Hormone Validation Information, Determination of Reference Intervals, and Comparison of Results for Serum T_4 and TSH Concentrations Between Two Commercial Laboratories

Validation of serum T4 and TSH assays for cats: Serum T_4 and feline TSH concentrations were measure by two commercial laboratories in this study.[1,2] In both laboratories, serum T_4 concentration was measured by a homogenous enzyme immunoassay (EIA) on an automated biochemistry analyzer, whereas feline TSH was measured by chemiluminescent enzyme immunoassay (CEIA) using a canine TSH (cTSH) assay.[3,4]

We previously reported[5] the procedure used to validate the T_4 and cTSH assays for use in cats with the Antech Diagnostics Laboratory. For this study, we also validated the T_4 and cTSH assays cTSH assays for use on cats using the IDEXX reference laboratory, which we describe as follows.

Assay of serial dilutions of a feline serum pool containing high concentrations of T_4 or TSH resulted in inhibition curves with slopes parallel to those of the standard curves for both hormones. For the T_4 assay, the intra-assay coefficient of variation (CV), calculated by assay of 4 replicates of 5 serum pools (with concentrations ≈0.7 µg/dL, 1.5 µg/dL, 2.5 µg/dL, 5.5 µg/dL, 12 µg/dL), was 3.3%, whereas the interassay CV, calculated by assay of the same 5 serum pools on 4 consecutive days, was 5.8%. For validation of the cTSH assay for feline serum, the intra-assay CV, calculated by assay of 4 replicates of 5 serum pools (with concentrations ≈ 0.04 ng/mL, 0.10 ng/mL, 0.25 mg/mL, 0.40 ng/mL and 2.0 ng/ml), was 4.3%. The interassay CV for the cTSH assay, calculated by assay of the same 5 serum pools on 4 consecutive days, was 5.8%. The sensitivity (i.e., limit of quantification) for the T4 assay was 0.5 µg/dL and 0.03 ng/mL for TSH.

Data S1— Supporting information for "Efficacy of Low-dose (2 millicurie) versus Standard-dose (4 millicurie) Radioiodine Treatment for Cats with Mild to Moderate Hyperthyroidism"

Determination of reference intervals for serum T4 and TSH assays in two laboratories: For both Antech and IDEXX laboratories, reference intervals (RI) for T_4 and TSH concentrations were established by the nonparametric method of percentile estimates with confidence intervals to determine the central 95th percentile interval (i.e., 2.5 through 97.5th percentile range) for results from clinically normal cats.[6] To be included, these cats had to be ≥ 7 years of age and considered healthy based on an unremarkable client history, physical examination (i.e., none had palpable thyroid nodules), complete blood count, serum chemistry profile, and urinalysis. Results of qualitative and quantitative thyroid scintigraphy[7] were also normal in all of these control cats.

For the Antech T_4 and TSH assays, 160 clinically normal cats (aged 7-18 years) were used to establish RIs, whereas 165 clinically normal cats (aged 7-19 years) were used to establish RIs for the IDEXX assays. For the Antech assays, the calculated RI for T_4 ranged from 0.9 to 3.9 μg/dL and the RI for cTSH ranged from 0.03 to 0.3 ng/mL. Similarly, for the IDEXX assays, RIs for T4 and TSH ranged from 0.9 to 3.8 μg/dL and 0.03 to 0.25 ng/mL, respectively. In both laboratories, 95% of normal cats had serum TSH values ≤0.2 ng/mL. Only 2 cats of 160 cats had values slightly higher than 0.3 ng/dl measured at Antech laboratories (0.35 and 0.38 ng/mL), whereas the highest normal TSH concentration measured at IDEXX was 0.29 ng/mL. Therefore, an upper RI of 0.3 ng/mL for TSH concentration was selected for both laboratories.

Comparison of serum T4 and TSH assays in two laboratories: For comparison, serum samples from 462 hyperthyroid, euthyroid, and hypothyroid cats were divided into 2 aliquots and submitted both to Antech Diagnostics and IDEXX Reference Laboratories for analysis of serum T_4 and TSH concentrations. The serum concentration of both hormones reported from each reference laboratory were compared and analyzed by use of the Wilcoxon

signed rank test. No significant differences were found between the serum concentrations of T_4 (median, 5.9 µg/dl vs. 6.0 µg/dl; $P = 0.062$) or TSH (0.02 vs. 0.02; $P = 0.349$) measured by the two laboratories.

Bland-Altman bias plots[8] were used to compare overall agreement of the serum concentrations of T_4 or TSH reported in each laboratory. Overall, close comparison of results was found with the bias plot analysis, both for serum T_4 (Figures 1 and 2) as well as for TSH (Figures 3).

