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TRUFORMA® Point-of-Care

Canine Pancreatic Lipase (cPL) Assay

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Key Messages

- Accurate and precise measurement of canine pancreatic lipase (cPL) levels is needed to diagnose and manage pancreatitis in dogs.
- The TRUFORMA® platform uses innovative bulk acoustic wave (BAW) technology to provide a non-optical and fluorescence-free detection system for diagnostic use at the point of care (POC) in veterinary clinics.
- The TRUFORMA cPL assay is a fully quantitative, POC assay that eliminates reference lab testing workflows, including shipping and additional sample handling.
- The dynamic range of the TRUFORMA cPL assay allows the quantification of high and low concentrations of serum cPL without dilution or additional processing steps, which is vital for accurate and complete diagnosis of acute and chronic pancreatitis in canines.
- The high precision and correlation to a reference laboratory assay shown for the TRUFORMA cPL assay provides veterinarians with accurate and reliable diagnostic results at the POC, creating opportunities for improved patient treatment and real-time client communication.

Introduction

Accurately diagnosing pancreatitis in dogs can be extremely challenging in veterinary practice. Histopathology has traditionally been viewed as the gold standard method for diagnosing pancreatitis but is invasive and patient complications are a concern, especially if the animal has relevant comorbidities.[1] Abdominal ultrasound is another option readily available in many general practices, but the clinical utility of this method can be limited by equipment capabilities and/or ultrasonographer experience. A third option, serum-based assays for the detection of canine pancreatic lipase (cPL) have become increasingly common in the veterinary landscape. Current cPL analysis options at the point of care (POC) have been shown to deliver variable performance.[1,2] While reference laboratory testing can provide accurate results, this necessitates shipping samples, and the associated turnaround times can limit early initiation of appropriate treatment. This is especially important for animals presenting with acute abdomen syndrome where delayed decision making can increase the risk for adverse patient outcomes.[3] The TRUFORMA platform, which uses BAW sensor technology, now offers a fully quantitative immunoassay specific for cPL at the POC. Rapid, precise, and accurate measurement of serum cPL levels will aid veterinarians in differentiating healthy dogs from those with pancreatitis.

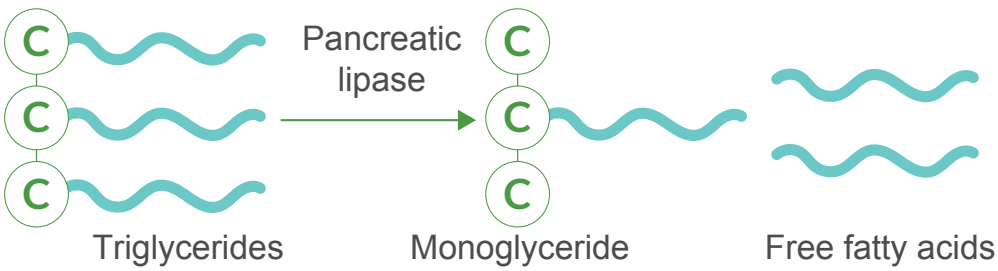
The objectives of this study were to:

- Determine analytical performance attributes for the TRUFORMA cPL assay.
- Describe how the TRUFORMA cPL assay differs from other currently available assays.
- Compare TRUFORMA cPL assay performance with an assay used as part of the standard of care at veterinary diagnostic laboratories to identify canine pancreatitis.

Clinical Significance of Canine Pancreatic Lipase (cPL) Testing

Lipases are a class of enzymes that catalyze the hydrolysis of fats and can be found in various tissues depending on the organism. While certain lipases can be found diffusely, others can be tightly regulated and generated only in specific tissues. Canine pancreatic lipase (cPL) originates solely from pancreatic acinar cells and is vital for the metabolism of dietary fats. cPL is a water-soluble enzyme that is secreted as an inactive molecule along with its cofactor colipase.[4] In the presence of fats, pancreatic lipase binds to colipase and a conformational change occurs, activating the enzymatic function of cPL. Activated pancreatic lipase can hydrolyze dietary triglycerides at their ester bonds to generate free fatty acids that can be absorbed by enterocytes (**Figure 1**).[4]

