IDEXX **Catalyst One™ Chemistry** Analyzer for In-house Measurement of Total Thyroxine (TT_4) Concentration in Serum from Dogs and Cats

Authors:

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Abstract

Background: The IDEXX Catalyst One™ Chemistry Analyzer is a bench-top chemistry analyzer that uses dry-slide technology to measure biochemical analytes. IDEXX Laboratories, Inc. is introducing an additional analyte to the platform, Catalyst® Total T_4 Test, which measures total thyroxine (TT_4) using a novel immunoassay method.

Objectives:

1. Validate the clinical results of the Catalyst Total T_4 Test, for canine and feline serum samples, through comparison to a method used at veterinary reference laboratories: the DRI® Thyroxine (T_4) Assay.[1]
2. Evaluate precision and interfering substances performance.

Methods:

1. Canine and feline serum samples from both healthy and clinical patients were analyzed with the Catalyst Total T_4 Test and a reference method for thyroxine.[1] Results from both instruments were compared using regression analysis with calculation of R-squared (R^2), slope and mean bias for both species. The clinical interpretations, based on the reference intervals and guidelines used at a commercial reference laboratory,[2] were then compared.
2. A precision study, according to Clinical and Laboratory Standards Institute (CLSI) guidelines,[3] was conducted with control fluids at two concentrations to determine total precision performance by determining the coefficient of variation (CV). The impact of interfering substances (hemoglobin, bilirubin and lipids) was studied, according to CLSI guidelines.[4]

Results:

1. The Catalyst Total T_4 Test showed an excellent correlation to the reference method (canine R^2 0.95; feline R^2 0.97). The slope for both species was 0.95. For the canine results, there was a very slight negative mean bias (-0.01 μg/dL) and for the feline results there was a slight positive mean bias (0.1 μg/dL).
2. The average CV was 9.2%. There was minimal impact from the interfering substances assessed.

Conclusion:

Based on these results, the Catalyst Total T_4 Test provides accurate and reliable total thyroxine analysis in serum samples from dogs and cats, as compared to a reference method.

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Introduction

Thyroid disorders are encountered commonly in veterinary practice. As thyroid disorders typically respond well to treatment and carry a good prognosis, it is important for the clinician to routinely screen patients with suspected or potential thyroid disease. We recommend that TT_4 be used as the initial screening test for thyroid disorders. The test is most often used to exclude the diagnosis of hypothyroidism in dogs or to screen for hyperthyroidism in cats. Additionally, regular monitoring of TT_4 concentration is used to assess the efficacy of treatment in both species.

The Catalyst® Total T_4 Test comprises a new immunoassay system from IDEXX Laboratories that is designed to measure TT_4 concentrations in serum or lithium heparin plasma samples from dogs (0.5–10 µg/dL; 6.4–128.7 nmol/L) and cats (0.5–20 µg/dL; 6.4–257.4 nmol/L) without the need to dilute the sample. This test uses ELISA technology in a new Catalyst slide format. It is designed to produce prompt, reliable and accurate test results in the veterinary clinic.

The objective of this study was to conduct a comprehensive comparison of TT_4 concentrations determined by the Catalyst Total T_4 Test, using an IDEXX Catalyst One™ Chemistry Analyzer, and TT_4 concentrations determined by DRI® Thyroxine (T_4) Assay, a method used by reference laboratories.[1,2] In this study, the DRI T_4 assay is deemed to be the reference method.

Method Comparison

Materials and Methods: Serum samples were collected from 75 dogs and 85 cats, including a mixture of healthy animal and clinical patients. All samples were analyzed using both the DRI T_4 assay (run on a clinical chemistry analyzer used in veterinary reference laboratories)[5] and the Catalyst Total T_4 Test (run on the IDEXX Catalyst One analyzer). Each sample was run twice on the reference method and an average was calculated for use in the comparison. Each sample was also run once on three Catalyst One analyzers to give a total of three comparisons per sample (a grand total of 480 comparisons).

Samples were retained at 4°C and each comparison was completed within 4 hours. All testing was completed in a randomized order. Both assays were performed according to the manufacturer's specifications.

The clinical interpretation of the results from both methods were compared using the current recommendations from a veterinary reference laboratory.[2]

The IDEXX Catalyst One analyzer uses algorithms to suppress results when it encounters problems with either sample quality or the consumable. This is not an automated feature for the reference method. In this study, 7 results (1.5% of all comparisons; 4 canine and 3 feline) were suppressed by the Catalyst One analyzer and the comparisons were excluded from the analysis.

