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# TRUFORMA® Point-of-Care Canine and Feline Thyroid-Stimulating Hormone (TSH) Assay

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## Key Messages

- Accurate and precise measurements of canine and feline thyroid-stimulating hormone (TSH) levels in dogs and cats are needed for the diagnosis of thyroid disease.
- The TRUFORMA platform uses innovative bulk acoustic wave (BAW) technology to provide a non-optical and fluorescence-free detection system for diagnostic use at the point of care (POC) in veterinary clinics.
- The extended dynamic range of the TRUFORMA canine and feline TSH assay allows the quantification of high and low concentrations of TSH with the same consumable, which is vital for accurate diagnosis of canine and feline thyroid disease.
- The high level of precision and correlation of the TRUFORMA canine and feline TSH assay to a predicate reference laboratory assay provides veterinarians with accurate and reliable diagnostic results at the POC, improving patient treatment and offering real-time client communication.
- The TRUFORMA feline TSH assay is currently the only feline-optimized TSH assay available at either reference laboratory or POC settings.

# Introduction

Accurately diagnosing thyroid dysfunction can be a challenge in veterinary practice due to the complexity and costs of current methodologies performed at reference laboratories as well as the variability in performance of the available POC immunoassays.[1] Although canine-optimized TSH assays are available at reference laboratories, there is a need for both a feline-optimized TSH assay to accurately diagnose and monitor feline thyroid disease, and accurate, precise canine and feline TSH assays at the veterinary POC. The TRUFORMA platform, which uses BAW sensor technology, was developed to accurately differentiate healthy and diseased animals at the POC in veterinary practice, with a canine and feline TSH assay that provides veterinarians with rapid, reliable, and accurate measurement of TSH at the POC for the first time.

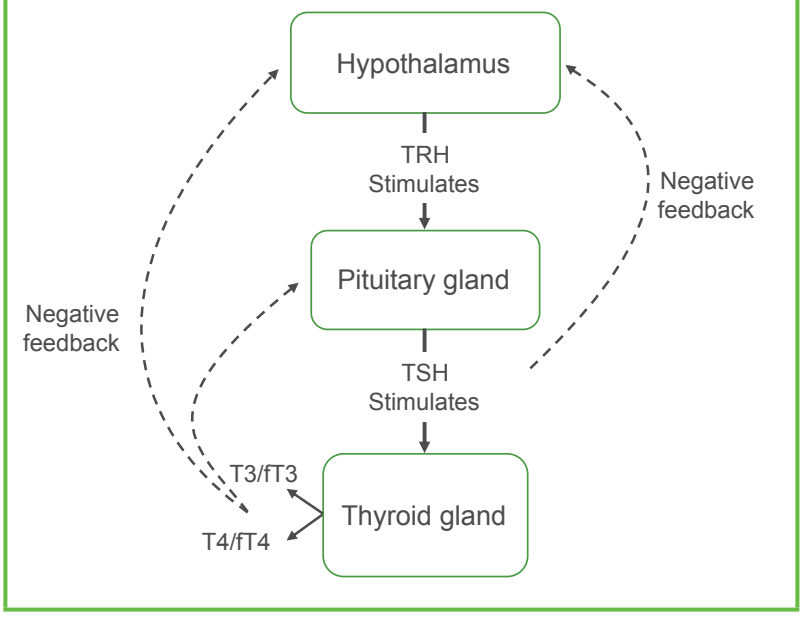
The objectives of this study were to:

- Determine analytical and clinical performance attributes for the TRUFORMA TSH assay.
- Demonstrate how the TRUFORMA canine and feline TSH assay differs from the currently available assays.
- Compare TRUFORMA canine and feline TSH assay performance to an assay used as part of the standard of care at veterinary laboratories.

# Clinical Significance of TSH Testing

TSH, a glycoprotein produced by thyrotrope cells in the anterior pituitary gland, stimulates the thyroid gland to synthesize and secrete thyroxine (T4) and triiodothyronine (T3). Under normal conditions, TSH synthesis and secretion is modulated by the negative feedback regulatory mechanism on the pituitary gland in response to high T4 and T3 levels (**Figure 1**). TSH secretion is also stimulated by thyrotropin-releasing hormone (TRH), which is released by the hypothalamus as part of the negative feedback regulatory mechanism.