For the serum TSH assays, comparison between the 2 laboratories shows very good agreement for concentrations within the reference interval (0.03 to 0.3 ng/ml), extending up to twice the upper threshold of reference limit (Figures 4 and 5). However, marked heteroscedasticity was found at higher TSH concentrations (Figure 3). Furthermore, no cat was differently classified by the 2 laboratories – all cats with TSH concentrations ≤0.3 ng/mL measured by one laboratory also had TSH concentrations ≤0.3 ng/mL measured by the other laboratory. Similarly, all cats with TSH concentrations >0.3 ng/mL measured by one laboratory also had TSH concentrations >0.3 ng/mL measured by the other laboratory, despite the increasing disagreement between laboratory measurements as TSH concentrations increased (Figure 3).

Footnotes and References

1. Antech Diagnostics, Lake Success, NY USA.
2. Idexx Reference Laboratories, Westbrook, ME USA.
3. DRI® Thyroxine (T_4) assay, Microgenics Corporation, Freemont, CA, USA.
4. Immulite Canine TSH, Siemens Healthcare Diagnostics Products. Tarrytown, NY, USA.
5. Peterson ME, Guterl JN, Nichols R, et al. Evaluation of serum thyroid-stimulating hormone concentration as a diagnostic test for hyperthyroidism in cats. *J Vet Intern Med* 2015;29:1327-1334.
6. Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. Clin Chem 1971;17:275-284.
7. Peterson ME, Guterl JN, Rishniw M, et al. Evaluation of quantitative thyroid scintigraphy for diagnosis and staging of disease severity in cats with hyperthyroidism: comparison of the percent thyroidal uptake of pertechnetate to the thyroid-to-salivary ratio and thyroid-to-background ratios. Vet Radiol Ultrasound 2016;57:427-440.
8. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307-310.

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Figure 1

Limits of agreement (LOA) plot showing agreement in T4 measurements between 2 laboratories in 462 cats. Solid lines represent the 95% LOA, dashed line represents perfect agreement.

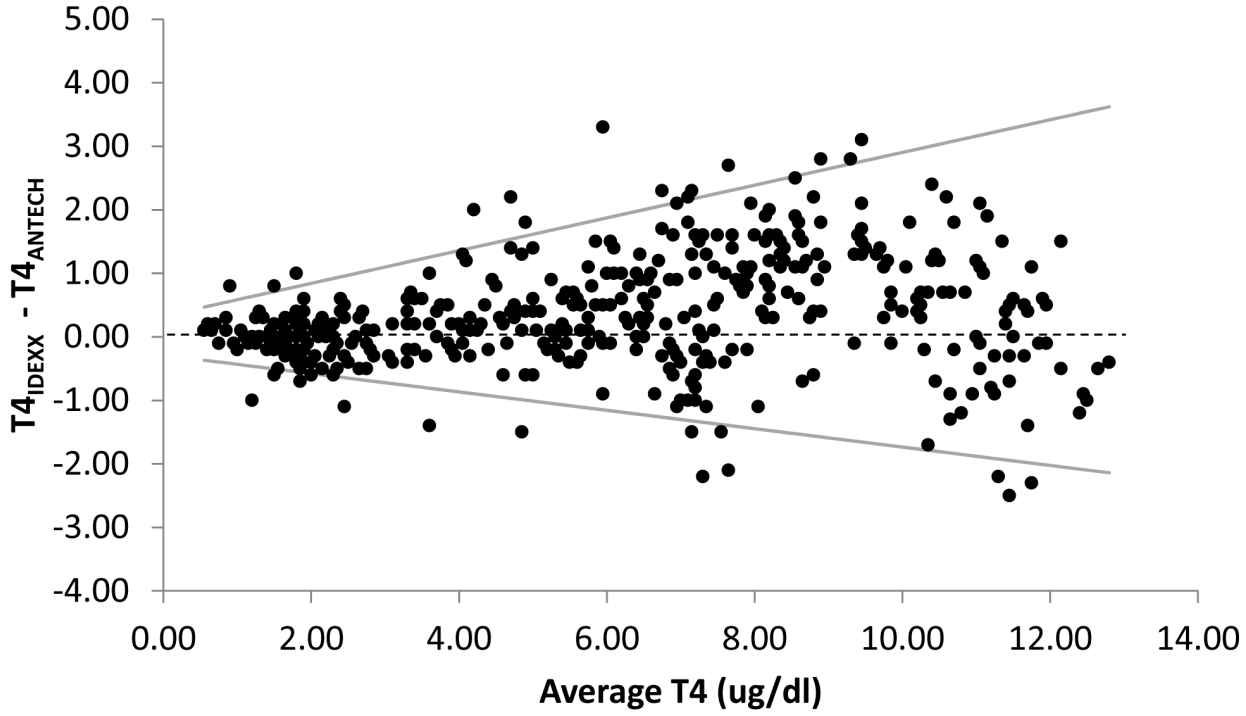

Figure 2

Normalized limits of agreement (LOA) plot showing the percent difference in T4 measurements between 2 laboratories in 462 cats. Solid lines represent the 95% LOA, dashed line represents perfect agreement.

