

Development and validation of a radioimmunoassay for the measurement of canine pancreatic lipase immunoreactivity in serum of dogs

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Objective—To develop and validate a radioimmunoassay (RIA) for measuring canine pancreatic lipase immunoreactivity (cPLI) in serum obtained from dogs.

Sample Population—Serum samples from 47 healthy dogs.

Procedures—Canine pancreatic lipase (cPL) was purified from pancreatic specimens of dogs. Antibodies against cPL were raised in rabbits and purified by use of affinity chromatography. A tracer was produced by iodination of cPL with ^{125}I . An RIA was established and validated by determination of sensitivity, working range, dilutional parallelism, spiking recovery, and intra- and interassay variability. A reference range for cPLI in serum was established by use of the central 95th percentile for samples obtained from 47 healthy dogs.

Results—Sensitivity and upper limit of the working range were 0.88 and 863 $\mu\text{g/L}$, respectively. Observed-to-expected ratios for serial dilutions ranged from 84.9 to 116.5% for 4 samples. Observed-to-expected ratios for spiking recovery ranged from 82.8 to 128.6% for 4 samples. Coefficients of variation for intra-assay variability for 4 serum samples were 18.3, 4.2, 3.5, and 8.9%, whereas interassay coefficients of variation were 29.2, 6.2, 3.9, and 4.4%, respectively. The reference range was 4.4 to 276.1 $\mu\text{g/L}$.

Conclusions and Clinical Relevance—We conclude that the RIA described is sensitive, linear, accurate, precise, and reproducible, with limited accuracy in the high end of the working range and limited precision and reproducibility in the low end of the working range. Additional studies are needed to evaluate whether this degree of accuracy, precision, and reproducibility will negatively impact clinical use of this assay. (*Am J Vet Res* 2003;64:1237–1241)

Evaluation of serum lipase activity has been used to diagnose pancreatitis in humans and dogs for several decades.¹⁻⁶ However, measurement of serum lipase activity lacks sensitivity and specificity in dogs and humans with exocrine pancreatic disorders.^{1,7-9} After

total pancreatectomy, serum lipase activity decreases in dogs.^{9,10} However, substantial serum lipase activity is still detectable in these dogs, suggesting that there are extrapancreatic sources of serum lipase.^{9,10} Several studies^{1-3,11-13} have documented that dogs with experimentally induced or naturally occurring pancreatitis can have increases in serum lipase activity; however, other dogs with pancreatitis have little or no increases in serum lipase activity.¹

Several conditions in dogs have been associated with an increase in serum lipase activity. Renal disease, hepatic disease, lymphosarcoma, hemangiosarcoma of the heart, adenocarcinoma of the small intestine, amyloidosis in multiple organs, heat stress, muscular exercise, or administration of prednisone or dexamethasone have all been attributed as causes for increases in serum lipase activity.^{1,14-16} Although it is possible that localized areas of inflammation in the pancreas may have been missed in some of these dogs, it seems unlikely that this would be the case for all of the dogs described. One explanation for the poor specificity of serum lipase activity for diagnosis of pancreatitis in dogs is that lipase is also synthesized and secreted by cells of other organs, and assays for measurement of lipase activity in serum may detect lipases of other tissues in the serum. Immunohistochemical analysis of 38 types of tissue in dogs revealed that pancreatic lipase could only be detected in pancreatic acinar cells, suggesting that pancreatic lipase is specific for the exocrine portion of the pancreas.¹⁷ To test this hypothesis, an immunoassay for the measurement of canine pancreatic lipase (cPL) in serum was needed.

One of the major breakthroughs in the field of medical diagnostics was the development of radioimmunoassays (RIAs) in the 1960s.¹⁸ The general principles of RIAs are easy to understand and have been described elsewhere.¹⁹ Diagnostically, RIAs are of special interest because they allow measurement of the concentration of a specific analyte. In contrast, catalytic assays, such as an assay to measure lipase activity, measure the function of an analyte. Several analytes with differing molecular structures may have the same or similar function; thus, catalytic assays may not be able to differentiate among these analytes. Gastric lipase and pancreatic lipase are good examples of analytes with structural differences that have a similar function. To use these analytes as organ-specific markers, the concentration of the specific analyte must be quantified by a specific assay. Therefore, the goals of the study reported here were to develop and validate an

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RIA for the measurement of cPL immunoreactivity (cPLI) in serum obtained from dogs.

