TRUFORMA®

Cobalamin and Folate Test

FOR THE QUANTITATIVE
DETECTION OF
COBALAMIN AND FOLATE
LEVELS IN CANINE AND FELINE 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
Plymouth, MN 55447 USA
www.zomedica.com

INTENDED USE

The TRUFORMA® Cobalamin and Folate Test is used for the quantitative detection of cobalamin and folate levels in canine and feline serum. These two B vitamins are often measured together to aid in the diagnosis and monitoring of intestinal disease, small intestinal microbial imbalance (dysbiosis), or exocrine pancreatic insufficiency (EPI).

OVERVIEW

Cobalamin (vitamin B_{12}) and folate (vitamin B_9) are both water-soluble vitamins involved in proliferative cellular processes like amino acid metabolism and DNA synthesis. While both are absorbed in the small intestine, serum folate levels are dependent on the absorptive function of the jejunum (proximal small intestine) whereas serum cobalamin levels reflect the absorptive function of the ileum (distal small intestine). As such, reduced serum levels of folate or cobalamin may indicate insufficient nutrient absorption in the jejunum and ileum, respectively, while diffuse malabsorption is often indicated by decreased serum cobalamin and folate levels.

An additional pattern between these two analytes (high folate, low cobalamin) has clinical significance and can be attributed to either jejunum dysbiosis or EPI. Certain bacteria that naturally reside in the small intestine can synthesize folate while binding cobalamin, making it inaccessible for absorption. When this population of bacteria multiply uncontrollably, serum levels of folate can become significantly elevated while cobalamin levels decrease resulting in the dysbiotic state.

This test employs both an offboard Sample Diluent step and a single-use cartridge containing the assay for the detection of cobalamin and folate in serum. The offboard preparation step is required to release cobalamin and folate molecules from binding proteins in the serum so they can be quantified accurately when the mixture is added to the cartridge.

The TRUFORMA Cobalamin and Folate Test works as a duplexed competitive immunoassay with individual Bulk Acoustic Wave (BAW) sensors coated with either cobalamin- or folate-analyte (solid phases). During the test, serum is processed to release bound folate and cobalamin molecules. This processed serum is then combined with biotin-labeled folate-binding protein (FBP) and a complex consisting of intrinsic factor (IF) and a biotinylated, anti-IF antibody, that flow across the BAW biosensor surface. FBP and IF complex bind to released folate and cobalamin molecules in the serum, preventing FBP and IF complex from binding to their respective analyte-decorated surfaces of the bio-sensor. After unbound FBP and IF complex are removed with a wash step, a streptavidin-enzyme conjugate is flowed over the sensor surface, where it is immobilized via streptavidin-biotin interactions. After several additional wash steps, an enzyme substrate is exposed to the BAW biosensor surface where the enzyme converts the substrate to an insoluble product. This insoluble material accumulates on the biosensor surface and is measured as a shift in frequency by the BAW biosensor.

The change in frequency for each resonator is used to separately calculate the concentration of folate and cobalamin present in the sample using an analyte-specific calibration curve. As a competitive immunoassay, a sample containing a low concentration of analyte results in a higher amount of insoluble material on the solid phase, thus a larger signal. Concentrations of serum folate and cobalamin are inversely proportional to the FBP and IF complexes bound to the solid phase and the magnitude of the signal. Folate and cobalamin concentrations that fall outside of normal reference ranges may be indicative of small intestinal disease.

PRECAUTIONS & WARNINGS

- Wear gloves anytime the Sample Diluent or Sample Diluent-Serum mixture is being handled.
- 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 two new disposable bulb pipettes 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 and feline 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.
- Rarely, high globulin samples (>80 mg/mL) may form a highly viscous solution when mixed with the Sample Diluent that prevents cartridge loading. In such cases, contact Customer Success (734-369-2555).

STORAGE & HANDLING

- Store cartridges at refrigerated temperature (2–8 °C / 35–46 °F).
- Do not freeze cartridges.
- Serum samples may be stored under refrigerated conditions (2–8 °C / 35–46 °F) for up to 2 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. Cobalamin and Folate Cartridges (1 per sample required)
2. Disposable Bulb Pipettes (2 per sample required)
3. Sample Diluent Tubes (1 per sample required)
4. Instructions for Use

SERUM COLLECTION

Collect blood in blood collection tubes without anticoagulant. After complete clotting has occurred, separate serum by centrifugation.

The TRUFORMA Cobalamin and Folate Test has been tested with red-top tubes 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.

