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Comparison of 2 assays for measuring serum total thyroxine concentration in dogs and cats

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Abstract

Background: No gold standard assay for serum total thyroxine (TT4) concentration in small animals exists. The Microgenics DRI TT4 (MTT4) assay is used by most reference laboratories.

Hypothesis/Objectives: IDEXX Catalyst Total T4 (CTT4) and Immulite 2000 TT4 (ITT4) results will agree with MTT4 results.

Animals: Residual small animal sera were randomized before reanalysis (dogs, CTT4 versus MTT4: n = 176, ITT4 versus MTT4: n = 74; cats, CTT4 versus MTT4: n = 319, ITT4 versus MTT4: n = 79).

Methods: Validation and method comparison study. Serum TT4 concentration was measured on all analyzers. Pairwise Pearson correlation, cumulative sum linearity test, regression, and Bland-Altman method were performed.

Results: CTT4 versus MTT4 in dogs: constant bias (y-intercept) was 0.10 μg/dL (95% confidence interval [CI], 0.05-0.15), proportional bias (slope) was 0.86 μg/dL (95% CI, 0.83-0.89); in cats, constant bias was 0.13 μg/dL (95% CI, 0.08-0.20) and proportional bias was 1.01 μg/dL (95% CI, 0.98-1.03), but the test for linearity failed. Bland-Altman plots identified increasing disagreement with increasing serum TT4 concentrations.

ITT4 versus MTT4 in dogs, constant bias was 0.14 μg/dL (95% CI, 0.04-0.22) and 0.22 μg/dL (95% CI, 0.09-0.33) for cats; proportional bias was 0.76 (95% CI, 0.72-0.80) for dogs and 0.71 (95% CI, 0.69-0.74) for cats.

Conclusions and Clinical Importance: Differences in CTT4 and MTT4 results affect interpretation at higher serum TT4 concentrations. The ITT4 proportional bias will underestimate serum TT4 concentrations in dogs and cats, compared to MTT4. Serial TT4 measurements should be done using the same assay.

Abbreviations: ASVCP, American Society for Veterinary Clinical Pathology; C1, IDEXX Catalyst One Analyzer; CDx, IDEXX Catalyst Dx Analyzer; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; CTT4, Catalyst Total T4 Test; CUSUM, cumulative sum; CV, coefficient of variation; CVi, coefficient of variation, individual; EIA, enzyme immunoassay; ITT4, Immulite 1000 chemiluminescent TT4 assay; MTT4, Microgenics DRI human TT4 EIA assay; TT4, total thyroxine.

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1 | INTRODUCTION

Thyroid disorders are important endocrine diseases in companion animals with a prevalence of up to 0.8% in dogs[1] and 2% in cats.[2] The diagnosis of hypothyroidism in dogs is complicated by a number of factors including euthyroid sick syndrome, which causes a decrease in the measured serum total thyroxine (TT4) concentration in the presence of non-thyroidal illness.[3,4] Euthyroid sick syndrome also may complicate the diagnosis of hyperthyroidism in cats.[5] Radioimmunoassay and chemiluminescent techniques have been validated for measurement of TT4 in dogs and cats.[6,7] The Microgenics DRI TT4 enzyme immunoassay (EIA) designed for use in humans (MTT4; Microgenics Corporation, Fremont, California) is the assay most commonly used by commercial reference laboratories. This assay recently has been validated for use in healthy cats,[8] but validation has not been published in dogs. The previously developed Microgenics CEDIA TT4 EIA for humans was validated for cats and dogs against radioimmunoassay.[9] Validation of the Immulite 1000 chemiluminescent TT4 assay (Siemens Healthineers USA) has been published in both dogs[10] and cats.[2] These studies frequently have been cited in other published studies of thyroid function in dogs and cats.[11-14] The Immulite 2000 TT4 assay (ITT4; Siemens Healthineers USA) has been used to measure TT4 in dogs and cats, but full validation of this assay has not been published.[15,16] The MTT4 and ITT4 assays are both commonly utilized in commercial laboratories. The MTT4 assay has been evaluated for linearity, precision, accuracy, analytical range, and detection limits in healthy animals and those affected with hypothyroidism or hyperthyroidism (G. Bilbrough, personal communication), but in most cases this information has not been published in the peer-reviewed literature.

A novel dry-slide TT4 assay (CTT4; Catalyst Total T4 Test, IDEXX Laboratories, Inc, Westbrook, Maine) recently was introduced for in-clinic use using 2 bench top point-of-care analyzers (IDEXX Catalyst One Analyzer [C1]; IDEXX Catalyst Dx Analyzer [CDx]). The CTT4 assay has been compared previously to the MTT4 assay using sera from untreated hyperthyroid and ^{131}I-treated hyperthyroid cats with favorable agreement, coefficient of variation (CV), and linearity.[17] Guidelines from the American Society for Veterinary Clinical Pathology (ASVCP) state that comparative testing for quality assurance between established methods and newly developed methods should be performed to assess whether constant bias or proportional bias exist that could interfere with clinical interpretation of results.[18]

We hypothesized that the CTT4 and ITT4 in dogs and cats would be equivalent to the MTT4 for clinically relevant TT4 test results. Our objectives were (1) to compare serum concentrations of TT4 determined using MTT4 run on an Olympus AU 400 analyzer (Beckman-Coulter, Brea, California) to results of the CTT4 run on the C1 or CDx analyzers and (2) to compare the ITT4 run on the Immulite 2000 analyzer (Siemens Healthineers USA) to the MTT4 run on the Olympus AU 400 analyzer. Because no true gold standard assay has been established for measurement of TT4 in dogs and cats, for the aims of this study, the ITT4 and CTT4 were compared to the commonly used MTT4 assay.