**Figure 1.** Hypothalamic-Pituitary Thyroid Axis




fT3, free triiodothyronine; fT4, free thyroxine; T3, triiodothyronine; T4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone

## Canine TSH

Serum total T4 and free T4 (fT4) concentrations, in conjunction with serum TSH concentrations, are currently recommended for the assessment of canine thyroid gland function in dogs.[2] Clinical studies have shown that high serum TSH concentrations correlate with the diagnosis of canine hypothyroidism when serum total T4 or fT4 concentrations are low.[3-6]

## Feline TSH

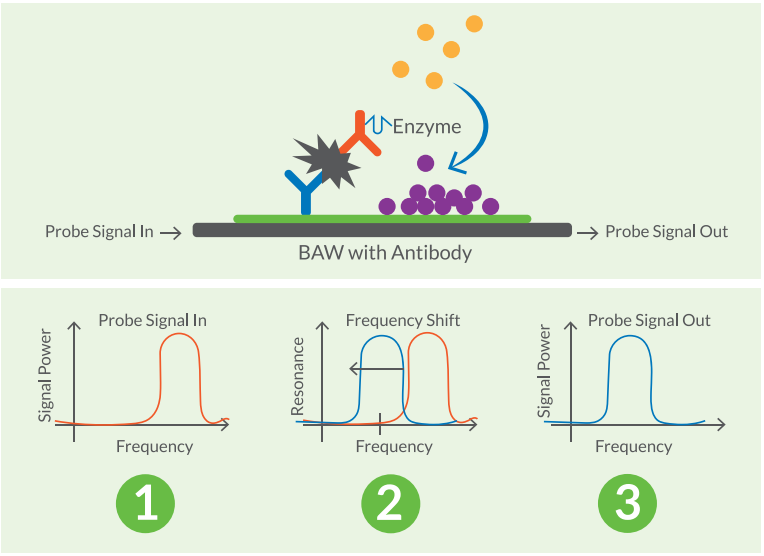
Hyperthyroidism in cats can be diagnosed directly by assessment of circulating concentrations of T4, or indirectly with TSH concentrations.[7] Studies have shown that high T4 or suppressed TSH levels are indicative of hyperthyroidism.[8] Cats are often screened for hyperthyroidism through the measurement of total T4, but cats with hyperthyroidism may have total T4 concentrations within the reference range, necessitating further testing of fT4 and TSH.[8] However, a valid species-optimized TSH assay is not available for cats, thereby limiting the utility of feline TSH testing. The currently available canine-optimized TSH test has been evaluated for feline use, but is not able to distinguish between low and low-normal TSH levels in cats due to its analytical sensitivity. This creates a need for a feline-optimized TSH assay to accurately diagnose and monitor feline thyroid disease as well as to prevent iatrogenic hypothyroidism.[8] Additionally, measurement of TSH concentration is commonly used as a first-line discriminatory test of thyroid function in humans.[9] A feline-verified TSH test is poised to improve diagnosis and exclusion of feline hyperthyroidism.

- Accurate and reliable measurements of canine and feline TSH levels are needed for the diagnosis of thyroid disease.
- A valid feline-optimized TSH assay is not currently available.
- A high-performance veterinary POC immunoassay for TSH, with published performance data, is needed.

# TRUFORMA Platform

The TRUFORMA platform uses BAW sensor technology to provide a nonoptical 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 biosensors consist of multiple resonators, each composed of a piezoelectric material subjected to an electrical field. The resonators can be coated with detection reagents, such as antibodies and nucleic acids, for immunoassay and molecular testing. Whereas 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 2**). Veterinary medical professionals will be the first to use the BAW sensor technology in a POC diagnostic setting due to human diagnostic test premarket approval timelines.

