For decades serum lipase activity has been used for diagnosing pancreatitis in dogs and humans. However, serum lipase activity is neither sensitive nor specific for pancreatitis in either species. Lipases are produced by many cell types in the body, and serum lipase activity may be affected by many extrapancreatic conditions.

An ELISA for the measurement of cPLI concentrations has been developed and analytically validated. In that study, a reference range of 2.2 to 102.1 μg/L was established by use of the central 95th percentile of serum cPLI concentrations for 74 healthy dogs. Serum cPLI concentrations in 25 dogs with exocrine pancreatic insufficiency were all less than the lower limit of the reference range, which suggested that at least in this group of dogs, serum cPLI concentration is limited in origin to the exocrine pancreas.

For a cPLI assay to be clinically useful in veterinary practice, cPLI concentrations must be unaffected by short-term storage, such as would be encountered during shipping and processing of serum and plasma samples. Furthermore, because many laboratories accept both serum and plasma for determination of biochemical panels, it is important to know whether an assay yields comparable values for both types of samples. In addition, being able to measure cPLI concentrations in serum and plasma would allow clinicians to limit the volume of blood collected from small or severely ill patients. Finally, studies have revealed that serum lipase activity increases in dogs after administration of prednisone to dogs on serum canine pancreatic lipase immunoreactivity concentrations for 74 healthy dogs. Serum cPLI concentrations in 25 dogs with exocrine pancreatic insufficiency were all less than the lower limit of the reference range, which suggested that at least in this group of dogs, serum cPLI concentration is limited in origin to the exocrine pancreas.

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tation of glucocorticoids. Thus, it is important to know the effects of prednisone administration on serum cPLI concentration. Because of the multitude of dogs that receive prednisone in veterinary practice, it is important to know the effect of prednisone administration on serum cPLI concentrations. Therefore, the objectives of the study reported here were to evaluate the short-term stability of serum cPLI concentration in samples stored at various storage temperatures, to compare serum and plasma cPLI concentrations in samples obtained from the same dog, and to examine the effects of prednisone administration on serum cPLI concentration in dogs.

**Materials and Methods**

**Sample population**—For the evaluation of stability of cPLI concentrations, 8 randomly selected canine serum samples that were submitted to the Gastrointestinal Laboratory at Texas A&M University for analysis but that were not completely used during the analysis and were scheduled to be discarded were obtained. Similarly, for the comparison between results for plasma and serum cPLI concentrations, 30 matched serum and plasma samples (ie, serum and plasma samples obtained from the same dog and at the same time) that were submitted to the Clinical Pathology Laboratory at Texas A&M University for analysis but that were not completely used during the analysis and were scheduled to be discarded were obtained. For evaluation of the effects of long-term prednisone administration on serum cPLI concentrations, 6 young adult heterozygous (carrier) female dogs with X-linked hereditary nephritis that were being used for comparative studies of Alport syndrome in humans were included in the study reported here. The dogs had mild glomerular proteinuria (urine protein-to-creatinine ratio ranged from 0.95 to 3.64) attributable to their genetic disorder but were otherwise healthy. Use of these dogs and this portion of the study were reviewed and approved by the Laboratory Animal Care and Use Committee at Texas A&M University.

**Stability evaluation**—Each of the 8 serum samples was divided into 20 aliquots (30 µL/ aliquot). Five aliquots for each sample were stored at each of 4 temperatures (room temperature [approx 24°C], 4°C, −20°C, and −80°C). An aliquot from each sample and temperature group was analyzed to determine the cPLI concentration on days 0, 3, 7, 14, and 21 (day 0 = day the samples were divided into aliquots).

**Comparison of serum and plasma concentrations of cPLI**—Matched serum and plasma samples for 30 randomly selected dogs were obtained and analyzed for cPLI concentration. The correlation between serum and plasma cPLI concentrations was determined.

**Effect of long-term prednisone administration on serum cPLI concentrations**—Pretreatment serum samples were obtained from each of the 6 dogs on days 0 and 14. Prednisone was administered (2.2 mg/kg, PO, q 24 h) on days 15 through 42. Serum samples were obtained during prednisone treatment on days 28 and 42. Additional serum samples were obtained after cessation of prednisone treatment on days 56 and 70.