2 | MATERIALS AND METHODS

This study was designed as a method-comparison study. All samples were obtained from residual dog and cat serum samples remaining after primary diagnostic testing at a reference laboratory (IDEXX Laboratories, Inc) between September 23, 2015, and October 26, 2015. All samples had serum TT4 concentration determined at the time of submission by the Microgenics DRI TT4 assay designed for humans (Microgenics, Freemont, California) and run on an Olympus AU 400 analyzer (Beckman-Coulter) following the normal laboratory protocol. All samples were stored at −80°C for up to 33 days until aliquoted. IDEXX Laboratories approved the use of these residual serum samples for research purposes.

Samples with insufficient volume were excluded. Individual samples were removed from storage, thawed at room temperature for 5 minutes, and transferred into new tubes that were labeled only with random numbers by 1 investigator (E.D.S.W.), and stored again at −80°C for 3.5 to 7.5 months. Batches of serum samples later were thawed and tested using multiple assays (CTT4 versus MTT4 and ITT4 versus MTT4 study arms) at the same time without intervening freeze-thaw cycles. Samples therefore were subjected to 2 freeze-thaw cycles (1 after initial sample submission for randomization and another after sample randomization) before the final round of testing, which took place a maximum of 233 days after initial sampling. To ensure that a full range of TT4 concentrations would be included, the tubes containing residual serum were organized into groups, which contained low, low-normal, high-normal, and high TT4 concentrations, respectively, based on initial MTT4 results and clinical cut points from the reference laboratory (Table 1). Samples were chosen with the aim of achieving a minimum of 40 samples per typical TT4 concentration group for each species for the comparison between the IDEXX Catalyst TT4 assay run on either the C1 or CDx analyzer (IDEXX Laboratories) and the MTT4 assay, and a minimum of 20 samples per typical TT4 concentration group for the ITT4 to MTT4 comparison.

The 3 assay systems utilized consisted of the Microgenics DRI TT4 assay for humans (Microgenics, Freemont) run on the Olympus AU 400 analyzer (Beckman-Coulter), the IDEXX Catalyst TT4 assay run on either the C1 or CDx analyzer (IDEXX Laboratories), and the Immulite 2000 TT4 assay run on the Immulite analyzer (Canine TT4, Siemens Healthineers USA). The MTT4 assay was run twice for all samples, when the samples were tested at the time of submission, and again for this study. Reportable ranges for the MTT4 are 0.5-15 μg/dL

TABLE 1 Study population concentration groups for dog and cat TT4

Concentration groups for TT4	
Dog **Total sample size**	**Cat**
CTT4 versus MTT4: n = 176	CTT4 versus MTT4: n = 319
ITT4 versus MTT4: n = 74	ITT4 versus MTT4: n = 79
Low: <1.00 μg/dL (<12.87 nmol/L)	**Low**: <0.80 μg/dL (<10.30 nmol/L)
CTT4 versus MTT4: n = 34	CTT4 versus MTT4: n = 20
ITT4 versus MTT4: n = 22	ITT4 versus MTT4: n = 21
Low normal:1.00-2.50 μg/dL (12.87-32.18 nmol/L)	**Low normal**: 0.80-3.00 μg/dL (10.30-38.62 nmol/L)
CTT4 versus MTT4: n = 75	CTT4 versus MTT4: n = 175
ITT4 versus MTT4: n = 22	ITT4 versus MTT4: n = 22
High normal: >2.50-4.00 μg/ dL (>32.18-51.49 nmol/L)	**High normal**: >3.00-4.70 μg/ dL (>38.62-60.50 nmol/L)
CTT4 versus MTT4: n = 31	CTT4 versus MTT4: n = 42
ITT4 versus MTT4: n = 19	ITT4 versus MTT4: n = 19
High: >4.00 μg/dL (>51.49 nmol/L)	**High**: 4.70 μg/dL (>60.50 nmol/L)
CTT4 versus MTT4: n = 36	CTT4 versus MTT4: n = 82
ITT4 versus MTT4: n = 11	ITT4 versus MTT4: n = 17

Note: Samples for this study were divided into low, low normal, high normal, and high TT4 concentration clinical cut-point groups for the dog and cat for the purposes of choosing a well-represented distribution of concentrations based on MTT4.
Abbreviations: CTT4, Catalyst Total T4 Test; ITT4, Immulite 1000 chemiluminescent TT4 assay; MTT4, Microgenics DRI human TT4 EIA assay.

(6.44-193.08 nmol/L) in dogs and 0.5-20 μg/dL (6.44-257.44 nmol/L) in cats. The reference laboratory performs dilutions above 12 μg/dL (154.46 nmol/L) in cats.[17] The CTT4 reportable range is 0.5-10 μg/dL (6.44-128.72 nmol/L) for dogs and 0.5-20 μg/dL (6.44-257.44 nmol/L) for cats. The ITT4 reportable range for dogs and cats is 0.5-15 μg/dL (6.44-193.08 nmol/L). The C1 and CDx platforms were treated as the same platform for the purposes of this study. Previously, IDEXX Laboratories completed Catalyst Total T4 method comparisons, separately for dog (n = 213) and cat samples (n = 213), which showed a very strong correlation (dog, r = .99; cat, r = .97) and minimal bias (dog slope, 0.97; intercept, 0.05 μg/dL [0.64 nmol/L]; cat slope, 0.97; intercept, 0.11 μg/dL [1.42 nmol/L]) between the 2 catalyst analyzers (G. Bilbrough, personal communication). All assays were performed by experienced laboratory technicians following the manufacturer instructions. Laboratory personnel were blinded to the original TT4 concentration measured by MTT4.