**Figure 2.** BAW Technology in the TRUFORMA TSH Immunoassay



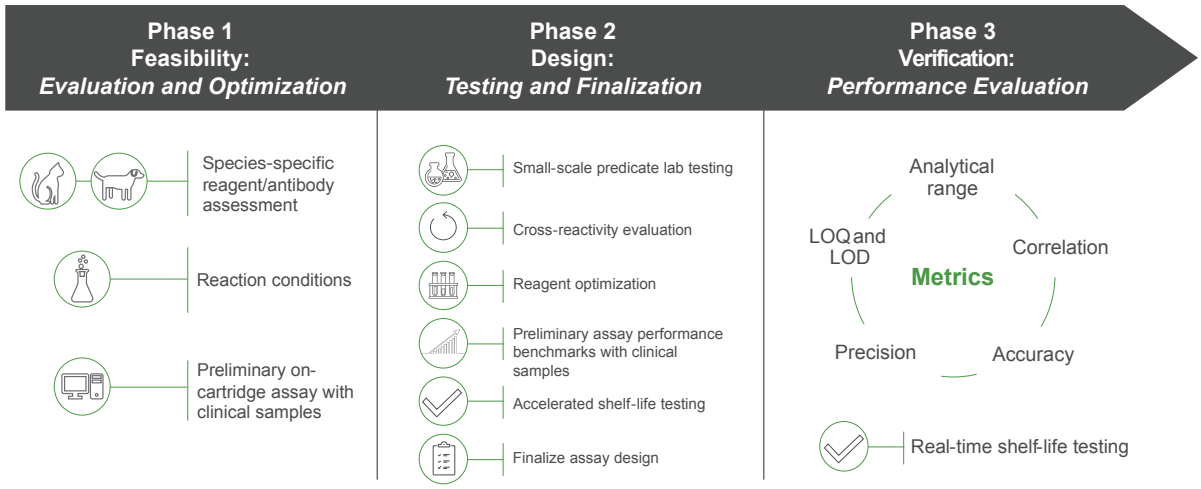

The TRUFORMA TSH assay is a sandwich immunoassay in which the BAW sensor is coated with a monoclonal capture antibody. Antigen present in the sample binds to a second monoclonal antibody in solution as well as the capture antibody. Enzyme binds to this complex that is now immobilized on the sensor surface. After several wash steps, an enzyme substrate is exposed to the BAW biosensor surface, and enzyme converts the substrate to an insoluble product that accumulates on the BAW biosensor surface. This is measured as a shift in frequency by the BAW biosensor. The signal is proportional to the amount of analyte present.

BAW, bulk acoustic wave; TSH, thyroid-stimulating hormone

# TSH Assay Development Overview

The TRUFORMA canine and feline TSH assay was developed specifically for use in dogs and cats. Twenty-five monoclonal antibody pairs were screened to determine an antibody pair with optimum performance for canine and feline testing. Using industry standard recommendations for bioanalytical method validation and the Clinical and Laboratory Standards Institute (CLSI) guidelines on method comparison and bias estimation (EP09c)[10], the TRUFORMA assay performance requirements were chosen to meet or exceed reference laboratory performance to provide unparalleled performance at the POC. The three phases of the canine and feline TSH assay development were designed to create a high-quality and reliable POC assay, and included feasibility evaluation and optimization with species-optimized assessment, design, and testing of preliminary assay performance and performance verification (**Figure 3**).

**Figure 3.** Overview of the TRUFORMA TSH Assay Development




LOD, limit of detection; LOQ, limit of quantitation; TSH, thyroid-stimulating hormone

