Element i+™ Total T4 Technical Summary

Introduction

Total thyroxine (T4) is the primary hormone produced by the thyroid gland and plays a central role in regulating metabolism. Measurement of Total T4 is a cornerstone of veterinary endocrine diagnostics in dogs and cats. In dogs, decreased Total T4 concentrations are supportive of hypothyroidism, while in cats, increased Total T4 concentrations are the principal indicator of hyperthyroidism.

Assay Overview

Intended use and reportable range

The Element i+ Total T4 assay is an in vitro diagnostic test for the quantitative determination of T4 in canine and feline serum or lithium heparin plasma. Results are intended to be used as an aid in the assessment of thyroid function, including evaluation of dogs suspected of hypothyroidism and cats suspected of hyperthyroidism, and for monitoring therapeutic response.

Reportable range: 0.7–20.0 µg/dL, 9-257 nmol/L

Principle of measurement

Competitive immunoassay performed on a proprietary planar waveguide cartridge. Endogenous Total T4 competes with surface bound antigen for binding to a fluorophore labeled antibody, generating an inverse fluorescence signal quantified via lot specific calibration.

Point of care platform

The assay runs on the Element i+ Analyzer with single use cartridges (50 µL sample). Results display automatically upon completion.

Materials and Methods

Specimens and reagents

Matrix: Canine/feline serum or lithium heparin plasma (other anticoagulants not evaluated)
Cartridge: Element i+ Total T4 cartridge with dried reagent pellet and lot specific calibration
Analyzer: Element i+ Analyzer

Method comparison

A method comparison study followed CLSI EP09c guidelines using canine and feline patient samples spanning the reportable range. The IMMULITE 2000 reference laboratory Total T4 immunoassay served as the comparative procedure. Feline (250) and canine (337) samples spanning the reportable range (0.7-20 µg/dL) were tested on both reference method and Element i+. Statistical analyses included Passing–Bablok regression and Bland–Altman difference analysis.

Precision and Reproducibility

A precision study was executed using a 15 × 2 × 3 design (15 days, two runs per day, three replicates per run) to estimate repeatability and within-laboratory precision. A reproducibility study was conducted using a 5 × 5 × 3 design (five days, five replicates per day, three instruments) to estimate repeatability and between-laboratory reproducibility. Study designs and statistical analyses were based on CLSI EP05-A3, with precision target of ≤15%, and reproducibility target of ≤20% for TT4 concentration >1.0 µg/dL.

Interference testing

A dedicated interference study evaluated the effect of common endogenous substances on Total T4 measurement using the Element i+ assay, following CLSI EP07 principles. Canine and feline serum or lithium heparin plasma samples were tested at clinically relevant Total T4 concentrations representative of the reference interval. Acceptance criteria required that the difference between interferent spiked samples and paired controls not exceed ±15%.

Interferents and concentrations tested

Protein:	8 g/dL
Bilirubin:	35 mg/dL
Cholesterol:	600 mg/dL
Hemoglobin:	1,000 mg/dL
Triglycerides:	1,000 mg/dL

Cross-reactivity

Cross-reactivity with structurally related thyroid hormones, iodothyronine derivatives, antithyroid agents, and commonly co-administered pharmaceuticals was evaluated following the CLSI EP07 framework. Test compounds were evaluated at supra-therapeutic concentrations to challenge assay specificity. Acceptance criteria were defined based on percent change relative to control samples to exclude clinically significant analytical interference.

Results

Based on Passing–Bablok regression, overall bias was <20% within the 95% confidence interval for samples ≥1.0 µg/dL (13 nmol/L) (per product insert). Agreement with the established laboratory method supports use of common, species-appropriate interpretive reference intervals consistent with reference-laboratory testing for canine and feline thyroid disease.

The regression plots comparing mean dose values from the IMMULITE® 2000 and Element i+ demonstrate strong performance, with excellent correlation and minimal bias observed for both species.

