ZOMEDICA®

TRUFORMA® Point of Care

Canine NT-proBNP Assay

Senthuran Atputhanathan, Sr. Research and Development Scientist, Zomedica

T. J. Barclay, DVM, Senior Professional Services Veterinarian, Zomedica

MaryJane Been, Research and Development Scientist, Zomedica

Key Messages

- Accurate and precise measurement of endogenous canine N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels is essential for assessing cardiac health in dogs. Produced by the heart's ventricles in response to increased wall stress, elevated NT-proBNP levels serve as an indicator of heart disease or heart failure.
- The TRUFORMA platform employs innovative bulk acoustic wave (BAW) technology to deliver a non-optical, fluorescence-free detection system for point-of-care (POC) diagnostics in veterinary clinics.
- The dynamic range of the TRUFORMA platform for the canine NT-proBNP assay allows for the quantification of varying concentrations of NT-proBNP in plasma or serum, aiding in the assessment of heart disease likelihood. Low NT-proBNP levels suggest a low likelihood of heart disease, while intermediate levels point to a possible presence of heart disease, requiring further evaluation. High NT-proBNP levels indicate a strong likelihood of heart disease, necessitating additional diagnostics to classify the condition.
- The canine NT-proBNP assay enables the quantification of NT-proBNP levels in plasma or serum at the POC without dilution or additional processing, allowing veterinarians to reduce processing time and focus on diagnosing conditions like myxomatous mitral valve disease (MMVD) and dilated cardiomyopathy (DCM).
- NT-proBNP measurement aids veterinarians in differentiating between cardiac and respiratory origins of symptoms like coughing or labored breathing. Furthermore, NT-proBNP testing can be valuable during routine wellness checks, especially for dog breeds with a genetic predisposition to heart conditions.
- The TRUFORMA canine NT-proBNP assay demonstrates high precision and strong correlation with reference laboratory assays, offering veterinarians accurate and reliable diagnostic results at the POC. This enables better treatment decisions and facilitates real-time communication with clients.

Introduction

Advancements in diagnostic technology have revolutionized veterinary care, enabling more efficient and timely assessments of animal health. POC testing platforms are increasingly essential in providing rapid diagnostic results directly at the site of patient care, reducing the time between sample collection and clinical decision-making. One such breakthrough in POC diagnostics is the use of BAW sensor technology, which offers a non-optical, fluorescence-free detection system capable of delivering high precision and sensitivity. This innovative approach is particularly valuable for assessing biomarkers such as NT-proBNP, a critical indicator of cardiac health in canine patients. The ability to measure NT-proBNP levels accurately at the POC can assist veterinarians in diagnosing heart disease and heart failure, offering the potential for improved patient outcomes through faster diagnosis and intervention. The integration of BAW sensor technology in the TRUFORMA platform provides a promising solution to meet these needs, enhancing the efficiency of veterinary care.

The objectives of this study were to:

- Determine the analytical performance attributes of the TRUFORMA Canine NT-proBNP assay.
- Describe the distinctions between the TRUFORMA Canine NT-proBNP assay and other currently available assays.
- Compare the performance of the TRUFORMA Canine NT-proBNP assay with that of an assay used as part of the standard of care in veterinary diagnostic laboratories for diagnosing canine heart disease.

Clinical Significance of Canine NT-proBNP

Canine NT-proBNP is a biomarker with significant clinical relevance for diagnosing and managing heart disease in dogs. It is produced by the heart's ventricles in response to increased wall stress, making it a useful indicator of cardiac dysfunction. Elevated NT-proBNP levels are commonly associated with conditions such as myxomatous mitral valve disease (MMVD) and dilated cardiomyopathy, which are prevalent in certain dog breeds. Measurement of NT-proBNP concentrations allows veterinarians to distinguish between cardiac and non-cardiac causes of symptoms such as coughing, difficulty breathing, and exercise intolerance. Additionally, NT-proBNP testing can aid in monitoring the progression of heart disease, guiding treatment decisions, and assessing the effectiveness of therapeutic interventions. By providing a non-invasive and reliable tool for early detection of cardiac dysfunction, NT-proBNP testing enhances the overall management of canine heart disease, improving patient outcomes and quality of life.

