Understanding lipase assays in the diagnosis of pancreatitis in veterinary medicine

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ABSTRACT
Pancreatitis commonly occurs in humans, dogs, and cats. For both veterinary and human health-care professionals, measurement of serum pancreatic lipase concentration or activity provides useful support for a diagnosis of pancreatitis. In this Currents in One Health manuscript, we will discuss commonly used lipase assays in veterinary medicine, namely catalytic colorimetric and immunological lipase assays. We highlight potential diagnostic pitfalls associated with analytical specificity, assay validation, and sample condition interferences. Catalytic lipase assays may detect extrapancreatic lipases. In addition, we propose a decision tree for interpretation of lipase assays in the context of a clinical patient.

As is the case in humans, pancreatitis is the most common disease of the exocrine pancreas in dogs and cats. Pancreatic histopathology was traditionally considered the gold standard for diagnosing pancreatitis, but it is rarely performed antemortem in veterinary clinical practice. This is due to its invasiveness and high costs in addition to other inherent limitations, such as the potential to miss localized pancreatic lesions and detection of abnormalities that may be clinically irrelevant. Therefore, the diagnosis of pancreatitis in dogs and cats is often based on an overall assessment of clinical data, including history, clinical signs, physical examination findings, and clinicopathologic and diagnostic imaging findings in addition to measurement of pancreatic lipase concentrations or activity in serum or plasma.

Under normal physiologic conditions, the vast majority (approx 99%) of pancreatic lipase and colipase is released from the apical pole of the pancreatic acinar cell into the pancreatic juice for subsequent digestion of dietary fat. Less than 1% diffuses from the basolateral aspect of the acinar cells into circulation. However, when the pancreas is inflamed, apical secretion is blocked and a large amount of pancreatic lipase is released into the vascular space via the basolateral aspect of the cell. Therefore, measurement of pancreatic lipase in circulation can be used as a noninvasive diagnostic marker for acinar cell inflammation or damage during pancreatitis.

There are numerous methods available for measuring serum lipase, and these can be broadly divided into the following categories: (1) catalytic methods (eg, spectroscopy, turbimetry, titrmetry, and colorimetry), which measure lipase activity through either decreased substrate concentration or increased hydrolytic product concentration, and (2) immunological methods (eg, ELISA and radioimmunoassay), which measure the concentration of a specific lipase independently from its lipolytic activity. The number and breadth of available tests and methodologies can make it challenging to select and understand the appropriate use of each assay in clinical practice. The purpose of this article is to review the various pancreatic lipase assays that are available and, importantly, potential pitfalls or diagnostic challenges associated with the effects of extrapancreatic lipases, analytical assay validation, and sample condition interferences. This will help veterinarians understand appropriate assay selection for clinical patients. We will also provide a decision tree that will help to integrate interpretation of lipase assays in the context of an individual patient. A detailed review of the many digestive and metabolic lipases is addressed in the companion Currents in One Health by Lim et al, AJVR, August 2022.

Analytical Specificity
Analytical specificity is something we do not commonly discuss in clinical practice, but it is of significant importance when an appropriate diagnostic test is being selected. The analytical specificity is distinct but interrelated to the diagnostic specificity of an assay. The report ed diagnostic sensitivities and specificities to diagnose pancreatitis in dogs and cats are summarized (Supplementary Tables S1 and S2). Briefly, the diagnostic specificity represents the percentage of nondiseased individuals who are identified by the assay as being negative for
the disease. Analytical specificity in contrast is the ability of an assay to measure the particular substance that it is designed to detect, without also detecting structurally similar compounds that have different physiologic roles. When an assay is not analytically specific, it often produces false positives, thus decreasing diagnostic specificity. The analytical specificity of a lipase assay could be affected by the detection of extrapancreatic lipases such as lipoprotein lipase or hepatic lipase, among others (see companion Currents in One Health manuscript by Lim et al, AJVR, August 2022).