# Assay Verification Results

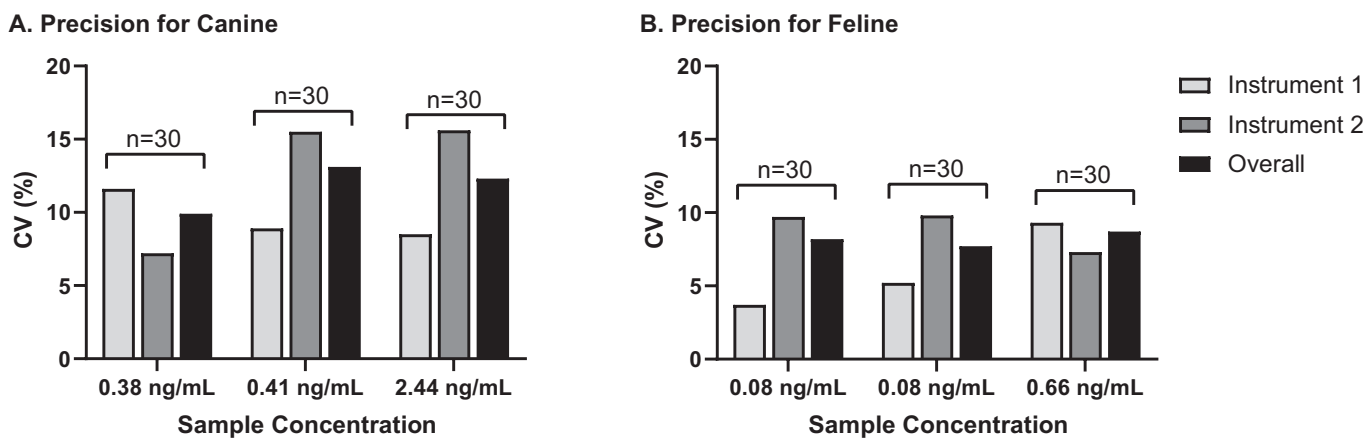
The TRUFORMA canine and feline TSH assay performance was compared with that of the reference-laboratory predicate, Siemens IMMULITE® 2000 TSH assay, an automated chemiluminescent immunoassay. Spiked, normal, and diseased canine and feline samples were analyzed.

## Analytical Precision

Analytical precision was evaluated by measuring the variability in assay results (between-run percentage coefficient of variation [%CV]) under the normal operating conditions in the laboratory. Precision was evaluated by testing three different serum samples with varying TSH concentrations for both the canine and feline versions of the assay. Each sample was tested with five replicates on three separate days on two different instruments, for a total of 90 results for each species. Observed %CV was calculated.

The TRUFORMA TSH assay demonstrated an overall %CV of < 20% across the dynamic range for both canine (**Figure 4A**) and feline serum samples (**Figure 4B**). For each sample, the overall %CV was comparable to the %CV for each instrument, indicating repeatability across instruments. A ± 25% between-runs %CV is recommended and is considered a quality %CV for measuring assay precision in a ligand binding assay.[11]

**Figure 4.** Precision of the TRUFORMA TSH Assay




%CV was calculated for three serum samples for (**A**) canine and (**B**) feline samples using 90 runs per species. One statistical outlier was removed from the feline data set following CLSI EP05-A3 guidelines. CV, coefficient of variation; TSH, thyroid stimulating hormone.

## Time to Test Results, Dynamic Range, Limit of Quantitation

Time to Test Results (TTR) refers to the amount of time for TRUFORMA to initiate the test and generate the result. The POC testing of the TRUFORMA platform provides more timely and actionable results, which can improve patient care through faster clinical decision making and communication with pet owners.

Dynamic range refers to the span of test result values that can be accurately measured, and limit of quantitation (LOQ) refers to the lowest (lower limit of quantification [LLOQ]) and highest (upper limit of quantification [ULOQ]) analyte concentrations that can be reliably detected with predefined accuracy and precision.

Currently available commercial canine TSH assays cannot accurately measure TSH concentrations at levels expected for cats with hyperthyroidism, or some older euthyroid cats, because they are below the LLOQ.[8]

The TRUFORMA TSH assay had an LLOQ for canine serum samples comparable to that of the IMMULITE Canine TSH assay (**Table 1**). The LLOQ for the TRUFORMA feline test was determined to be 0.008 ng/mL (8 pg/mL) (**Table 1**). The TRUFORMA TSH assay's extended dynamic range (fold change = 1250) allows the quantification of both clinically high and clinically low canine and feline TSH concentrations using the same consumable. The ability of this assay to quantify measurements down to single-digit pg/mL levels illustrates the high sensitivity of BAW sensor technology. This assay can be used to improve patient diagnosis and treatment at the POC without the need to send samples to a reference laboratory.

