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Pancreas-specific lipase concentrations and amylase and lipase activities in the peritoneal fluid of dogs with suspected pancreatitis

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ABSTRACT

Diagnosing acute pancreatitis in the dog can be challenging. The aim of this study was to determine the concentrations of pancreas-specific lipase immunoreactivity (cPLI), and the activities of amylase and lipase, in the peritoneal fluid from a population of dogs diagnosed with acute pancreatitis based on clinical signs, ultrasonographic findings and serum cPLI concentrations. In a prospective study, cPLI concentrations, and amylase and lipase activities, were measured in the peritoneal fluid of 14 dogs with pancreatitis and 19 dogs with non-pancreatic disease.

The sensitivity and specificity of peritoneal fluid cPLI concentration (cut-off value 500 μg/L) were 100.0% (95% confidence interval, CI, 80.7–100.0%) and 94.7% (95% CI 76.7–99.7%), respectively. The sensitivity and specificity of peritoneal fluid amylase (cut-off value 1050 U/L) and lipase activities (cut-off value 500 U/L) were 71.4% (95% CI 44.5–90.2%) and 84.2% (95% CI 62.8–95.8%) for amylase activity, and 92.9% (95% CI 69.5–99.6%) and 94.7% (95% CI 76.7–99.7%) for lipase activity, respectively. In conclusion, peritoneal fluid cPLI concentration was highly sensitive as a complementary diagnostic tool in a group of dogs with suspected acute pancreatitis. Peritoneal fluid lipase activity was not as sensitive as cPLI concentration, but may also support a diagnosis of acute pancreatitis in dogs.

Introduction

Acute pancreatitis (AP) is a common disease in dogs presenting with non-specific clinical signs, such as anorexia, vomiting, weakness and abdominal discomfort. The frequent lack of specific routine laboratory abnormalities makes the diagnosis challenging (Hess et al., 1998; Steiner, 2003). Abdominal ultrasonography is commonly used as an aid in the diagnosis of AP. Common ultrasonographic findings in dogs include an enlarged, irregular, hypoechoic, or even mass-like, pancreas with peripancreatic hyperechoic mesentery and peritoneal effusion. Other abnormalities may include gastroparesis, corrugation of the duodenum, distended, hypomotile intestines (functional ileus) and biliary distension due to extrahepatic biliary obstruction (Hecht and Henry, 2007).

Although these ultrasonographic parameters may support a diagnosis of AP, their validity has not been assessed critically in dogs. The sensitivity of abdominal ultrasonography in cases of fatal AP in dogs has been reported as 68% (Hess et al., 1998). Abdominal ultrasonography has also been shown to have only fair agreement with serum canine pancreatic lipase immunoreactivity (cPLI), which is the most sensitive and specific non-invasive test for diagnosing AP in dogs (Kook et al., 2014).

Historically, serum amylase and lipase activities have been used for the diagnosis of pancreatitis in dogs (Brobst et al., 1970; Mia et al., 1978; Strombeck et al., 1981). However, there are extrapancreatic sources of amylases and lipases (Simpson et al., 1991). Pancreatic biopsy is considered to be the most definitive diagnostic test for pancreatitis, although lesions can be missed even with multiple biopsies (Newman et al., 2004), and obtaining pancreatic biopsies is invasive.

Assays for the measurement of serum cPLI are commonly used as diagnostic tools, since they specifically measure lipase from pancreatic acinar cells (Steiner et al., 2002; Huth et al., 2010). One of the most common assays used to measure cPLI concentrations in dogs is the Spec cPL assay. In one study, Spec cPL had a specificity of 97.5% for diagnosis of AP in dogs, while in another large clinical trial the specificity was estimated to be 77% using a Bayesian model. The reported sensitivity of this assay ranged between 63.6 and 82%.

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However, many of the dogs in these studies had only mild pancreatitis on histopathological examination (Steiner et al., 2008; Neilson-Carley et al., 2011; McCord et al., 2012). More recent studies have shown that Spec cPL is most useful in cases of moderate to severe pancreatitis, with sensitivities and specificities (cut-off value 400 μg/L) in these cases in the ranges 71.0–77.8% and 80.5–100%, respectively (Trivedi et al., 2011; Mansfield et al., 2012; McCord et al., 2012).