Materials and Methods

Serum samples—Serum samples were obtained from 47 healthy dogs owned by volunteers. These dogs did not have a history of clinical disease, as reported by the owners. However, additional diagnostic tests were not performed to establish gastrointestinal health in these dogs. All owners signed an informed owner consent document before enrollment of their dog into the study.

Production and purification of polyclonal antibodies—

Two New Zealand White rabbits were used to produce antibodies directed against cPL. Each rabbit received repeated inoculations with purified cPL emulsified with a commercially available adjuvant^a or incomplete Freund's adjuvant.^b The protocol for antibody production was reviewed and approved by an animal care and use committee.

Briefly, a preinoculation blood sample was collected from each rabbit. Rabbits were then inoculated with an emulsion consisting of 100 µg of cPL in 500 µL of PBS solution (pH, 7.2)^c and 500 µL of a commercially available adjuvant.^b The emulsion was divided into 4 equal volumes and injected at 4 sites in each rabbit. Blood samples were collected 2, 4, and 6 weeks after the initial inoculation. Antibody concentrations were evaluated by use of a simplified RIA. After evaluation of the antibody concentration in the sample obtained 6 weeks after the initial inoculation, both rabbits received booster inoculations with an emulsion consisting of 200 µg of cPL in 500 µL of PBS solution (pH, 7.2)^c and 500 µL of the commercially available adjuvant. The emulsion was divided into 4 equal volumes and injected at 4 sites in each rabbit. Antibody concentrations were evaluated 2, 4, and 6 weeks after the booster injection (8, 10, and 12 weeks after the initial inoculation). The antibody response was not as strong as we had expected; thus, we requested a change for the antibody-production protocol, which was granted.

Approximately 6 weeks later, both rabbits were inoculated with an emulsion consisting of 200 µg of cPL in 500 µL of PBS solution (pH, 7.2) and 500 µL of incomplete Freund's adjuvant.^b Rabbits were reinoculated at 2-week intervals (2 and 4 weeks, emulsion consisting of 200 µg of cPL in 500 µL of PBS solution [pH, 7.2] and 500 µL of incomplete Freund's adjuvant; 6, 8, 10, 12, 14, and 16 weeks, emulsion consisting of 100 µg of cPL in 500 µL of PBS solution [pH, 7.2] and 500 µL of incomplete Freund's adjuvant). In addition, 1 of the rabbits received additional booster inoculations at 18 and 20 weeks. A blood sample was collected from each rabbit by venipuncture of an ear vein prior to every booster injection.

Both rabbits were euthanized 2 weeks after the last booster inoculation. Rabbits were exsanguinated by intracardiac aspiration. Serum was separated, harvested, and stored frozen at -20°C until further use. Evaluation of samples obtained at the time of exsanguination revealed that serum from the rabbit that received the fewest booster injections had a maximum binding of 40.1% (serum dilution, 1 in 500), whereas serum obtained from the rabbit that received the most booster injections had a maximum binding of 68.1% (serum dilution, 1 in 500). Because of the superior antibody titer, serum from the rabbit that received the most booster injections was used for development of the RIA.

Polyclonal antibodies were purified by use of affinity chromatography. Briefly, an affinity chromatography column^d was prepared for cPL in accordance with the manufacturer's instructions. Rabbit anti-cPL serum was subjected to lipoprotein precipitation; then, pH of the serum was adjusted by addition of a solution consisting of 75mM Tris-HCl^e and 150mM NaCl^f (pH, 8.0), and the buffered serum was applied

to the column. After absorbance (measured at 280 nm) of the eluent from the column had returned to baseline values, the column was washed with a solution consisting of 100mM glycine^g and 500mM NaCl (pH, 3.0). The purified polyclonal antibody was then buffered in PBS solution (pH, 7.2),^c concentrated to form a solution that contained approximately 1 mg/mL, frozen, and stored at -80°C. Specificity of the purified antibody was documented in another report¹⁷ by immunolocalization of cPL in specimens of 38 types of tissues obtained from 2 clinically normal dogs.