Canine: slope ≈ 0.95, r = 0.95
Feline: slope ≈ 1.06, r = 0.96

The Bland-Altman analysis, performed using a single replicate from each platform, indicated good agreement and no clinically significant systematic bias upon visual inspection of the difference plots. This conclusion is quantitatively supported by the following data:

Canine Samples: Mean bias of 0.11 (95% Limits of Agreement: -1.4 µg/dL to 1.6 µg/dL)
Feline Samples: Mean bias of -0.07 (95% Limits of Agreement: -1.5 µg/dL to 1.4 µg/dL)

These findings reinforce that the Element i+ Total T4 assay provides results consistent with the reference immunoassay, enabling continuity in clinical interpretation between in-hospital and reference laboratory. workflows.

Figure 1:
Regression plot of canine serum samples Element i+ vs. reference method. Red dotted line represents 95% Confidence Interval to the Regression Line.

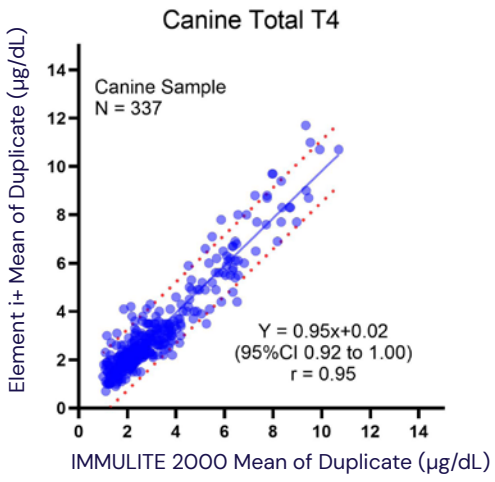


Figure 2:
Regression plot of feline serum samples Element i+ vs. reference method. Red dotted line represents 95% Confidence Interval to the Regression Line.

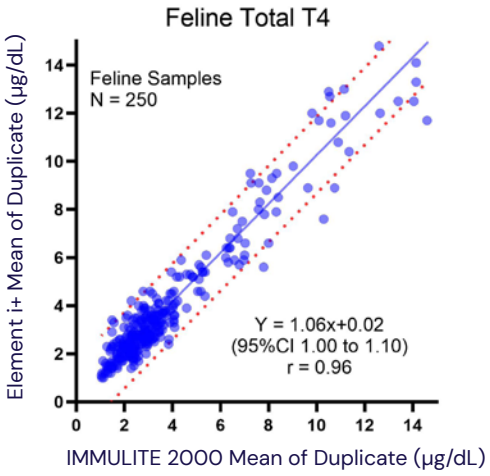


Figure 3:
Bland-Altman bias plot of normal range for canine samples dose comparison of Immulite 2000 vs. Element i+.

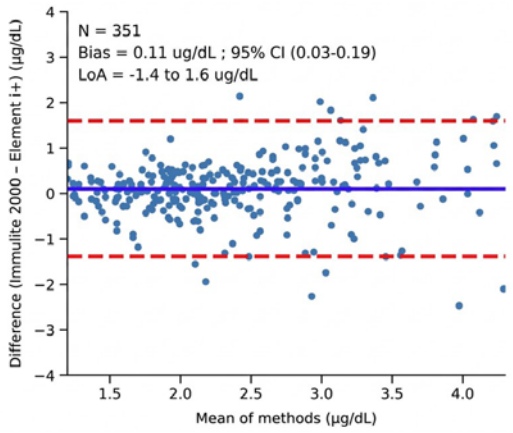
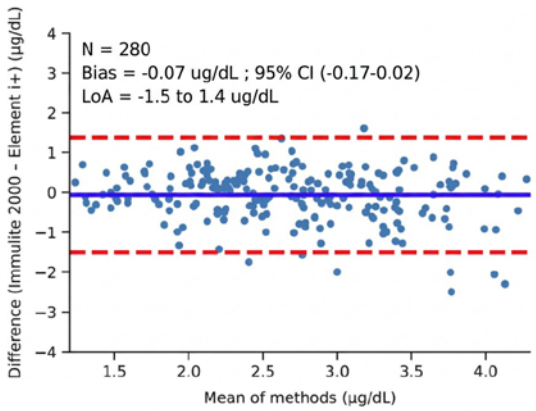


Figure 4:
Bland-Altman bias plot of normal range for feline samples dose comparison of Immulite 2000 vs. Element i+.