Canine NT-proBNP Testing

Diagnostic Applications:

- Differentiation of Cardiac and Respiratory Conditions: Elevated NT-proBNP levels are associated with cardiac diseases, while normal or low levels suggest respiratory causes when accompanied by symptoms such as coughing and difficulty breathing. This distinction aids in accurate diagnosis and treatment planning.
- Monitoring Disease Progression: Serial NT-proBNP measurements can track disease progression in conditions like MMVD, assisting in predicting the risk of congestive heart failure (CHF) development.

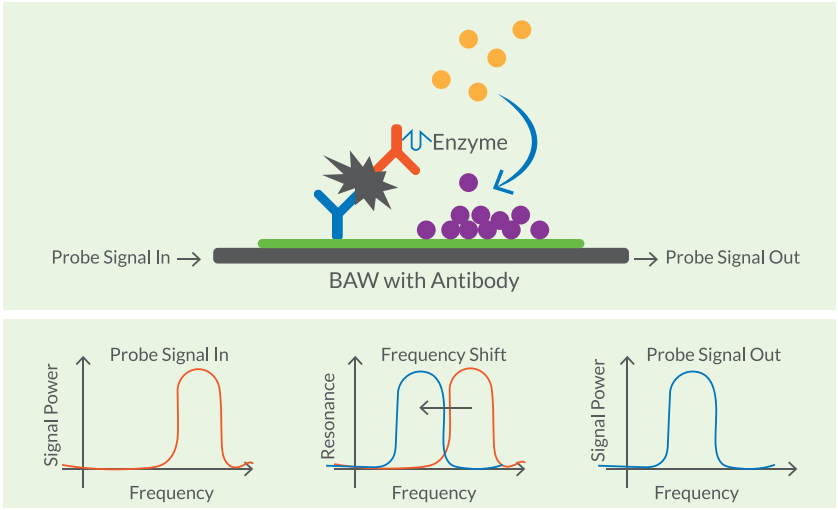
Clinical Considerations:

- Breed-Specific Variations: Certain breeds, such as Labrador Retrievers, Newfoundlands, and Greyhounds, may exhibit false positives due to breed-specific variations.[1,2,3] Additionally, kidney disease can influence NT-proBNP levels, necessitating careful interpretation of results.[4]
- Complementary Use: NT-proBNP testing should be used in conjunction with other diagnostic tools, such as thoracic radiographs and echocardiography, to provide a comprehensive assessment of cardiac health.

TRUFORMA PLATFORM

The TRUFORMA platform uses BAW sensor technology to provide a non-optical and fluorescence-free detection system for diagnostic use at the point of care. 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 biological detection reagents such as antibodies or nucleic acids for immunoassay and molecular testing, respectively. 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 1). 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 1. BAW Technology in the TRUFORMA canine NT-proBNP Immunoassay