Immunological Lipase Assays

Immunological assays specifically measure the concentration of pancreatic lipase in a biological sample (pancreatic lipase immunoreactivity [PLI] in dogs [cPLI] and cats [fPLI]), using species-specific and anti-pancreatic lipase–specific antibodies. Commercial send-out ELISAs (Spec cPL and Spec fPL; Idexx Laboratories) use a set of monoclonal antibodies produced against recombinant pancreatic lipase (Figure 1). Monoclonal antibodies are used for commercial assays, as they are more specific than polyclonal antibodies and recognize only a single epitope on an antigen. Therefore, no other lipases are expected to show cross-immunoreactivity. This is supported by data showing no significant increase in postheparin cPLI and fPLI concentrations (as measured by Spec cPL and Spec fPL) as well as minimal detection of pancreatic lipase in dogs with exocrine pancreatic insufficiency (EPI). Heparin is used to measure analytic specificity because it causes release of extrapancreatic lipases into the circulation. Thus, an increase in lipase concentration or activity after administration of heparin indicates detection of extrapancreatic lipases by an assay. Dogs with EPI serve as a naturally occurring model for studies of analytical specificity of lipase assays, as they have lost > 90% of the pancreatic secretory capacity, resulting in low to negligible serum trypsin-like immunoreactivity and PLI concentrations. Various validation studies have shown the Spec cPL assay to be precise, linear, stable, and reproducible. It has also been reported to be unaffected by hemolysis, icterus, or lipemia. Thus, the Spec cPL and fPL assays are considered analytically specific, and peer-reviewed publications document good analytical validation of the Spec cPL assay.

Spec cPL ELISA

Figure 1—Principle of Spec pancreatic lipase immunoreactivity in dogs (cPL) ELISA: (a) sample is added into ELISA wells precoated with capture antibody (monoclonal anti-canine pancreatic lipase [cPL] 7E11), (b) capture antibody binds to antigen (cPL) with high specificity, (c) horseradish peroxidase (HRP)-conjugated detection antibody (blue; monoclonal anti-cPL 4G11) binds to the immobilized antigen, and (d) HRP catalyzes the conversion of TMB substrate into colored products.
Measurement of cPLI and fPLI concentrations is generally considered to be a sensitive and specific diagnostic test for pancreatitis in dogs and cats, respectively, depending on the diagnostic criteria and cutoff values used. The reported diagnostic sensitivities and specificities to detect pancreatitis in dogs and cats are summarized (Supplementary Tables S1 and S2). These values vary widely depending on the criteria set by researchers for the diagnosis of pancreatitis (ie, histopathologic findings, clinical signs, and ultrasonographic findings), the type of pancreatitis (ie, acute, chronic, or acute on chronic), severity (ie, mild, moderate, or severe), and the diagnostic cutoff values used. In general, severe forms of pancreatitis are associated with a higher diagnostic sensitivity, whereas mild cases of pancreatitis are associated with a lower diagnostic sensitivity. Pancreatic tumors in dogs and cats are often accompanied by pancreatitis of variable severity, thus causing an increase in measured serum pancreatic lipase. Cytology or biopsy may therefore be needed to distinguish pancreatic neoplasms (rare) from pancreatitis.

In addition, rapid point-of-care semiquantitative tests (SNAP cPL and SNAP fPL; Idexx Laboratories) are available. The reported diagnostic sensitivities and specificities range from 74% to 100% and 59% to 78%, respectively, in dogs. With higher sensitivity compared to specificity, the SNAP PL test is useful as a screening tool to quickly rule out pancreatitis, with the recommendation for abnormal test results to be followed up by a Spec PL in the laboratory. Recently, 2 new point-of-care immunoassays (VetScan cPL Rapid Test; Zoetis; Vcheck cPL and Vcheck fPL; Bionote USA Inc) were developed for the measurement of pancreatic lipase concentrations in dogs and cats.

The VetScan cPL uses polyclonal antibodies that are directed against canine pancreatic lipase in a lateral flow immunoassay format (Figure 2). Similar to the SNAP cPL, this test uses a sandwich ELISA format that detects pancreatic lipase in a sample using a colloidal gold-conjugated detection antibody and an immobilized capture antibody in the test well. This test is quantified by a dedicated reader. The manufacturer of this test reported that it is affected by hemolysis but not icterus, lipemia, or anticoagulants. One study showed that this assay correlated well with the Spec cPL (intraclass correlation coefficient = 0.96). A subsequent analytical validation of this assay showed that 61% of test samples did not adhere to the manufacturer’s specifications (results within 60 µg/L of the actual pancreatic lipase concentration) and showed poor linearity, repeatability, and reproducibility. Additionally, it was also shown to be affected by lipemia in 1 study. A criticism of both of these studies was that the VetScan cPL was performed in a laboratory environment, which did not allow for direct comparison with the SNAP cPL.