SAMPLE DILUENT INSTRUCTIONS

The TRUFORMA Cobalamin and Folate Test requires a manual offboard step to denature the serum sample prior to cartridge loading and device processing.

To accommodate this workflow, each box of ten (10) TRUFORMA Cobalamin and Folate cartridges contains:

- Ten (10) Sample Diluent tubes filled with 50 µL of Diluent.
- Ten (10) 150 µL double-bulb pipettes for the denature step.
- Ten (10) 150 µL double-bulb pipettes for cartridge loading.

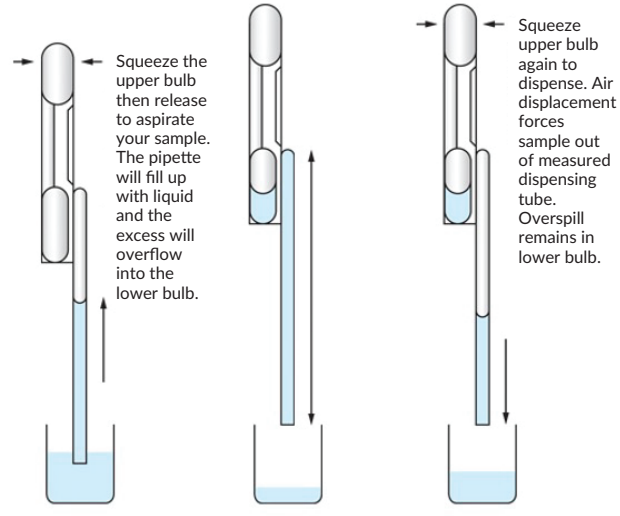
To prepare serum samples for testing with the TRUFORMA Cobalamin and Folate Test, please follow the below instructions while wearing disposable gloves when handling the Sample Diluent.

1. Retrieve the serum to be analyzed and one (1) Sample Diluent tube with two (2) 150 µL double-bulb pipettes from the cartridge box.

2. Perform a short spin of the Sample Diluent tube in a centrifuge or gently tap the tube on a benchtop to remove any liquid from the cap and then open the tube.

3. Aspirate 150 µL of serum into one of the double-bulb pipettes by squeezing the top bulb completely, placing the tip in the serum, and then releasing to allow liquid to fill the length of the pipette stem (**Figure 1**).
   a. Excess serum will overflow into the lower bulb and indicates the target volume (150 µL) has been reached.

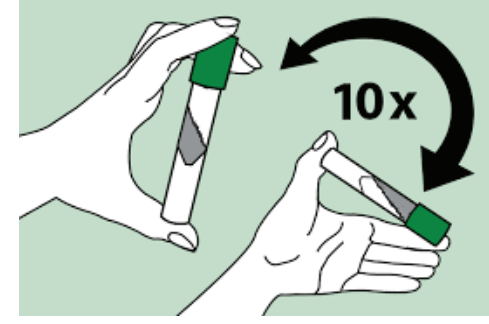
Figure 1: Transfer Volume with a Double-Bulb Pipette



4. Dispense the serum from the double-bulb pipette into the Sample Diluent tube by fully squeezing the upper bulb again.
   a. Ensure all serum is expelled directly into the buffer for optimal sample denaturing.
   b. Dispose of the double-bulb pipette.

5. Close Sample Diluent tube and invert ten (10) times (**Figure 2**).

Figure 2:



Invert Diluent-Serum Mixture Ten Times

6. Perform a short spin of the Sample Diluent containing the Diluent-serum mixture in a centrifuge or gently tap the tube on a benchtop to remove any liquid from the cap and then open the tube.

7. The sample is now ready to be added to the cartridge. Proceed to the TEST PROCEDURE section and follow the instructions using a fresh double bulb pipette to add the Sample Diluent-Serum mixture to the cartridge.

