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TRUFORMA™ Point-of-Care Canine and Feline Total Thyroxine (tT4) Assay

Ashley Wood, Director of Research & Development, Diagnostics, Zomedica

Matthew Ryder, Staff Research & Development Scientist, Qorvo Biotechnologies

Ian Harmon, Staff Research & Development Scientist, Qorvo Biotechnologies

Casey Wegner, Sr. Director of Research & Development, Partnerships, Zomedica

Key Messages

- Accurate and precise measurements of canine and feline total thyroxine (tT4) levels are needed for the diagnosis of thyroid disease.
- The TRUFORMA™ platform uses innovative bulk acoustic wave (BAW) technology to provide a nonoptical and fluorescence-free detection system for diagnostic use at the point-of-care (POC) in veterinary clinics.
- The dynamic range of the TRUFORMA™ canine and feline tT4 assay allows the quantification of high and low concentrations of tT4 within the same assay, which is vital for accurate diagnosis of canine and feline thyroid disease.
- The high precision and correlation to a reference laboratory assay shown for the TRUFORMA™ canine and feline tT4 assay provide veterinarians with accurate and reliable diagnostic results at the POC, providing opportunities for improved patient treatment and real-time client communication.

Introduction

Accurately diagnosing thyroid dysfunction can be a challenge in veterinary practice due to the complexity and costs of current methodologies for immunoassays performed at a reference laboratory and the variability in performance of the available POC testing.[1] The TRUFORMA™ platform, which uses BAW sensor technology, was developed to accurately differentiate between healthy animals and those with thyroid dysfunction at the POC in veterinary practice. The canine and feline tT4 assay provides veterinarians rapid, reliable, and accurate measurement of tT4 at the POC.

The objectives of this study were to:

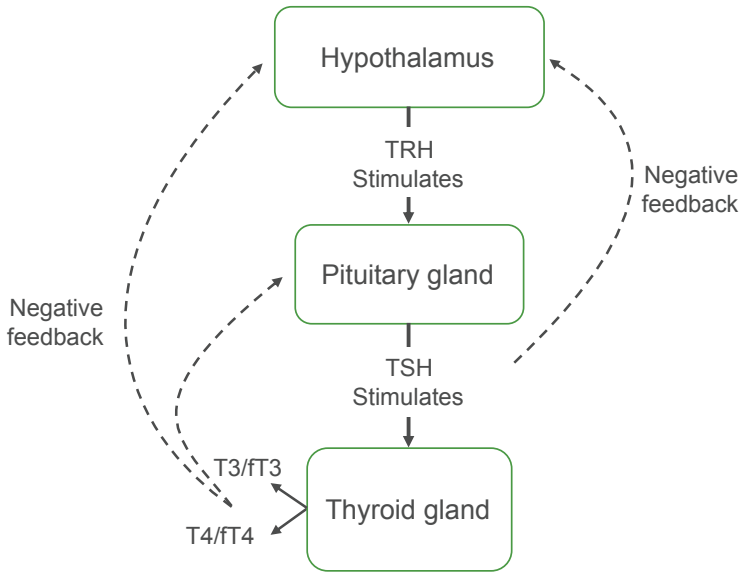
- Determine analytical performance attributes for the TRUFORMA™ tT4 assay.
- Describe how the TRUFORMA™ canine and feline tT4 assay differs from available assays.
- Compare TRUFORMA™ canine and feline tT4 assay performance with an assay used as part of the standard of care at veterinary diagnostic laboratories.

Clinical Significance of tT4 Testing

Thyroxine (T4) and triiodothyronine (T3) are synthesized and secreted by the thyroid gland in response to stimulation by thyroid-stimulating hormone (TSH), a glycoprotein produced by thyrotrope cells in the anterior pituitary gland (**Figure 1**). T4 and T3 are involved in the negative feedback regulatory mechanisms, which control release of thyrotropin-releasing hormone (TRH) and TSH from the hypothalamus and pituitary gland, respectively.