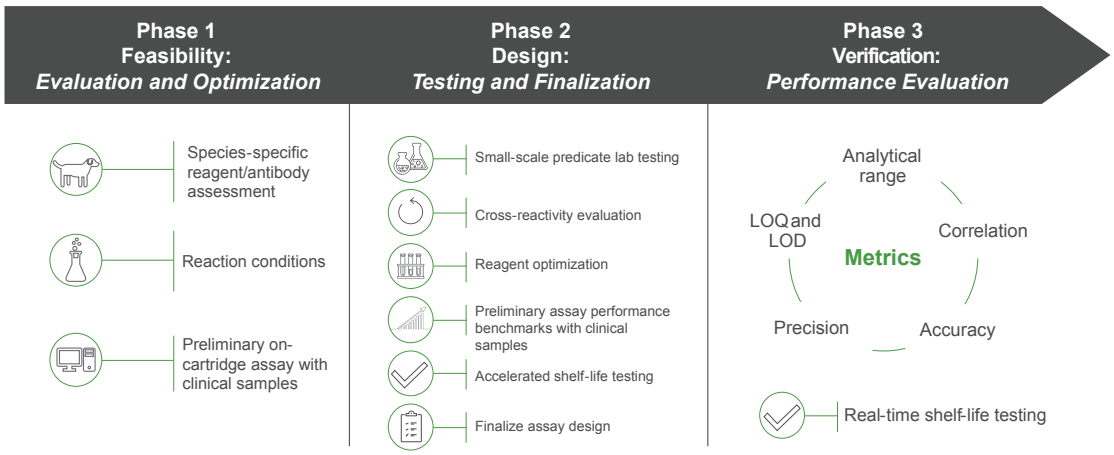


The TRUFORMA canine NT-proBNP assay is a sandwich immunoassay in which the BAW sensor is coated with a monoclonal capture antibody (blue). The antigen, canine NT-proBNP, present in the sample (gray), binds to the monoclonal detection antibody in solution (orange), and this complex is recognized by the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

Canine NT-proBNP Assay Development Overview

The TRUFORMA Canine NT-proBNP assay utilizes an antibody pair specifically selected for its non-overlapping epitopes, ensuring precise detection of the target biomarker. Furthermore, conducting TRUFORMA testing at the POC reduces issues associated with canine NT-proBNP degradation. Assay performance requirements were defined using industry-standard recommendations for bioanalytical method validation and the Clinical and Laboratory Standards Institute (CLSI) guidelines on method comparison and bias estimation (EP09c), ensuring the assay meets or exceeds the capabilities of reference laboratories and delivers exceptional performance at the POC.[5,6] The assay development process encompassed three key phases to create a high-quality and dependable POC diagnostic tool: feasibility evaluation and optimization with species-specific assessments, the design and testing of preliminary performance, and analytical performance verification (**Figure 2**).

Figure 2. Overview of the TRUFORMA Canine NT-proBNP Assay Development



LOD, limit of detection; LOQ, limit of quantitation.

Assay Verification Results

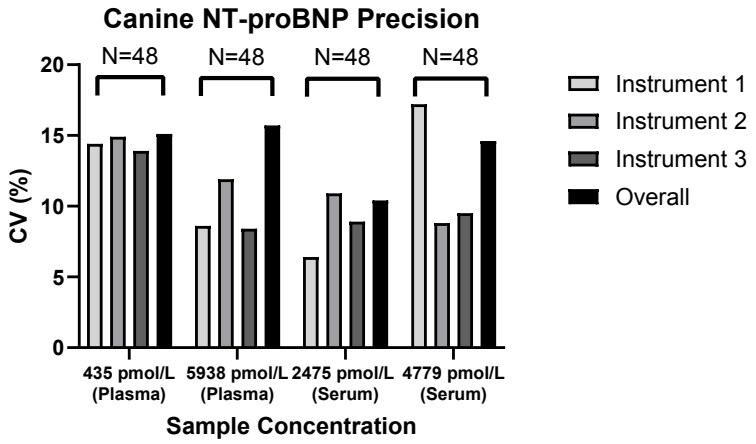
The analytical performance of the TRUFORMA Canine NT-proBNP assay was evaluated in comparison to the CardioPet proBNP Test performed at the IDEXX reference laboratory. Samples from both healthy and diseased dogs were utilized for testing, with matched EDTA plasma and serum samples analyzed independently. Each matrix was compared separately with the predicate assay to assess the overall agreement between the TRUFORMA Canine NT-proBNP assay and the reference laboratory.