Figure 2—Principle of lateral flow immunoassay: (a) add drop(s) of sample in sample well (S), (b) add buffer to sample well, (c) capillary action moves sample across lateral flow test, (d) cPL in sample binds to colloidal-gold anti-cPL detection antibodies (blue) in conjugate pad, (e) sample enters test well (T) and antibody-antigen complex binds to immobilized anti-cPL capture antibodies (green), and (f) colloidal-gold anti-cPL detection antibodies (blue) bind to immobilized anti-IgG antibodies (orange). Adapted from “COVID-19 Serologic Diagnostic Test Through Antibody Detection.” Created with BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates.
not mimic the intended patient-side use of the assay. Further, in a small-scale study, point-of-care repeatability as expressed by coefficient of variation was subsequently shown to be low (mean, 17%; range, 4.7% to 32.6%). This indicates a greater-than-recommended variation in test results when a single sample is repeatedly measured. While repeated measurement of the same sample is of no relevance to clinical practice, this finding is clinically important, as it shows that the sample may randomly result in a different diagnostic bin (ie, normal, equivocal, or suggestive of pancreatitis). This also has implications when pancreatic lipase concentrations are being monitored, as differences in results may reflect variation in assay performance rather than changes in pancreatic inflammation.

The Vcheck cPL and FPL use antibodies against canine and feline pancreatic lipase, respectively. It is unknown whether these antibodies are polyclonal or monoclonal antibodies, which may affect binding specificity. Similar to the VetScan cPL, the Vcheck assay uses a lateral flow immunoassay format to detect pancreatic lipase by fluorescence also using a dedicated reader (Figure 2). The manufacturer states that Vcheck FPL is affected by sample hemolysis and hematocrit (for whole blood samples), where a low hematocrit causes falsely high values and a high hematocrit causes falsely low values. This information is not available for Vcheck cPL. It is also unclear whether this test is affected by lipemia or hyperbilirubinemia, although preliminary data suggest that the assay is unaffected. A partial validation of this canine assay showed poor linearity, precision, and reproducibility. Additionally, another peer-reviewed study confirmed the poor repeatability of this assay with more than half of the samples showing an unacceptably high coefficient of variation (>20%). Thus, this version of the assay should be interpreted cautiously. Vcheck cPL and FPL have recently been replaced by a newer version (Vcheck cPL 2.0 and FPL 2.0). As with all new assays, they should undergo robust analytical validation before they can be recommended for use in a clinical setting.

Lastly, new send-out commercial cPLI and fPLI ELISAs (Laboklin GmbH & Co KG) have been developed in-house. The in-house cPLI ELISA uses polyclonal antibodies that are directed against recombinant canine pancreatic lipase. Information on the type of antibodies used for the in-house fPLI ELISA is unavailable. The analytical validations of these assays show acceptable reproducibility and acceptable to poor but acceptable repeatability, with a reported linearity that is outside of the ideal range of 90% to 110%. Thus, further evaluation of this assay is needed before routine clinical use can be recommended. Immunological lipase assay development, use, and research in veterinary medicine also has significant translational value. This will likely further expand as more research is performed into the presence of extrapancreatic lipases in nonpancreatic disease conditions and their effects on nonimmunological lipase assays.

Catalytic Colorimetric Lipase Assays

Catalytic colorimetric lipase assays measure serum lipase activity by measuring the rate at which hydrolytic reaction products are formed. The formation of hydrolytic products usually causes a color change, and the more intense the color change, the higher the lipase activity. Substrates used in lipase activity assays can be glycerol derived or synthetic carboxylic esters. For full hydrolytic activity of lipases, the presence of bile salts, colipase, and calcium in optimal concentrations is required. Serum lipase activity commonly included in chemistry profiles in human and veterinary medicine lacks specificity for pancreatic lipase. This is important because it means that these biomarkers may be elevated in nonpancreatic disease. It is also important to inquire about the methodology your laboratory uses if lipase is included on your routine serum chemistry panel, as the analytical specificity varies significantly. Early catalytic colorimetric lipase assays used 1,2-diglyceride as a substrate. However, because many tissues and organs also synthesize and secrete lipases, measurement of serum lipase activity by use of 1,2-diglyceride lacks analytical specificity for pancreatic lipase and is not considered useful for the diagnosis of pancreatitis. Researchers have tried to circumvent this shortcoming by using other substrates (eg, 1,2-o-dilauryl-rac-glycerol-3-glutaric acid-(6'-methylresorufin) ester [DGGR] and triolein that are preferentially hydrolyzed by pancreatic lipase as well as optimizing assay conditions to favor hydrolytic activity of pancreatic lipase over other lipases. Since then, catalytic colorimetric assays have been widely used for the diagnosis of pancreatitis because of their ease of measurement, inexpensiveness, and lack of need for specialized equipment or expertise.