Once secreted, >99% of T4 is bound to plasma proteins and <1% is unbound or free T4 (fT4). Both protein-bound and fT4 levels in the blood are measured with tT4 testing, whereas fT4 testing measures unbound hormone only.

Figure 1. Hypothalamic-Pituitary-Thyroid Axis



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

Canine tT4

Measurement of serum tT4 and fT4 concentrations, in conjunction with serum TSH concentrations, are currently recommended for the assessment of canine thyroid gland function.[2] Measurement of tT4 may be used as both a screening test for hypothyroidism and to monitor hypothyroid treatment.

Feline tT4

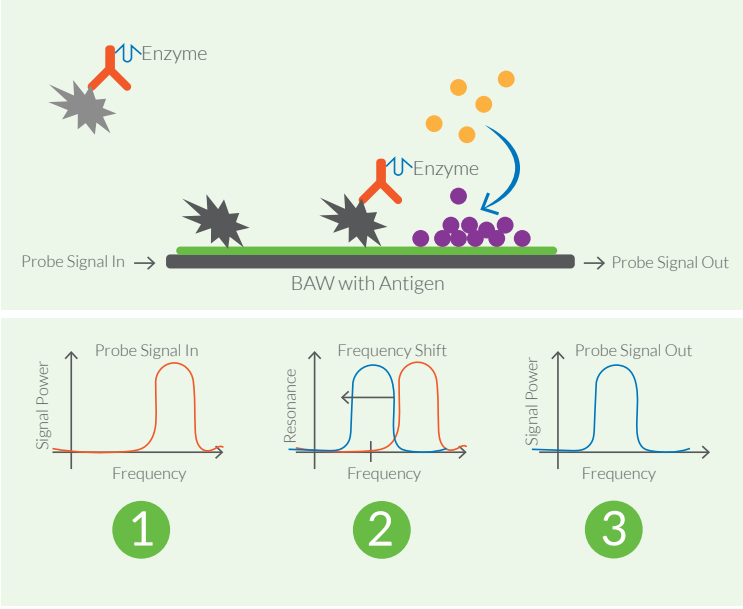
Serum tT4 measurements are recommended alone or in conjunction with fT4 or TSH testing for cats that are suspected to have hyperthyroid disease.[3] In addition, tT4 concentrations are used to monitor treatment of hyperthyroid disease.

- Accurate and reliable measurements of canine and feline tT4 levels are needed for the diagnosis of thyroid disease.
- A high-performance veterinary POC immunoassay for tT4, 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 POC in clinics. 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™ tT4 Immunoassay