Analytical Precision

Analytical precision was assessed 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 two canine EDTA plasma samples and two canine serum samples, each with varying NT-proBNP concentrations, for a total of four samples. Each sample was tested in twelve replicates on four separate days using three

different instruments, resulting in 48 measurements for each sample. The TRUFORMA canine NT-proBNP assay demonstrated an overall %CV of <20% (**Figure 3**). 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 a quality %CV of ±20% is expected within the assay range.[5]

Figure 3. Precision of the TRUFORMA Canine NT-proBNP Assay



%CV was calculated for two separate canine EDTA plasma and serum samples with varying canine NT-proBNP concentrations, totaling 96 runs per matrix. One statistical outlier was removed from the plasma sample dataset at 5938 pmol/L, following CLSI EP05-A3 guidelines.[7]

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

Time to Test Results (TTR) refers to the amount of time elapsed from initiating the TRUFORMA test to generation of the final result. The POC testing of the TRUFORMA platform provides more timely and actionable results compared to sending to a reference lab, which can improve patient care through faster clinical decision making and communication with pet owners (**Table 1**). 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 metrics. The LLOQ of the TRUFORMA Canine NT-proBNP assay was determined to be 100 pmol/L and the ULOQ was established at 10,000 pmol/L (**Table 1**). Overall, the dynamic range of the TRUFORMA canine NT-proBNP assay allows the quantification of clinically high and low canine NT-proBNP concentrations.

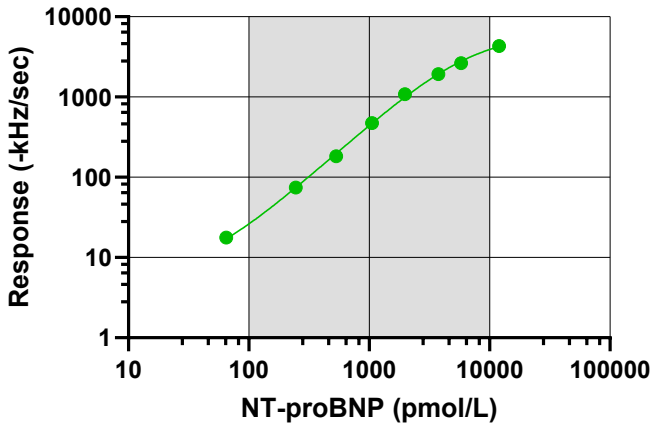
Table 1. Summary of TTR and Dynamic Range for the TRUFORMA Canine NT-proBNP test

	TRUFORMA	CardioPet (IDEXX)
TTR	< 16.0 Minutes	1 - 2 Days*
Dynamic Range (pmol/L)	100 – 10,000	250 – 10,000

*Does not include shipping time to reference laboratory
TTR Time to Test Results

Nine calibrators with known canine NT-proBNP concentrations were tested using TRUFORMA Canine NT-proBNP assay cartridges. Each calibrator was run in eight replicates across eight instruments, with the average value used to generate a standard curve. Separate standard curves were generated for plasma and serum samples. The reportable ranges of the TRUFORMA canine NT-proBNP assay demonstrate linear performance within the clinically relevant range for both plasma and serum (**Figure 4**).

Figure 4. Standard Curve of the TRUFORMA Canine NT-proBNP Assay



Nine calibrators with known concentrations of canine NT-proBNP were used to generate a standard curve for canine analysis. A representative standard curve for plasma is shown here. The shaded region represents the reported dynamic range of the TRUFORMA Canine NT-proBNP Assay for canine EDTA plasma and serum samples.