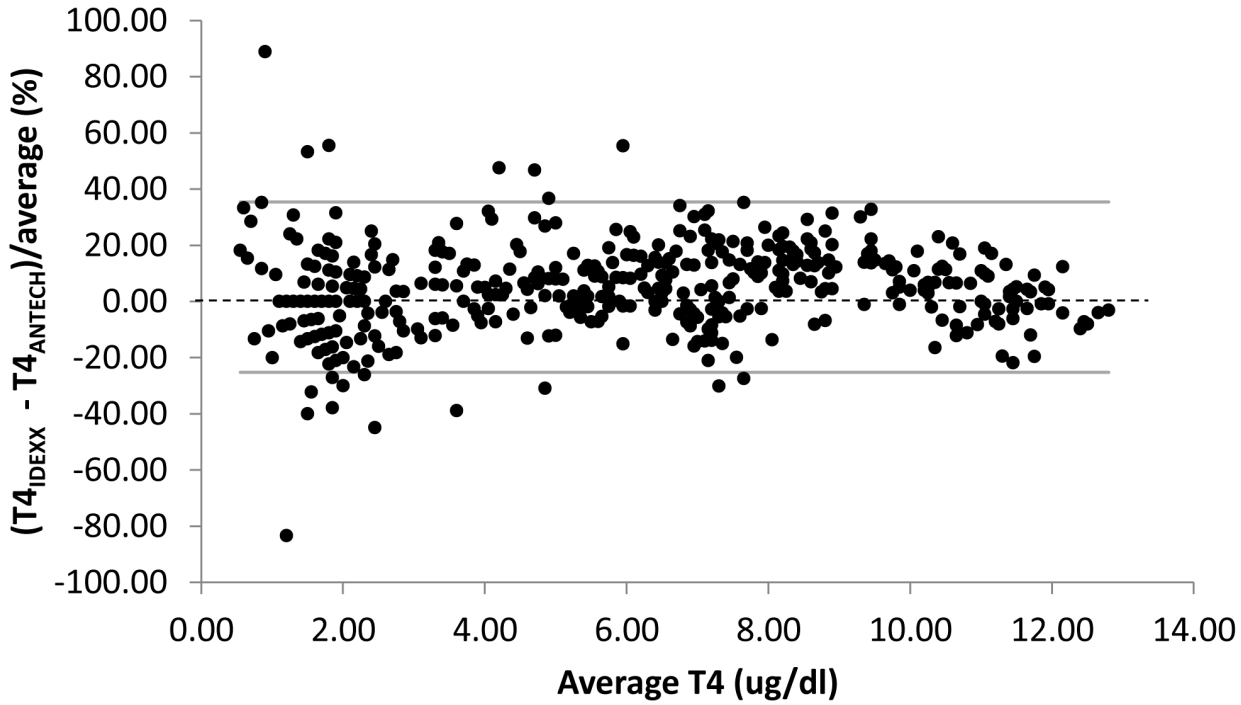

Figure 3

Limits of agreement (LOA) plot showing agreement in TSH measurements between 2 laboratories in 462 cats, with the TSH concentration plotted on a base-2 logarithmic scale. Dashed line represents perfect agreement.