Figure 1. cPL Enzymatic Activity



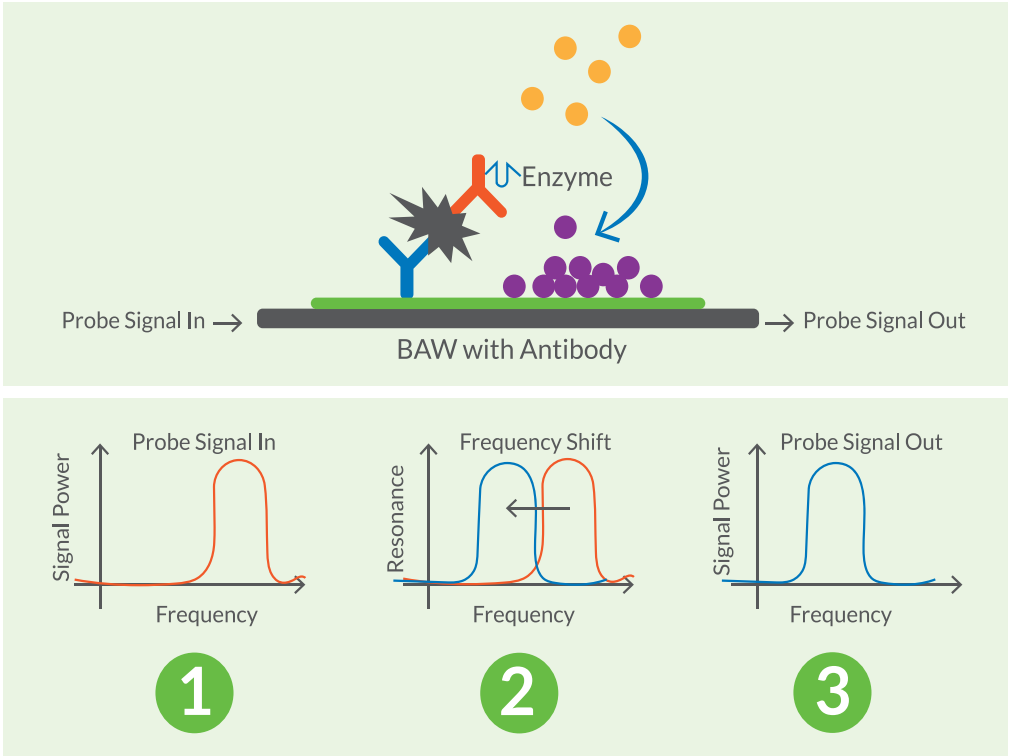
Canine Pancreatic Lipase

Under normal homeostatic conditions, the majority of pancreatic lipase is released from apical pancreatic acinar cells directly into the pancreatic juice while less than 1% is released from basolateral pancreatic acinar cells. In the case of pancreatitis however, apical secretion can be inhibited, and a significant amount of pancreatic lipase may be released from the basolateral side into the vascular space. Therefore, measurement of pancreatic lipase in serum is very specific[5,6] and provides information regarding inflammation or damage of the pancreas[7] and other possible underlying gastrointestinal diseases.

TRUFORMA Platform

The TRUFORMA platform uses BAW sensor technology to provide a non-optical and fluorescence-free detection system for diagnostic use at the POC. BAW technology is extremely reliable and precise, and has been well tested in products across industries such as telecommunications and aerospace. Functionalized BAW sensors consist of multiple resonators, each composed of a piezoelectric material subjected to an electrical field. The resonators can be coated with detection reagents, such as antibodies, for immunoassay testing. Whereas most current enzyme-based immunoassays use optical sensors to detect the generation of luminescence or color change, BAW biosensors used as part of TRUFORMA assays measure both binding events and the insoluble product that is generated by the enzymes that accumulate on the sensor surface, thereby creating a frequency shift in resonance proportional to the mass accumulated on the sensor (**Figure 2**). Veterinary medical professionals were the first to use the BAW sensor technology in a POC diagnostic setting though this technology subsequently received emergency use authorization (EUA) for rapid COVID-19 antigen testing in humans.