DGGR was introduced 20 years ago as a substrate that is more specific for pancreatic lipase than 1,2-diglyceride. It was initially optimized for use in people and then later introduced into veterinary medicine. Under alkaline conditions and with the presence of colipase, bile salts, and calcium, pancreatic lipase cleaves DGGR into triglyceride and glutaric acid that is conjugated to a bluish-purple methyleirosorufin dye. This unstable conjugated glutaric acid is spontaneously hydrolyzed to form glutaric acid and methyleirosorufin. The formation of methyleirosorufin is measured at a wavelength of 580 nm (570 to 590 nm) at 37 °C, and its rate of formation is proportional to the lipase activity in the sample.

Various analytical studies to determine the utility of DGGR-based lipase assays as a diagnostic modality for pancreatitis have been carried out, initially in human medicine and later in veterinary medicine. This assay is generally only available as a send-out test through your serum chemistry provider, although some in-house wet chemistry analyzers are also capable of using this substrate. Most of these studies have focused on the validation and diagnostic sensitivity and specificity of the lipase assay for pancreatitis but not analytical specificity for pancreatic lipase. Studies have shown that the DGGR-based lipase assay is unaffected by sample freezing, lactic acidosis, or expertise.

In summary, catalytic colorimetric lipase assays are widely used in human medicine and are becoming increasingly popular in veterinary medicine. However, their use is limited by the lack of specific targeting and the potential for false-positive results due to the presence of extra-pancreatic lipases. Further research is needed to develop more specific and sensitive assays for the diagnosis of pancreatitis in veterinary medicine.
glycerol, anticoagulants (EDTA dipotassium salt, sodium citrate, lithium heparin, and sodium fluoride), or carboxyl ester lipase but is variably affected by hemoglobin and lipemia.\textsuperscript{3,5,37} Similar to Spec cPL and fPL, the diagnostic accuracies of the DGGR-based lipase assay vary widely depending on criteria for diagnosing pancreatitis, types of pancreatitis and its severity, and cutoff values of the assays used.\textsuperscript{14-16,23,35} The reported diagnostic sensitivities and specificities to detect pancreatitis in dogs and cats are summarized (Supplementary Tables S1 and S2). Many of these studies have also compared either the correlation or agreement of a DGGR-based lipase assay with other established methods for measuring serum lipase activity (eg, turbidimetry or 1,2-diglyceride-based assays), PLI concentrations (eg, Spec cPL or Spec fPL), or other diagnostic modalities (eg, ultrason or histopathology).\textsuperscript{14,21,35,37,40-42} Caution should be utilized when these results are being interpreted, as correlation is not a suitable statistical analysis for method comparison. Therefore, high agreement does not necessarily mean that the results are interchangeable.