It is not uncommon for dogs with AP to have peritoneal effusions. In human beings and dogs with pancreatitis, amylase and lipase activities are increased in peritoneal fluid (Geokas and Rinderknecht, 1974; Dubick et al., 1987; Frossard et al., 2000; Guija De Arespacochaga et al., 2006). In a study comparing lipase activity in the peritoneal fluid of dogs diagnosed with different diseases, there was significantly higher lipase activity in the peritoneal fluid of dogs with pancreatitis than in dogs with conditions of non-pancreatic etiology (Guija De Arespacochaga et al., 2006).

The utility of measurement of peritoneal fluid cPLI concentrations to aid in the diagnosis of pancreatitis has not yet been evaluated. The aim of this study was to establish the sensitivity and specificity of the Spec cPL to determine cPLI concentrations in the peritoneal fluid in dogs with suspected AP. In addition, peritoneal fluid amylase and lipase activities were evaluated in the same population of dogs.

Materials and methods

Study design

Dogs with peritoneal effusions as shown by abdominal ultrasonography were prospectively enrolled. Each dog had a physical examination, complete blood count, serum biochemistry profile, abdominal ultrasonography, and peritoneal fluid biochemistry and cytology (Idexx Laboratories). Serum and peritoneal fluid cPLI concentrations were measured using the Spec cPL assay (Idexx Laboratories), and activities of peritoneal fluid amylase (Stanbio Alpha-Amylase LiquiColor, Stanbio Laboratory) and lipase (Diazyme Lipase Assay Kit, Diazyme Laboratories) were determined. The study was approved by the Veterinary Specialty Hospital of San Diego Research Advisory Committee (approval number 6, date of approval 1 June 2011) and informed owner consent was obtained before enrolling any dog in the study.

Concentrations of cPLI and activities of amylase and lipase in peritoneal fluid were compared between dogs with AP and dogs with non-pancreatic disease (NP). Since a priori cut-off values for measurements in peritoneal fluid were not available, sensitivity and specificity were calculated based on a receiver operating characteristic (ROC) curve with a goal of maximizing sensitivity, while reaching a specificity >80%.

Selection of dogs with acute pancreatitis

Dogs were included in the AP group if they had (1) a serum cPLI concentration >400 μg/L, consistent with a diagnosis of pancreatitis; (2) at least two of the following clinical signs consistent with AP: lethargy, inappetence, weakness, vomiting, abdominal pain and/or diarrhea; (3) complete resolution of clinical signs suspected to be secondary to AP; and (4) a real-time ultrasonographic findings supportive of AP without concurrent disease: pancreatic enlargement, pancreatic hypoechogenicity, irregular margins, peri-pancreatic fluid, hyperechoic surrounding mesentery and/or changes to the adjacent intestines. Dogs were excluded from this experimental group if there was evidence of concurrent systemic disease known to cause peritoneal effusion.

Selection of dogs with non-pancreatic disease

Dogs were included into the NP group if they had a final diagnosis of a NP disease, a serum cPLI concentration (<200 μg/L) that did not support a diagnosis of pancreatitis and results of abdominal ultrasound examination that were not consistent with AP.

Statistical analysis

Statistical analyses were performed using Prism 6 for Windows (GraphPad). The data for peritoneal fluid cPLI concentrations, and amylase and lipase activities, were assessed for normality using the D'Agostino and Pearson omnibus normality test. Significance was set at $P < 0.05$. Peritoneal fluid cPLI concentrations and amylase activities were compared between groups using Mann–Whitney tests. Peritoneal fluid lipase activities were compared between groups using a Student's *t* test. Sensitivity and specificity were calculated based on ROC curves with a goal of maximizing sensitivity, while reaching a specificity >80%.

Results

Thirty-three dogs were enrolled into the study and categorized as either AP ($n = 14$) or NP ($n = 19$). The AP group consisted of eight castrated males and six spayed females. The median age of AP dogs was 8 years (range 3–12 years). Breeds in the AP group were mixed breed ($n = 3$), Yorkshire terrier ($n = 2$), Cocker spaniel ($n = 2$), Miniature Schnauzer ($n = 2$), and one of each Jack Russell terrier, Samoyed, Shetland sheepdog, Corgi and Golden retriever. The NP group consisted of 13 castrated males and six spayed females. NP dogs had a median age of 9 years (range 4–15 years). Breeds in the NP group included mixed breed ($n = 4$), Golden retriever ($n = 2$), and one each of Border collie, Wheaten terrier, Scottish deerhound, Sharpei, Shih-tzu, Doberman, Corgi, Coonhound, Hungarian Viszla, Yorkshire terrier, Australian shepherd, Husky and Bichon Frise.