Quality control was performed weekly for the Catalyst Dx and Catalyst One for 2 concentrations. The Olympus AU 400 analyzer had daily calibration and quality control runs for 5 concentrations. The Immulite 2000 had daily calibration and quality control runs performed for

3 concentrations. All samples were run in duplicate on the CTT4, MTT4, and ITT4.

The method-comparison analysis was performed as recommended in ASVCP guidelines[18,19] using commercial statistical software (Stata SE, version 14.1, College Station, Texas; MedCalc version 16.8.4, Ostend, Belgium) on the whole data set. Differences between analytic results for paired methods were calculated as raw difference and percentage deviation from the MTT4 concentration and are not reported. Samples with paired results differing by >50% (strongly discordant results) were assessed to determine whether there was possible laboratory error or if the difference was related to results beyond detection limits.

2.1 | Validation

Validation was performed for the CTT4 and MTT4 assays in dogs. To determine precision, guidelines from the Clinical and Laboratory Standards Institute (CLSI) EP05 were utilized.[17,20] For intra-assay variability, 5 replicate TT4 measurements were performed on 3 serum pools on the same day and CV was calculated. Inter-assay variability was calculated from 20 replicate samples taken from 4 serum pools analyzed on 5 days. Linearity and limits of the blank also were determined using CLSI guidelines which have been described in depth using the same protocol.[17]

2.2 | Statistical analysis

Statistical analyses included calculation of the Pearson correlation coefficient (r), and the Passing-Bablok linear regression to measure constant and proportional bias.[21,22] Cumulative sum (CUSUM) linearity was assessed in the Passing-Bablok regression because of the assumption of linearity for this regression model.[23] If the CUSUM test for linearity indicated significant deviation, then piecewise/segmented regression was performed, which determined a *y*-intercept and slope for an initial linear segment, a breakpoint/knot, and a slope for a second linear segment proceeding from the breakpoint.[24]

Bland-Altman plots were performed to compare the difference in measured serum TT4 concentration to the average concentration for the MTT4 versus CTT4 and MTT4 versus ITT4 comparisons in both cats and dogs.[25,26]

3 | RESULTS

The specimens for all method comparisons were drawn from samples submitted from the northeast United States. Signalment, other demographic information, and history pertinent to submitted samples were not available for evaluation. Sample size information for each species and concentration group is summarized in Table 1.

3.1 | Dogs

For the CTT4 in dogs, the intra-assay CV for low, medium, and high TT4 concentrations were 2.44% at 0.88 μg/dL (11.33 nmol/L), 6.5% at 2.2 μg/dL (28.32 nmol/L), and 7.12% at 3.96 μg/dL (50.97 nmol/L), and the inter-assay CV was 2.31% at 0.88 μg/dL (11.33 nmol/L), 9.7% at 2.3 μg/dL (29.61 nmol/L), and 8.7% at 4.0 μg/dL (51.49 nmol/L). The limit of blank for the MTT4 was 0.65 μg/dL (8.37 nmol/L). The limit of blank for the CTT4 was 0.82 μg/dL (10.56 nmol/L). The CTT4 assay was linear from 0.5 to 10 μg/dL (6.44-128.72 nmol/L) for both the C1 and CDx. For the MTT4 in dogs, the inter-assay CV were 13.81% at 1.09 μg/dL (14.03 nmol/L), 8.99% at 2.03 μg/dL (26.13 nmol/L), and 3.55% at 3.82 μg/dL (49.17 nmol/L). The intra-assay CVs for MTT4 were 3.2% at 0.41 μg/dL (5.28 nmol/L), 2.9% at 1.98 μg/dL (25.49 nmol/L), and 3.2% at 5.53 μg/dL (71.18 nmol/L). The MTT4 assay was linear from 0.44 to 12.81 μg/dL (5.66-164.89 nmol/L). Intra-assay variability for the ITT4 was reported as a mean of 13.75% with an inter-assay mean variability of 14.25% and a lower limit of detection of 0.25 μg/dL (3.22 nmol/L).[27]

3.1.1 | CTT4 versus MTT4

For the CTT4 versus MTT4 method comparison, MTT4 values ranged from 0.52 to 13.23 μg/dL (6.69-170.30 nmol/L) with a median of 1.99 μg/dL (25.62 nmol/L; Table 2). Correlation between the CTT4 and MTT4 results was excellent (r = .98; Table 2). No significant deviation from linearity was detected using the Passing-Bablok regression, and the y-intercept of the regression equation indicating constant bias was 0.10 μg/dL; 95% confidence interval (CI), 0.05-0.15 (1.29 nmol/L; 95% CI: 0.64-1.93 nmol/L) which did not include 0 (Figure 1A, Table 2). The slope of the Passing-Bablok regression was 0.86 (95% CI, 0.83-0.89; Figure 1A, Table 2) which

did not include 1, indicating proportional bias was present. Based on the lower value of the CI interval for the biases, users of the CTT4 should expect no more than a 0.80 μg/dL (10.30 nmol/L) discrepancy between the 2 assays at TT4 concentrations <5 μg/dL (64.36 nmol/L). On the Bland-Altman plot (Figure 1B), there was an average absolute difference of 0.21 μg/dL (2.70 nmol/L). Greatest disagreement in TT4 results between the 2 analyzers was noted at MTT4 concentrations >3 μg/dL (38.62 nmol/L).

