

Evaluation of a novel, sensitive thyroid-stimulating hormone assay as a diagnostic test for thyroid disease in cats

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OBJECTIVE

Clinicians commonly use thyroid-stimulating hormone (TSH) concentrations to diagnose thyroid disorders in humans and dogs. In cats, canine TSH chemiluminescent immunoassays (CLIA) assays are commonly used to measure TSH, but these TSH-CLIA cannot measure low TSH concentrations (< 0.03 ng/mL) and therefore cannot distinguish between low-normal concentrations and truly low TSH concentrations (characteristic of hyperthyroidism). Our aim was to evaluate a novel TSH assay based on bulk acoustic wave (BAW) technology that has lower functional sensitivity (0.008 ng/mL) than TSH-CLIA.

ANIMALS

169 untreated hyperthyroid cats, 53 cats treated with radioiodine (¹³¹I), 12 cats with chronic kidney disease (CKD), and 78 clinically healthy cats.

METHODS

Serum concentrations of T₄, TSH-CLIA, and TSH-BAW were measured in all cats. Untreated hyperthyroid cats were divided into 4 severity groups (subclinical, mild, moderate, and severe), whereas ¹³¹I-treated cats were divided into euthyroid and hypothyroid groups.

RESULTS

Test sensitivity, specificity, and positive predictive value for identifying hyperthyroidism were higher for TSH-BAW (90.5%, 98.9%, and 86.9%) than TSH-CLIA (79.9%, 76.7%, and 21.7%; *P* < .001). Test sensitivity for identifying ¹³¹I-induced hypothyroidism was only 45.5% for T₄ versus 100.0% for both TSH-CLIA and TSH-BAW (*P* = .03), whereas TSH-BAW had a higher positive predictive value (100%) than did either TSH-CLIA (81.2%) or T₄ (71.9%).

CLINICAL RELEVANCE

Serum TSH-BAW alone or together with T₄ is a highly sensitive and specific diagnostic test for evaluating feline hyperthyroidism and iatrogenic hypothyroidism. Finding low serum TSH-BAW concentrations is most useful for diagnosing subclinical and mild hyperthyroidism, in which serum T₄ remains within or only slightly above the reference interval.

Key Words: thyrotropin, thyrotrophin, thyroxine, hyperthyroidism, hypothyroidism

Thyroid scintigraphy is considered the gold standard for the diagnosis of hyperthyroidism in cats.¹⁻⁴ Unfortunately, because of the expense and the special licensing required to perform nuclear imaging, few veterinary practices have access to the equipment needed to obtain thyroid scintigraphic images. Most clinicians screen for feline hyperthyroidism by measuring serum total T₄ concentrations.

However, roughly 10% of hyperthyroid cats have serum T₄ concentrations that remain within the upper half of the reference interval because of early (subclinical) disease, fluctuating T₄ concentrations, or concurrent nonthyroidal illness.^{1,5,6}

Thyroid-stimulating hormone (TSH; or thyrotropin) is used in human patients as a first-line test for screening overt and subclinical hyperthyroidism.⁷⁻¹⁰ Investigators have evaluated the diagnostic utility of serum TSH concentrations in identifying feline hyperthyroidism, with TSH most commonly measured by a commercial reference laboratory using a chemiluminescent immunoassay (CLIA) canine TSH

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assay (Immulite Canine TSH assay; Siemens).^{1,6,11,12} The diagnostic utility of serum TSH-CLIA in cats with hyperthyroidism is limited, however, because these assays cannot measure very low TSH concentrations (< 0.03 ng/mL) and therefore cannot distinguish between low-normal concentrations (found in approx 25% of clinically normal cats)⁶ from the truly low TSH concentrations characteristic of hyperthyroidism.^{7,8}