CARTRIDGE LOADING

Figure 3

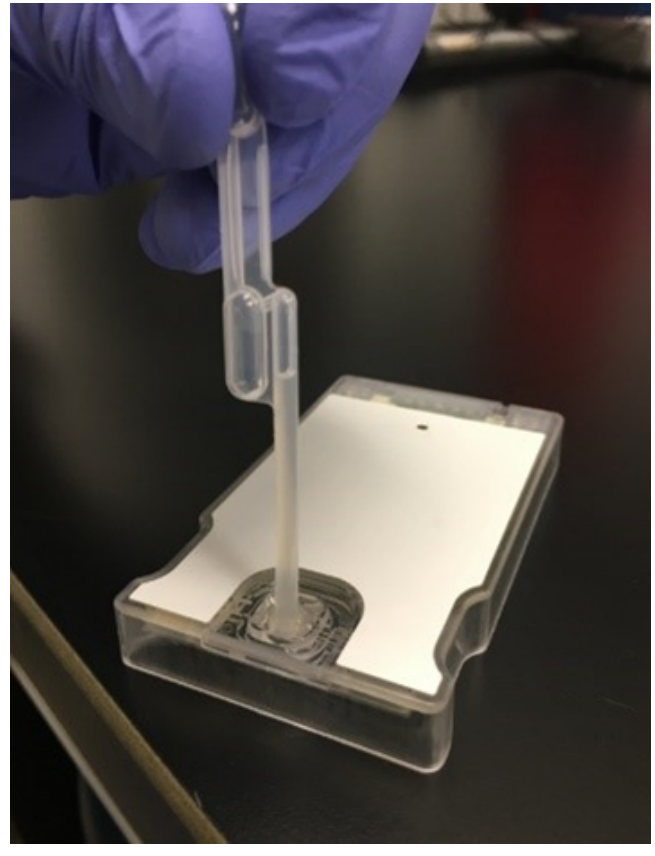


Figure 4

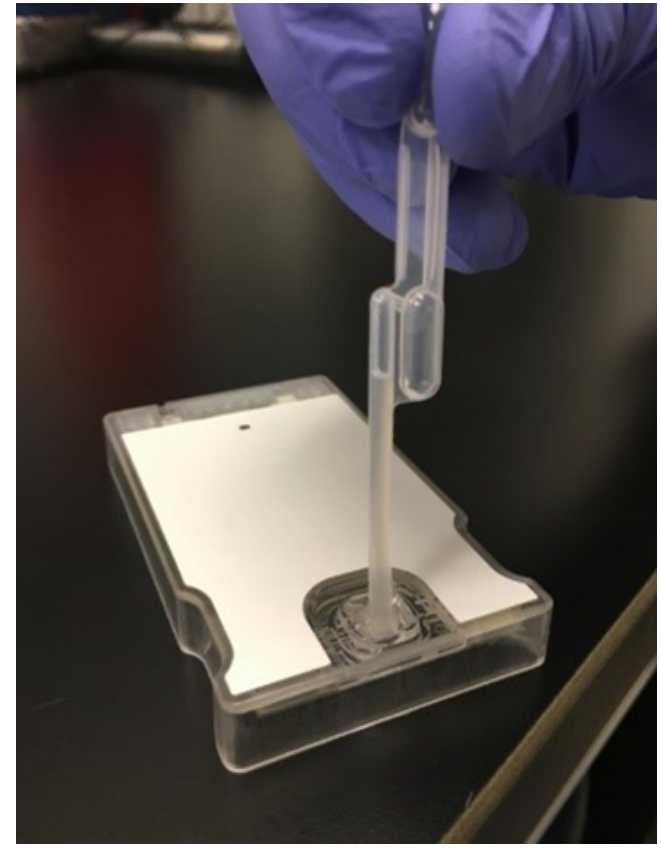


Figure 5

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 a supplied bulb pipette, dispense the Serum-Sample Diluent mix (see SAMPLE DILUENT INSTRUCTIONS) into the sample port (approximately 150 µL).

Recommended technique for adding sample: With Serum-Sample Diluent filled bulb pipette (**Figure 3**), wet the sample port filter by placing the bulb pipette tip on top of the sample port filter and lightly squeeze the pipette (**Figure 4**), 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 5.**

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 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 accordance with local waste guidelines.

EXPECTED VALUES

The reference ranges listed in **Table 2** are those established for canine and feline patient populations. Test results should be interpreted in conjunction with a patient's clinical signs.

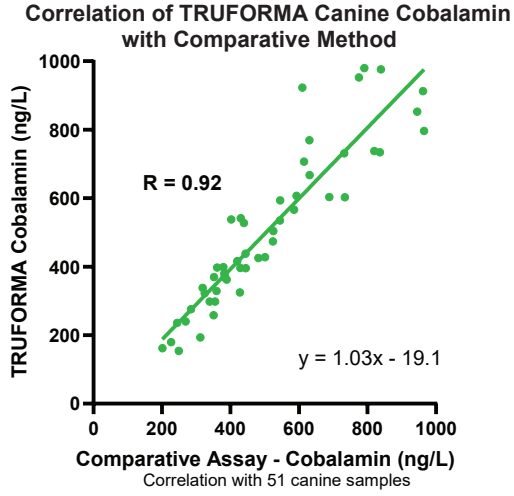
Table 1: TRUFORMA Dynamic Range

Analyte	Canine and Feline Dynamic Range
Cobalamin (ng/L)	150 - 1000
Folate (µg/L)	2 - 50