Figure 2. BAW Technology in the TRUFORMA cPL Immunoassay

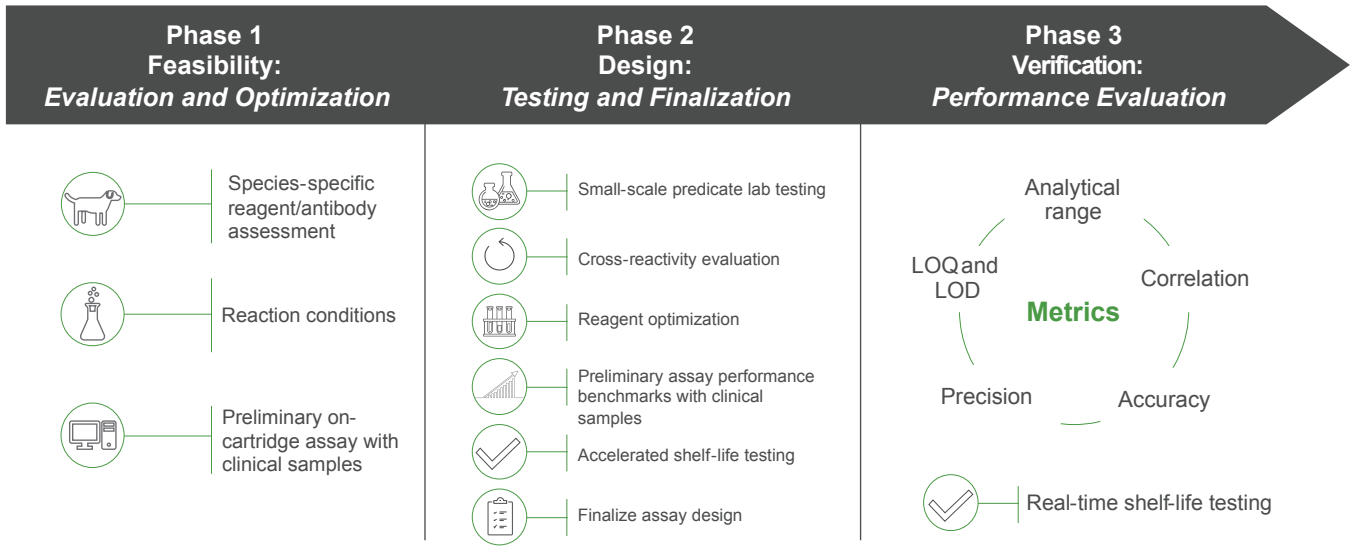


The TRUFORMA cPL assay is a sandwich immunoassay in which the BAW sensor is coated with a monoclonal capture antibody (blue). Antigen present in the sample (gray) binds to a monoclonal detection antibody in solution (orange) and this complex is recognized by the capture antibody on the sensor surface. After several wash steps, an enzyme substrate (yellow) is added and bound enzyme converts the substrate to an insoluble product (purple) that accumulates on the BAW biosensor surface. This is measured as a shift in frequency by the BAW biosensor and the signal is proportional to the amount of analyte present in the sample. BAW, bulk acoustic wave; cPL, canine pancreatic lipase.

cPL Assay Development Overview

The TRUFORMA cPL assay uses a pair of monoclonal antibodies that were selected for optimal performance. Using the industry standard recommendations for bioanalytical method validation[8] and the Clinical and Laboratory Standards Institute (CLSI) guidelines on method comparison and bias estimation,[9] the TRUFORMA assay performance requirements were chosen to meet or exceed reference laboratory performance in order to provide unparalleled performance at the POC. The three phases of the cPL assay development were designed to provide a high-quality and reliable POC assay and included feasibility evaluation and optimization with species-specific assessment, design and testing of preliminary assay performance, and performance verification (**Figure 3**).

Figure 3. Overview of the TRUFORMA cPL Assay Development



cPL, canine pancreatic lipase; LOD, limit of detection; LOQ, limit of quantitation.

Assay Verification Results

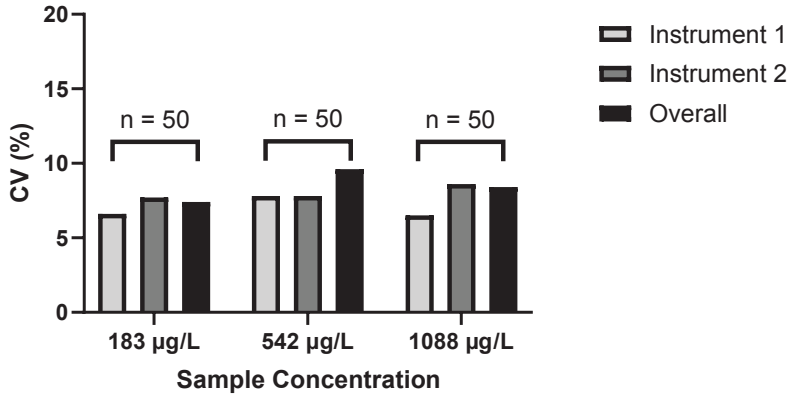
The TRUFORMA cPL assay analytical performance was evaluated and compared with the reference laboratory predicate Spec cPL® Test (Texas A&M School of Veterinary Medicine & Biomedical Sciences, Gastrointestinal Laboratory), a plate-based quantitative enzyme-linked immunosorbent assay (ELISA).[10] Samples from healthy and diseased canines as well as spiked canine serum samples were analyzed.