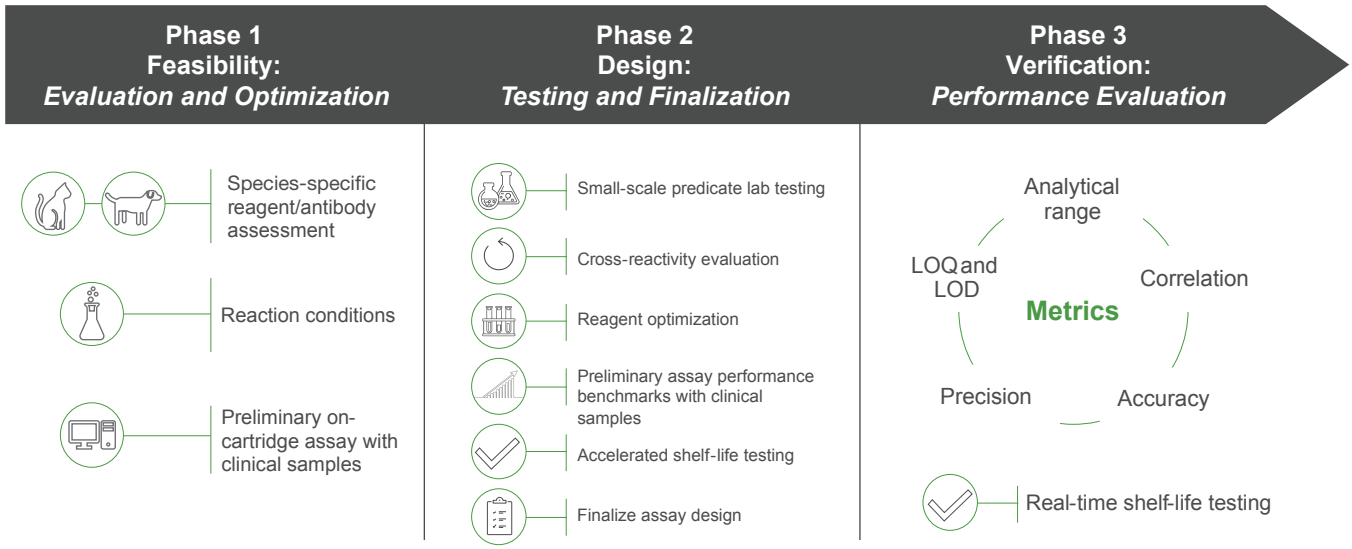


The TRUFORMA™ tT4 assay is a competitive immunoassay in which the BAW sensor is coated with antigen (dark gray). Antigen present in the sample (light gray) binds to an antibody-enzyme conjugate in solution and prevents the antibody from binding to the antigen-coated biosensor. After several wash steps, an enzyme substrate is exposed to the BAW biosensor surface and bound 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 in the sample. BAW, bulk acoustic wave; tT4, total thyroxine.

tT4 Assay Development Overview

The TRUFORMA™ canine and feline tT4 assay is a competitive immunoassay that uses a monoclonal anti-T4 antibody, which was selected for optimum performance for canine and feline testing. Using the industry standard recommendations for bioanalytical method validation[4] and the Clinical and Laboratory Standards Institute (CLSI) guidelines on method comparison and bias estimation (EP09c),[5] the TRUFORMA™ assay performance requirements were chosen to meet or exceed reference laboratory performance to provide unparalleled performance at the POC. The 3 phases of the canine and feline tT4 assay development were designed to provide a high-quality and reliable POC assay and included feasibility evaluation and optimization with species-specific assessment, design with testing of preliminary assay performance, and performance verification (**Figure 3**).

Figure 3. Overview of the TRUFORMA™ tT4 Assay Development



LOD, limit of detection; LOQ, limit of quantitation; tT4, total thyroxine.

Assay Verification Results

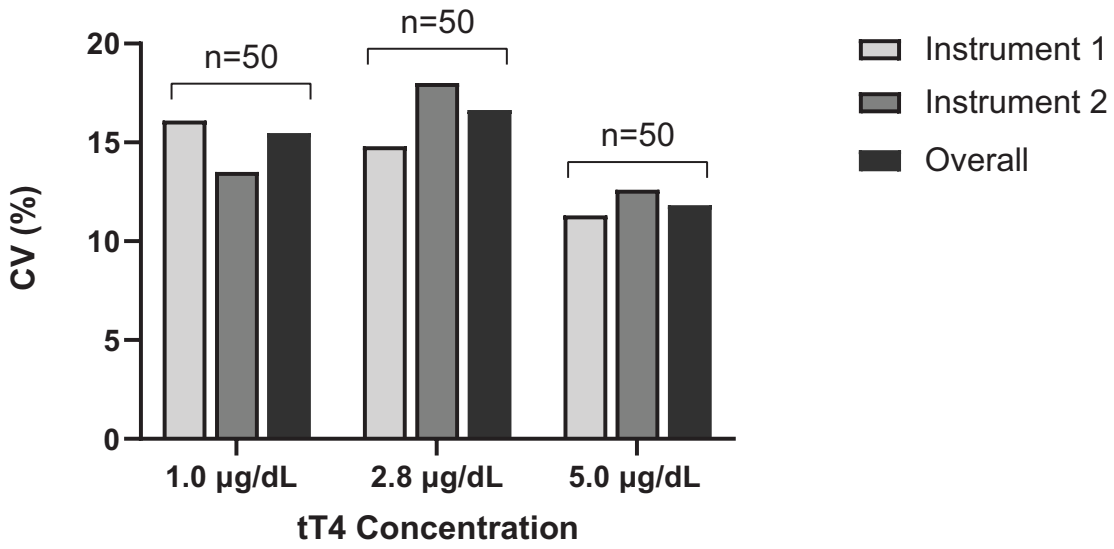
The TRUFORMA™ canine and feline tT4 assay analytical performance was evaluated and compared with the reference-laboratory predicate Siemens IMMULITE® 2000 Canine Total T4 assay. This is an automated solid-phase chemiluminescent competitive immunoassay that has been independently validated for feline use.[7] Normal, diseased, diluted, and spiked 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 3 serum samples with varying tT4 concentrations. Each sample was tested with 5 replicates on 5 separate days on 2 different instruments for a total of 150 results. Observed %CV was calculated.