3.1.2 | ITT4 versus MTT4

Strongly discordant results were observed in 9 dogs with MTT4 concentrations in the range of 0.52-0.84 μg/dL (6.69-10.81 nmol/L), median 0.56 μg/dL (7.21 nmol/L), but with ITT4 concentrations of <0.50 μg/dL (6.44 nmol/L). These results were excluded from the analysis and 65 dog results were included. For the ITT4 versus MTT4 method comparison, MTT4 values ranged from 0.83-11.07 μg/dL (10.68-142.49 nmol/L) with a median of 2.63 μg/dL (33.85 nmol/L). No significant deviation from linearity was found (CUSUM test; P = .41) and correlation between the ITT4 and MTT4 on regression was excellent (r = .99; Table 2). The y-intercept was 0.14 μg/dL indicating constant bias; 95% CI, 0.04-0.22 μg/dL (1.80 nmol/L; 95% CI, 0.51-2.83 nmol/L; Figure 2A, Table 2). The slope of the regression was 0.76 (95% CI, 0.72-0.80), indicating significant proportional bias was present (Figure 2A, Table 2). The Bland-Altman plots showed a difference of −2 μg/dL (−25.74 nmol/L) from the mean for the ITT4 compared to the MTT4 at TT4 concentrations >6 μg/dL (77.23 nmol/L) indicating greater disparity at higher concentrations (Figure 2B), consistent with the proportional bias. Enough disparity was present to alter interpretation at concentrations >3 μg/dL (38.62 nmol/L).

TABLE 2 Method comparison of canine and feline TT4 results by the Passing-Bablok regression including sample size (n), MTT4 value, non-reference value (either CTT4 or ITT4) depending on the row, correlation (r) value, constant bias (y-intercept), proportional bias (slope), and cumulative sum (CUSUM) linearity

	n	MTT4 observed range	Non-reference method observed range	r	Constant bias (y-intercept)	Proportional bias (slope)	CUSUM linearity
Dog							
CTT4 versus MTT4	176	0.52-13.23 μg/dL	0.54-12.28 μg/dL	0.98	0.10 μg/dL (95% CI: 0.05-0.15 μg/dL)	0.86 (95% CI: 0.83-0.89)	No significant deviation (P = .63)
ITT4 versus MTT4	64[a]	0.83-11.07 μg/dL	0.60-9.70 μg/dL	0.99	0.14 μg/dL (95% CI: 0.04-0.22 μg/dL)	0.76 (95% CI: 0.72-0.80)	No significant deviation (P = .41)
Cat							
CTT4 versus MTT4	319	0.59-19.77 μg/dL	0.60-18.10 μg/dL	0.99	0.13 μg/dL (95% CI: 0.08-0.20 μg/dL)	1.01 (95% CI: 0.98-1.03)	Piecewise regression performed[b]
ITT4 versus MTT4	58[c]	0.73-10.12 μg/dL	0.59-7.10 μg/dL	0.99	0.22 μg/dL (95% CI: 0.09-0.33 μg/dL)	0.71 (95% CI: 0.69-0.74)	No significant deviation (P = .30)

