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BRIEF COMMUNICATION

Partial validation of the Vcheck canine pancreatic lipase assay

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Abstract

Measurement of canine pancreatic lipase immunoreactivity (cPLI) is used for diagnosing pancreatitis in dogs. Because pancreatitis can be a life-threatening disease with severe complications, an in-house cPLI test would be valuable to obtain rapid test results. The aim of this study was to evaluate a point-of-care cPLI test, Vcheck cPL. Precision, determined according to EP15, and linearity under dilution were determined and judged against preset quality goals. Results from the Vcheck cPL were compared with a previously validated cPLI ELISA, Spec cPL. In a retrospective study, cPLI results from dogs with and without acute pancreatitis, as determined by pancreatic ultrasound examination, were investigated to assess the performance of the assay in a clinical setting. Statistical analysis included the Mann–Whitney test, Chi-square test, and Passing–Bablok regression analysis with a significance level of 0.05. Precision of the assay was acceptable, with intra-, inter-, and total coefficients of variation (CV%) less than 12.1%, 6.4%, and 12.1%, respectively. Results from the linearity study indicated that the method was acceptably linear at lower concentrations but not in the high-concentration range. The method comparison study revealed that Vcheck generally measured higher concentrations compared with Spec cPL, and that the methods should not be used interchangeably. Dogs with acute pancreatitis had significantly higher cPLI concentrations compared with dogs without pancreatitis ($P < 0.01$), but there was a marked overlap in cPL concentrations between the two groups.

KEYWORDS
canine pancreatic lipase immunoreactivity, method validation, pancreatitis

Pancreatitis is a common disease in dogs that can be classified as acute or chronic.[1,2] Due to the large variation in clinical signs, the diagnosis of the disorder remains challenging as it may mimic other diseases.[3,4] Measurement of serum or plasma canine pancreatic lipase (cPL), an enzyme that is synthesized in pancreatic acinar cells, is recommended as part of the diagnostic work-up in dogs with suspected pancreatitis.[2,5,6] A validated canine-specific cPL ELISA (Spec cPL) is available at referral laboratories, but because acute pancreatitis is a highly dynamic process that can lead to life-threatening complications quickly, an in-house cPLI test would be valuable for obtaining rapid test results.[2,4,7,8] The aim of this study was to evaluate a point-of-care cPLI test, Vcheck cPL (BioNote, Inc., Hwaseong-si, Gyeonggi-do, Republic of Korea), by performing the following studies: (a) precision, (b) linearity under dilution, (c) comparison to a previously validated cPLI ELISA, Spec cPL, and (d) investigating the performance of the assay in a clinical setting by a retrospective clinical study where cPLI concentrations from dogs with and without acute pancreatitis were determined. In the latter study, it was

hypothesized that cPLI concentrations would be higher in dogs with acute pancreatitis diagnosed by ultrasound examination than in dogs without pancreatitis.

The Vcheck cPL is a quantitative fluorescent immunoassay using canine-specific antibodies. The measurement range is 50–2000 μg/L, and the required sample volume is 25 μL of serum. A new test device is used each time a sample is analyzed, and the analysis of one sample takes approximately 5 minutes. Calibrators are provided by the manufacturer, and calibration is recommended every 60 days. According to the manufacturer, a concentration <200 μg/L indicates that pancreatitis is unlikely (negative result), a value between 200 and 400 μg/L is considered equivocal (gray zone), and a value >400 μg/L is consistent with pancreatitis (positive result).

For the validation experiments, left-over canine serum samples submitted to the Clinical Pathology Laboratory, University Animal Hospital (UDS) at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, were used. Samples used in the study were taken as part of the routine diagnostic work-up, and ethical approval was not required. Dog owners approved that left-over samples could be used for research purposes when their dog was admitted to the University Animal Hospital.

Blood samples were collected between January and July 2022, and sera were stored at −20°C for a maximum of 3 weeks. To be included, samples had to be free from visible hemolysis, lipemia, and icterus. Two canine control samples, provided by the manufacturer, were analyzed daily prior to the analysis of the study sample. One of the authors (P.J.) performed all the analyses.