**Measurement of cPLI concentrations**—The method for analysis has been described elsewhere. Briefly, 96-well flat-bottom ELISA plates were coated (200 ng/well) with affinity purified anti–cPLI antibodies in 100 µL of carbonate-bicarbonate buffer (pH, 9.4). Plates were incubated at 37°C for 1 hour with constant shaking. Plates were washed 4 times with PBS solution (pH, 7.2). Nonspecific binding was blocked by the addition of a commercially available blocking solution (200 µL/well) and incubation at 37°C for 1 hour with constant shaking. Plates were again washed 4 times with PBS solution. A PBS solution with 1% bovine serum albumin and 0.05% Tween (buffer A) was used as a control sample. Unknown and control samples were diluted 1 in 200 in buffer A. Standard and unknown samples were loaded (in duplicate) into wells of the plates (100 µL/well). Plates were then incubated at 37°C for 1 hour but without shaking. After wells were washed, biotinylated anti–cPLI antibodies were added (100 ng/well), and plates were incubated at 37°C for 1 hour with constant shaking. After wells were washed, horseradish peroxidase–labeled streptavidin (200 ng/well) was added. Plates were again incubated for 1 hour at 37°C with constant shaking. After another wash cycle with PBS solution, the plates were developed by the addition of 3,3′,5,5′-tetramethylbenzidine dihydrochloride substrate solution (1) and incubation for 15 minutes. The color reaction was stopped by the addition of 4M acetic acid and 0.5M sulfuric acid, and the absorbance of each well was measured at a wavelength of 450 nm.

Standard curves were calculated by use of a 4-parameter curve with the following equation:

\[
y = \frac{(A - D)}{(1 + |x/C|^g)} + D
\]

where y is the absorbance at a wavelength of 450 nm, A is the y-axis value corresponding to the asymptote at low values of the x-axis, D is the y-axis value corresponding to the asymptote at high values of the x-axis, x is the cPLI concentration, C is the x-axis value corresponding to the midpoint between A and D, and B is the value that describes how rapidly the curve transitions from the asymptotes in the center of the curve. All 4 parameters were calculated by use of an algorithm based on the Levenberg-Marquardt method.

**Statistical analysis**—Each data set was tested for normality by use of a Kolmogorov-Smirnov normality test. A statistical software package was used for
all analyses, and values of \( P < 0.05 \) were considered significant.

For the stability evaluation, serum cPLI concentrations were compared by use of a repeated-measures ANOVA. For the comparison between plasma and serum cPLI concentrations, data were analyzed by use of a Wilcoxon signed rank test, and the correlation between serum and plasma cPLI concentrations was determined. For evaluation of the effects of long-term prednisone administration on serum cPLI concentrations, mean serum cPLI concentrations for the 6 time points (days 0, 14, 28, 42, 56, and 70) were compared by use of a repeated-measures ANOVA. In addition, the mean difference of the mean serum cPLI concentration of the 2 pretreatment samples (days 0 and 14) and the serum cPLI concentration after prednisone administration for 4 weeks (day 42) was compared by use of a paired t test.

**Results**

Mean serum cPLI concentrations for each of the 8 randomly selected serum samples did not vary significantly in samples stored at room temperature (approx 24°C), \(-20^\circ C\), or \(-80^\circ C\) and analyzed on days 0, 3, 7, 14, or 21. However, there was some variation for each serum sample (Table 1).

For the 30 paired samples, serum cPLI concentrations ranged from 0 to 257.0 µg/L (mean ± SD, 64.1 ± 69.8 µg/L) and plasma cPLI concentrations ranged from 0 to 255.6 µg/L (mean, 62.1 ± 69.8 µg/L). The median values did not differ significantly (\( P = 0.48 \)) between serum and plasma cPLI concentrations. There was a strong correlation (Spearman \( r = 0.977; P < 0.001 \)) between serum and plasma cPLI concentrations (Figure 1).

For the dogs receiving long-term administration of prednisone, serum cPLI concentrations for all dogs and all time points ranged between 11.9 and 68.0 µg/L, which was within the reference range of 2.2 to 102.1 µg/L (Table 2). Mean serum cPLI concentrations for the 6 dogs were 30.9, 33.9, 32.1, 31.7, 26.3; and 29.6 µg/L on days 0, 14, 28, 42, 56, and 70, respectively. Repeated-measures ANOVA revealed that mean serum cPLI concentration did not differ significantly (\( P = 0.43 \)) over time (Figure 2). Also, a paired t test revealed that the mean serum cPLI concentration did not differ significantly (\( P = 0.35 \)) between the mean value for the 2 pretreatment samples (days 0 and 14) and the value at the end of 4 weeks of oral administration of prednisone (day 42; Figure 3).

**Discussion**

The findings of the study reported here indicated that the serum cPLI concentration was stable when stored for at least 21 days at temperatures that samples are routinely exposed to during shipping and storage until analysis. Because the serum cPLI concentration was stable when samples were stored at room temperature, this would suggest that samples should be stable even when they are not frozen for transport. There was some variation of results for each of the samples (Table 1). The mean CV for samples among time points ranged from 6.5% to 21.4% (overall mean CV, 13.1%) and was highest for samples with the lowest and highest serum cPLI concentrations. This finding was similar to the interassay variability detected during the analytic validation of the assay.