A more sensitive TSH assay with a lower limit of quantification has recently been developed for in-clinic use on a point-of-care (POC) diagnostic platform (TRUFORMA Feline TSH assay; Zomedica Inc). Instead of chemiluminescence, this novel TSH assay uses bulk acoustic wave (BAW) resonators as biosensors that measure mass.^{13,14} The sensors are coated with monoclonal antibodies that capture TSH molecules as the sample flows over the sensor surface. Monoclonal detection antibodies also recognize the TSH molecules and recruit an enzyme that converts a substrate to an insoluble product. Direct binding and the product of this enzymatic reaction create mass on the sensor surface that leads to a shift in resonance frequency that is converted to a TSH concentration. Increased resonance frequency of the BAW biosensors leads to increased assay sensitivity, facilitating the detection of very low concentrations of TSH in the sample. Consequently, this TSH assay can measure concentrations substantially lower than 0.03 ng/mL, the lower end of the reportable range of the TSH-CLIA used by most veterinary reference laboratories.^{6,11,15}

Radioiodine (¹³¹I) is considered the treatment of choice for feline hyperthyroidism due to its high efficacy and minimal side effects.^{16,17} However, iatrogenic hypothyroidism develops in a relatively high proportion (20% to 60%) of ¹³¹I-treated cats,^{18,19} which can worsen existing azotemia and decrease survival time.¹⁹⁻²¹ Therefore, identifying ¹³¹I-induced hypothyroidism allows clinicians to supplement with levothyroxine, thereby reducing the negative consequences of hypothyroidism. Recent studies²¹⁻²⁵ illustrate the utility of serum TSH-CLIA measurements for the diagnosis of iatrogenic hypothyroidism in cats, but serum TSH-BAW has not yet been evaluated as a diagnostic test for hypothyroidism.

In this study, we sought to evaluate this novel TSH assay by comparing serum TSH-BAW and TSH-CLIA concentrations in clinically healthy cats, cats with varying severity of untreated hyperthyroidism, cats treated with ¹³¹I, and cats with naturally occurring chronic kidney disease (CKD). To that end, we evaluated and compared the sensitivity and specificity and positive predictive values (PPV) and negative predictive values (NPV) of serum TSH-BAW and TSH-CLIA concentrations as diagnostic tests for hyperthyroidism and ¹³¹I-induced iatrogenic hypothyroidism in cats.

Methods

Animals

We collected serum from 169 cats with hyperthyroidism (diagnosis confirmed by thyroid scintigraphy)^{2,3} and 53 cats previously treated for hyperthyroidism at the Animal Endocrine Clinic from

June 2020 to June 2023. We also collected serum from 78 clinically normal cats and 12 cats with CKD examined during the same period.

Hyperthyroid cats—Hyperthyroid cats were referred to our clinic for treatment with ¹³¹I. Of these 169 hyperthyroid cats, 104 (61.5%) had never received methimazole, whereas 65 (38.5%) had been treated with methimazole for periods of 1 week to 3.5 years (median, 220 days). In all methimazole-treated cats, the drug was discontinued \geq 7 days (median, 8 days) before evaluation.

To be eligible for inclusion, all untreated hyperthyroid cats underwent an evaluation to confirm the diagnosis that included a review of the past medical record, complete physical examination, routine laboratory testing (CBC, serum biochemical profile, complete urinalysis), determination of serum thyroid hormones (total T₄, T₃, and TSH),^{6,24} and qualitative and quantitative thyroid scintigraphy.^{2,3} All hyperthyroid cats were then treated with ¹³¹I, using a previously described dosing algorithm.¹⁸ In all cats, we calculated a dose (severity) score, which incorporated the cats' thyroid tumor volume, serum thyroid hormone (T₄ and T₃) concentrations, and ^{99m}Tc-pertechnetate uptake value measured with quantitative thyroid scintigraphy.^{18,25} Based on their severity score, each cat was then assigned to 1 of 4 severity groups: subclinical hyperthyroidism (average severity score < 1.5), mild hyperthyroidism (1.5 to 1.79), moderate hyperthyroidism (1.8 to 2.49), or severe hyperthyroidism (\geq 2.5), as previously defined.^{18,25}

Cats treated with ¹³¹I—We also collected serum from 53 previously hyperthyroid cats that had undergone ¹³¹I treatment¹⁸ a minimum of 5 to 12 months (median, 6.5 months) before blood samples were drawn.