Precision was determined by analyzing five canine serum samples in triplicate daily over 5 days and was expressed as coefficients of variation (CV%). Samples were aliquoted and stored at −20°C during the experiment, and one aliquot was thawed each day of the experiment and thoroughly mixed prior to analysis. In accordance with previous validation studies of canine cPLI assays, a variability of ≤10% was considered desirable, a variability of 10% to ≤20% acceptable, and a variability of >20% was considered unacceptable.[9,10] Intra-, inter-, and total variations were determined according to EP15.[11,12]

In the study of linearity under dilution, two canine serum samples with high cPL concentrations that had been stored at −20°C for a period of maximum 2 months, were thawed and pooled, in order to obtain one sample with a concentration close to the upper limit of the assay's measurement range. The high-concentration sample was manually diluted using a serum sample with low cPL concentration to obtain six dilutions (80%, 60%, 40%, 20%, 10%, and 5% of the high-concentration sample). Samples were analyzed in triplicate and random order on a single day. Results were assessed graphically by plotting the observed concentration (*O*) against the calculated expected concentration (*E*) and calculating the *O*/*E* ratio. An *O*/*E* ratio of 0.8–1.2 was considered acceptable.

The method comparison study was performed with 42 canine serum samples that were analyzed on the Vcheck using one replicate on the day of arrival at the laboratory and then stored at −20°C for a maximum of 18 weeks. Samples were shipped frozen to a referral laboratory (Vet Med Labor GmbH, Division of IDEXX Laboratories, Kornwestheim, Germany) and analyzed with the previously validated cPLI ELISA, Spec cPL.[7] The measurement range of Spec cPL was 30–2000 μg/L. Results were visually inspected on a scatter plot, and a Passing–Bablok regression analysis was performed to derive regression data. The recommended cut-off values for diagnosing pancreatitis were identical for Spec cPL and Vcheck, and agreement between the two assays regarding the classification of results (negative, gray zone, or positive) were compared using the Spec cPL as the reference method.

A retrospective study was performed to evaluate cPLI concentrations measured by Vcheck in dogs with and without acute pancreatitis. Medical records from dogs admitted to the University Animal Hospital, Swedish University of Agricultural Sciences, between the 1 September 2020 and the 15 August 2021 were examined. The inclusion criterion was that a cPLI measurement and a pancreatic ultrasound examination had been performed within 24 hours of each other. If a dog fulfilled this criterion on more than one occasion, data were obtained only from the first visit. Ultrasound examinations were performed as part of the routine diagnostic work-up at the Diagnostic Imaging Clinic at the University Animal Hospital. Dogs were allocated to have acute pancreatitis or no pancreatitis based on the written report from the radiologist. Animals that were diagnosed with chronic pancreatitis based on the ultrasound examination, as well as cases where the radiology report was inconclusive, were excluded. If a dog had acute on chronic pancreatitis, it was included.

Additional data collected from medical records included sex, age, and weight. Non-normal distributions were detected by visual inspection of histograms and normal quantile plots for cPLI, age, and weight. Statistical analyses included the Mann–Whitney test and Chi-square test for quantitative and qualitative data, respectively. The significance level was set to 0.05. Statistical analyses were performed using statistical software for Microsoft Excel (Analyze-it Software, Ltd, Leeds, UK) and Minitab (Minitab 2020, LLC, State College, PA, USA). cPLI results >2000 μg/L were set to 2001 μg/L and cPLI concentrations <50 μg/L were set to 49 μg/L for the analyses.

The precision study showed that the Vcheck cPL met the preset criteria for at least acceptable precision (Table 1). The high and low serum pools used in the linearity study had measured cPL concentrations of 1921 and 62 μg/L, respectively. When reviewing data from the dilution study, it was judged that the measured concentration of the undiluted high-concentration sample was falsely low. Due to this, the expected concentration of the undiluted sample was calculated based on the dilution factors and mean result of the four samples with the lowest concentrations. With this approach, the expected concentration of the undiluted sample was estimated to be 2902 μg/L. The O/E ratios for samples diluted to 60%, 40%, 20%, and 5% of the calculated original concentration were within acceptable limits (0.83–0.98). The 10% dilution had an O/E ratio of 0.76, slightly below the acceptable limit. For the two samples with the highest concentrations, O/E ratios were unacceptably low (0.66 and 0.71) (Figure 1).