Precision and reproducibility (EP05-A3)

The data, summarized in Table 1 and 2, demonstrate good analytical precision and reproducibility across clinically relevant Total T4 concentrations. These results confirm that the Element i+ Total T4 assay meets or exceeds performance expectations under routine operating conditions.

Table 1: Summary of Precision Results

SAMPLE	N	MEAN (µg/dL)	TOTAL PRECISION %CV
1C	85	1.8	15
2C	86	3.4	10
3C	90	9.2	9
4F	89	9.0	11
5F	90	16.4	10
6F	90	18.3	8

Table 2: Summary of Reproducibility Results

SAMPLE	N	MEAN (µg/dL)	BETWEEN SITE %CV	REPRODUCIBILITY %CV
1C	72	1.8	3.8	17
2C	73	3.8	2.8	12
3C	75	9.1	2.5	8
4F	75	8.7	1.8	11
5F	73	15.3	3.5	9
6F	75	16.6	2.4	10

Interfering substances

The results for interfering substance testing are summarized in Table 3. For both low and high Total T4 samples, all substances produced on average ≤ 15% change versus controls. Precision (CV%) remained within acceptable limits across replicates. None of the tested substances caused clinically significant interference with the Element i+ Total T4 assay. The assay demonstrates robustness against common endogenous interferents, including hemolysis, icterus, lipemia, and hyperproteinemia.

Table 3: Summary of Interfering Substances

INTERFERENT	CONCENTRATION TESTED	RESULT
Protein	8 g/dL	No significant effect
Bilirubin	35 mg/dL	No significant effect
Cholesterol	600 mg/dL	No significant effect
Hemoglobin	1000 mg/dL	No significant effect
Triglycerides	1000 mg/dL	No significant effect

Cross Reactivity

Minimal analytical cross-reactivity was observed with structurally related thyroid hormones, iodothyronine derivatives, antithyroid agents, and commonly co-administered pharmaceuticals, with all tested substances producing ≤1% change in measured Total T4 concentrations. An exception was tetraiodothyroacetic Table 4 below shows the results.

Table 4: Summary of Cross Reactivity

TEST REACTANT		% CROSS-REACTIVITY
Tetraiodothyroacetic acid	10 µg/dL	7%
Triiodothyronine (T3)	100 µg/dL	<1%
Triiodothyroacetic acid	1000 µg/dL	<1%
Monoiodotyrosine	1000 µg/dL	<1%
Diiodo-L-tyrosine	1000 µg/dL	<1%
Methimazole	1000 µg/dL	<1%
5,5'-Diphenylhydantoin	1000 µg/dL	<1%
Phenylbutazone	1000 µg/dL	<1%
6-n-Propyl-2-thiouracil	1000 µg/dL	<1%

Conclusion

The Element i+ Total T4 assay demonstrates robust analytical performance. It shows excellent agreement with the reference method, evidenced by strong linear correlation (r=0.95 for canine, r=0.96 for feline) and minimal, clinically insignificant bias. The mean bias was just 0.11 µg/dL (canine) and 0.07 µg/dL (feline), with 95% Limits of Agreement falling within approximately ±1.5 µg/dL. Furthermore, the assay has acceptable precision, reproducibility, and minimal analytical interference.

Taken together, these characteristics of accuracy and reliability support confident, same-visit thyroid testing in dogs and cats.