TRUFORMA®

Equine Insulin Test

FOR THE QUANTITATIVE
DETECTION OF INSULIN
IN EQUINE EDTA 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
Plymouth, MN 55447 USA
www.zomedica.com

INTENDED USE

The TRUFORMA® Insulin Test is used for the quantitative detection of insulin levels in equine plasma to screen for insulin dysregulation (ID).

OVERVIEW

Insulin is a polypeptide hormone that is produced by the pancreatic islets of Langerhans and serves to reduce blood glucose levels in addition to other functions relating to energy production.

This test employs a single-use cartridge containing an assay for the detection of insulin in equine plasma. The "Default" protocol is recommended for general screening and testing of samples anticipated to be below 400 µU/mL. The "Auto Dilute" protocol is recommended as a follow up test for samples >400 µU/mL. During the "Auto Dilute" test the sample is automatically diluted on the cartridge to extend the dynamic range to 1500 µU/mL.

The TRUFORMA Insulin Test is a sandwich immunoassay, utilizing a biotin-labeled anti-insulin monoclonal detection antibody (dAb) and a monoclonal capture antibody immobilized onto a Bulk Acoustic Wave (BAW) sensor solid phase. If the "Auto Dilute" protocol is selected, the sample is first diluted automatically on the cartridge. Following this step, both run protocols follow the same steps. During the test, sample is mixed with dAb allowing the detection antibodies to bind insulin molecules.This mixture then flows across the biosensor surface and the dAb-insulin complex is captured by the immobilized antibody on the sensor surface. Next a streptavidin-enzyme conjugate is flowed over the sensor surface where it is immobilized to the biotin-labeled 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 insulin present in the sample using the stored calibration curve. As a sandwich immunoassay, a sample containing a high concentration of insulin results in a higher amount of dAb-biotin-streptavidin-enzyme complex attached to the solid phase and a higher signal. Thus, concentrations of plasma insulin are proportional to the amount of insulin that is bound to the solid phase. Insulin concentrations that fall outside of the normal reference range may be indicative of abnormal levels of insulin as a result of an endocrine disorder.

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 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 4 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. Insulin Cartridge(s)
2. Disposable Bulb Pipette(s)
3. Instructions for Use

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 Insulin 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.

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. Follow on screen prompts and select either "Plasma (Default)" or "Auto Dilute Plasma (>400 µU/mL)" depending on the anticipated insulin result range (see **Table 1** for run protocol dynamic ranges)

7. 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.

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

USING BULB PIPETTE

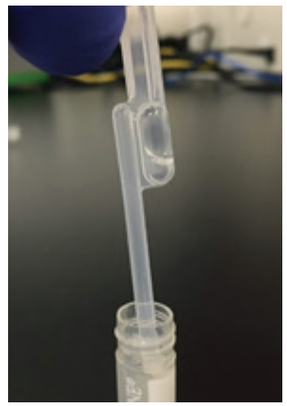


Figure 1

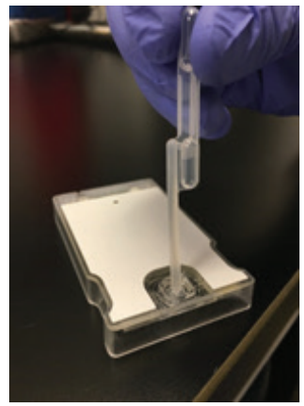


Figure 2

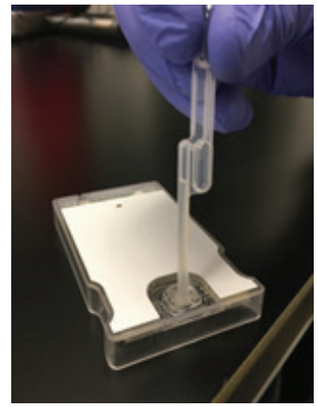


Figure 3

REFERENCE RANGES

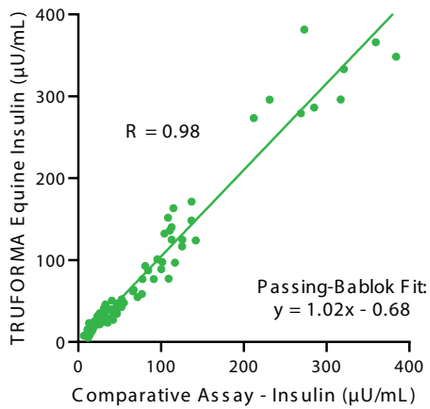
Use the QR code below for the insulin reference ranges established for equine patient populations. Test results should be interpreted in conjunction with a patient's clinical signs.

Table 1: TRUFORMA Insulin Dynamic Ranges

Run Protocol	Dynamic Range	Recommended Use
Plasma (Default)	**7.5-400 µU/mL**	Wellness screening, Oral Sugar Test, monitoring of horses historically <400 µU/mL
Auto Dilute Plasma	**100-1500 µU/mL**	Follow up testing of samples >400 µU/mL, monitoring of horses historically >400 µU/mL

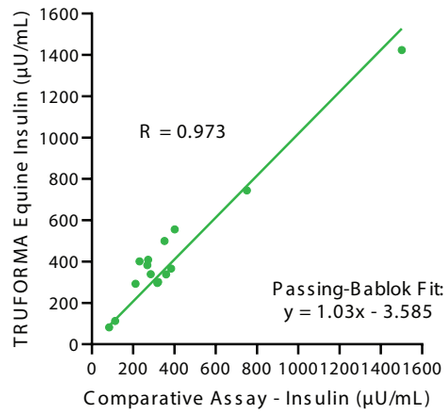
Insulin Reference Ranges

Figure 4: Correlation of TRUFORMA Equine Insulin (Default) with Comparative Method



Correlation with 114 equine samples

Figure 5: Correlation of TRUFORMA Equine Insulin (Auto Dilute) with Comparative Method



Correlation with 15 equine samples

PERFORMANCE DATA

Test results are reported in micro- units per milliliter (µU/mL) and unless otherwise noted, all data was generated using equine plasma samples collected in K_2 EDTA tubes. The dynamic range for the "Default Plasma" and "Auto Dilute Plasma" test protocols are outlined in **Table 1**, additional performance metrics are detailed in the TRUFORMA Insulin Assay White Paper available on myZomedica.com.

PRECISION

Precision was evaluated by testing six equine plasma samples with varying insulin concentrations. For the "Default" protocol each sample was tested with twelve replicates on four separate days across three different instruments for a total of 144 results. Precision ranged from 7.1-10.2%. For the "Auto Dilute" protocol each samples was tested with twelve replicates on 2 separate days across three different instruments for a total of 72 results. Precision ranged from 5.6%-6.8%.

CORRELATION

Correlation analysis evaluates the agreement and commutability of a new test method with a comparative or reference method. Insulin levels were measured in 114 equine plasma samples for the "Default" protocol (**Figure 4**) and 15 samples using the "Auto Dilute" protocol (**Figure 5**) using both the TRUFORMA system and radioimmunoassay (RIA). All samples were analyzed on the same freeze-thaw cycle for both methods, and the results were used to generate correlation statistics.

The TRUFORMA system will display the insulin plasma concentration results and interpretation guidelines for test results are available on myZomedica.com.

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901-8209-1000 Rev B