The TRUFORMA™ tT4 assay demonstrated an overall %CV of <20% (**Figure 4**). 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.[4]

Figure 4. Precision of the TRUFORMA™ tT4 Assay



%CV was calculated for 3 serum samples with varying tT4 concentrations using 150 runs. One statistical outlier was removed from the 5.0 µg/dL data set following CLSI EP05-A3 guidelines. CV, coefficient of variation; tT4, total thyroxine.

Dynamic Range and Limit of Quantitation

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 LOQ [LLOQ]) and highest (upper LOQ [ULOQ]) analyte concentrations that can be reliably detected with predefined accuracy and precision.

The TRUFORMA™ tT4 assay had an LLOQ for both canine and feline serum samples that was slightly lower than that of the IMMULITE® Canine Total T4 assay (**Table 1**). The TRUFORMA™ tT4 assay's dynamic range (fold change >60) allows the quantification of both clinically high and clinically low canine and feline tT4 concentrations using the same consumable. This enhanced dynamic range can improve the ability to diagnose and treat at the POC without the need to send samples to a reference laboratory.

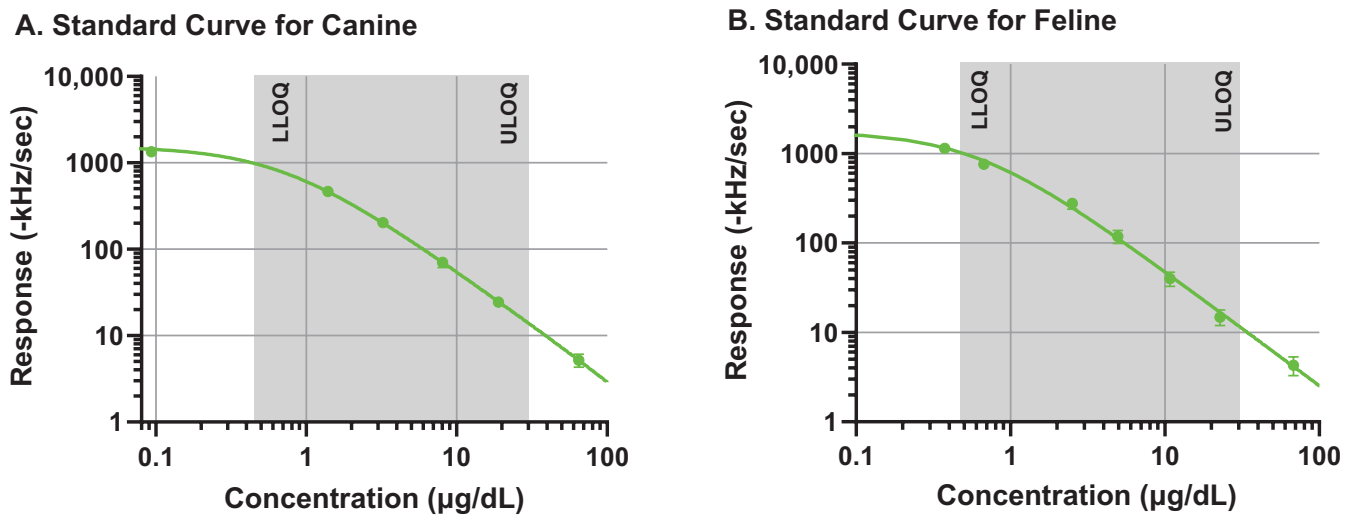
Table 1. Summary of the Dynamic Range for the TRUFORMA™ Canine and Feline tT4 Assay Compared With the Siemens IMMULITE® Canine Total T4 Assay