For each species, correlation plots were constructed with calculation of R-squared (R^2), slope and mean bias. R^2 is a statistical technique that evaluates the relationship between two series of events, and the slope of this correlation directly speaks to the overall bias. In this context, an R^2 of one and a slope of one are considered ideal. The mean bias was calculated using results within the appropriate dynamic range (canine 0.5–10.0 µg/dL; feline 0.5–20.0 µg/dL). Additionally, to expose any range-specific bias, plots were made graphing the average of TT_4 values obtained using the two methods on the *x*-axis, versus their difference, plotted on the *y*-axis.

Results: The results are shown in figure 1 (canine) and figure 2 (feline). The comparison of the clinical interpretations is shown in table 2 (canine) and table 3 (feline). There was excellent correlation between the two methods and strong concordance on the clinical interpretation.

Precision Analysis and Interfering Substances Study

Materials and Methods: Precision was assessed according to Clinical and Laboratory Standards Institute (CLSI) EP05-A method guidelines.[3] Two levels of control fluid, IDEXX Catalyst® TT_4 Low Control and IDEXX Catalyst Advanced Control, were assayed on the Catalyst One™ Chemistry Analyzer. There were two replicates run on three Catalyst One instruments in the morning and afternoon for five days, for a total of 20 replicates of each fluid per instrument. Total precision was calculated per CLSI EP5-A method guidelines.

Interference caused by the presence of hemoglobin, bilirubin or lipids was assessed according to CLSI EP07-A2 method guidelines.[4] Canine serum samples, which were visibly clear of interferents, were collected and pooled. Aliquots of the pooled sample were then prepared and spiked with varying concentrations of the substances shown in table 1. Each aliquot was run in duplicate on five Catalyst One analyzers in a random order.

Interference	Spiking Material	Number of Levels of Interfering Substance
Hemolysis	Lysed canine red blood cells to produce hemoglobin	5
Lipemia	Intralipid 30%	5
Icterus	Ditaurobilirubin (DTB; a synthetic bilirubin derivative)	6

Table 1: Preparation of aliquots of the pooled sample to assess common interfering substances.

Results: The average CV, across the three Catalyst One analyzers, for fluid with a mean concentration of 2.3 µg/dL was 9.7%. The same figure for fluid with a mean concentration of 4.0 µg/dL was 8.7%. This new method shows a precision similar to other methods described in the veterinary literature.[6] The results of the interfering substances study are shown in table 4. The common interfering substances examined here have a minimal impact on the reported TT_4 concentrations.

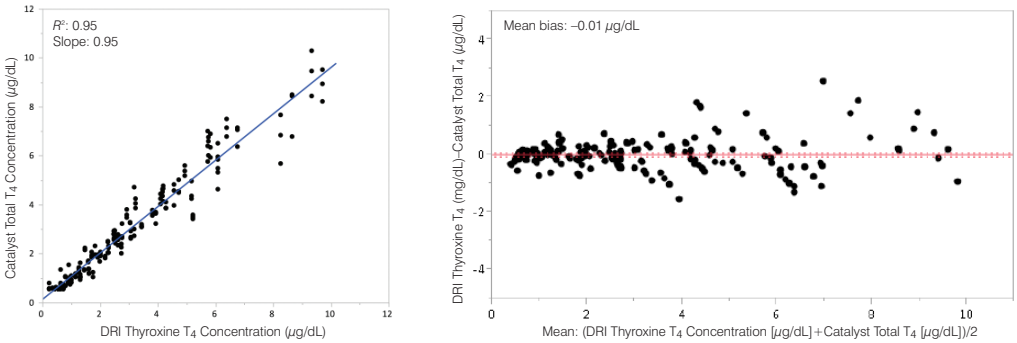

Figure 1. Linear regression graph and bias plot of pairwise comparisons (n=221) of serum TT_4 concentrations in canine samples measured by the two assays. The line of best fit for the data is indicated in the linear regression graph, with the slope and R-squared (R^2). The bias plot shows the mean bias (solid line) with 95% upper and lower confidence intervals (dashed lines).