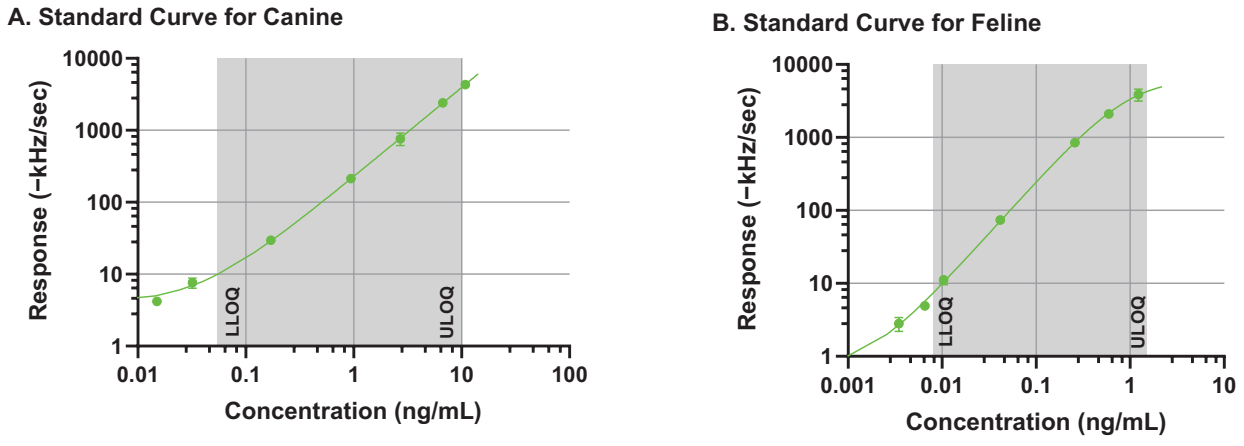
**Table 1:** Standard Curves of the Canine and Feline TRUFORMA TSH Assay

| Test | TRUFORMA | | | IMMULITE[12] |
|---|---|---|---|---|
| | Canine | Feline | Combined | Canine |
| TTR, min | < 18.0 | < 20.0 | N/A | 65.0 |
| Dynamic range, ng/mL | 0.05-10.0 | 0.008-1.5 | 0.008-10.0 | 0.03-12.0 |
| LLOQ, ng/mL | 0.050 | 0.008 | 0.008 | 0.03 |
| ULOQ, ng/mL | 10.0 | > 1.5 | 10.0 | 12.0 |

N/A, not applicable; LOQ, limit of quantitation; TSH, thyroid-stimulating hormone; TTR, time-to-results; ULOQ, upper limit of quantification

For each species, seven calibrators with known concentrations of TSH were tested using the TRUFORMA TSH cartridge. Each calibrator was run with six replicates across three different instruments, and the average value was used to generate a standard curve. The linearity and reportable range of the TRUFORMA TSH assay illustrates linear performance within the clinically relevant range for the canine and first feline-optimized TSH assay." (**Figure 5**).