We divided these cats into 1 of 3 thyroid categories based on serum T₄ and TSH-CLIA concentrations by the reference laboratory: euthyroid (T₄, 1.0 to 3.8 μ g/dL; TSH \leq 0.30 ng/mL), overtly hypothyroid (T₄ < 1.0 μ g/dL; TSH > 0.30 ng/mL), and subclinically hypothyroid (T₄, 1.0 to 3.8 μ g/dL; TSH > 0.30 ng/mL), as previously defined.^{24,25} Because of the limited number of ¹³¹I-induced hypothyroid cats evaluated, we combined the 11 subclinical and overt groups into a single iatrogenic hypothyroid group. As with the untreated hyperthyroid cats, thyroid scintigraphy was used to help confirm the diagnosis in these hypothyroid cats.²¹

Cats with CKD—We collected serum from 12 cats with previously diagnosed CKD. For inclusion in this study, these cats had to have moderate azotemia, defined as serum concentration > 2.5 mg/dL (range, 2.6 to 6.5 mg/dL; median, 2.9 mg/dL; and IQR, 2.8 to 3.8 mg/dL), together with a urine specific gravity value < 1.030 (range, 1.010 to 1.029; median, 1.016; and IQR, 1.012 to 1.020). All of these cats had clinical signs (ie, polyuria or polydipsia) and physical exam findings (eg, small kidney size) consistent with CKD. None had any clinical evidence of thyroid disease (ie, none had palpable thyroid nodules) or a history of hyperthyroidism.

Clinically normal cats—We recruited 78 clinically (and clinicopathologically) healthy cats at the time of routine evaluation which were used as euthyroid controls to establish reference intervals for serum TSH-BAW concentrations. To be eligible, these cats had to be ≥ 7 years of age and considered healthy based on an unremarkable owner history and normal results on physical examination, routine laboratory testing (ie, CBC, serum biochemistry profile, and urinalysis), and serum T_4 concentration.

Ethics approval was obtained from the Institution's Animal Care and Use Committee. All owners provided informed consent.

Assays for serum T_4 and TSH concentrations

Blood samples were collected from all cats, placed into plain serum tubes (ie, containing no gel separator), and then centrifuged (1,500 X *g* for 10 to 15 minutes) within 1 hour after collection; serum was separated and stored at 4 °C until assayed for serum T_4 and TSH determinations, generally within 24 hours of collection. Serum total T_4 concentration was measured by a homogenous enzyme immunoassay [DRI Thyroxine (T4) assay; Microgenics Corp], and serum TSH was measured by CLIA (Immulite Canine TSH; Siemens Healthcare Diagnostics Products), validated for use in cats as previously described.⁶ Both serum T_4 and TSH-CLIA were assayed by a commercial laboratory (IDEXX Reference Laboratories).

On the same blood sample, serum TSH-BAW was also measured by use of a POC testing device (TRUFORMA Feline TSH assay; Zomedica Inc). This in-clinic testing was performed according to the manufacturer's instructions. In brief, we removed a refrigerated test cartridge from its pouch and placed the cartridge on a flat surface. Using the supplied blub pipette, we then added approximately 115 μ L of serum into the sample port of the cartridge and inserted the loaded cartridge into the POC device. After completion of the test (< 20 minutes), the instrument ejected the disposable cartridge, and we recorded the test results displayed on the user interface of the POC device.

Validation of the TSH-BAW assay—The TSH-BAW assay is a sandwich immunoassay that uses monoclonal capture and detection antibodies selected for high affinity and specificity for recombinant canine TSH, based on manufacturer specifications and binding affinity measurements (Octet; Creative Biolabs). Twenty-five commercially available monoclonal antibody pairs were screened to determine an antibody pair with optimum performance for low to high concentrations of TSH in feline sera.

Assay precision was evaluated by determining both within-run (same day) and between-run (separate days) assay variability. For within-run precision, 5 replicates of 3 feline sera pools containing low-normal to high serum concentrations of TSH-BAW (0.06, 0.10, and 0.68 ng/mL, respectively) were sequentially run on the same day; the within-run coefficients of variation (CVs) were 3.8%, 4.7%, and 7.3%, respectively (average, 5.2%). Between-run precision

was tested with samples from 3 feline sera pools containing low to high serum concentrations of TSH-BAW (0.008, 0.056, and 0.787 ng/mL, respectively) run on 6 separate days. The between-run CVs were 9.9%, 2.6%, and 7.3% respectively (average, 6.6%).