Triolein is another substrate that has been introduced as being more pancreatic lipase specific than traditionally used substrates.\textsuperscript{43} It was optimized for use in veterinary medicine using a proprietary colorimetric dry chemistry method by Fujifilm Corporation and is marketed as v-LiP-P.\textsuperscript{40} This assay is generally available as a point-of-care assay, but some serum chemistry service providers may also offer this assay as a send-out test. Fewer analytical studies are available for the triolein-based lipase assay. One study\textsuperscript{44} has demonstrated good reproducibility for canine samples. While studies on DGGR-based lipase assays have focused on diagnostic test agreement, all of the studies looking at the triolein-based lipase assay have focused on the correlation between results of the triolein and other established methods of measuring serum lipase activity (eg, DGGR and 1-octadecyl 2,3-diacyl glycerol)\textsuperscript{38,40} or PLI concentrations.\textsuperscript{38-40} Good correlations were reported between triolein- and DGGR-based lipase assays in dogs and cats and with Spec cPL in dogs.\textsuperscript{38,40} In one of these studies,\textsuperscript{38} samples that were hemolyzed, icteric, and lipemic were excluded from analysis. This likely affected the results, as the triolein-based lipase assay is influenced by hemolysis and icterus.\textsuperscript{12} This is especially noteworthy, as many patients with pancreatitis are icteric. In dogs, the reported sensitivity and specificity in 1 retrospective study\textsuperscript{44} using a combination of compatible clinical signs, Spec cPL, and ultrasonographic findings for the diagnosis of acute pancreatitis were 100% and 89.5%, respectively (Supplementary Table S1). In cats, the reported sensitivity and specificity in 1 study\textsuperscript{19} using Spec fPL as the gold standard were 66.7% and 93.2%, respectively (Supplementary Table S2). In that study,\textsuperscript{19} the reported Pearson correlation coefficient was 0.70 (n = 138) for triolein-based lipase assay and Spec fPL. When looking at cats with a serum Spec fPL in the normal or gray zone (< 5.4 µg/L), the correlation was −0.0132 (n = 117).\textsuperscript{19} Seven cats with an increased serum lipase activity (> 30 U/L) had a serum Spec fPL that was inconsistent with a diagnosis of pancreatitis (< 3.5 µg/L), indicating that lipases other than pancreatic lipase could have been interfering with the triolein-based lipase assay.\textsuperscript{39} It should be noted that this study, like many other studies on pancreatitis, was based on an imperfect “standard” (Spec fPL) for the diagnosis of pancreatitis; thus, the true positive cats were unknown. To date, no studies have looked at the effects of other lipases on the triolein-based lipase assay.

### Effects of Extrapancreatic Lipases on Analytical Specificity of Lipase Assays

Lipase assays play an important role in the diagnosis or exclusion of pancreatitis in dogs and cats, but clinicians should be cognizant to not interpret results in isolation (Figure 3). In a recent large study,\textsuperscript{45} of 1,360 critically ill dogs admitted to the intensive care unit for various reasons, only 30% of dogs with an increased serum lipase activity (defined as a value of 3 times the upper limit of the reference interval) had a clinical diagnosis of acute pancreatitis. Several studies have reported increased serum lipase concentrations or activities in primary extrapancreatic disorders such as renal, gastrointestinal, cardiac, or endocrine diseases; neoplasia; infections; intervertebral disc disease; and portal hypertension or even chronic use of corticosteroids.\textsuperscript{2,26,45-60}

Although the exact cause for elevated lipase concentrations or activities is unknown, it could be due to several reasons, such as the presence of concurrent subclinical or clinical pancreatitis in study cohorts, either as a comorbidity or sequela of the primary disease of interest. An accurate determination of concurrent pancreatitis, especially in the face of similar clinical signs, is challenging due to the inherent nature of the traditional gold standard (ie, histopathology) for a diagnosis of pancreatitis. These increases could also be due to a true increase of pancreatic lipase in circulation without histopathologic evidence of pancreatitis in the collected samples. The increase of lipase concentration or activity in animals with kidney disease is unclear and requires further study. However, there are studies of both dogs and cats with experimentally induced kidney failure that showed no increases in serum PLI concentrations, suggesting that decreased glomerular filtration rate and decreased urinary clearance alone are unlikely to cause an increase in pancreatic lipase in serum.\textsuperscript{45,51,62} Additionally, endogenous and exogenous interferences can affect analytical specificity of lipase assays. For immunological assays, cross-reactivity against endogenous extrapancreatic lipases potentially expressing similar epitopes could be of concern. As for catalytic assays, substrate specificity and extrapancreatic lipases contributing to serum lipase activity are important considerations.

Although pancreatic lipase accounts for most of the serum lipase activity in healthy animals, extrapancreatic lipases can contribute up to 25% of serum lipase activity, as demonstrated in a study\textsuperscript{63} of
Figure 3 — Decision tree for interpretation of lipase assays in the context of a clinical patient.