Radioiodination of cPL—The chloramine T method²⁰ was used to iodinate cPL²¹ with ¹²⁵I to produce a tracer solution; the procedure was performed at room temperature (approx 20°C). Briefly, a miniature stir bar^h (8 × 1.5 mm) was placed in a polypropylene test tubeⁱ (75 × 12 mm), and 10 µL of 250mM sodium phosphate^j (pH, 7.5) was added to the tube. The tube was suspended over a stir plate,^k and 10 µL of ¹²⁵I^l (in the form of sodium iodide; 0.1 mCi/µL at time of production) was added by use of a Hamilton syringe.^m This was followed in rapid succession by the addition of 10 µg of cPL in 10 µL of PBS solution (pH, 7.2),^c 10 µL of a solution (2 mg/mL) of chloramine Tⁿ in 50mM sodium phosphate (pH, 7.5), 100 µL of a solution (400 mg/mL) of sodium metabisulfite^o in 50mM sodium phosphate (pH, 7.5), and 860 µL of a solution (2 mg/mL) of potassium iodide^p in 50mM sodium phosphate (pH, 7.5). The iodinated-protein fraction was separated from free iodide by use of size-exclusion chromatography on a disposable column^q in accordance with the manufacturer's instructions. **Radioimmunoassay buffer (RIAB)**; consisting of 50mM sodium phosphate [pH, 7.5], 5 g of bovine serum albumin/L, and 0.2 g of sodium azide^r) was used as the mobile phase. Fractions (1 mL) were collected, and the fraction containing the peak protein concentration was identified. The tracer solution was adjusted to contain approximately 40,000 counts/min and; stored in a refrigerator at 4°C until further use.

Procedure for cPLI-RIA—All samples were assayed in duplicate in 12 × 75-mm polypropylene tubes.ⁱ Tracer solution (100 µL) was added to the first set of duplicate tubes; 100 µL of tracer solution and 200 µL of RIAB were added to the next set of duplicate tubes; and 100 µL of tracer solution, 100 µL of anti-cPL antibody solution (approx 1 mg of purified monospecific anti-cPL antibody/mL diluted at 1 in 10,000 with RIAB), and 100 µL of RIAB were added to the third set of tubes (designated B₀). The following 9 sets of tubes were used for the standard solutions; 100 µL of tracer solution, 100 µL of antibody solution, and 100 µL of a standard solution (512, 256, 128, 64, 32, 16, 8, 4, or 2 µg of cPL/L in RIAB) were added to each tube. The standard solutions were prepared from concentrated pure cPL in PBS solution (pH, 7.2). Protein concentration of the concentrated cPL solution was determined by measurement of absorbance at 280 nm and use of the specific absorbance of cPL of 1.11 for a 1-mg/mL solution of cPL.²⁰ All subsequent tubes were used for unknown samples; 100 µL of tracer solution, 100 µL of antibody solution, and 100 µL of sample were added to each of these tubes. Tubes were mixed by use of a vortexer¹ and incubated for 2 hours at room temperature (approx 20°C). After incubation, 100 µL of carrier serum (consisting of 1 mL of normal rabbit serum mixed with 99 mL of RIAB) and 1 mL of a commercially available precipitation solution^u were added to each tube, except the first set of tubes that contained only tracer solution. Tubes were then mixed by use of the vortexer and centrifuged^v at 3,000 × g for 20 minutes at 4°C. Supernatant was carefully decanted from all tubes, except the first set that contained only tracer solution, and all tubes were counted for 1 minute in a gamma counter.^w Values for the standard solutions were plotted by use of log/logit

transformation of the data. For the log/logit transformation, cPL concentrations were recorded on the x-axis in a logarithmic fashion, and values on the y-axis were calculated by use of the following equation:

$$y = \log_e \left(\frac{B_{\text{standard}}/B_0}{1 - (B_{\text{standard}}/B_0)} \right)$$

where B_{standard} is the counts per minute for each standard solution, and B_0 is the counts per minute for the third set of tubes (ie, 100 μ L of tracer solution, 100 μ L of anti-cPL antibody solution, and 100 μ L of RIAB).