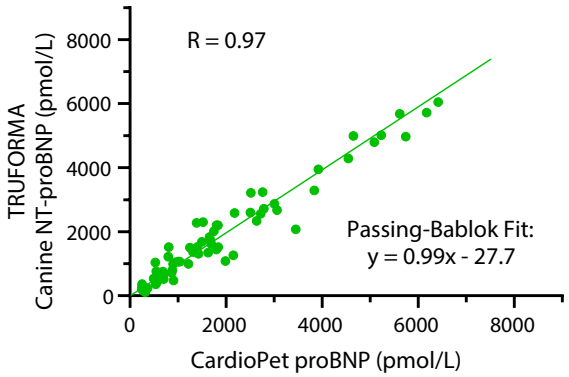
Assay Correlation Between the TRUFORMA Canine NT-proBNP and CardioPet proBNP Assays

Passing-Bablok and Bland-Altman bias plot analysis were used to evaluate the agreement and commutability of the TRUFORMA Canine NT-proBNP assay with the comparative IDEXX CardioPet test. A total of 70 canine K_2 EDTA plasma samples and 71 serum samples were run on the same freeze-thaw cycle using both the TRUFORMA and CardioPet tests. The instruments report concentrations based on their respective standard curves, and these results were used to generate correlation and bias plots. One sample reading <100 pmol/L on TRUFORMA was removed from the analysis.

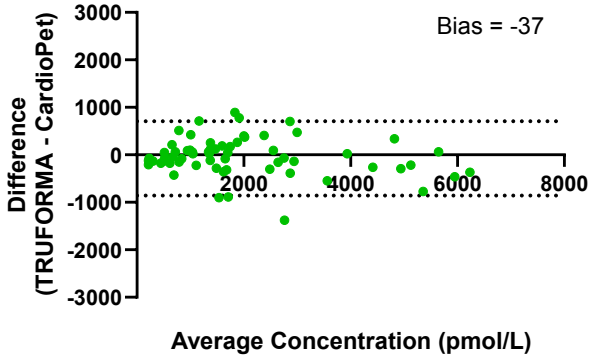
The TRUFORMA Canine NT-proBNP assay demonstrated high correlation with the CardioPet assay with both plasma (**Figure 5A**; R=0.97) and serum (**Figure 5C**; R=0.95) samples. Bias analysis revealed scatter with no apparent bias within the dynamic range, which is relevant for clinical decision-making regarding potential MMVD and predicting the risk of CHF development (**Figures 5B and 5D**).

Figure 5. Correlation and Bias of the TRUFORMA Canine NT-proBNP Assay Compared to the IDEXX CardioPet Canine NT-proBNP Assay

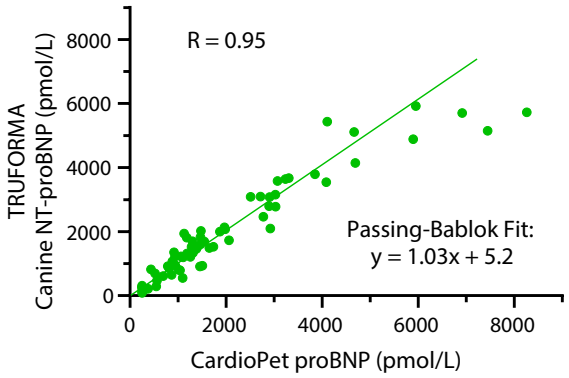
(A) Plasma Correlation



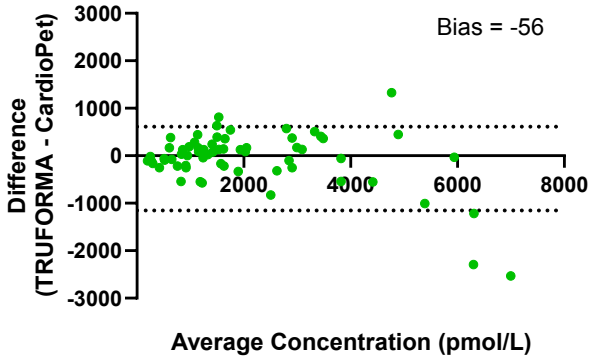
(B) Plasma Bland-Altman Bias Plot



(C) Serum Correlation



(D) Serum Bland-Altman Bias Plot



Correlation analysis was performed by comparing the results from the TRUFORMA Canine NT-proBNP and IDEXX CardioPet assays using 70 canine plasma samples (A) and 71 canine serum samples (C). Bland-Altman bias plots were generated by plotting the mean concentrations against the percentage difference (TRUFORMA – CardioPet) for canine plasma (B) and serum samples (D). Dotted lines represent the 95% limits of agreement.