Accuracy was checked with dilutional linearity and parallelism. To perform these studies, hypothyroid cat serum containing a high concentration of TSH was serially diluted (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128) with hyperthyroid cat serum containing an undetectable concentration of TSH (< 0.008 ng/mL). The TSH-BAW assay showed dilutional linearity between 0.92 and 0.007 ng/mL, with a 19% CV for the cumulated back-calculated TSH concentrations (**Supplementary Figure S1**). Additionally, the TSH-BAW assay was able to distinguish a feline sample with a low (0.008 ng/mL) serum TSH concentration from one with a low-normal (0.012 ng/mL) serum TSH concentration ($P = .0022$).

Potential cross-reactivity with other pituitary glycoprotein hormones that have a structure similar to TSH, such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH),²⁶ was also assessed. To that end, known amounts of FSH and LH were added to TSH-depleted serum and tested in triplicate using the TSH-BAW assay. For both FSH and LH, < 0.001% cross-reactivity was observed in the assay. In addition, since serum LH concentrations are known to increase in neutered cats,²⁷ we tested 51 young cats (1 to 5 years) of both sexes (25 male and 26 female) that were either intact ($n = 22$) or neutered ($n = 29$). No difference in serum TSH-BAW concentrations was detected between intact and neutered cats (median [IQR], 0.054 ng/mL [0.04 to 0.07 ng/mL] vs 0.046 ng/mL [0.03 to 0.07 ng/mL]; $P = 0.52$), again suggesting there is no detectable cross-reactivity with LH in the TSH-BAW assay.

The limit of quantitation (functional sensitivity) of the TSH-BAW assay, defined as concentration that results in a CV of less than 20%,^{28,29} was calculated at 0.008 ng/mL. The upper limit of quantification for the TSH-BAW assay was calculated at 1.5 ng/mL.

Data analysis—Data were assessed for normality by the D'Agostino-Pearson test and by visual inspection of graphical plots.³⁰ Data were not normally distributed; therefore, all analyses used nonparametric tests. Results are reported as median (IQR, 25th to 75th percentile) and are represented graphically as scatter dot plots. For all analyses, statistical significance was defined as $P \leq .05$.

For serum T_4 and TSH-CLIA concentrations, we used reference intervals that were previously established in clinically normal cats in our clinic.⁶ For serum TSH-BAW concentrations, reference intervals were established by the robust method using Box-Cox transformed data from the results of our 78 clinically normal cats.^{31,32} The lower reference limit thus determined for serum TSH-BAW was 0.10 ng/mL (90% CI, 0.008 to 0.124 ng/mL), whereas the upper reference limit was 0.30 ng/mL (90% CI, 0.21 to 0.42 ng/mL).

All statistical analyses were performed using proprietary statistical software (GraphPad Prism, version 10.1 [GraphPad Software] and MedCalc

Statistical Software, version 22.013). For serum TSH-CLIA, undetectable concentrations were defined as < 0.03 ng/mL and assigned an arbitrary concentration of 0.02 ng/mL for data analysis, as previously described.^{6,11} For serum TSH-BAW, undetectable concentrations were defined as < 0.008 ng/mL and assigned an arbitrary concentration of 0.004 ng/mL for data analysis. For serum TSH-CLIA, all undetectable concentrations (< 0.03 ng/mL or 0.02 ng/mL) were considered low; for TSH-BAW, only concentrations below the lower endpoint of the reference interval (0.01 ng/mL) were considered low.