Validation of cPLI-RIA—The assay was validated by determining assay sensitivity, working range, linearity, accuracy, precision, and reproducibility. Assay sensitivity, maximum concentration detectable by the assay, dilutional parallelism, spiking recovery, and intra- and interassay variability were evaluated. Assay sensitivity was determined by use of 10 duplicates of the third set of tubes (ie, 100 μ L of tracer solution, 100 μ L of anti-cPL antibody solution, and 100 μ L of RIAB) and calculation of the mean and SD of the raw counts for these 10 duplicates; the value for the mean minus 3 times the SD was compared with values for the standard solutions. The lower limit of the working range was defined as the assay sensitivity rounded up to the next integer. Upper limit of the working range was established by evaluating the maximum concentration detectable by the assay. Ten duplicate sets of samples, each of which contained 5,000 μ g of cPL/L in RIAB, were measured, and mean and SD were calculated; value for the mean plus 3 times the SD of the 10 sets of duplicates (and, hence, the maximum concentration) was determined by comparison with values for the standard solutions.

Serum samples were selected that contained concentrations that would be in various areas of the working range of the assay. Because a volume of less than 1 mL was available for most serum samples, samples were combined for this portion of the validation. However, no more than 2 serum samples were combined for any 1 test.

Dilutional parallelism was evaluated by use of 4 serum samples. Three of the serum samples were evaluated undiluted and at dilutions of 1 in 2, 1 in 4, 1 in 8, and 1 in 16. The fourth sample had the lowest cPLI concentration and was only evaluated undiluted and at dilutions of 1 in 2 and 1 in 4.

Spiking recovery was determined by adding concentrations of 0, 1, 2, 4, 8, 16, 32, 64, 128, and 256 μ g of cPL/L of

RIAB to each of 4 serum samples. Intra-assay variability was determined by evaluating 4 serum samples 10 times within the same run of the assay. Interassay variability was determined by evaluating 4 samples in 10 consecutive runs of the assay.

A reference range for cPLI in canine serum was established. The cPLI concentration was measured in serum samples obtained from 47 clinically normal dogs, and the central 95th percentile was calculated.

Results

Iodination resulted in tracer solution with specific radioactivity between 25 and 40 μ Ci/mg of cPL immediately after radioiodination. Approximate binding for the assay was 63%. The assay had reproducible linear results for the logarithmically transformed standard solutions (Fig 1). Sensitivity of the assay was 0.88 μ g/L, resulting in a lower limit of the working range of 1 μ g/L. Maximum detectable concentration was 863.2 μ g/L, resulting in an upper limit of the working range of 863 μ g/L. Observed-to-expected ratios for serial dilution of 4 serum samples ranged from 84.9 to 116.5% (Table 1). Observed-to-expected ratios for spiking recovery of 4 serum samples with 10 spiking concentrations ranged from 82.8 to 128.6% (mean \pm SD, 106.6 \pm 12.6%; Table 2).

Coefficients of variation for intra-assay variability were determined for 4 serum samples. Mean \pm SD concentration of the 4 samples was 7.1 \pm 1.3, 38.3 \pm 1.6, 95.5 \pm 3.3, and 362.5 \pm 32.3 μ g/L; intra-assay coefficients of variation for the samples were 18.3, 4.2, 3.5, and 8.9%, respectively. Coefficients of variation for interassay variability were determined for 4 serum samples. Mean \pm SD concentration of the 4 samples was 2.4 \pm 0.7, 38.6 \pm 2.4, 87.1 \pm 3.4, and 355.3 \pm 15.7 μ g/L; interassay coefficients of variation for the samples were 29.2, 6.2, 3.9, and 4.4%, respectively.