Test	TRUFORMA™			IMMULITE®
	Canine	Feline	Combined	Canine
Dynamic range, µg/dL	0.45 - >30	0.47 - >30	0.45 - >30	0.5 - 15
LLOQ, µg/dL	0.45	0.47	0.45	0.5
ULOQ, µg/dL	>30	>30	>30	15

LLOQ, lower limit of quantitation; tT4, total thyroxine; ULOQ, upper limit of quantitation.

For each species, 7 calibrators with known concentrations of tT4 were tested using the TRUFORMA™ tT4 cartridge. Each calibrator was run with 9 replicates across 3 different instruments, and the average value was used to generate a standard curve. The linearity and reportable range of the TRUFORMA™ tT4 assay illustrates linear performance within the clinically relevant range for the TRUFORMA™ tT4 assay (**Figure 5**).

Figure 5. Standard Curves of the TRUFORMA™ Canine and Feline tT4 Assay



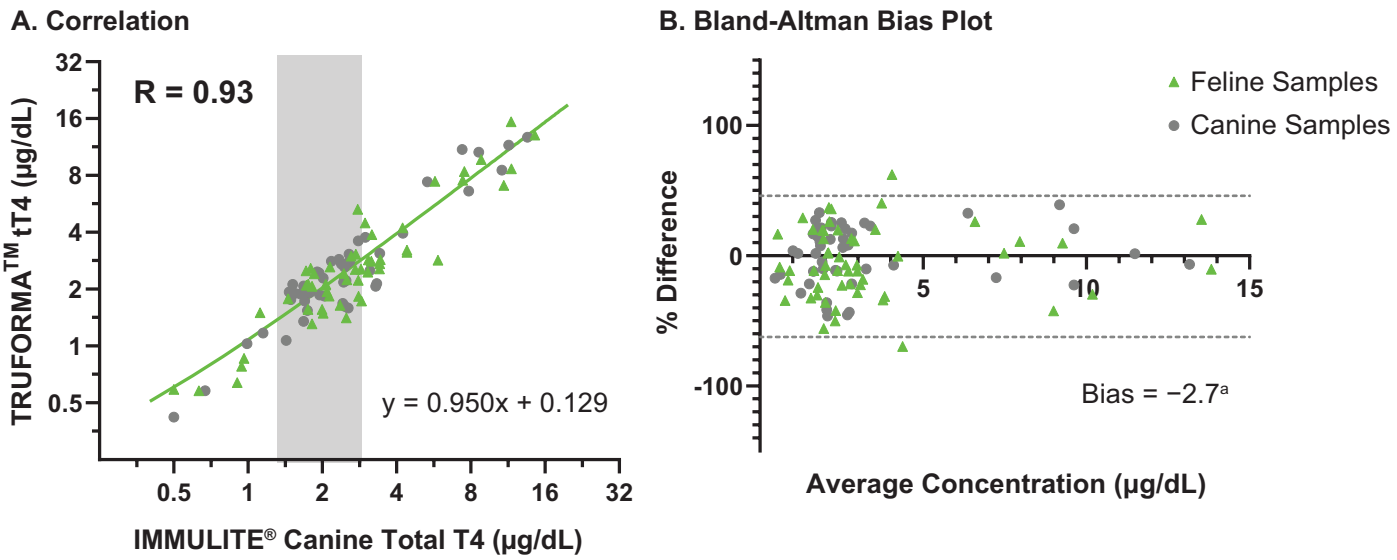
Seven calibrators with known concentrations of tT4 were used to generate a standard curve for (**A**) canine and (**B**) feline analysis. The shaded region represents the reported dynamic range. LLOQ, lower limit of quantitation; tT4, total thyroxine; ULOQ, upper limit of quantitation.