We compared how serum hormone concentrations differed among the hyperthyroid cats, ¹³¹I-treated euthyroid cats, ¹³¹I-treated hypothyroid cats, clinically normal cats, and cats with CKD with the Kruskal-Wallis test, followed by the Conover post hoc test.³³ The differences in the prevalence of within the reference interval serum TSH-CLIA and TSH-BAW concentrations among the different groups of cats were analyzed by use of the χ^2 tests and 2-sample z test.³⁴ Finally, sensitivity, specificity, PPV, and NPV for diagnosing hyperthyroidism and iatrogenic hypothyroidism were calculated³⁵ for each serum hormone concentration alone (T_4 , TSH-CLIA, and TSH-BAW) and for combinations of test results (T_4 plus TSH-CLIA and T_4 plus TSH-BAW). For the calculation of predictive values, we used a pretest probability (eg, population disease prevalence) of 7.5% for hyperthyroidism in mature and senior cats^{36,37} and 20% for ¹³¹I-induced hypothyroidism.^{18,25} The McNemar test³⁸ was used to determine whether differences existed between the sensitivity and specificity for T_4 , TSH-CLIA, or TSH-BAW concentrations as diagnostic tests for hyperthyroidism and hypothyroidism in cats.

Results

Animal characteristics

Hyperthyroid cats—Hyperthyroid cats ranged in age from 4 to 18 years (median, 12 years; IQR, 10 to 14 years) and included 89 (52.7%) females and 80 (47.3%) males. Most cats were domestic short-haired, medium-haired, or long-haired cats ($n = 148$; 87.6%). Other breeds included Main Coon (5 cats), Siamese (4 cats), American Shorthair (3 cats), Russian Blue (2 cats), and American Bobtail, Burmese, Ragdoll, Norwegian Forest Cat, Scottish Fold, Siberian Forest Cat, and Tonkinese (1 cat each).

Based on the severity score calculated for these 169 cats,¹⁸ 40 had subclinical hyperthyroidism, 55 had mild hyperthyroidism, 37 had moderate hyperthyroidism, and 37 had severe hyperthyroidism (**Supplementary Table S1**).

Cats treated with ¹³¹I—Radioiodine-treated cats ranged in age from 6 to 21 years (median, 13 years; IQR, 12 to 15 years) and included 31 (58.5%) females and 22 (41.5%) males. Forty-six of these cats (87%) were domestic short- or long-haired cats. Other breeds included Siberian (2 cats) and American Shorthair, Siamese, Chartreux, Ragdoll, and Burmese (1 cat

each). Forty-two cats became euthyroid, whereas 11 developed iatrogenic hypothyroidism after ¹³¹I.

Cats with CKD—Cats suffering from CKD ranged in age from 8 to 20 years (median, 15 years; IQR, 12 to 17 years) and included 4 (33%) males and 8 (67%) females. Ten (83%) of these cats were domestic shorthair; other breeds were Siamese (1 cat) and Persian (1 cat).

Clinically normal cats—Clinically normal cats ranged in age from 7 to 20 years (median, 15 years; IQR, 8 to 15 years) and included 40 (51.3%) females and 38 (48.7%) males. Of the clinically normal cats, 70 (89.7%) were domestic short-haired, medium-haired, or long-haired; the remainder were American Shorthair (2 cats), Ragdoll (2 cats), and Bengal, Bombay, Persian, and Siamese (1 cat each). These cats were used to establish reference intervals for the TSH-BAW assay.

Serum T_4 , TSH-CLIA, and TSH-BAW concentrations

Hyperthyroid cats—All 4 hyperthyroid severity groups had higher T_4 concentrations than clinically normal, ¹³¹I-treated, and CKD cats ($P < .0001$; Supplementary Table S1). However, 20/40 (50%) of the cats with subclinical hyperthyroidism had serum T_4 concentrations within the reference interval (**Figure 1**). Serum T_4 concentrations progressively increased with increasing severity of hyperthyroidism ($P < .0001$).

All 4 hyperthyroid severity groups had lower serum TSH-CLIA concentrations than the clinically normal, ¹³¹I-treated, and CKD cats ($P < .0001$; Supplementary Table S1). More hyperthyroid cats had undetectable serum TSH-CLIA concentrations (< 0.03 ng/mL) than clinically normal cats (137/169 [81.1%] vs 20/78 [25.6%]; $P < .0001$; **Figure 2**). Of the hyperthyroid cats, 9/40 (22.5%) with subclinical hyperthyroidism, 10/55 (18.2%) with mild hyperthyroidism, 5/37 (13.5%) with moderate hyperthyroidism, and 8/37 (21.6%) with severe hyperthyroidism had measurable serum TSH-CLIA concentrations (≥ 0.03 ng/mL). We found no difference in the prevalence of measurable serum TSH-CLIA concentrations between the 95 hyperthyroid cats with subclinical to mild disease and the 74 cats with moderate to severe disease (19/95 [20%] vs 13/74 [17.6%]; $P = .69$).