Serum cPLI concentrations were measured in samples obtained from 47 clinically normal dogs (Fig 2). Serum cPLI concentration ranged from 3.7 to

Table 1—Dilutional parallelism of canine pancreatic lipase immunoreactivity (cPLI) as measured by use of a newly developed radioimmunoassay (RIA)

Sample	Dilution	Observed (μ g/L)	Expected (μ g/L)	O:E (%)
1	1 in 1	7.01	NA	NA
	1 in 2	3.22	3.50	92.0
	1 in 4	1.65	1.75	94.2
2	1 in 1	31.87	NA	NA
	1 in 2	14.53	15.94	91.2
	1 in 4	7.67	7.97	96.3
	1 in 8	4.41	3.98	110.7
	1 in 16	1.75	1.99	87.9
3	1 in 1	89.95	NA	NA
	1 in 2	44.77	44.98	99.5
	1 in 4	22.81	22.49	101.4
	1 in 8	10.59	11.24	94.2
	1 in 16	4.77	5.62	84.9
4	1 in 1	485.05	NA	NA
	1 in 2	282.47	242.53	116.5
	1 in 4	139.35	121.26	114.9
	1 in 8	65.28	60.63	107.7
	1 in 16	32.13	30.32	106.0

O:E = Ratio of observed concentration to expected concentration. NA = Not applicable.

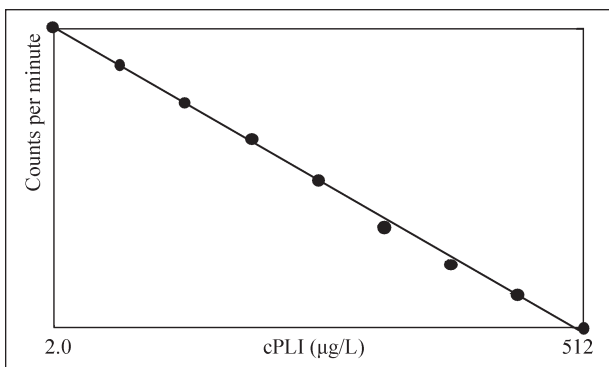


Figure 1—Representative standard curve for a newly developed radioimmunoassay (RIA) for measurement of canine pancreatic lipase immunoreactivity (cPLI) in serum. Concentrations of the standard solutions are plotted in a logarithmic fashion on the x-axis, whereas values on the y-axis are calculated by use of the following equation: $y = \log_e \left(\frac{B_{\text{standard}}/B_0}{1 - (B_{\text{standard}}/B_0)} \right)$, where B_{standard} is the counts per minute for each standard solution, and B_0 is the counts per minute for a set of tubes that contained 100 μ L of tracer solution, 100 μ L of anti-cPL antibody solution, and 100 μ L of RIA buffer.

Table 2—Spiking recovery of cPLI as determined by use of a newly developed RIA

Sample	Spiking concentration (µg/L)	Observed (µg/L)	Expected (µg/L)	O:E (%)
1	0	1.9	NA	NA
	1	2.4	2.9	82.8
	2	4.1	3.9	105.1
	4	5.1	5.9	86.4
	8	9.6	9.9	97.0
	16	20.0	17.9	111.7
	32	40.8	33.9	120.4
	64	73.8	65.9	112.0
	128	157.3	129.9	121.1
	256	329.3	257.9	127.7
2	0	16.0	NA	NA
	1	17.0	17.0	100.0
	2	16.2	18.0	90.0
	4	20.9	20.0	104.5
	8	24.6	24.0	102.5
	16	34.9	32.0	109.1
	32	59.0	48.0	122.9
	64	101.9	80.0	127.4
	128	174.5	144.0	121.2
	256	349.9	272.0	128.6
3	0	37.8	NA	NA
	1	42.5	38.8	109.5
	2	38.4	39.8	96.5
	4	37.5	41.8	89.7
	8	44.2	45.8	96.5
	16	57.0	53.8	105.9
	32	75.6	69.8	108.3
	64	123.7	101.8	121.5
	128	201.3	165.8	121.4
	256	321.7	293.8	109.5
4	0	207.3	NA	NA
	1	216.5	208.3	103.9
	2	204.8	209.3	97.8
	4	207.0	211.3	98.0
	8	188.0	215.3	87.3
	16	218.0	223.3	97.6
	32	231.2	239.3	96.6
	64	266.3	271.3	98.2
	128	383.5	335.3	114.4
	256	524.2	463.3	113.1

828.4 µg/L (mean ± SD, 75.7 ± 129.2 µg/L). The reference range calculated by use of the central 95th percentile was 4.4 to 276.1 µg/L.