Assay Correlation Between TRUFORMA™ tT4 Assay and Siemens IMMULITE® Canine Total T4 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. A total of 101 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™ tT4 assay showed high correlation (R = 0.93) with the Siemens IMMULITE® Canine Total T4 assay (**Figure 6A**), with no apparent bias (bias, −2.7%; 95% CI, −7.6% to 2.2%) (**Figure 6B**) using canine and feline samples.

Figure 6. Correlation and Bias of TRUFORMA™ tT4 Assay Compared With the Siemens IMMULITE® Canine Total T4 Assay



(**A**) Correlation studies were performed comparing the results from the TRUFORMA™ tT4 and Siemens IMMULITE® Canine Total T4 assays using 47 canine samples and 54 feline samples. Dotted lines represent the 95% CI for the linear regression line; shaded region represents the IMMULITE® Canine Total T4 reference range.[7] (**B**) Bland-Altman bias plots were generated by plotting the mean concentration vs the % difference ([TRUFORMA™ – IMMULITE®]/mean) for canine and feline 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 bias. tT4, total thyroxine.

Cross-Reactivity

Known amounts of T4 (3.5 µg/dL) and potential cross-reactants were added to depleted serum and tested with ≥4 replicates using the TRUFORMA™ tT4 assay.

No significant cross-reactivity was observed in the TRUFORMA™ tT4 assay, and no cross-reactants interfered with the reported tT4 concentrations (**Table 2**).

Table 2. Summary of Cross-Reactivity for the TRUFORMA™ tT4 Assay

Material	Concentration, µg/dL	Cross-Reactivity, %[a]
D-T4	10	8.45
Tetraiodothyroacetic acid	10	4.97
L-T3	100	1.58
	25	0.41
3,5-Diiodo-L-tyrosine	1000	ND
Methimazole	1000	ND
5,5′-Dephenylhydantoin	1000	0.02
Phenylbutazone	1000	ND
D-T3	25	ND
	1	ND
L-T4	100	105.25
	12	100.00

[a]Values observed for TRUFORMA™ cross-reactivity are equal or below those reported for the IMMULITE® 2000.[7] ND, not detectable; T3, triiodothyronine; tT4, total thyroxine.

Conclusions

The TRUFORMA™ canine and feline tT4 assay demonstrated high precision as a POC diagnostic platform, with a wider dynamic range than the reference laboratory assay. The TRUFORMA™ canine and feline tT4 assay's extended dynamic range allows the quantification of both high and low tT4 concentrations within the same assay, which is vital for accurate diagnosis of canine and feline thyroid disease and allows for diagnostic confidence for tT4 concentrations near the decision thresholds. In addition, because tT4 measurements are often used to monitor the efficacy of treatment of thyroid disease, the availability of the TRUFORMA™ tT4 assay at the POC provides the opportunity for timely adjustments in medication dosing. Once reference ranges are established for the TRUFORMA™ tT4 assay, sensitivity and specificity results will be reported from third-party validation studies.

The TRUFORMA™ canine and feline tT4 assay provides veterinarians with accurate and reliable diagnostic results at the POC, allowing for improved client communication and patient treatment.

Acknowledgments

The authors would like to give special thanks to Elizabeth George, Phil Henning, and Brenna Maus for their hard work and dedication to development and data collection and to Kevin Gorman for valuable contributions to assay design and paper preparation. Third-party writing assistance was provided by Health Interactions, Inc.

Abbreviations and Acronyms

BAW Bulk acoustic wave
CLSI Clinical and Laboratory Standards Institute
CV Coefficient of variation
fT3 Free triiodothyronine
fT4 Free thyroxine
LOD Limit of detection
LOQ Limit of quantitation
LLOQ Lower limit of quantitation
ND Not detectable
POC Point-of-care
T3 Triiodothyronine
T4 Thyroxine
tT4 Total thyroxine
TRH Thyrotropin releasing hormone
TSH Thyroid-stimulating hormone
ULOQ Upper limit of quantitation

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