All AP dogs had at least two clinical signs consistent with AP. The most common clinical signs in dogs with AP were lethargy (11/14, 79%), inappetence (10/14, 71%), abdominal pain (9/14, 64%) and vomiting (8/14, 57%). The most common clinical signs in the NP group were lethargy, inappetence, abdominal pain, vomiting and weakness. Diagnoses in dogs in the NP group included hepatic and/or splenic hemangiosarcoma ($n = 4$), hepatopathy with secondary portal hypertension ($n = 3$), portal thrombosis ($n = 2$), hepatic sarcoma ($n = 2$), protein-losing enteropathy ($n = 1$), aspiration pneumonia with secondary sepsis ($n = 1$), perforated small intestinal stromal tumor ($n = 1$), right-sided congestive heart failure ($n = 1$), urethral tear ($n = 1$), splenic hematoma ($n = 1$), ruptured gallbladder mucocele ($n = 1$) and sepsis of unknown origin ($n = 1$).

On the basis of biochemistry and cytology, peritoneal fluid in dogs with AP was characterized as a transudate ($n = 1$), modified transudate ($n = 5$) or non-septic exudate ($n = 8$). Changes suggestive of infection or neoplasia were not present in any sample from the AP group, and the majority of cells were non-degenerate neutrophils, with smaller percentages of macrophages and small lymphocytes. In the NP group, peritoneal fluid was characterized as a transudate ($n = 4$), modified transudate ($n = 7$), septic exudate ($n = 1$), non-septic exudate ($n = 2$) and hemorrhagic effusion ($n = 5$).

In the AP group, the median peritoneal fluid amylase activity was 2396 U/L (range 255–5179 U/L). In the NP group, the median peritoneal fluid amylase activity was 515 U/L (range 114–1724 U/L). The sensitivity and specificity of peritoneal fluid amylase activity (cut-off value 1050 U/L) for a diagnosis of AP were 71.4% (95% confidence interval, CI, 44.5–90.2%) and 84.2% (95% CI 62.8–95.8%), respectively ($P = 0.0002$; Figs. 1 and 2).

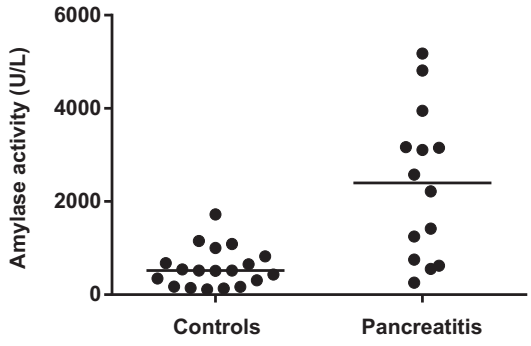

Fig. 1. Comparison of peritoneal fluid amylase activities of dogs with acute pancreatitis (AP) and dogs with non-pancreatic diseases (NP). Bars represent the medians for each group. There was a significant difference between the two groups ($P = 0.0002$).

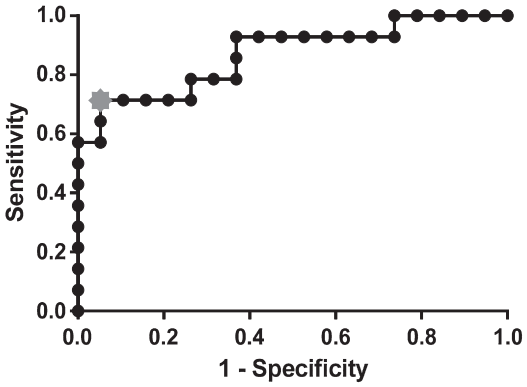

Fig. 2. Receiver operator characteristic (ROC) curve for amylase activity in canine peritoneal fluid. The gray star shows the approximate location of the cut-off value of 1050 U/L chosen for a specificity of >80% at optimal sensitivity.