Analytical Precision

Analytical precision was evaluated by measuring the variability in assay results (between-run percentage coefficient of variation [%CV]) under normal operating conditions in the laboratory. Precision was evaluated by testing three spiked canine samples with varying cPL concentrations. Each sample was tested with five replicates over five separate days on two different instruments, for a total of 150 results. Observed %CV was calculated.

The TRUFORMA cPL assay demonstrated an overall %CV of <10% (**Figure 4**). For each sample, the overall %CV was comparable to the %CV for each instrument, indicating repeatability across instruments. For a ligand-binding assay, a ± 25% between-runs %CV is recommended at the upper and lower limits of the dynamic range while within the range, a quality %CV is ± 20%.[8]

Figure 4. Precision of the TRUFORMA cPL Assay



%CV was calculated for 3 serum samples with varying cPL concentrations using 150 runs. CV, coefficient of variation; cPL, canine pancreatic lipase.

Time to Test Results (TTR), Dynamic Range, and Limit of Quantitation

Time to Test Results (TTR) refers to the amount of time elapsed from TRUFORMA initiating the test to generation of the final result. The POC testing of the TRUFORMA platform provides more timely and actionable results compared to the reference lab, which can improve patient care through faster clinical decision making and communication with pet owners.

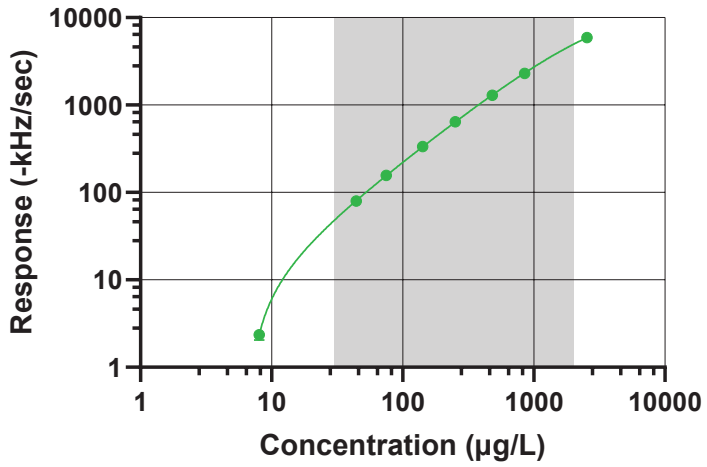
Dynamic range refers to the span of test result values that can be accurately measured by an assay, and limit of quantitation (LOQ) refers to the lowest (lower limit of quantitation [LLOQ]) and highest (upper limit of quantitation [ULOQ]) analyte concentrations that can be reliably detected with predefined accuracy and precision. The LLOQ of the TRUFORMA cPL assay was determined to be 30 µg/L while the ULOQ was established at 2000 µg/L. Overall, the dynamic range of the TRUFORMA cPL assay allows the quantification of clinically high and low cPL concentrations (**Table 1**).

Table 1. Summary of TTR and Dynamic Range for the TRUFORMA cPL Assay Compared with the Spec cPL Test

	TRUFORMA	Spec cPL Test (TX A&M)
TTR	<20.0 Minutes	2 – 3 Days*
Dynamic Range (µg/L)	30 – 2000	30 – 2000

TTR, time to test results; *Turnaround time after receipt of sample as posted on vetmed.tamu.edu

Figure 5. cPL Standard Curve



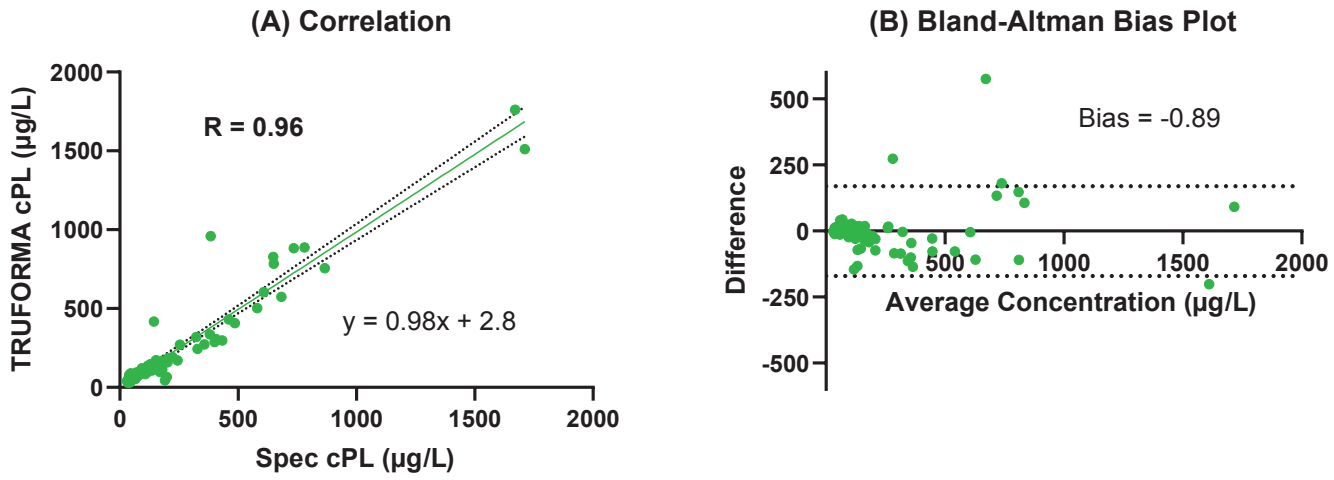
Eight calibrators with known concentrations of cPL were used to generate a standard curve. The shaded region represents the dynamic range of the TRUFORMA cPL Assay. cPL, canine pancreatic lipase.