All 4 hyperthyroid severity groups had lower serum TSH-BAW concentrations than clinically normal, ¹³¹I-treated, and CKD cats ($P < .0001$; Supplementary Table S1). More hyperthyroid cats had low or undetectable serum TSH-BAW concentrations (< 0.01 ng/mL) than clinically normal cats (154/169 [91.1%] vs 1/78 [1.3%]; $P < .0001$; **Figure 3**). Of the hyperthyroid cats, 11/40 (27.5%) with subclinical hyperthyroidism, 2/55 (3.6%) with mild hyperthyroidism, 1/37 (2.7%) with moderate hyperthyroidism, and 1/37 (2.8%) with severe hyperthyroidism had serum TSH-BAW concentrations within the reference interval. Cats with subclinical hyperthyroidism had higher serum TSH-BAW concentrations than the other 3 hyperthyroid groups (Supplementary Table S1). Cats with subclinical to

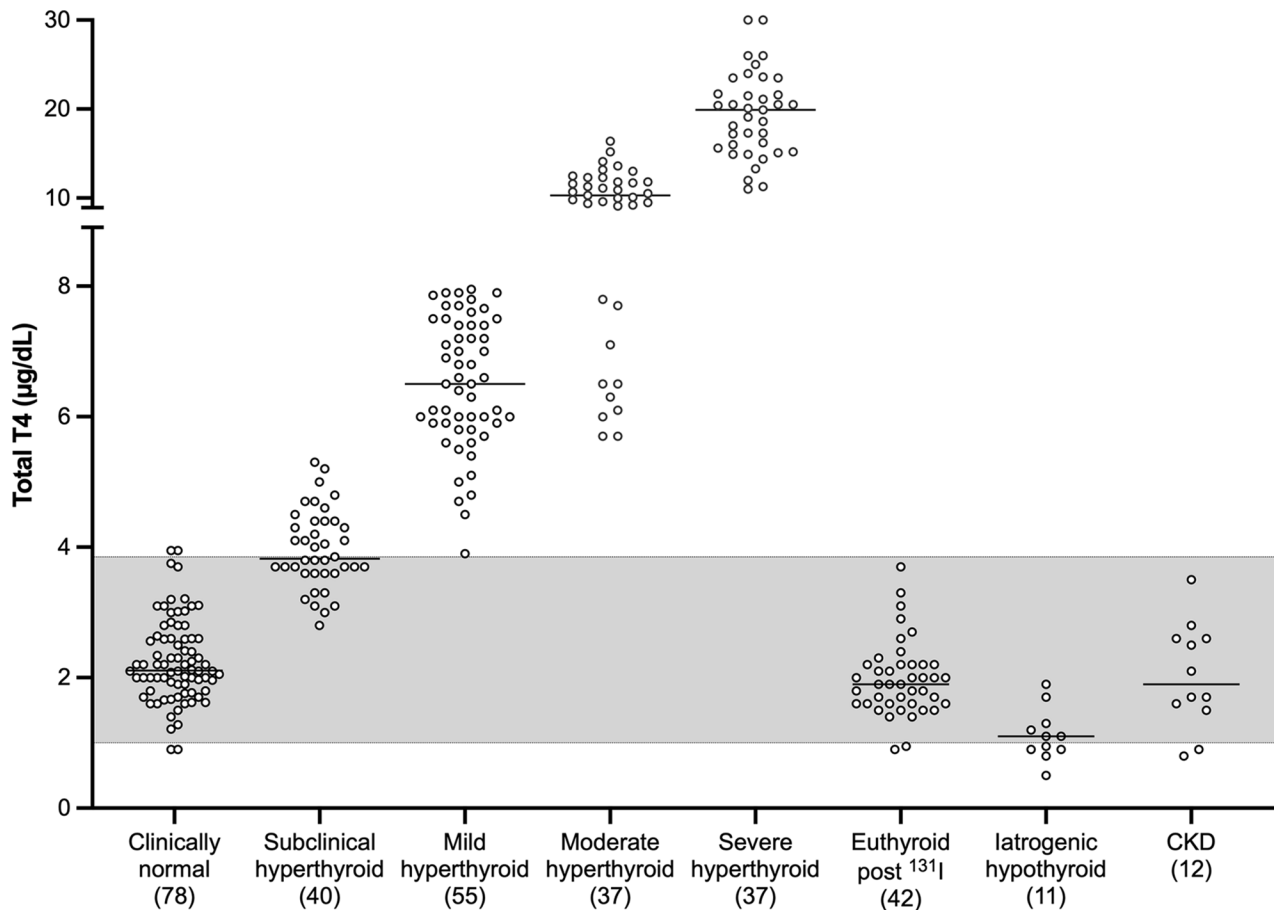


Figure 1—Serum T₄ concentrations in clinically normal cats, untreated hyperthyroid cats (divided into 4 severity groups), ¹³¹I-treated cats (divided into euthyroid and iatrogenic hypothyroid groups), and cats with chronic kidney disease (CKD). Horizontal lines represent the median for each group, and the shaded box represents the reference interval.

mild hyperthyroidism had a higher prevalence of within the reference interval serum TSH-BAW concentrations than did cats with moderate to severe disease (13/95 [13.7%] vs 2/74 [2.7%]; $P = .013$).

More hyperthyroid cats had serum TSH-CLIA concentrations within the reference interval (≥ 0.3 to 0.3 ng/mL) than had serum TSH-BAW concentrations within its reference interval (≥ 0.01 to 0.30 ng/mL) (32/169 [18.9%] vs 16/169 [9.5%]; $P = .019$; Figures 2 and 3).

Cats with ¹³¹I-induced hypothyroidism—