Abbreviations: CTT4, Catalyst Total T4 Test; ITT4, Immulite 1000 chemiluminescent TT4 assay; MTT4, Microgenics DRI human TT4 EIA assay.
[a]Nine of 74 samples excluded due to ITT4 <0.50 μg/dL but MTT4 0.50-0.84 μg/dL.
[b]Piecewise regression was performed for this regression.
[c]Nineteen of 79 samples excluded due to ITT4 <0.50 μg/dL but MTT4 0.52-0.93 μg/dL, with an additional 3 samples excluded for other reasons.
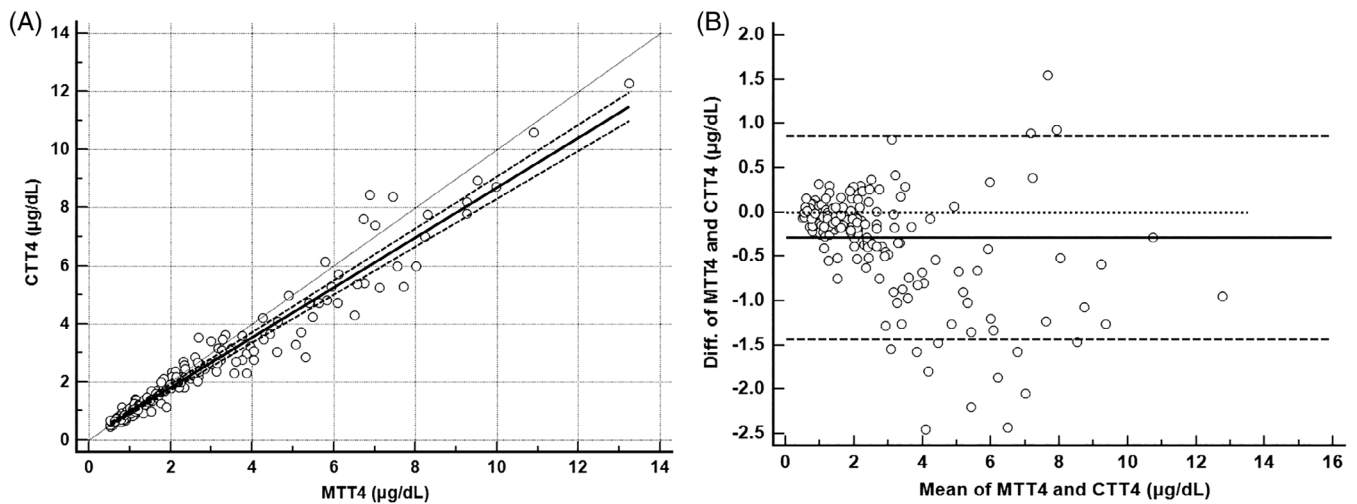
FIGURE 1   A, Passing-Bablok regression of canine CTT4 versus MTT4. The regression equation for the Passing-Bablok regression was $y = 0.103 + 0.860x$. The solid line represents the data regression line with dashed lines representing the confidence intervals. B, Bland-Altman plot of CTT4 versus MTT4 for dogs. The solid line represents the mean difference (-0.28 μg/dL [-3.60 nmol/L]) and the dashed lines represent the mean difference ±1.96 SD (-1.43-0.86 μg/dL [-18.41-11.07 nmol/L]). CTT4, Catalyst Total T4 Test; MTT4, Microgenics DRI human TT4 EIA assay
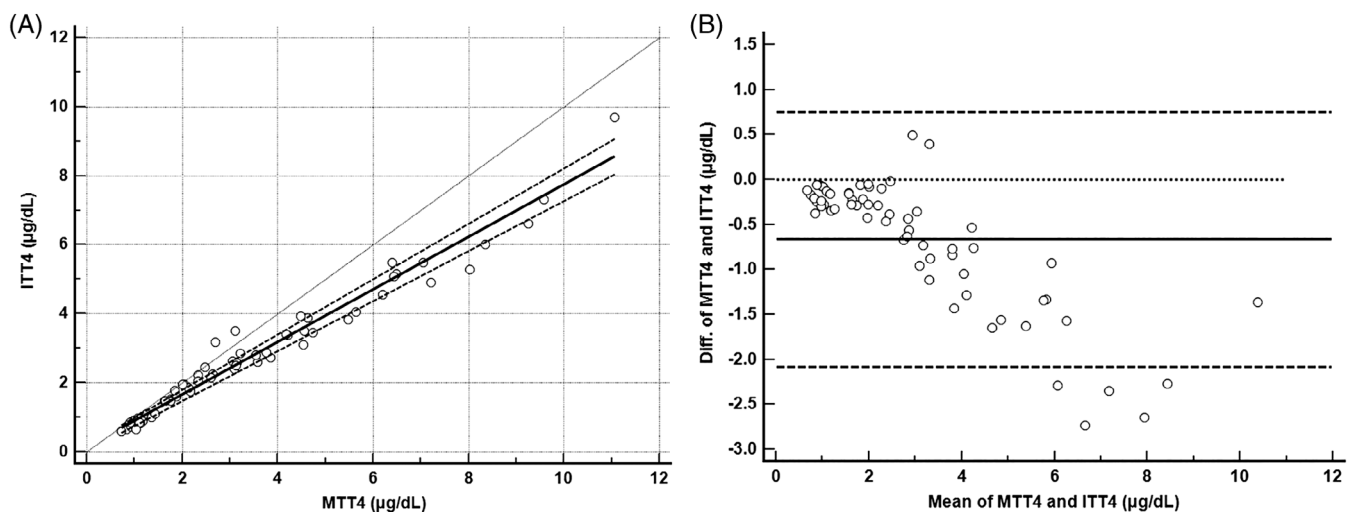

FIGURE 2   A, Passing-Bablok regression of canine ITT4 versus MTT4. The regression equation for the Passing-Bablok regression was $y = 0.144 + 0.762x$. The solid line represents the data regression line with dashed lines representing the confidence intervals. B, Bland-Altman plot of ITT4 versus MTT4 for dogs. The solid line represents the mean difference (-0.66 μg/dL [8.50 nmol/L]) and the dashed lines represent the mean difference ±1.96 SD (-2.08-0.75 μg/dL [-26.77-9.65 nmol/L]). ITT4, Immulite 1000 chemiluminescent TT4 assay; MTT4, Microgenics DRI human TT4 EIA assay

3.2 | Cats

The CTT4 assay previously has been validated for cats. Intra-assay variability had a mean of 2.8%, inter-assay variability had a mean of 6.6%, and the assay was linear from 0 to 11.65 μg/dL (0-149.96 nmol/L).[17] Published CV data from MTT4 validation in cats showed inter- and intra-assay variability <12%, and the assay was linear from 0.44 to 11.73 μg/dL (5.66 to 150.99 nmol/L).[8] Published CV data from ITT4 validation in cats showed intra-assay variability of 14.5% at a mean of 1.3 μg/dL (16.73 nmol/L), 8.2% at a mean of 5.2 μg/dL (66.93 nmol/L),

and 4.5% at a mean of 11.9 μg/dL (153.18 nmol/L). The interassay variability was 14.9% at a mean of 1.3 μg/dL (16.73 nmol/L), 9.0% at a mean of 5.2 μg/dL (66.93 nmol/L), and 7.0% at a mean 11.9 μg/dL (153.18 nmol/L) and a lower limit of detection of 0.3 μg/dL (3.86 nmol/L).[2]