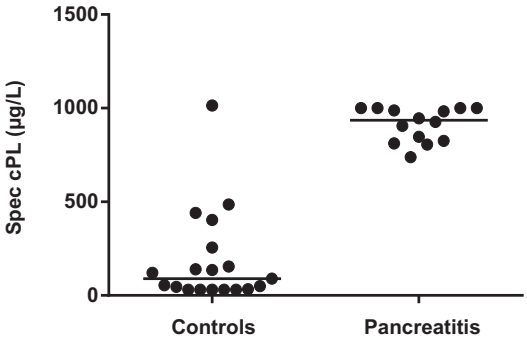

Fig. 5. Comparison of peritoneal fluid cPLI concentrations as measured by Spec cPL between dogs with acute pancreatitis (AP) and dogs with non-pancreatic diseases (NP). Bars represent the medians for each group. There was a significant difference between the two groups ($P \leq 0.0001$).

The mean ± standard deviation (SD) peritoneal fluid lipase activity in dogs with AP was 801 ± 245 U/L. The mean ± SD value for peritoneal fluid lipase activity in NP dogs was 197 ± 151 U/L. The sensitivity and specificity of peritoneal fluid lipase activity (cut-off value 500 U/L) for a diagnosis of AP were 92.9% (95% CI 69.5–99.6%) and 94.7% (95% CI 76.7–99.7%), respectively ($P \leq 0.0001$; Figs. 3 and 4).

In dogs with AP, the median peritoneal fluid cPLI concentration was 936 µg/L (from 738 µg/L to >1000 µg/L, the upper working limit of the Spec cPL). In the NP group, the median peritoneal fluid cPLI concentration was 89 µg/L (from 30 µg/L to >1000 µg/L). The sensitivity and specificity of peritoneal fluid cPLI concentration as measured by the Spec cPL assay (cut-off value 500 µg/L) for a diagnosis of AP were 100% (95% CI 80.7–100.0%) and 94.7% (95% CI 76.7–99.7%), respectively ($P \leq 0.0001$; Figs. 5 and 6). One dog from the NP group had an increased peritoneal fluid cPLI concentration above the cut-off value of 500 µg/L (cPLI >1000 µg/L); this dog had bile peritonitis. Three dogs in the NP group had peritoneal fluid cPLI concentrations 400–500 µg/L; the diagnoses in these three dogs were perforated gastrointestinal stromal tumor of the small intestine (cPLI 403 µg/L), hepatopathy with secondary portal hypertension (cPLI 440 µg/L) and hepatic sarcoma (cPLI 485 µg/L).

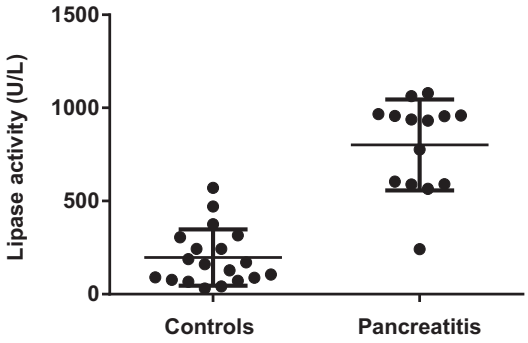

Fig. 3. Comparison of peritoneal fluid lipase activities between dogs with acute pancreatitis (AP) and dogs with non-pancreatic diseases (NP). Bars represent the mean and standard deviation (SD) for each group. There was a significant difference between the two groups ($P \leq 0.0001$).

Discussion

Peritoneal fluid cPLI concentration measured with the Spec cPL was highly sensitive and specific for a diagnosis of AP, with a sensitivity of 100% and a specificity of 94.7% at a cut-off value of 500 µg/L. This is the first study to report cPLI concentrations in the peritoneal fluid of dogs. Although it may seem plausible that dogs with increased serum cPLI concentrations would have higher peritoneal fluid cPLI concentrations, pancreatic lipase is a large protein that cannot freely cross serosal barriers or capillary walls. Thus, increases in cPLI concentrations in peritoneal fluid may not be reflected in serum cPLI concentrations.