Of the 42 samples in the method comparison study, six results were outside the measurement range of one or both methods and were excluded from the comparison. A scatter plot of the remaining 36 samples revealed that Vcheck generally measured higher concentrations compared with Spec cPL (Figure 2). Because the linearity study indicated that Vcheck was not accurate at high concentrations, two samples with markedly increased cPLI concentrations (≥1068 µg/L with Spec cPL) were excluded from the Passing–Bablok regression analysis. When regression analysis was performed on the remaining 34 samples, it revealed a proportional error with a slope of 1.31 (95% CI 1.07–1.53). The intercept was 0.11 µg/L (95% CI −37.4–32.2).

When interpreting results according to the cut-off values specified by the manufacturers, the Vcheck cPL and Spec cPL classified results identically in 36/42 (86%) of the cases. Four samples that were negative with Spec cPL had a result in the gray zone with Vcheck, and two samples in the gray zone with Spec cPL had a positive result with Vcheck.

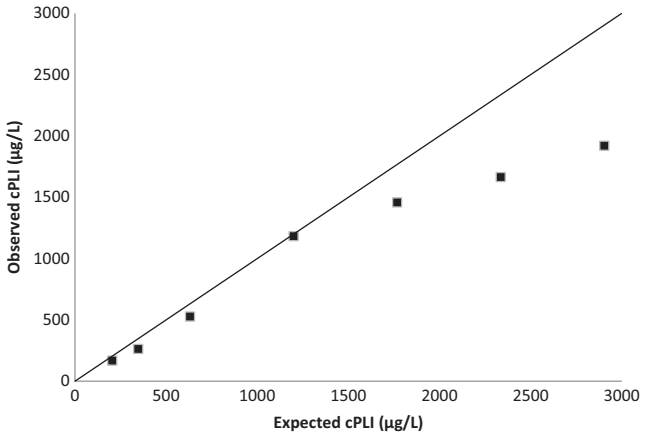

FIGURE 1 Linearity under dilution of a high serum sample, diluted with a low serum sample, measured with Vcheck cPL. The concentration of the high undiluted sample was estimated to be 2902 µg/L, which was calculated based on dilution factors and the mean concentration of the four samples with the lowest concentrations. The solid line represents $y = x$

In the retrospective study reviewing medical records, 189 dogs met the inclusion criterion. A total of 30 dogs were excluded either due to chronic pancreatitis (n = 6) or inconclusive ultrasound reports (n = 24). Of the remaining dogs, 21 were judged to have acute pancreatitis, and 138 dogs did not have pancreatitis. cPLI concentrations were significantly higher in dogs with acute pancreatitis (Figure 3; Table 2), but there was considerable overlap between the two groups. Dogs interpreted to have acute pancreatitis had cPLI concentrations ranging from <50 to >2000 µg/L, and dogs without pancreatitis had cPLI concentrations ranging from <50 to 1581 µg/L.

The validation experiments performed in this study yielded variable results. Precision was good or acceptable for all tested samples, which is in contrast with a previous study where precision was unacceptably high (>20%) in five out of nine tested samples.[9] The reason for this discrepancy is not clear. The two studies were conducted in different years at different laboratories with different instruments and reagent batches, and it would not be possible to identify a single explanatory cause. It is advisable that each laboratory introducing the Vcheck cPL conducts an in-house precision study to ensure acceptable performance before using the assay to analyze patient samples. It should further be noted that in the current study, all analyses were performed by a single person, and it is likely that the assay will be less precise in the clinical setting where it is handled by different operators. The intra-assay variation was considerably higher than the interassay variation for four of the five tested samples in the precision study. According to the guidelines, the interassay variance is recommended to be set to 0 in these cases since all variance is explained by the within-assay variation.[12] The higher intra-assay variation can be explained by the fact that individual test devices were used every time a sample was analyzed and that most of the imprecision was due to the test devices and not dependent on whether samples were analyzed on the same or different days.