**Potential pit-falls:**
- Extrapancreatic lipases (e.g., hepatic lipase or lipoprotein lipase) may influence the results of catalytic lipase assays. The concentrations of extrapancreatic lipases may be altered in various physiologic or non-pancreatic disease states
  - Clinical action: consider submitting an immunologic lipase assay to verify a positive result
- Pancreatic lipase has a short half life – samples should be taken as close to the time of diagnostic imaging as possible.
- Assays that have suboptimal (*) analytic validation should be used with caution
- (*) should not be used to sequentially monitor pancreatic lipase concentration
- (**) do not evaluate in hemolyzed or lipemic serum (common) – assay results are affected

*Due to a lack of analytical validation and clinical data the VCheck PL has been excluded*

Acute pancreatitis may be a primary or secondary disease process. Clinicians should be cognizant to rule out other causes of a patient's clinical signs, which may require alternate treatments.

**Proposed interpretive criteria:**

Criteria for **clinically probable acute pancreatitis**:
- > cut-off value for pancreatic lipase concentration or activity
- ≥ 2 sonographic features of acute pancreatitis (or CTA evidence of acute pancreatitis). ²

Criteria for **clinically suspect acute pancreatitis**:
- > cut-off value for pancreatic lipase concentration or activity + < 2 sonographic features of acute pancreatitis. ²
- Or equivocal pancreatic lipase concentration or activity + ≥ 2 sonographic features of acute pancreatitis
- Clinical action: repeat imaging &/or pancreatic lipase measurement over time is indicated. Sonographic features of acute pancreatitis can lag behind elevated pancreatic lipase increase. Consider pancreatic fine needle aspirate.

**Unlikely to be acute pancreatitis:**
- Within reference interval pancreatic lipase concentration or activity
- < 2 sonographic features of acute pancreatitis
pancreatectomized dogs. The hydrolytic activities of extrapancreatic lipases, such as hepatic lipase, lipoprotein lipase, and carboxyl ester lipase, can lead to the analytical nonspecificity of catalytic assays that measures serum lipase activity. While most lipases have a substrate preference, they often share lipolytic activity for the same substrate. For example, while triglyceride is predominantly hydrolyzed by pancreatic lipase and gastric lipase, it can also be hydrolyzed, to a certain extent, by hepatic, lipoprotein, and carboxyl ester lipase. Therefore, initial catalytic colorimetric lipase assays established with 1,2-diglyceride (one of the hydrolytic products of triglyceride) as a substrate are not specific for pancreatic lipase. The 2 substrates that are said to be more pancreatic lipase specific are DGGR and triolein.

Early studies suggested that DGGR, a synthetic substrate, is specific for the measurement of pancreatic lipase because it is poorly hydrolyzed by many other lipases apart from pancreatic lipase. Subsequently, its analytical specificity for pancreatic lipase was supported by its noninterference by in vitro spiking of samples with carboxyl ester lipase, a lipolytic enzyme commonly released in concert with pancreatic lipase during pancreatitis. However, the specificity of DGGR for pancreatic lipase was questioned when an abstract reported that 69% (33/48) of dogs with EPI had DGGR lipase activity within the reference interval. This suggests that DGGR-based lipase assays can be affected by lipolytic activities of extrapancreatic lipases. The exact sources of lipase activity measured by the DGGR-based lipase assay in these dogs were not further investigated in that abstract. In the absence of pancreatic lipase in EPI patients, gastric lipase secretion can increase 3- to 4-fold to contribute up to 30% of dietary fat digestion. In vitro studies showed that gastric lipase and lipoprotein lipase can hydrolyze DGGR as a substrate. The clinical significance of gastric lipase on the DGGR lipase assay is unclear and warrants further study.

A recent study investigated the effect of heparin-inducible lipases on DGGR-based lipase assays as well as Spec cPL and fPL in 6 dogs and 6 cats. Ten minutes post-heparin injection, plasma lipase activities increased significantly in both dogs and cats, albeit remaining in their respective reference intervals, whereas Spec cPL and fPL concentrations did not change significantly. Hepatic and lipoprotein lipases, displaced by exogenous heparin administration, were most likely responsible for this increase in plasma lipase activity. The exact lipase responsible for this increase was not specifically investigated. A recent study published in abstract format attempted to further evaluate potential impacts of documented hepatic pathology on a DGGR lipase assay. This study reported no relationship between hepatic pathology and DGGR lipase. However, the study design permitted that DGGR lipase results could be used from any time period within 4 months of the postmortem. Because lipase has a relatively short half-life (approx 2 hours), significant changes could have occurred between the time of DGGR measurement and collection of histopathologic samples at necropsy. Studies investigating the clinical significance of hepatic and lipoprotein lipase on lipase assays are currently underway and may lead to caution in interpreting catalytic lipase assays for the diagnosis of pancreatitis.