This study showed that there was a good correlation between serum and peritoneal cPLI concentrations, making the Spec cPL another potentially useful diagnostic test on peritoneal fluid samples

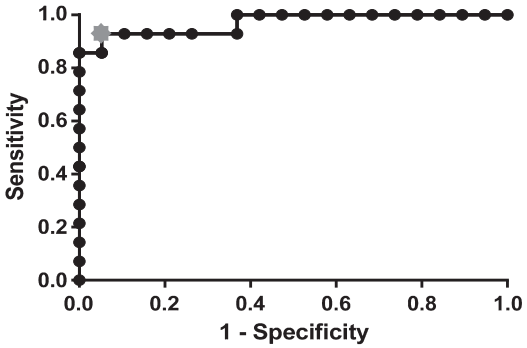

Fig. 4. Receiver operator characteristic (ROC) curve for lipase activity in canine peritoneal fluid. The gray star shows the approximate location of the cut-off value of 500 U/L chosen for a specificity of >80% at optimal sensitivity.

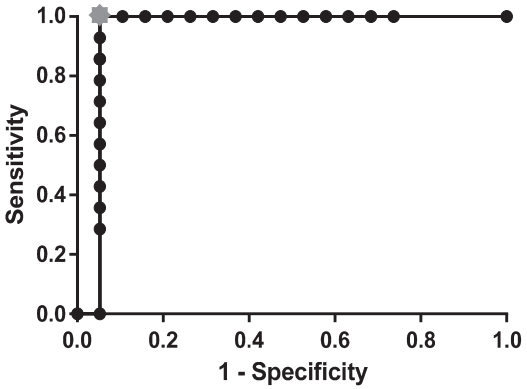

Fig. 6. Receiver operator characteristic (ROC) curve for cPLI concentrations in canine peritoneal fluid. The gray star displays the approximate location of the cut-off value of 500 µg/L chosen for a specificity of >80% at optimal sensitivity.

from dogs. As seen in this study, dogs with peritoneal fluid effusions secondary to AP, or from NP conditions, can present with similar clinical signs. Abdominal ultrasonography has low sensitivity, even in fatal cases of AP, and shows only fair agreement with serum Spec cPL results (Hess et al., 1998; Kook et al., 2014).

Serum cPLI concentration is the most sensitive and specific non-invasive test available for diagnosing AP in dogs; however, the reported sensitivity of this assay is 63.6–82.0% (Steiner et al., 2008; Trivedi et al., 2011; McCord et al., 2012). Peritoneal fluid Spec cPL may be most useful in cases presenting with clinical signs and abdominal ultrasonography that are suspicious for AP, but have equivocal serum Spec cPL results (cPL values 200–400 μg/L). Future studies should investigate the utility of the Spec cPL in peritoneal fluid in this population of dogs.

One of the most common and challenging scenarios encountered by emergency veterinary clinicians is differentiating dogs with severe forms of AP from dogs with septic peritonitis, where the clinical signs and peritoneal fluid cytology results can be similar, especially early in the disease process. Comparison of peritoneal effusion and peripheral blood glucose and lactate concentrations is a useful tool, but can give equivocal results in some cases (Bonczynski et al., 2003). Our study included one dog with septic peritonitis secondary to gastrointestinal perforation. The peritoneal fluid cPLI concentration and peritoneal fluid lipase activity in this dog were 403 μg/L and 470 U/L, respectively. On the basis of the values defined in our study, this dog was negative for AP. Assessing peritoneal fluid cPLI concentrations in clinical situations such as this could be useful, but cannot be recommended for definitive differentiation of such cases until more animals have been evaluated. Future studies investigating cut-off values for peritoneal fluid cPLI concentrations and peritoneal fluid lipase activities to differentiate dogs with AP from dogs with septic peritonitis are warranted.