**Figure 5.** Standard Curves of the Canine and Feline TRUFORMA TSH Assay



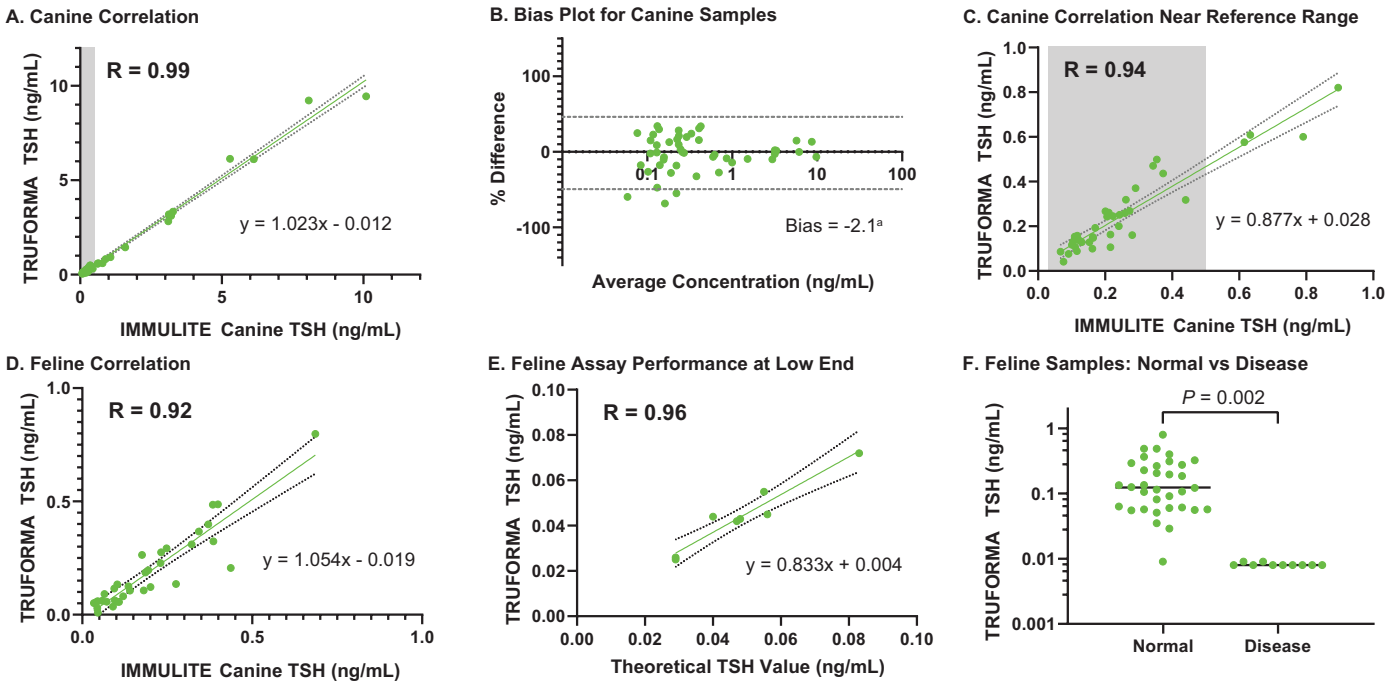

Seven calibrators with known concentrations of TSH were used to generate a standard curve for (**A**) canine and (**B**) feline analysis. The shaded region represents the reported dynamic range. LLOQ, lower limit of quantitation; TSH, thyroid-stimulating hormone; ULOQ, upper limit of quantification.

## Assay Correlation Between the TRUFORMA TSH Assay and the Siemens IMMULITE TSH Assay

Assay correlation and Bland-Altman bias plot analysis evaluate the agreement and commutability of a new test method with a comparative or reference method. 91 serum samples were run on the same freeze-thaw cycle on the TRUFORMA and Siemens IMMULITE devices. The instrument reports concentrations based on the standard curve, and results were used to generate correlation and bias plots.

The TRUFORMA TSH assay showed high correlation (R = 0.99) to the Siemens IMMULITE canine TSH assay (**Figure 6A**) with no apparent bias (mean bias, −2.1%; 95% CI, −9.0 to 4.9%) (**Figure 6B**) across the canine dynamic range. High correlation (R = 0.94) was maintained when focused on the most clinically important portion of the dynamic range (**Figure 6C**). Feline samples showed relatively high correlation (R = 0.92) across the dynamic range to the Siemens IMMULITE Canine TSH assay, which was not developed for feline testing (**Figure 6D**). The Siemens IMMULITE Canine TSH test is occasionally used to support feline hyperthyroid testing as it has been evaluated for feline use despite its lack of ability to effectively distinguish between low and low-normal TSH levels in cats.[8] Dilution studies demonstrated high correlation (R = 0.96) within the most clinically important portion of the feline dynamic range (**Figure 6E**) and, together, the optimized reagents and improved analytical sensitivity for the TRUFORMA feline TSH test demonstrated statistically significant (*P* = 0.002) discrimination between healthy and hyperthyroid patient samples (**Figure 6F**).