Interfering Substances

Known amounts of canine NT-proBNP at approximately 2000 pmol/L was combined with known amount of an endogenous interfering substance and tested in six replicates using the TRUFORMA Canine NT-proBNP assay. The following endogenous interfering substances were tested: 37 mmol/L Triglycerides, 342 µmol/L Bilirubin (unconjugated), 342 µmol/L Bilirubin (conjugated), 2 g/L hemoglobin. No endogenous interfering substances tested had an impact to the results outside the precision of the assay for both EDTA plasma and serum samples.

Conclusions

The TRUFORMA Canine NT-proBNP assay demonstrated high precision across a wide dynamic range, ensuring the reliability of canine NT-proBNP results at the point of care (POC) in under 16 minutes. The dynamic range allows for the quantification of NT-proBNP at clinical decision points essential for diagnosing conditions such as MMVD and DCM. Optimized for canine EDTA plasma and serum samples at the POC, the TRUFORMA Canine NT-proBNP assay enables immediate testing without the need for significant sample handling or shipping, which can delay results and the timely confirmation of disease or treatment. Additionally, offering testing in both plasma and serum matrices provides greater flexibility for veterinarians.

By providing accurate and reliable diagnostic results at the POC in under 16 minutes, the TRUFORMA Canine NT-proBNP assay empowers veterinarians to make more rapid, informed patient diagnoses, improving treatment outcomes and enhancing communication with clients.

Abbreviations and Acronyms

BAW	Bulk Acoustic Wave
CHF	Congestive Heart Failure
CLSI	Clinical and Laboratory Standards Institute
CV	Coefficient of Variation
EUA	Emergency Use Authorization
LLOQ	Lower Limit of Quantitation
LOD	Limit of Detection
LOQ	Limit of Quantitation
MMVD	Myxomatous mitral valve disease
NT-proBNP	N-terminal pro-B-type natriuretic peptide
POC	Point of Care
TTR	Time to Test Results
ULOQ	Upper Limit of Quantitation

References

1. Viera de Lima G and de Silveira Ferreira F. N-terminal-pro brain natriuretic peptides in dogs and cats: A technical and clinical review. *Vet World.* 10: 1072-1082 2017.
2. Gomart S, Allaway D, Harrison M, Dickson D, Seo J, Ferasin L, Payne JR, Hezzell MJ, Borgeat K. Long-term biological variability and the generation of a new reference interval for plasma N-terminal pro-B-type natriuretic peptide in Labrador retrievers. *J Small Anim Pract.* 2020 Jun;61(6):368-373.
3. Sjöstrand K, Wess G, Ljungvall I, Häggström J, Merveille AC, Wiberg M, Gouni V, Lundgren Willesen J, Hanås S, Lequarré AS, Mejer Sørensen L, Wolf J, Tiret L, Kierczak M, Forsberg S, McEntee K, Battaille G, Seppälä E, Lindblad-Toh K, Georges M, Lohi H, Chetboul V, Fredholm M, Höglund K. Breed differences in natriuretic peptides in healthy dogs. *J Vet Intern Med.* 2014 Mar-Apr;28(2):451-7.
4. Miyagawa Y, Tominaga Y, Toda N, Takemura N. Relationship between glomerular filtration rate and plasma N-terminal pro B-type natriuretic peptide concentrations in dogs with chronic kidney disease. *Vet J.* 2013 Aug;197(2):445-50.
5. US Food and Drug Administration. Bioanalytical method validation. 2018.
6. CLSI. Measurement procedure comparison and bias estimation using patient samples. 3rd ed. CLSI guideline EP09c. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
7. CLSI. Evaluation of Precision of Quantitative Measurement Procedures. 3rd ed. CLSI guideline EP05-A3.