For most of the serum samples, this variation was of little diagnostic consequence. The mean for one of the samples for all determinations was within the refer-

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**Table 2**—Mean serum cPLI concentrations in 6 healthy dogs before (day 0 and 14), during (day 28 and 42), and after (day 56 and 70) oral administration of prednisone. *

<table>
<thead>
<tr>
<th>Dogs</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 42</th>
<th>Day 56</th>
<th>Day 70</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.3</td>
<td>28.3</td>
<td>25.6</td>
<td>25.3</td>
<td>29.4</td>
<td>29.2</td>
<td>30.4 ± 5.4</td>
</tr>
<tr>
<td>2</td>
<td>33.7</td>
<td>48.0</td>
<td>62.7</td>
<td>43.8</td>
<td>29.5</td>
<td>40.2</td>
<td>43.0 ± 11.8</td>
</tr>
<tr>
<td>3</td>
<td>63.7</td>
<td>66.5</td>
<td>44.8</td>
<td>68.0</td>
<td>50.7</td>
<td>46.1</td>
<td>56.6 ± 10.6</td>
</tr>
<tr>
<td>4</td>
<td>19.9</td>
<td>22.1</td>
<td>23.7</td>
<td>20.2</td>
<td>17.4</td>
<td>11.9</td>
<td>19.2 ± 4.2</td>
</tr>
<tr>
<td>5</td>
<td>18.6</td>
<td>31.8</td>
<td>14.3</td>
<td>19.3</td>
<td>13.6</td>
<td>24.5</td>
<td>20.3 ± 6.8</td>
</tr>
<tr>
<td>6</td>
<td>15.4</td>
<td>18.5</td>
<td>21.4</td>
<td>13.6</td>
<td>17.1</td>
<td>16.0</td>
<td>17.0 ± 2.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>30.9 ± 17.9</td>
<td>35.9 ± 18.2</td>
<td>32.1 ± 18.1</td>
<td>31.7 ± 20.6</td>
<td>26.3 ± 13.7</td>
<td>29.6 ± 14.2</td>
<td>31.1 ± 15.8</td>
</tr>
</tbody>
</table>

Mean serum cPLI concentrations were within the reference range of 2.2 to 102.1 µg/L for all dogs and all time points.

*Prednisone was administered (2.2 mg/kg, q 24 h, PO) on days 15 through 42.
ence range and was 79.5 µg/L. Two of the 8 samples had overall means at the boundary between the reference range and the diagnostic questionable range (overall mean, 104.3 and 106.3 µg/L, respectively). Naturally, some of the measurements for these 2 samples crossed the cutoff value between the reference and questionable ranges. Two more samples had mean serum cPLI concentrations that were within the diagnostic questionable range (overall mean, 153.1 and 177.1 µg/L, respectively). None of the 20 measurements for either of these 2 samples crossed the boundary of the diagnostic range for pancreatitis or the upper limit of the reference range.

Three samples had mean serum cPLI concentrations that were within the diagnostic range for pancreatitis (overall mean, 246.3, 290.0, and 407.8 µg/L, respectively). None of the 20 measurements for the highest sample crossed into the diagnostic questionable range. However, 1 of the 20 measurements for the sample with the middle mean value and 3 of the 20 measurements for the sample with the lowest mean value crossed into the diagnostic questionable range. For these 4 measurements, the clinical interpretation would have differed; however, all 4 of these measurements (189.1, 190.6, 193.4, and 199.9 µg/L, respectively) were barely within the diagnostic questionable range.

Analysis of these data revealed that interassay variability of this assay may have an effect on interpretation for values at the boundary between the 3 diagnostic ranges. In such cases, repeat measurement of serum cPLI concentration may be warranted.

We did not detect a significant difference between serum and plasma cPLI concentrations. Therefore, plasma can be substituted for serum at the clinician’s discretion.

Additionally, oral administration of 2.2 mg of prednisone/kg once a day for 4 weeks did not significantly affect serum cPLI concentration in these 6 dogs. Thus, comparable administration of glucocorticoids will not compromise the ability of clinicians to accurately interpret cPLI concentrations, which is an important consideration, especially considering the multitude of canine patients that receive prednisone. Analysis of these results also suggested that the increases in serum lipase activity reported after administration of glucocorticoids in other studies most likely reflect release of lipases from nonpancreatic sources. Additional studies are needed to evaluate whether a higher dose or more frequent administration of prednisone or the administration of other glucocorticoids may have a significant effect on serum cPLI concentration.

One limitation of the study reported here was the use of heterozygous (carrier) female dogs with X-linked hereditary nephritis. These dogs were chosen because they were available at the time, and the authors did not believe that the study justified the purchase of clinically normal dogs. Because no significant effect of long-term administration of prednisone was found in these dogs, it appears unlikely that the outcome would have differed for clinically normal dogs.

References