**Figure 6.** Correlation of TRUFORMA TSH Assay Compared with Siemens IMMULITE Canine TSH Assay




Correlation studies were performed comparing the results from the TRUFORMA TSH and IMMULITE TSH assays using (**A**) 47 canine samples and (**D**) 34 feline samples. Dotted lines represent the 95% CIs for the linear regression lines; the shaded region represents the IMMULITE Canine TSH reference range.[12] (**C**) The 37 canine samples near the IMMULITE reference range were analyzed independently to show correlation in the most clinically important region (< 1.0 ng/mL). (**B**) A Bland-Altman bias plot was generated by plotting the mean concentration vs the % difference ([TRUFORMA – IMMULITE]/mean) for the canine samples. Dotted lines represent 95% limits of agreement. [a] The 95% CI for the bias includes the line of equality for both canine and feline samples, indicating no significant bias. (**E**) To evaluate assay performance at the low end, eight feline samples were diluted 10-fold and run with the TRUFORMA TSH assay. Theoretical TSH values were determined based on the pre-dilution IMMULITE value. (**F**) All hyperthyroid feline samples (disease) were below the LLOQ for IMMULITE, and seven were below the LLOQ for TRUFORMA, therefore, these were not included in the correlation analysis. A total of 34 normal and 10 hyperthyroid samples were evaluated, and the TRUFORMA TSH value is shown; 0.008 ng/mL was used for samples below the LLOQ. An unpaired, two-tailed *t* test was used to calculate the *P* value. LLOQ, lower limit of quantitation; TSH, thyroid-stimulating hormone.

## Cross-Reactivity

Known amounts of TSH, and potential cross-reactants (follicle-stimulating hormone [FSH], luteinizing hormone [LH], and human chorionic gonadotropin [hCG]) were added to depleted serum and tested in triplicate using the TRUFORMA TSH assay.

No significant cross-reactivity was observed in the TRUFORMA TSH assay, and no cross-reactants interfered with the reported TSH concentrations (**Table 2**).

**Table 2:** Summary of Cross-Reactivity for the TRUFORMA TSH Assay

| | Material (ng/mL added) | Cross-Reactivity, % |
|---|---|---|
| TSH negative (0.003 ng/mL) | FSH (1000) | < 0.001 |
| | LH (1000) | < 0.001 |
| | hCG (1000) | < 0.001 |
| TSH positive (0.8 ng/mL) | FSH (1000) | < 0.001 |
| | LH (1000) | < 0.001 |
| | hCG (1000) | < 0.001 |

FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; TSH, thyroid-stimulating hormone

# Conclusions

The TRUFORMA canine and feline TSH assay demonstrated high analytical sensitivity, specificity, and precision as a POC diagnostic platform, with a wider dynamic range than the reference laboratory assay. The TRUFORMA canine and feline TSH assay's extended dynamic range allows the quantification of both high and low TSH concentrations with the same consumable for both species, which is vital for accurate diagnosis of canine and feline thyroid disease. In addition, the TRUFORMA feline TSH assay is the first available feline-optimized TSH assay.

The TRUFORMA canine and feline TSH assay provides veterinarians with accurate and reliable diagnostic results at the POC, allowing for improved client communication and patient treatment.

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# Abbreviations and Acronyms

**BAW** Bulk Acoustic Wave
**CLSI** Clinical and Laboratory Standards Institute
**CV** Coefficient of Variation
**fT3** Free Triiodothyronine
**fT4** Free Thyroxine
**FSH** Follicle-Stimulating Hormone
**hCG** Human Chorionic Gonadotropin
**LH** Luteinizing Hormone
**LOD** Limit of Detection
**LOQ** Limit of Quantitation
**LLOQ** Lower Limit of Quantification
**POC** Point of Care
**T3** Triiodothyronine
**T4** Thyroxine
**TRH** Thyrotropin-Releasing Hormone
**TSH** Thyroid-Stimulating Hormone
**TTR** Time to Test Results
**ULOQ** Upper Limit of Quantification

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