The results from the linearity study indicated that the method gives false low results at high cPLI concentrations, which could be due to antigen excess when cPL is present at such high levels that it prevents effective antigen–antibody crosslinking. To prevent falsely low results from being reported, the measurement range could be narrowed by lowering the upper limit to 1000 µg/L, for example. Even with this limitation, the

TABLE 1 Intra- and interassay and total variation for canine pancreatic lipase immunoreactivity (cPLI) measured with Vcheck.

		Intra-assay variation		Interassay variation		Total variation	
	Overall mean cPLI µg/L	SD (µg/L)	CV (%)	SD (µg/L)	CV (%)	SD (µg/L)	CV (%)
Sample1	170	20.7	12.1	*	*	18.7	12.1
Sample2	351	18.6	5.3	22.6	6.4	29.3	8.3
Sample3	462	45.5	9.8	*	*	45.5	9.8
Sample4	762	86.5	11.3	*	*	86.5	11.3
Sample5	796	77.9	9.8	*	*	77.9	9.8

Note: Each sample was analyzed in triplicate on five different days, and precision was determined according to EP15.[11,12]

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

*The intra-assay SD was high compared with the interassay SD for four of the samples, which masked the interassay variation in the ANOVA used by EP-15, precluding the calculation of interassay CV.

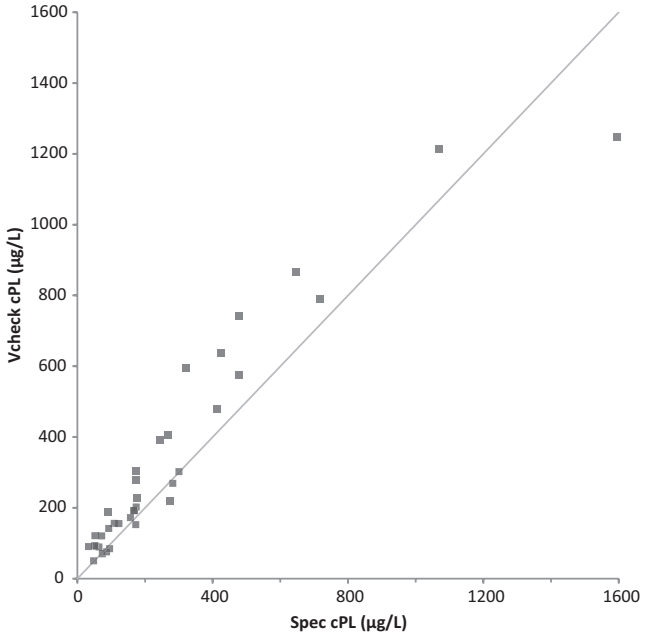

FIGURE 2 Results from a method comparison study between Spec cPL and Vcheck cPL illustrated in a scatter plot (n = 36). The solid line represents $y = x$. Six samples that were either above or below the measurement range of one or both methods were excluded.

