample with a Spec cPL of 120 µg/L, values ranged from 54 to 188 µg/L with an overall mean of 106 µg/L. One could argue that this high variability is not clinically important as it did not change the diagnosis since all 40 measurements were within the reference interval. However, for samples that are close to assay cutoff values, this high variability could have a much larger impact. For Sample 2, the intra-assay results showed that while the overall sample mean was close to the Spec cPL value, the variability was unacceptably high, which showed how the lack of repeatability could have an impact on clinical decisions. Sample 3 results also had highly variable repeatability with a sample mean that was close to the Spec cPL value. Overall, the assay fails basic sample repeatability and analyzer variability (nine of 12 intra-assay variability experiments showed a %CV of >20%).

The reproducibility of the VetScan cPL rapid assay was also poor often resulting in concentrations that were in different diagnostic bins for the same sample. Since there would be no reason to repeat the measurement for a sample in a clinical setting, a veterinarian could easily misdiagnose a patient as not having pancreatitis or as having pancreatitis based on this poor reproducibility.

Since Abaxis has not published any data on the repeatability or reproducibility of the VetScan cPL rapid assay, it is unclear why repeatability and reproducibility were poor in our study. One possible explanation is that the assay kits are affected by transport and storage conditions. According to the manufacturer, the VetScan cPL rapid test kits do not require refrigeration during storage. While the assay kits were kept at room temperature in our laboratory, it is conceivable that this could have affected repeatability and reproducibility. However, regardless of the reasons, the poor repeatability and reproducibility identified in our study suggest that under normal veterinary practice conditions, the VetScan cPL does not produce precise diagnostic results.

The limited correlation study (r = .722) could be considered high for two different clinical pathology variables that assess similar physiologic functions (e.g., serum creatinine and BUN concentrations to assess renal function), but would be considered poor for trying to correlate two assays that measure the same analyte. As the calculated bias of 40.66% µg/L between the two assays was apparently random with no proportional bias, it would not be possible to correct the results with a constant or proportional adjustment factor.

One limitation of this study is that all VetScan cPL results were only obtained with single measurements. As indicated in the introduction of this manuscript, validation studies for clinical pathology analytes often use duplicate or even triplicate measurements. However, the aim of this study was to describe analytical validation parameters under veterinary practice conditions, where a veterinarian would only run a patient sample on VetScan cPL once. Further studies using duplicate and/or triplicate measurements could provide further analytical validation data for this assay.

In summary, the Abaxis VetScan cPL assay for measurement of serum cPLI concentrations shows poor linearity, repeatability, and

![FIGURE 3](image-url) A Bland-Altman plot shows a mean bias of 40.7 µg/L (solid line) and 95% limits of agreement from −239.4 to 311.7 µg/L (dashed lines). Most variation between the results of the two different assays was random as demonstrated by the wide limits of agreement; and therefore, correction with a constant or proportional adjustment factor would not improve performance.
reproducibility when tested on a single occasion as usually performed by veterinarians in general practice. Intra- and especially inter-assay variability were poor, and most sample results were identified in more than one diagnostic bin upon repeat analysis. The manufacturer’s specification, suggesting that the VetScan cPL provides confirmed in more than one diagnostic bin upon repeat analysis. The intraspecimen variability were poor, and most sample results were identified by veterinarians in general practice. Intra- and especially intraspecimen variability were poor, and most sample results were identified by veterinarians in general practice. Intra- and especially intraspecimen variability were poor, and most sample results were identified by veterinarians in general practice. Intra- and especially intraspecimen variability were poor, and most sample results were identified by veterinarians in general practice. Intra- and especially intraspecimen variability were poor, and most sample results were identified by veterinarians in general practice. Intra- and especially intraspecimen variability were poor, and most sample results were identified by veterinarians in general practice. Intra- and especially intraspecimen variability were poor, and most sample results were identified by veterinarians in general practice.

DISCLOSURES

All authors are employed by or affiliated with the Gastrointestinal Laboratory at Texas A&M University, which performs cPLI testing on a fee-for-service basis. Dr Steiner also serves as a paid consultant for IDEXX Laboratories, the manufacturer of Spec cPL and SNAP cPL.

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