Iatrogenic hypothyroid cats had lower serum T₄ concentrations and higher TSH-CLIA and TSH-BAW concentrations ($P < .0001$) than all of the other groups (ie, the clinically normal cats, hyperthyroid cats, ¹³¹I-treated euthyroid cats, and cats with CKD; Supplementary Table S1).

Five hypothyroid cats (45.5%) had T₄ concentrations below the reference interval, whereas 6 had low-normal concentrations (1.0 to 1.9 µg/dL; Figure 1). All 11 (100.0%) cats had high serum concentrations measured by both TSH-CLIA and TSH-BAW (Figures 2 and 3).

Cats made euthyroid after ¹³¹I treatment—The ¹³¹I-treated euthyroid cats had lower serum T₄

concentrations and higher TSH-CLIA and TSH-BAW concentrations ($P < .0001$) than hyperthyroid cats (Supplementary Table S1). However, ¹³¹I-treated euthyroid cats had higher serum T₄ concentrations and lower TSH-CLIA and TSH-BAW concentrations ($P < .0001$) than hypothyroid cats.

Forty ¹³¹I-treated euthyroid cats (95.2%) had T₄ concentrations within its reference interval (Figure 1). Two euthyroid cats (4.8%) had slightly low serum T₄ concentrations (0.9 and 0.95 µg/dL); both had normal serum concentrations measured by TSH-CLIA and TSH-BAW that were within reference interval.

Thirty-six ¹³¹I-treated euthyroid cats (85.7%) had TSH-CLIA concentrations within the reference interval, whereas 6 (14.3%) had TSH-CLIA concentrations below its reference interval (< 0.03 ng/mL; Figure 2). By comparison, 39 cats (92.9%) had TSH-BAW concentrations within the reference interval, whereas only 3 (7.1%) had TSH-BAW concentrations below its reference interval (< 0.01 ng/mL; Figure 3). The prevalence of within the reference interval concentrations measured by TSH-CLIA and TSH-BAW (85.7% vs 92.9%) did not differ ($P = .48$).