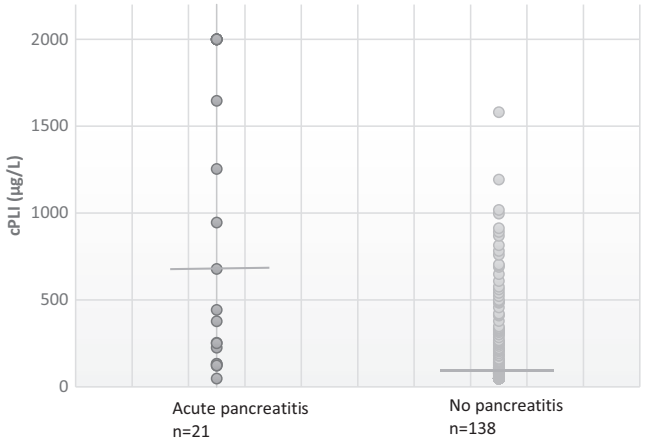

FIGURE 3 Dot plot of canine pancreatic lipase immunoreactivity (cPLI) results from a retrospective study of dogs with acute pancreatitis (n = 21) and without pancreatitis (n = 138). cPLI results >2000 µg/L were set to 2001 µg/L, and cPLI concentrations <50 µg/L were set to 49 µg/L in the figure. Each dot represents an individual cPLI result, and when two or more dogs had the same cPLI result, dots are superimposed in the figure. In the acute pancreatitis group, seven dogs had cPLI concentrations >2000 µg/L. In the no pancreatitis group, 46 dogs had cPLI concentrations <50 µg/L. Median cPLI concentration was 679 µg/L (interquartile range 180 to >2000 µg/L) in dogs with acute pancreatitis and 96 µg/L (interquartile range <50–306) in dogs with no pancreatitis. Median values are shown as horizontal lines in the figure.

test should be able to give information regarding whether or not acute pancreatitis is likely to be present. However, it would not be possible to monitor cPLI results in the high-concentration range using this assay.

TABLE 2 Sex and median age, median weight, and median canine pancreatic lipase immunoreactivity (cPLI) result in 159 dogs with and without acute pancreatitis, as determined by pancreatic ultrasound examination.

Parameter/ variable	Acute pancreatitis (n = 21)	No pancreatitis (n = 138)	*P*-value
Sex (male/ female)	9/12	72/66	0.43
Age (years)	7.6	5.0	0.10
Weight (kg)	10.3	11.4	0.78
cPLI (µg/L)	679 (180 to >2000)	96 (<50–306)	<0.01

Note: For cPLI, the interquartile range (IQR) is provided within parentheses.

Diluting high-concentration samples could be an option if exact results are wanted, but a thorough investigation of such a procedure should be carried out before introducing it in the clinical setting. This was not investigated in the current validation study. The current study neither investigated the possible presence of a clinically relevant prozone effect, where a sample with cPL concentration >2000 µg/L would be reported to be less than 2000 µg/L by the assay.

The method comparison showed a positive bias for the Vcheck assay compared with Spec cPL, and when judging how the two assays categorized samples (negative, gray zone, or positive), 6/42 (14%) samples were categorized higher with Vcheck compared with the Spec cPL. This implies that the cut-off values for Vcheck cPL should probably be higher than for Spec cPL, and results from the two methods should not be used interchangeably. There were no cases with positive results on Vcheck and negative results on Spec cPL, indicating that the presence of a gray zone is helpful for preventing misinterpretations by clinicians. In a previous study where results from the Spec cPL and Vcheck were compared, Spec cPL measured higher concentrations compared with Vcheck.[9] A possible explanation to why the opposite was found in our study could have been that alterations had been made by manufacturers; for example, by changing the calibration material.

Analysis of cPLI using Vcheck was offered as a routine diagnostic test at the University Animal Hospital from 2019, and when evaluating data retrospectively, it was shown that cPLI results were significantly higher in dogs with acute pancreatitis compared with dogs without pancreatitis, as hypothesized. There was an overlap in cPLI concentrations between the two groups, and cPLI should not be used as a standalone test in the diagnosis of pancreatitis. Instead, it is recommended to use information from both the history, clinical signs, and a panel of diagnostic tests such as CBC, biochemistry analysis, urinalysis, and results of imaging (such as abdominal ultrasound and/or advanced imaging) to rule out or diagnose other diseases that can show similar clinical signs.[2] A weakness of the current study was that ultrasound examinations were performed without a standardized protocol by radiologists that were not blinded to history and clinical findings. Furthermore, pancreatic ultrasound examination, used as the standard method in the current study, may give both false-positive and -negative results.[4,13,14] Another limitation of this study was that the effects of lipemia, icterus, and hemolysis were

not assessed. This might be of specific importance in dogs assessed for possible pancreatitis as these interferences are encountered frequently in this group of patients.

In conclusion, the Vcheck cPL had acceptable precision but was not acceptably linear over the whole measurement range. The method comparison study showed that Vcheck and Spec cPL could not be used interchangeably and that specific cut-off values need to be determined for the Vcheck cPL.

Dogs with acute pancreatitis had higher cPLI concentrations compared with dogs without pancreatitis, but there was an overlap between the groups, and cPLI should not be used as a standalone test in the diagnosis of pancreatitis.

DISCLOSURE

The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

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