Discussion

In the study reported here, we developed and validated an RIA for the measurement of cPLI in serum obtained from dogs. The assay has a wide working range (1 to 863 µg/L). Observed-to-expected ratios for serial dilutions were between 84.9 and 116.5%, which is acceptable and suggests that the assay is linear.

Observed-to-expected ratios for spiking recovery were between 82.8 and 128.6%. Observed-to-expected ratios typically were < 100% when < 10 µg of pure cPLI was added to the samples and > 100% when higher concentrations of cPLI were added.

Analysis of these findings suggests that the assay overestimates larger concentrations of spiked protein. Overestimation was higher for samples with a lower baseline serum cPLI concentration than for samples with a higher baseline serum cPLI concentration. It is unknown what could have caused this overestimation of spiked cPLI concentrations. One possible explanation is that the standard solutions for RIAs are plotted by use of a log-logit transformation. This leads to consistent underestimation of the concentration of the highest standard solution. For example, the highest standard solution in 1 assay (512 µg/L) was extrapolated from the results to provide a value of 433 µg/L. Underestimation of the highest standard solution leads to overestimation of samples with a high concentration. Whether this phenomenon sufficiently explains the degree of the observed overestimation is unknown.

We are not aware of any generally accepted minimum performance criteria for data of spiking recovery of RIAs. However, consistency of observed-to-expected recovery ratios is more important than the values of these ratios themselves. Consistent overestimation of samples would not have an impact on the clinical use of such an assay, because all samples of the same concentration would be overestimated in the same manner. The assay described here consistently overestimates serum cPLI concentrations in the upper area of the working range; thus, we hypothesize that the limited accuracy of the assay reported here does not negatively affect the clinical use of this assay. However, this hypothesis will have to be tested in studies conducted on the clinical performance of this assay.

The intra-assay coefficient of variation for 3 of the 4 serum samples was < 10%, which suggests that the assay is precise. The coefficient of variation for the serum sample with a mean cPLI concentration of 7.1 µg/L was 18.3%, which was slightly higher than the maximum value of 10 to 15% we hoped to achieve for the assay. However, it should be pointed out that a serum cPLI concentration of 7.1 µg/L in an assay with a working range of 1 to 863 µg/L is extremely low. Samples with concentrations at extreme values are not commonly used for validation of an assay, but they were used here because the lower area of the working range of the assay may be of interest for evaluation of a specific group of patients, namely dogs with exocrine pancreatic insufficiency. It should be mentioned that

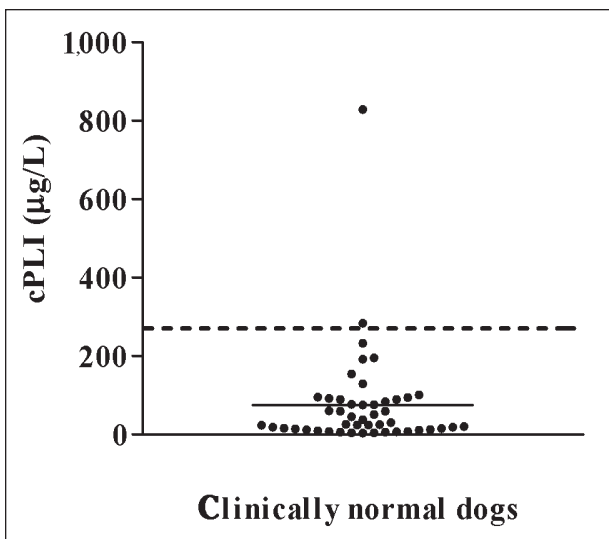


Figure 2—Scatterplot of the serum cPLI concentrations for 47 clinically normal dogs, as determined by use of a newly developed RIA. Each symbol represents the value for a specific dog. Mean value (solid horizontal line) and upper limit of the reference range (dotted horizontal line) were calculated.

although the coefficient of variation for this sample was 18.8%, the range of measured concentrations for this sample was 4.6 to 9.1 µg/L. Whether this degree of variability would change interpretation for a clinical sample remains unknown. Thus, it remains to be determined whether this degree of precision will be sufficient to prevent misinterpretation of results for serum cPLI concentrations in dogs with suspected exocrine pancreatic insufficiency.