3.2.1 | CTT4 versus MTT4

The MTT4 results ranged from 0.59 to 19.77 μg/dL (7.59 to 254.48 nmol/L) with a median result of 2.14 μg/dL (27.55 nmol/L). Although

correlation between the CTT4 and MTT4 was excellent (r = .99), the CUSUM test for linearity identified significant deviation from linearity (P < .01; Table 2) and piecewise regression analysis was performed. The calculated y-intercept (indicating constant bias) and slope (indicating proportional bias) of the initial linear segment included 0 (y-intercept = −0.15 μg/dL; 95% CI, −0.52 to 0.22 μg/dL; −1.93 nmol/L; 95% CI, −6.69 to 2.83 nmol/L) and 1 (slope = 1.25; 95% CI, 0.93-1.56), respectively, until the MTT4 breakpoint of 1.80 μg/dL (95% CI, 0.94-2.66 μg/dL; 23.17 nmol/L; 95% CI, 12.10-34.24 nmol/L). The second linear segment from 1.80 μg/dL (23.17 nmol/L) had a slope of 0.94 (95% CI, 0.93-0.96), indicating significant proportional bias in concentrations

greater than the breakpoint. On the Bland-Altman plot (Figure 3B), greatest variation in TT4 results between the 2 analyzers was noted in MTT4 concentrations >11 μg/dL (141.59 nmol/L).

3.2.2 | ITT4 versus MTT4

After excluding a single strongly discordant result (>6-fold difference) as a possible laboratory error, 19 cats had MTT4 concentrations ≥0.50 μg/dL (6.44 nmol/L) but with ITT4 concentrations of <0.50 μg/dL (6.44 nmol/L; MTT4 range, 0.63-0.93 μg/dL [8.11-11.97 nmol/L;
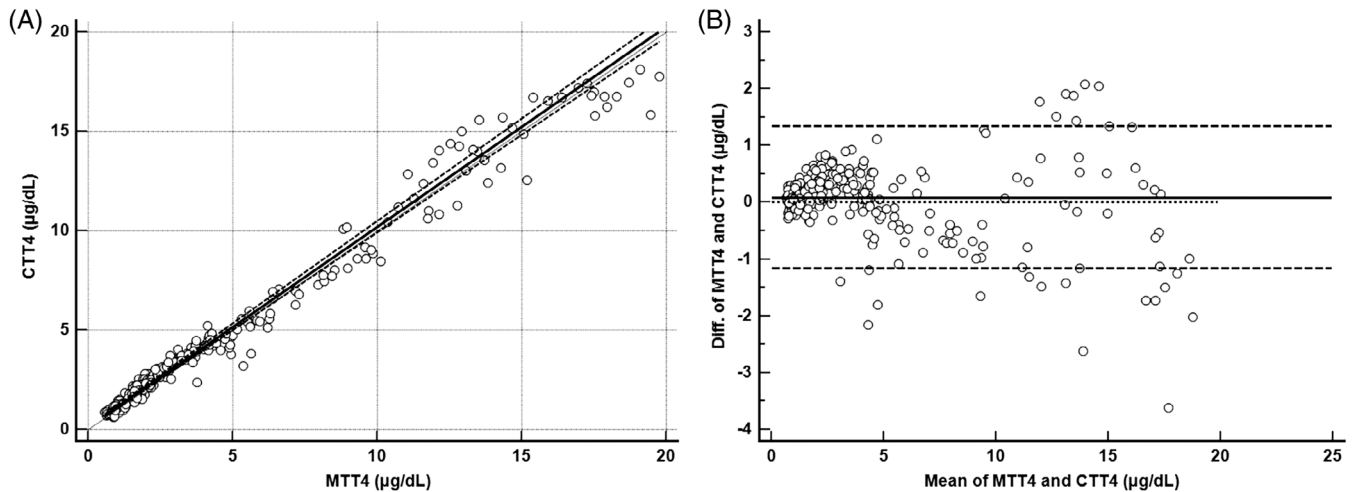

FIGURE 3   A, Passing-Bablok regression line of feline CTT4 versus MTT4 is shown but the assumption of linearity was violated. Piecewise regression analysis yielded a slope of 1.25 (95% CI, 0.93-1.56) indicating a tendency to overestimate TT4 concentration until the MTT4 breakpoint of 1.80 μg/dL (95% CI, 0.94-2.66 μg/dL) [23.17 nmol/L; 95% CI, 12.10-34.24 nmol/L]. The second linear segment from 1.80 μg/dL [23.17 nmol/L] had a slope of 0.94 (95% CI, 0.93-0.96), indicating proportional bias with subsequent underestimation of TT4 concentrations. B, Bland-Altman plot of CTT4 versus MTT4 for cats. The solid line represents the mean difference (0.10 μg/dL [1.29 nmol/L]) and the dashed lines represent the mean difference ±1.96 SD (−1.19-1.30 μg/dL [−15.32-16.73 nmol/L]). CI, confidence interval; CTT4, Catalyst Total T4 Test; MTT4, Microgenics DRI human TT4 EIA assay
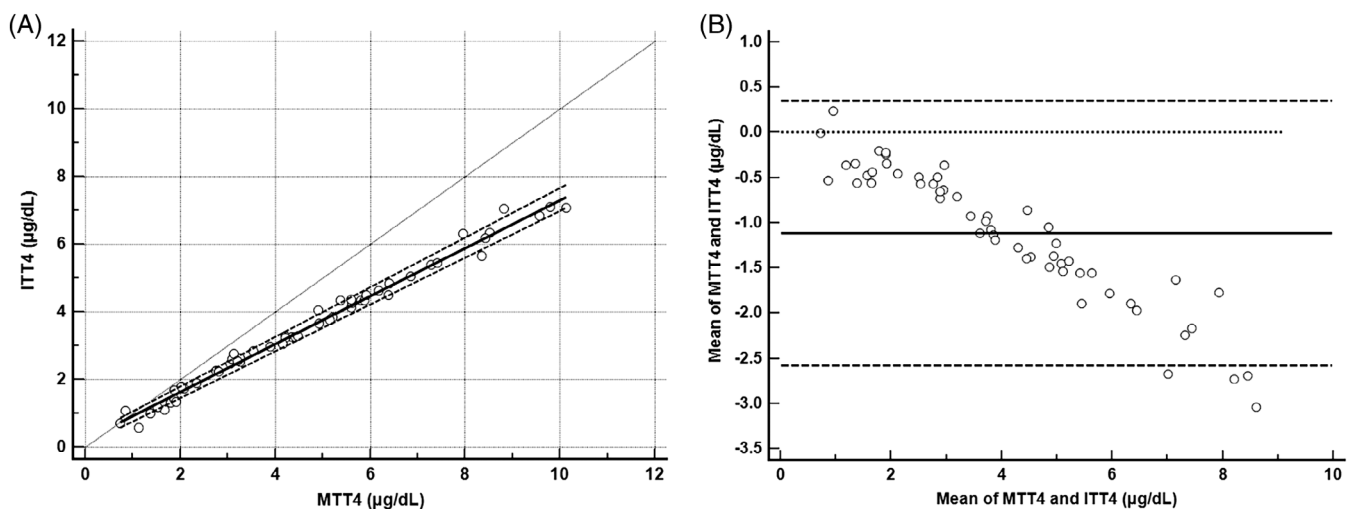

