TRUFORMA®

eACTH Test
Instructions for Canine Use

FOR THE QUANTITATIVE DETECTION OF ENDOGENOUS ADRENOCORTICOTROPIC HORMONE (eACTH) LEVELS IN CANINE PLASMA

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® eACTH Test is used for the quantitative detection of endogenous adrenocorticotropic hormone (eACTH) levels in canine and equine plasma to assess adrenal function. This document provides instructions for use with canine plasma.

OVERVIEW

eACTH is a hormone that is produced by the pituitary gland that stimulates adrenal gland production and release of cortisol into the blood.

This test employs a single-use cartridge containing the assay for the detection of eACTH in canine plasma. The TRUFORMA eACTH Test works as a sandwich immunoassay, utilizing a biotin-labeled anti-ACTH detection antibody (dAb) and a capture antibody immobilized onto a Bulk Acoustic Wave (BAW) sensor solid phase. During the test, the sample is diluted and flows across the biosensor surface. eACTH molecules present in the sample bind to the sensor surface. A biotin labeled detector antibody then flows across the surface and binds to any immobilized eACTH. Next, a streptavidin-enzyme conjugate is flowed over the sensor surface, where it is immobilized to the detector antibody via the streptavidin-biotin interaction. After 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 surface and is measured as a shift in frequency by the BAW biosensor. The signal is proportional to the amount of enzyme present on the BAW biosensor surface. The change in frequency is used to calculate the concentration of eACTH present in the sample using the stored calibration curve. As a sandwich immunoassay, a sample containing a high concentration of eACTH results in a higher amount of dAb-biotin-streptavidin-enzyme complex attached to the solid phase and a larger signal. Thus, concentrations of plasma eACTH are proportional to the amount of eACTH that is bound to the solid phase.

eACTH concentrations that fall outside of the normal reference range may be indicative of abnormal levels of eACTH as a result of an endocrine disorder.

PRECAUTIONS & WARNINGS

- Do not use the 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 the cartridge if it appears to be cracked, broken, leaking liquid or otherwise damaged.
- For veterinary in vitro diagnostic use of canine and equine plasma samples only.
- Use only K_2 EDTA plasma collection tubes for sample collection.
- 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 samples may be stored under refrigerated conditions (2–8 °C/35–46 °F) for up to 12 hours prior to testing.
- Plasma samples should be stored in plastic containers, not glass.
- 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. eACTH Cartridge(s)
2. Disposable Bulb Pipette(s)
3. Instructions for Use

PLASMA COLLECTION

Draw blood into K_2 EDTA blood collection tube. After collection, invert the tube up to 10 times and separate plasma by centrifugation. The eACTH molecule degrades rapidly when left at room temperature – refrigerate or test plasma immediately after collection.

The TRUFORMA eACTH Test has been tested with purple top 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.

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.

USING BULB PIPETTE

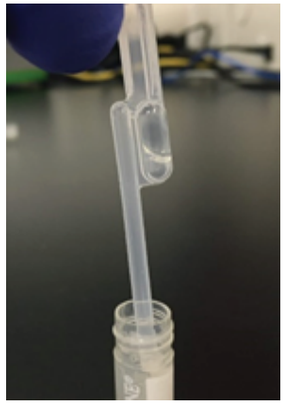


Figure 1

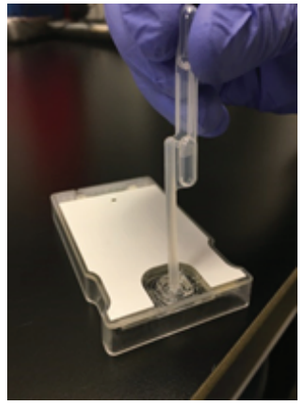


Figure 2

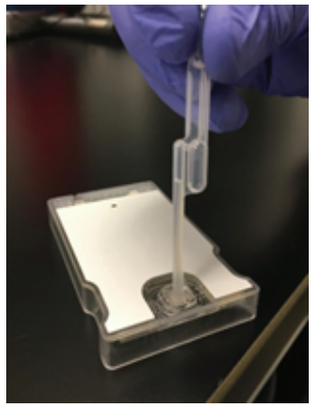


Figure 3

EXPECTED VALUES

The reference ranges listed in **Table 1** are those established for the canine patient population. Test results should be interpreted in conjunction with a patient's clinical signs.

Table 1: Reference Range

	TRUFORMA Canine
Reference Range (pg/mL)	**10.5 – 117.6**

Table 2: Dynamic Range

	TRUFORMA Canine
Dynamic Range (pg/mL)	**5.0 – 700.0**

Figure 4: Precision of Canine TRUFORMA eACTH

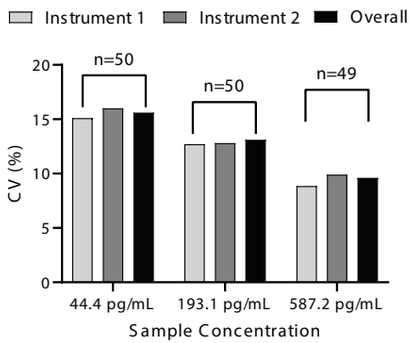
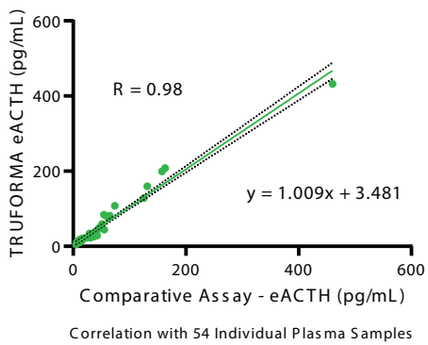


Figure 5: Correlation of Canine TRUFORMA eACTH with Comparative Method



Correlation with 54 Individual Plasma Samples

PERFORMANCE DATA

See Tables and Figures for data representative of the test's performance. Test performance results are reported in picograms per milliliter (pg/mL). Unless otherwise noted, all data were generated using canine plasma samples collected in purple top K_2 EDTA tubes. All performance data were generated on the TRUFORMA system unless otherwise noted. The dynamic range for the TRUFORMA eACTH Test is found in **Table 2**.

PRECISION

Precision was evaluated by testing three canine plasma samples, each with varying eACTH concentrations. Each sample was tested in five replicates on five separate days using two different instruments for a total of 149 results **(Figure 4)**.

CORRELATION

Correlation analysis evaluates the agreement and commutability of a new test method with a comparative or reference method. eACTH levels were measured in 54 canine plasma samples using TRUFORMA and a comparative assay **(Figure 5)**. All samples were analyzed on the same freeze-thaw cycle for both methods, and the results were used to generate correlation statistics in **Figure 5.**

The TRUFORMA system will display the eACTH plasma concentration results. Interpretation guidelines for test results are available on myZomedica.com.

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