TRUFORMA®

Canine NT-proBNP Test

FOR THE QUANTITATIVE
DETECTION OF NT-proBNP
IN CANINE EDTA
PLASMA AND SERUM

For Veterinary Use Only

READ ALL INSTRUCTIONS
BEFORE BEGINNING THE ASSAY

For Customer Assistance:
734-369-2555 (option 1)

Distributed and manufactured by:
Zomedica
Ann Arbor, MI 48108 USA
www.zomedica.com

INTENDED USE

The TRUFORMA® NT-proBNP Test is used for the quantitative detection of NT-proBNP levels in canine EDTA plasma or serum to aid in the diagnosis of cardiac disease.

OVERVIEW

NT-proBNP is a polypeptide hormone produced primarily by the cardiac ventricles in response to increased mycardial strain. It serves as a biomarker for cardiac function and elevated levels can be indicative of conditions such as congestive heart failure, assisting in the diagnosis and management of cardiac conditions in dogs.

This test employs a single-use cartridge containing an assay for the detection of NT-proBNP in canine plasma or serum. The TRUFORMA NT-proBNP Test is a sandwich immunoassay utilizing a biotin-labeled anti-NT-proBNP monoclonal detection antibody (dAb) and a monoclonal capture antibody immobilized onto a Bulk Acoustic Wave (BAW) sensor solid phase. During testing, the plasma or serum sample is mixed with dAb allowing the detection antibodies to bind to NT-proBNP molecules. This complex then flows across the biosensor surface, where the immobilized capture antibody binds the dAb-NT-proBNP complex to the sensor surface.

Next, a streptavidin-enzyme conjugate is introduced, binding to the biotin-labeled detection antibody through a streptavidin-biotin interaction. Following several wash steps, an enzyme substrate is exposed to the BAW biosensor surface. The enzyme converts the substrate into an insoluble product that accumulates on the BAW biosensor and is measured as a frequency shift by the sensor. The signal intensity, proportional to the amount of enzyme on the sensor, corresponds to NT-proBNP concentration in the sample and is calculated using a stored calibration curve.

In this sandwich immunoassay, higher NT-proBNP concentrations in the plasma or serum sample produce a higher dAb-biotin-streptavidin-enzyme complex accumulation on the solid phase, resulting in a stronger signal. Thus, plasma or serum NT-proBNP concentrations are proportional to the amount of NT-proBNP bound to the solid phase, with abnormal concentrations potentially indicating cardiac dysfunction or disease in canines.

PRECAUTIONS & WARNINGS

- Do not use a cartridge after the expiration date printed on the cartridge pouch.
- Do not remove the cartridge from pouch until ready for use.
- Use a new disposable bulb pipette for each test.
- Do not use a cartridge from a pouch that appears to be tampered with, torn or damaged.
- Do not use a cartridge if it appears to be cracked, broken, leaking liquid or otherwise damaged.
- For veterinary in vitro diagnostic use of canine plasma and serum samples only.
- As with any diagnostic test procedure, all other test procedures including the clinical status of the patient should be considered prior to final diagnosis.

STORAGE & HANDLING

- Store cartridges at refrigerated temperature (2-8 °C / 35-46 °F).
- Do not freeze cartridges.
- Plasma or serum samples may be stored under refrigerated conditions (2-8 °C / 35-46 °F) for up to 3 days prior to testing.
- Cartridges may be left at room temperature (20-25 °C / 68-77 °F) for up to 24 hours in the protective pouch, and up to 4 hours out of the protective pouch prior to use. If not used in this time period, dispose of the cartridge.

COMPONENTS

1. NT-proBNP Cartridge(s)
2. Disposable Bulb Pipette(s)
3. Instructions for Use

SAMPLE TYPE

Plasma or serum samples can be used with the TRUFORMA Canine NT-proBNP Test. The NT-proBNP peptide is more stable in EDTA plasma than serum, therefore, EDTA plasma is the preferred sample type.

PLASMA COLLECTION

Draw blood into K_2 EDTA blood collection tubes. After collection, gently invert the tube 8-10 times and separate plasma by centrifugation.

The TRUFORMA NT-proBNP Test has been tested with K_2 EDTA tubes and has not been tested with all types of blood collection tubes. Results may vary depending on the tube used due to the presence of additives such as clot activators, gels, or preservatives.

SERUM COLLECTION

Collect blood in blood collection tubes without anticoagulant. After complete clotting has occurred, separate serum by centrifugation. The TRUFORMA NT-proBNP Test has been tested with blood tubes without additives and has not been evaluated with all types of blood collection tubes. Results may vary depending on the tube used due to the presence of additives such as clot activators, gels, or preservatives.

TEST PROCEDURE

1. Confirm that the cartridge has not expired by reviewing the expiration date on the pouch label.

2. While wearing gloves, remove the cartridge from pouch and place on a flat surface. The pouch contains a desiccant that can be disposed of as normal waste.

3. Using the supplied bulb pipette, dispense the sample into the sample port (approximately 150 µL). Recommended technique for adding sample: With sample-filled bulb pipette (**Figure 1**), wet the sample port filter by placing the bulb pipette tip on top of the sample port filter and lightly squeeze the pipette (**Figure 2**), dispensing a small amount of sample. Lift bulb pipette tip off sample port filter and dispense remaining sample into sample port as shown in **Figure 3.**

4. Ensure all of the sample has been absorbed into the sample port with no pooling. Sample pooling in the port may be an indicator that the port has been overfilled. Do NOT overfill the sample port.