The interassay coefficient of variation for 3 of the 4 serum samples was < 10%. However, similar to the intra-assay variability, the interassay coefficient of variation for the sample with the lowest concentration (mean cPLI, 2.4 µg/L) was much higher than the 10 to 15% we targeted. Again, this sample, even more so than that used for evaluation of intra-assay variability, was in the extremely low area of the working range of the assay. Furthermore, the range of results that was measured spanned from 1.2 to 3.5 µg/L. The cutoff value established for dogs with exocrine pancreatic insufficiency will determine whether this degree of reproducibility will be sufficient for clinical use of the assay.

The RIA for cPLI described here was sensitive, linear, precise, and reproducible. The assay had a limited degree of accuracy in the high end of the working range of the assay and limited precision and reproducibility in the low end of the working range of the assay, which may limit the clinical usefulness of the assay. Whether this degree of accuracy, precision, and reproducibility will be sufficient for the diagnosis of exocrine pancreatic diseases remains to be determined in clinical studies. The reference range of serum cPLI concentrations of 4.4 to 276.1 µg/L is well within the working range of the assay of 1 to 863 µg/L. This should allow identification of dogs with decreased or increased serum cPLI concentrations. Also, the upper limit of the working range is more than 3 times the upper limit of the reference range, and the upper limit of the reference range is in the middle of the working range, where the assay has the best performance indices. Although the cutoff value for a diagnosis of pancreatitis in dogs will likely be slightly higher than the upper limit of the reference range, analysis of these findings indicates that the assay has the potential to be highly exact in distinguishing clinically normal dogs from dogs with pancreatitis. The clinical usefulness of this assay remains to be determined in future studies.

^aTiterMax Gold, CytRx Corp, Atlanta, Ga.

^bIncomplete Freund's adjuvant, Sigma Chemical Co, St Louis, Mo.

^c100mM sodium phosphate, 150mM NaCl, pH 7.2, BupHTM dry-blend buffers, Pierce Chemical Co, Rockford, Ill.

^dHiTrap NHS-activated column, Amersham Pharmacia Biotech, Piscataway, NJ.

^eTris-HCl, Sigma Chemical Co, St Louis, Mo.

^fNaCl, Sigma Chemical Co, St Louis, Mo.

^gGlycine, Sigma Chemical Co, St Louis, Mo.

^hMiniature stir bar, VWR Scientific, West Chester, Pa.

ⁱPolypropylene test tube, VWR Scientific, West Chester, Pa.

^jSodium phosphate, Sigma Chemical Co, St Louis, Mo.

^kStir plate, VWR Scientific, West Chester, Pa.

^{l125}I NEN, Life Sciences Products Inc, Boston, Mass.

^mHamilton syringe, VWR Scientific, West Chester, Pa.

ⁿChloramine T, Sigma Chemical Co, St Louis, Mo.

^oSodium metabisulfite, Sigma Chemical Co, St Louis, Mo.

^pPotassium iodide, Sigma Chemical Co, St Louis, Mo.

^qPD-10, Amersham Pharmacia Biotech, Piscataway, NJ.

^rBovine serum albumin, Sigma Chemical Co, St Louis, Mo.

^sSodium azide, Sigma Chemical Co, St Louis, Mo.

^tVortexer, VWR Scientific, West Chester, Pa.

^uN6 precipitation solution, Diagnostics Products Corp, Los Angeles, Calif.

^vGS-6R, Beckman Coulter, Anaheim, Calif.

^wRiastar, Packard Instrument Co, Meriden, Conn.

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