Table 2: Reference Ranges

Analyte	Canine Reference Range	Feline Reference Range
Cobalamin (ng/L)	251 - 908	>290
Folate (µg/L)	7.7 - 24.4	9.7 - 21.6

Figure 6: Canine Correlation



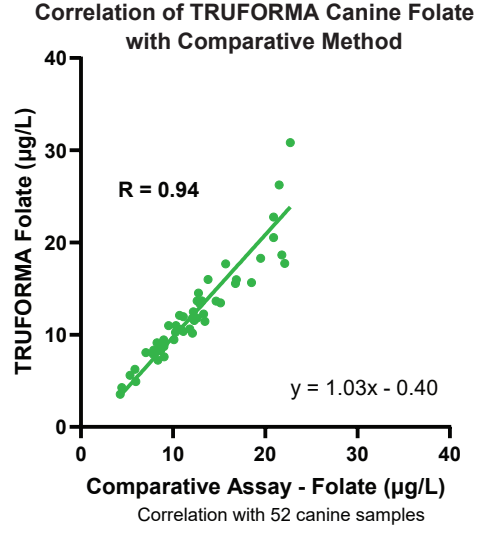
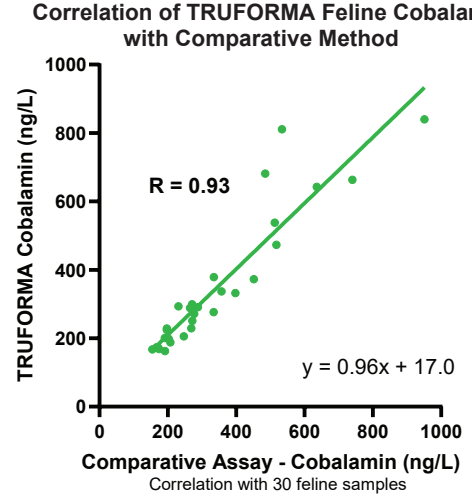
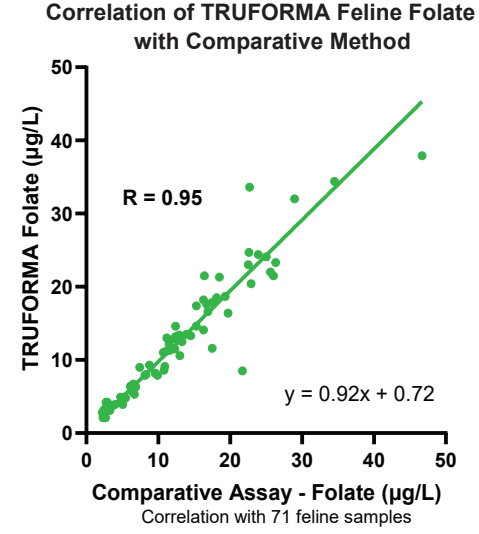


Figure 7: Feline Correlation





DYNAMIC RANGE AND REFERENCE RANGES

The reportable ranges for the TRUFORMA Cobalamin and Folate Test are found in **Table 1**. The normal ranges for cobalamin and folate listed in **Table 2** are those established for canine and feline patient population. Test results should be interpreted in conjunction with patient's clinical signs.

PRECISION

Seperate precision studies were performed for canine and feline samples. For canine a five-day precision analysis was run with 3 samples across 3 TRUFORMAs with a total of 50 replicates per control. The overall %CV for cobalamin was 5.8% (747 ng/L), 7.9% (301 ng/L), and 10.8% (203 ng/L) while for folate it was 9.0% (15.0 µg/L), 8.2% (10.4 µg/L), and 9.3% (6.4 µg/L). For feline samples a total of 50 replicates were obtained per control across multiple instruments. Overall %CV for cobalamin was 14.2% (292 ng/L), and 7.8% (679 ng/L) while for folate it was 14% (5.3 µg/L),9.7% (5.3 µg/L), and 5.8% (15.8 µg/L).

CORRELATION

Correlation analysis evaluates the agreement and commutability of a new test method with a comparative or reference method. Cobalamin and folate levels were measured in canine and feline serum samples using TRUFORMA and a comparative method. All samples were analyzed on the same freeze-thaw cycle for both methods, and results were used to generate correlation statistics in **Figures 6 & 7** for canine and feline samples respectively. The TRUFORMA System will display the cobalamin and folate serum concentration results. Interpretation of results are available on myZomedica.com.

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