5. Place the cartridge into the instrument by holding the sample port end with the cartridge label facing up and following the arrow indicated insert direction displayed on the cartridge label. Gently push the cartridge into the cartridge receiver until the instrument accepts and draws in the cartridge.

6. After the test is completed, the results will be displayed on the TRUFORMA system results screen and will also be electronically sent to myZomedica.com.

7. Dispose of the used cartridges and bulb pipettes in a biohazardous waste container.

PERFORMANCE DATA

Test results are reported in picomoles per liter (pmol/L) and unless otherwise noted, all data were generated using canine plasma samples collected in K_2 EDTA tubes or canine serum samples collected in red top tubes without gel barriers. The dynamic ranges for the TRUFORMA NT-proBNP Test are found in **Table 2** and additional performance metrics are detailed in the TRUFORMA NT-proBNP Assay White Paper available on myZomedica.com.

USING BULB PIPETTE

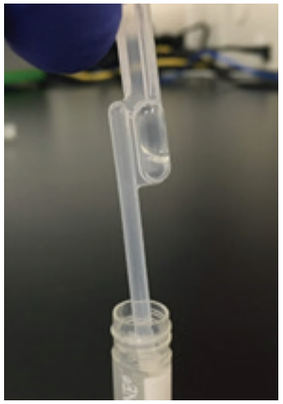


Figure 1

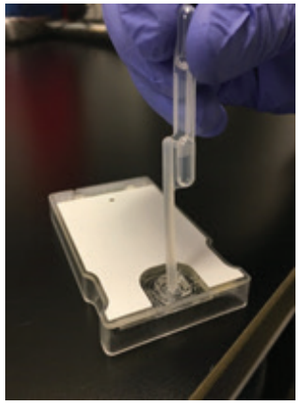


Figure 2

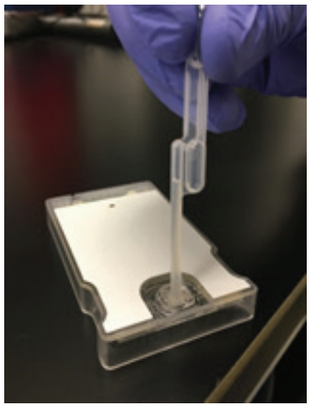


Figure 3

REFERENCE RANGES

The reference ranges listed in **Table 1** are those established specifically for canine patient populations. Test results should be interpreted in conjunction with the patient's clinical signs for an accurate assessment.

Table 1: Canine NT-proBNP Reference Range

TRUFORMA Canine NT-proBNP (pmol/L)	Interpretation
<900	Low likelihood of heart disease. Clinical signs are likely non-cardiac in origin.
900–1,800	Possible heart disease. Further diagnostics recommended to confirm.
>1,800	Probable heart failure. Further diagnostics indicated to classify disease

Cutoff values lower than those listed above are recommended for screening Doberman Pinschers for preclinical dilated cardiomyopathy.

Table 2: Canine NT-proBNP Dynamic Range

	TRUFORMA
LLOQ (pmol/L)	100
ULOQ (pmol/L)	10,000

Figure 4: Correlation of TRUFORMA Canine NT-proBNP with Comparative Method using EDTA Plasma Samples

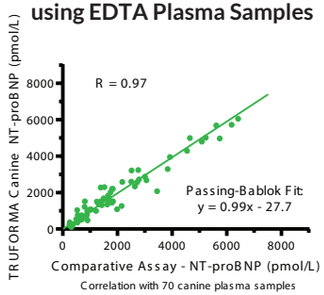
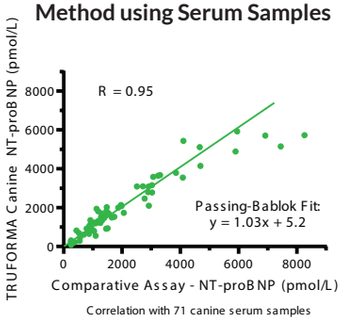


Figure 5: Correlation of TRUFORMA Canine NT-proBNP with Comparative Method using Serum Samples



PRECISION

Precision was evaluated by testing two canine 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. Plasma samples had mean NT-proBNP concentrations of 435 pmol/L and 5938 pmol/L and gave CVs of 15.4% and 16.2% respectively. Serum samples had a mean NT-proBNP concentration of 2475 pmol/L and 4779 pmol/L and gave CVs of 10.7% and 14.9% respectively.

CORRELATION

Correlation analysis assesses the agreement and commutability between a new test method and a comparative or reference method. For this study, NT-proBNP levels were measured in canine plasma (**Figure 4**) and serum samples (**Figure 5**) using both the TRUFORMA and a comparative assay. All samples underwent the same freeze-thaw cycle prior to analysis on both platforms, ensuring consistency. The results were then used to generate correlation statistics, as illustrated in **Figures 4** and **5**.

The TRUFORMA system displays NT-proBNP concentrations for plasma or serum samples, and interpretation guidelines for test results are available at myZomedica.com.

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