FIGURE 4   A, Passing-Bablok regression of feline ITT4 versus MTT4. The regression equation for the Passing-Bablok regression was y = 0.220 + 0.710x. The solid line represents the data regression line. B, Bland-Altman plot of ITT4 versus MTT4 for cats. The solid line represents the mean difference (−1.11 μg/dL [−14.29 nmol/L]) and the dashed lines represent the mean difference ±1.96 SD (−2.58-0.35 μg/dL [−33.21-4.51 nmol/L]). ITT4, Immulite 1000 chemiluminescent TT4 assay; MTT4, Microgenics DRI human TT4 EIA assay

median, 0.74 µg/dL [9.53 nmol/L]). The use of a designated low result of 0.5 µg/dL (6.44 nmol/L) for ITT4 for this many samples also impacted the regression equation, and these samples were excluded from further regression analysis. One cat had an ITT4 concentration >24 µg/dL (308.93 nmol/L) recorded (MTT4 = 17.26 µg/dL [222.17 nmol/L]). These results also were excluded from the analysis with the remaining 58 cats included in the study. The MTT4 concentrations ranged from 0.73-10.12 µg/dL (9.40-130.26 nmol/L) with a median of 4.35 µg/dL (55.99 nmol/L). No significant deviation from linearity was found (CUSUM test; P = .30), and correlation between the ITT4 and MTT4 was excellent (r = .99; Table 2). The y-intercept of the Passing-Bablok regression was 0.22 µg/dL; 95% CI, 0.09 to 0.33 [2.83 nmol/L; 95% CI, 1.16-4.25 nmol/L). The slope was 0.71 (95% CI, 0.69-0.74; Figure 4A, Table 2). Stronger proportional bias was evident at concentrations >8 µg/dL (102.98 nmol/L) on Bland-Altman plots.

4 | DISCUSSION

Our study suggests that in dogs with hypothyroidism, euthyroid sick syndrome, and drug-induced, breed- or age-related low serum thyroid hormone concentrations, or those receiving appropriate thyroid supplementation,[4] the results of CTT4 and MTT4 <3 µg/dL (38.62 nmol/L) are expected to be equivalent. At higher serum concentrations of TT4, such as in animals with functional thyroid tumors, those fed raw bone or bone meal diets or those that may have been over-supplemented with L-thyroxine, a difference should be anticipated, with slightly higher concentrations expected with the CTT4 assay compared to the MTT4 assay. For cats, the minimal bias present between the CTT4 versus MTT4 assays should not affect clinical decisions at concentrations <10 µg/dL (<128.72 nmol/L). At concentrations of TT4 ≥ 10 µg/dL (≥128.72 nmol/L) for cats, a significant difference should be expected between the 2 assays. This difference would not be expected to affect assessment of whether or not the patient had hyperthyroidism, but it potentially could affect assessment of treatment efficacy and calculation of radioactive iodine dose.[5] This finding was the result of marked heteroscedasticity and was similar to the results of a recent comparison of CTT4 versus MTT4 in 157 cats.[17] Although statistical evidence of nonlinearity was observed in the comparisons using cat sera, nonlinearity was not visually striking in the scatter or Bland-Altman plots. Nonlinear regression provided 2 separate intersecting segments, but the slopes or proportional bias related to each segment would be unlikely to affect the clinical interpretation of these results in cats.

The ITT4 versus MTT4 method comparison identified a constant bias in dogs and cats that did not contribute significantly to the total bias. This comparison did show moderate proportional bias in both dogs and cats. This finding particularly affected MTT4 results of <1 µg/dL (12.87 nmol/L) in which a number of corresponding ITT4 results were less than the reportable range of 0.5 µg/dL (6.44 nmol/L). This bias is unlikely to affect clinical decision-making, because results <1 µg/dL (12.87 nmol/L) and <0.5 µg/dL (6.44 nmol/L) are either compatible with hypothyroidism or the effect of concurrent illness. The

proportional bias seen in the Bland-Altman plots showed an increasing difference in dogs with TT4 concentrations >5 µg/dL (64.36 nmol/L). This difference would be unlikely to change clinical decisions regarding diagnosis of either iatrogenic oversupplementation or a functional thyroid tumor. The stronger proportional bias evident at values >8 µg/dL (102.98 nmol/L) on Bland-Altman plots would be unlikely to change clinical decisions regarding diagnosis of hyperthyroidism because patients would be considered hyperthyroid at this concentration regardless of the assay employed, although it might affect choice of dose for radioactive iodine treatment and assessment of treatment efficacy.