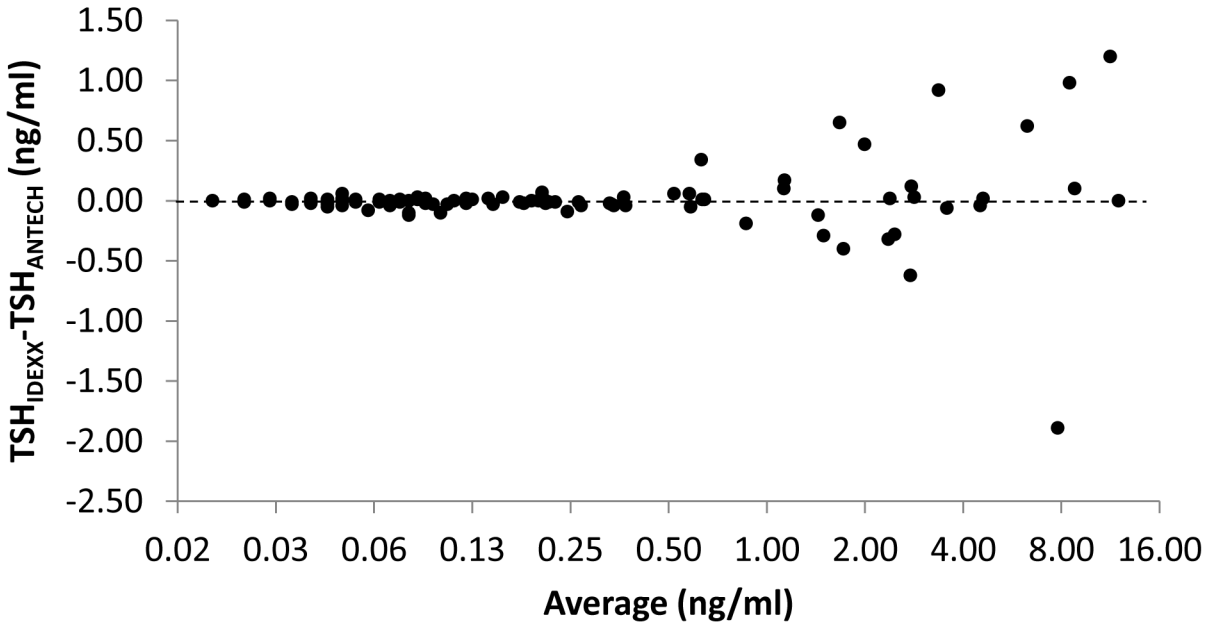

Figure 4

Same plot as Figure 3, but now indicating (closed box) the 437 cats with serum TSH values ≤ 2-times the upper reference limit (0.6 ng/dl; reference interval, 0.03-0.30 ng/ml).

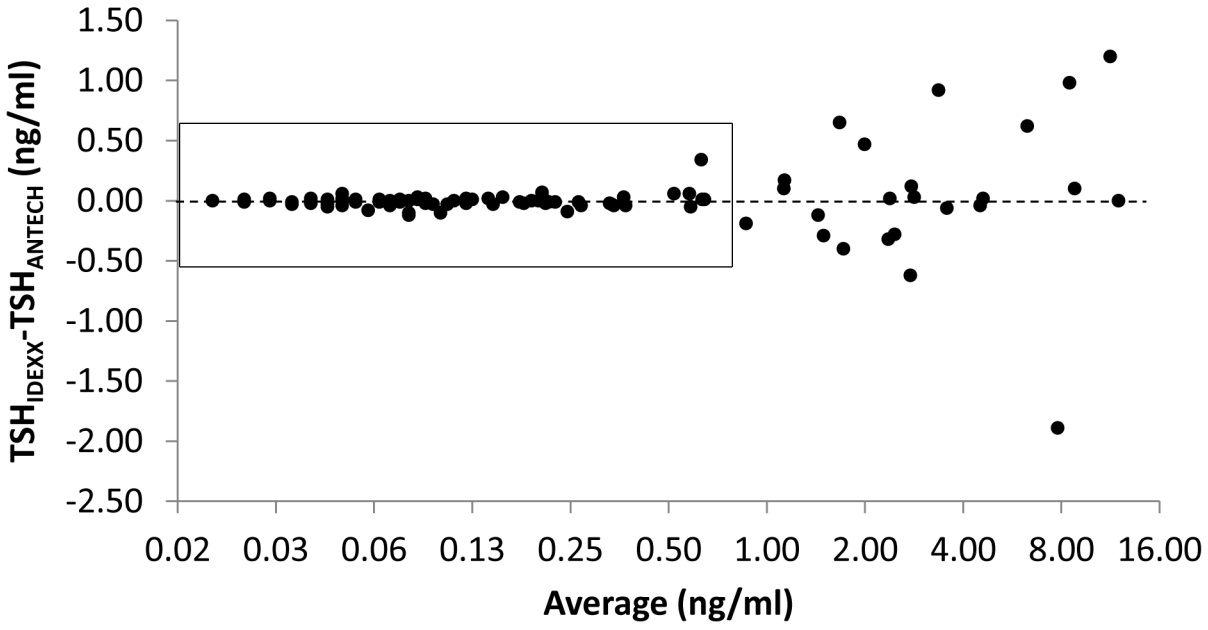

Figure 5

Detailed limits of agreement (LOA) plot showing agreement in TSH measurements between 2 laboratories in 436 cats with serum TSH values ≤ 2-times the upper reference limit (0.6 ng/ml), expanded from the boxed region in Figure 4. The serum TSH concentrations are plotted on a base-2 logarithmic scale. Solid lines represent the 95% LOA, dashed line represents perfect agreement.