Only one dog from the NP group had an increased peritoneal fluid cPLI concentration consistent with AP; this dog had bile peritonitis. Furthermore, this was the only dog with a value for peritoneal fluid lipase activity above the cut-off range. Free bile in the peritoneal cavity induces a strong inflammatory response, resulting in chemotaxis of inflammatory cells and increased vascular permeability. Bile peritonitis is usually diagnosed on the basis of findings of a ruptured gallbladder on ultrasonographic examination, identification of bile pigment crystals on peritoneal effusion cytology, and an effusion/serum bilirubin ratio >2.0 (Dempsey and Ewing, 2011). Additional diagnostic modalities would not be necessary for most bile peritonitis cases. Although this dog had a normal serum cPLI concentration and normal findings on pancreatic ultrasonography, the significant elevation in the peritoneal fluid cPLI concentration seen in this dog may have been due to early AP secondary to bile peritonitis. Evaluating more dogs with bile peritonitis would help to further elucidate the diagnostic utility of peritoneal fluid cPLI for diagnosing possible concurrent AP in these cases. Three dogs in the NP group had peritoneal fluid cPLI concentrations of 400–500 μg/L. In order to maximize sensitivity and specificity for diagnosis of AP in dogs, the cut-off value for peritoneal fluid cPLI concentrations (>500 μg/L) was set at a higher level than the cut-off value for serum cPLI concentrations (>400 μg/L).

Peritoneal fluid lipase activity had a sensitivity of 92.9% and a specificity of 94.7% at a cut-off value of 500 U/L for diagnosing AP in dogs. These results are similar to those of a previous study, in which dogs with AP had peritoneal fluid lipase activity >500 U/L (Guija De Arespacochaga et al., 2006). In the study by Guija De Arespacochaga et al. (2006), some dogs with NP disease had peritoneal fluid lipase activity >500 U/L; most of these dogs had a history of abdominal trauma and the authors suspected that the increased peritoneal fluid lipase activities in these cases were secondary to pancreatic injury. Future studies in dogs should include cases of abdominal trauma without septic peritonitis to further evaluate the impact of trauma on cPLI concentration and lipase activity in peritoneal fluid.

Effusions in this study were classified as transudates, modified transudates, exudates (septic and non-septic), hemorrhagic, urine or bile. In a previous study, increased concentrations of commonly occurring sample matrix components, including lipids, hemoglobin and bilirubin did not have any effect on the results of the Spec cPL assay (Huth et al., 2010). Although the previous study was performed on blood, effusions in our study had similar matrix components and results correlated well with serum Spec cPL results. Therefore, the various types of effusions in this study were not thought to have a substantial effect on peritoneal fluid Spec cPL assay results.

The limitations of our study included the small numbers of cases for each individual NP disease, making it difficult to draw conclusions about the diagnostic utility of the peritoneal fluid markers in differentiating AP from each specific NP disease. It would have been preferable if all ultrasonographic examinations were performed by a single board-certified radiologist using the same ultrasound machine. However, since many of the cases enrolled in the study were emergency admissions, it was not feasible to have a single radiologist perform the ultrasonographic examinations on all cases.

The grouping of dogs into AP or NP groups was done using the same criteria that veterinary clinicians would use in a clinical setting, including presenting clinical signs, resolution of clinical signs over time, abdominal ultrasonography and elevation of serum cPLI concentrations. A histopathologic diagnosis of pancreatitis would have allowed for more definitive grouping of dogs. However, this would have been more invasive, is rarely available in the clinical setting and lesions can be missed even if multiple biopsies are procured (Newman et al., 2004). The clinical signs, diagnostic work-up and follow-up support the correct grouping of the dogs in the present study.

Conclusions

Peritoneal fluid cPLI concentrations measured by Spec cPL in a group of dogs with suspected pancreatitis correlated well with a clinical diagnosis of AP based on clinical signs, serum Spec cPL results and abdominal ultrasonography. Peritoneal fluid cPLI concentrations should be considered as a complementary diagnostic tool in dogs with peritoneal effusion suspected of having AP. Peritoneal fluid lipase activity, albeit not as sensitive as cPLI concentration, may also support a diagnosis of AP in dogs. Future studies investigating cut-off values for peritoneal fluid cPLI concentration and peritoneal fluid lipase activity for differentiating dogs with AP from dogs with septic peritonitis would be clinically useful.

Conflict of interest statement

Joerg M. Steiner is Director of the Gastrointestinal Laboratory at Texas A&M University, which offers Spec cPL testing on a fee for service basis. He is also a paid consultant for IDEXX Laboratories. Jan Suchodolski is the Associate Director of the Gastrointestinal Laboratory at Texas A&M University, which offers Spec cPL testing on a fee for service basis. Jane Robertson is an employee of Idexx Laboratories, which also offers Spec cPL testing on a fee for service basis.

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