Our study had some limitations. First, because samples were drawn from residual blood sent to a reference laboratory, case information was not available to provide clinical background for the thyroid test results. Therefore, both false-positive and false-negative results may be present in the data set. However, the goal of our study was to compare the results of serum thyroid hormone concentrations as measured on separate machines. In this regard, the clinical status of the patient may be considered less relevant to the conclusions of the study. Sera were frozen at −80°C for up to 8.5 months and subjected to 2 freeze-thaw cycles. Previous studies have shown that dog and cat serum TT4 concentrations remain stable for 35 days at −20°C,[9] and that serum TT4 concentrations in humans remain stable for 6.5 years at −20°C,[28] but no published data are available to our knowledge on the effects of long-term storage or freeze-thaw cycles on serum TT4 concentrations in dogs and cats. Given that the MTT4 was repeated at the same time as the CTT4 and ITT4, no effect of storage or freeze-thaw cycle on the method comparison should have occurred. An additional limitation is that new reference intervals were not derived for the MTT4 and ITT4 assays in our study.

The Immulite 2000 TT4 assay (ITT4) was utilized in our study rather than the previously validated Immulite 1000 TT4 assay. The Immulite 1000 TT4 assay has been utilized most frequently in published studies of thyroid function in dogs and cats.[10-14] Very strong correlation was observed when the DPC Immulite 2000 Canine TT4 was compared previously to the Immulite 1000 Canine TT4 (r = .99).[29] However, differences that were not taken into account regarding individual machine performance could have been present. The CDx and C1 platforms were considered equivalent in our study although it is possible that differences may be inherent in running the CTT4 assays on these 2 machines. A direct comparison of the CTT4 to ITT4 was not performed. We recognize that the ITT4 is still in use in some markets, and that this comparison should be performed in the future.

Another limitation was the sample size for the different concentration and interval groups. For dogs, in the CTT4 versus MTT4 comparison, limited numbers of samples were available in the low and high reference ranges; therefore, the sample size was below our target of 40 samples in each concentration range, particularly in the <1.0 µg/dL (<12.87 nmol/L) group for dogs. These sample size limitations prevented assessment of bias at lower TT4 concentrations and therefore the relevance of agreement to overall bias between methods at these concentrations cannot be determined. For cats, limited samples

were available in the low range but this was not considered to influence the analysis. For the ITT4 versus MTT4 comparison, after exclusion of discordant results, small numbers of dog samples were available in the low, high normal, and high categories, and small numbers of cat samples were available in the low, high normal, and high categories, with these concentration intervals having <20 individual samples. We also recognize that a high number of discordant test results for both dogs and cats occurred between the ITT4 and the MTT4, particularly at low serum TT4 concentrations. It is impossible to know the cause of this discordance, but sample storage may have played a role. The ITT4 assays were performed 5 months after the MTT4 and CTT4 assays, and we hypothesize that these discrepant readings may have been a consequence of prolonged storage. This possibility compounds the limitations of the ITT4 to MTT4 comparison. No other clinically relevant effects of sample size were noted for this smaller arm of the study. Although the presence of lipemia, icterus, and hemolysis in samples was recorded, the effect of these sample characteristics on assay results was not assessed.

Another important consideration in the evaluation of assays is biological variation among animals (coefficient of variation, individual [CVi]). Quality assurance goals for TT4 attempt to show that the CV from assay results is not due simply to biological variation. Differences derived from CVi have been reported previously as 2.9% to 8.6% for cats[30] and 4.25% to 12.75% for dogs.[31] This suggests that the CTT4 in cats is just below the low threshold of quality goals (which is not a relevant difference), the MTT4 is within the range of quality goals, and the ITT4 also is within the range of historically assessed quality goals based on CVi. In dogs, the CTT4 and MTT4 are within the established quality goals for TT4, but the ITT4 CVi at 14.25% falls outside the quality goals.

With in-clinic testing, frequent quality control monitoring is necessary to maintain testing standards. The in-clinic CTT4 assay used in our study was performed in a reference laboratory setting by laboratory technicians with a high level of quality control. The results can only be appropriately extrapolated if recommended quality control is performed and staff members are well trained.

When measuring serum TT4 concentrations in dogs and cats, clinicians should consistently use 1 assay technique and laboratory for TT4 measurement, especially when following trends in individual patients. In the scenario in which test results from either an animal with euthyroid sick syndrome or hypothyroidism are compared between CTT4 testing and ITT4 testing, a thyroid stimulating hormone, free total thyroxine concentration, or both could be performed to aid in resolving inconsistencies between test results. Future studies are necessary to establish a gold standard assay for measurement of serum TT4 concentrations in dogs and cats. When an animal's serum TT4 concentration falls within the ranges discussed, test results from the CTT4 can be considered comparable to the MTT4 and ITT4 results in dogs and cats.

CONFLICT OF INTEREST DECLARATION

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

IDEXX Laboratories approved use of residual samples for research purposes.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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