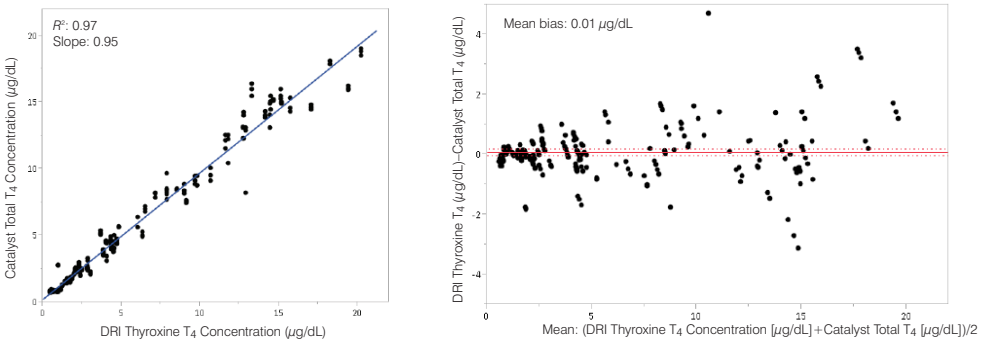

Figure 2. Linear regression graph and bias plot of pairwise comparisons (n=246) of serum TT_4 concentrations in feline samples measured by the two assays. The line of best fit for the data is indicated in the linear regression graph, with the slope and R-squared (R^2). The bias plot shows the mean bias (solid line) with 95% upper and lower confidence intervals (dashed lines).

Canine		DRI Thyroxine (T_4) Results		
		Low <1.0 µg/dL <13 nmol/L	Normal 1.0–4.0 µg/dL 13–51 nmol/L	High >4.0 µg/dL >51 nmol/L
Catalyst Total T_4 Results	Low <1.0 µg/dL <13 nmol/L	44	5	0
	Normal 1.0–4.0 µg/dL 13–51 nmol/L	5	111	0
	High >4.0 µg/dL >51 nmol/L	0	0	56

Table 2. Comparison of the clinical interpretation of the canine results based on the guidelines currently used by IDEXX Reference Laboratories.

Feline		DRI Thyroxine (T_4) Results		
		Low <0.8 µg/dL <10 nmol/L	Normal 0.8–4.7 µg/dL 10–60 nmol/L	High >4.7 µg/dL >60 nmol/L
Catalyst Total T_4 Results	Low <0.8 µg/dL <10 nmol/L	6	4	0
	Normal 0.8–4.7 µg/dL 10–60 nmol/L	11	126	0
	High >4.7 µg/dL >60 nmol/L	0	7	98

Table 3. Comparison of the clinical interpretation of the feline results based on the guidelines currently used by IDEXX Reference Laboratories.

Hemolysis		Lipemia		Icterus	
Hemoglobin concentration (mg/dL)	Catalyst Total T_4 average TT_4 concentration (µg/dL)	Intralipid concentration (mg/dL)	Catalyst Total T_4 average TT_4 concentration (µg/dL)	DTB concentration (mg/dL)	Catalyst Total T_4 average TT_4 concentration (µg/dL)
Not spiked	1.3	Not spiked	1.3	Not spiked	1.3
125	1.3	250	1.4	1	1.3
250	1.3	500	1.4	3	1.3
375	1.3	750	1.4	10	1.4
500	1.2	1000	1.4	20	1.3
				30	1.3

Table 4: Impact of interfering substances.

Discussion

In the feline samples, there was a strong agreement in the clinical characterization of the results. There were 7 cats reported to be high by the Catalyst Total T_4 Test and normal by the reference method. However, these cats had an average age of 13 years (range of 8–17 years; clinical symptoms not documented) and the reference method results (mean 4.3 μg/dL; range of 3.7–4.7 μg/dL) were well within the gray zone of 2.3–4.7 μg/dL, where the reference laboratory recommends further testing in old or symptomatic cats.

In the canine samples, examples of clinically discrepant results were also uncommon. Five results were normal by the reference method and low on Catalyst Total T_4 Test method. A further five were normal by the Catalyst Total T_4 Test method and low by the reference method. However, in all 10 of these comparisons, the higher result in the pair was in the lower part of the canine reference interval (sometimes called low normal; range of 1.0–2.0). Further testing, for example, with free T_4 and canine TSH, would have been recommended with either result (i.e., the apparent discrepancy would not have effected clinical outcome).

In all of the discrepant interpretations, both results were close to the appropriate threshold of the reference interval. This reflects the imprecision of both the reference method and the new method. As typical, the clinician must take care to not over interpret results that are close to the limits of a reference interval.

Differences may exist between the various methodologies for measuring TT_4, and any one result should be considered in light of the overall clinical picture and other clinicopathological data. It also highlights the need to use a single methodology for repeat testing, especially when monitoring therapy.

Conclusion

The new Catalyst Total T_4 Test produces accurate results when used to quantify TT_4 in serum samples from dogs and cats. This new immunoassay system demonstrates excellent correlation with the reference method and provides an accurate, reliable and convenient option for veterinarians who wish to diagnose and monitor animals with thyroid disease using their in-house laboratory.

References

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