Eight calibrators with known concentrations of cPL were tested using TRUFORMA cPL assay cartridges. Each calibrator was run with nine replicates across three different instruments, and the average value was used to generate a standard curve. The dynamic range of the TRUFORMA cPL assay illustrates linear performance within the clinically relevant range (**Figure 5**).

Assay Correlation Between the TRUFORMA cPL Assay and Spec cPL Test

Assay correlation and Bland-Altman bias plot analysis evaluate the agreement and commutability of a new test method with a comparative or reference method. A total of 165 individual canine serum samples were run on the same freeze-thaw cycle with the TRUFORMA cPL Assay and the Spec cPL Test and 95 generated results within the dynamic range for both methods. For valid results, each test method reports concentrations based on a standard curve, and the quantitative results were used to generate correlation and bias plots. The TRUFORMA cPL assay showed high correlation (R=0.96) with the Spec cPL Test (**Figure 6A**), while bias analysis depicted scatter with no apparent bias across the dynamic range (**Figure 6B**).

Figure 6. Correlation and Bias of the TRUFORMA cPL Assay Compared to the Spec cPL Test



(A) Correlation studies were performed comparing the results from the TRUFORMA cPL assay and Spec cPL Test using 95 canine serum samples. Dotted lines represent the 95% CI for the linear regression line. (B) A Bland-Altman bias plot was generated by plotting the mean cPL concentration vs. the difference (TRUFORMA - Spec cPL). Dotted lines represent 95% limits of agreement. cPL, canine pancreatic lipase; CI, confidence interval.

Cross-Reactivity

Known amounts of cPL and potential cross-reactants were added to depleted serum and tested in triplicate using the TRUFORMA cPL assay.

No significant cross-reactivity was observed in the TRUFORMA cPL assay, and no cross-reactants interfered with the reported cPL concentrations (**Table 2**).

Table 2. Summary of Cross-Reactivity for the TRUFORMA cPL Assay

Material	Concentration	Cross-Reactivity
Canine Pancreatic Lipase-Related Protein 1 (PNLIPRP1)	1000 µg/L	1.4%
Canine Gastric Triacylglycerol Lipase (LIPF)	2000 µg/L	1.1%

Conclusions

The TRUFORMA cPL assay demonstrated high precision as a POC diagnostic offering, with a dynamic range that permits quantification of cPL levels throughout the range necessary to evaluate canine pancreatitis. The TRUFORMA cPL assay provides veterinarians with accurate and reliable diagnostic results at the POC, allowing for improved client communication and patient treatment.

Acknowledgments

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Abbreviations and Acronyms

BAW	Bulk Acoustic Wave
CLSI	Clinical and Laboratory Standards Institute
cPL	Canine Pancreatic Lipase
CV	Coefficient of Variation
ELISA	Enzyme-Linked Immunosorbent Assay
EUA	Emergency Use Authorization
LLOQ	Lower Limit of Quantitation
LOD	Limit of Detection
LOQ	Limit of Quantitation
POC	Point of Care
TTR	Time to Test Results
ULOQ	Upper Limit of Quantitation

Figure Citations

Figure 1: Mhatre, Sveeta & Bhagit, Amita & Yadav, Raman. (2016). Pancreatic Lipase Inhibitor from Food Plant: Potential Molecule for Development of Safe Anti-obesity Drug. *MGM j. med. sci.* 3. 34-41. 10.5005/jp-journals-10036-1084.

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