Since hepatic and lipoprotein lipases share only about 30% amino acid sequence homology with pancreatic lipase, investigators were also interested in the effects of other lipases more closely related to pancreatic lipase on the DGGR-based lipase assays and Spec cPL. Pancreatic lipase–related protein (PLRP) 1 and 2 share a higher amino acid homology of 68% and 65%, respectively, with pancreatic lipase, in addition to having a similar catalytic triad. In 1 abstract, serum samples from dogs were spiked with increasing concentrations of recombinant human PLRP 1 and 2. Serum lipase activity as measured by DGGR-based assays increased up to 10-fold after addition of PLRP2 but not PLRP1. No changes in PLI concentration as measured by Spec cPL were observed with addition of either PLRP1 or 2. Follow-up studies by the authors have shown that PLRP2 is abundantly present not only in the canine pancreas but also in extrapancreatic organs, including the gastrointestinal tract and kidneys. However, at this time it remains unknown to what degree PLRP2 is released into circulation in dogs with gastrointestinal or renal diseases. It is interesting to note that dogs with renal disease or those receiving hemodialysis have increased serum DGGR lipase activity or PLI concentrations. The exact reason for this increase is unknown but has been attributed to concurrent presence of subclinical or clinical pancreatitis. In one of the studies, the increase in DGGR lipase activity was not correlated to the increase in plasma creatinine; thus, reduced GFR is unlikely to be the cause for increased lipase activity in circulation. Furthermore, the interference from heparin-inducible extrapancreatic lipases is considered unlikely, as sodium citrate was the main anticoagulant used for hemodialysis at the institution where this research was conducted.

Triolein is another substrate that is commonly used for measurement of pancreatic lipase activity. Triolein was initially proposed to be more specific because hepatic and lipoprotein lipase activities measured under assay conditions are negligible. Triolein is another substrate that is commonly used for measurement of pancreatic lipase activity. Triolein was initially proposed to be more specific because hepatic and lipoprotein lipase activities measured under assay conditions optimized for pancreatic lipase are negligible. Similar to DGGR-based lipase assays, the analytical specificity of the triolein-based lipase assay was questioned when dogs with EPI were shown to often have a normal serum lipase activity. In that study, only 58% and 78% of dogs with EPI had serum lipase activity within the lower 20% and 25% of the reference interval, respectively. In contrast, 98% and 100% of the same dogs with EPI had Spec cPL concentrations within the lower 20% and 25% of the reference interval, indicating that dogs with EPI have appropriately negligible serum PLI concentrations. Again, this shows that extrapancreatic lipases can hydrolyze triolein, but the source of this extrapancreatic lipase activity when triolein is used as a substrate has not yet been investigated.
Summary

The accurate measurement of pancreatic lipase activity or concentration is an important diagnostic tool for pancreatitis in dogs and cats. The most commonly used lipase assays in veterinary medicine are catalytic colorimetric lipase assays, followed by immunological lipase assay. Extrapancreatic lipases can affect the analytical specificity, thus influencing the diagnostic accuracies of lipase activity assays. The effect of extravapancreatic lipases on catalytic colorimetric lipase assays is not limited to traditionally used substrate (ie, 1,2-diglyceride) but also includes newer substrates, such as DGGR and triolein. Specifically, DGGR can be affected by hepatic lipase, lipoprotein lipase, PLRP2, and gastric lipase. Although the clinical significance of these extravapancreatic lipases is yet unknown, its interference should be considered when results from catalytic colorimetric lipase assays are interpreted. Conversely, immunological lipase assays (ie, cPLI or fPLI) are not affected by these extravapancreatic lipases (ie, hepatic lipase, lipoprotein lipase for cPLI and fPLI, and PLRP2 for cPLI).

Further studies are needed to determine whether more specific substrates are available or the specificity of the current substrates could be further optimized to decrease the impact of extravapancreatic lipases on the diagnostic accuracy of catalytic colorimetric lipase assays for pancreatitis in dogs and cats. Also, specific extravapancreatic diseases affecting lipase assays should be further investigated to determine the exact source of interference.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org