Cats with CKD—Cats with CKD had lower serum T₄ concentrations and higher TSH-CLIA and TSH-

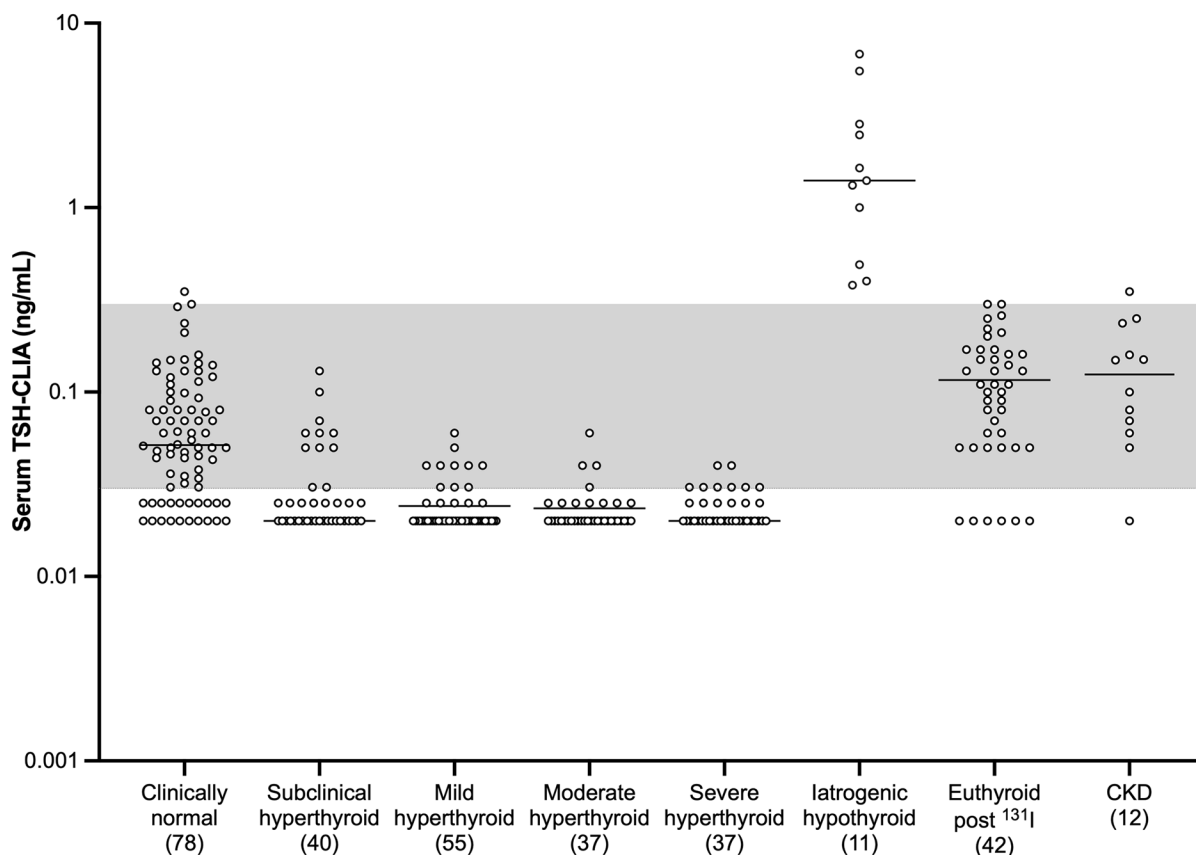


Figure 2—Serum thyroid-stimulating hormone chemiluminescent immunoassay (TSH-CLIA) concentrations in clinically normal cats, untreated hyperthyroid cats (divided into 4 severity groups), ¹³¹I-treated cats (divided into euthyroid and iatrogenic hypothyroid groups), and cats with chronic kidney disease (CKD). Horizontal lines represent the median for each group, and the shaded box represents the reference interval (0.03 to 0.3 ng/mL). Undetectable concentrations (< 0.03 ng/mL) are plotted as low concentrations of 0.02 to 0.025 ng/mL.

BAW concentrations ($P < .0001$) than hyperthyroid cats (Supplementary Table S1), but higher serum T_4 concentrations and lower TSH-CLIA and TSH-BAW concentrations ($P < .0001$) than hypothyroid cats (Supplementary Table S1).

Ten cats with CKD (83%) had T_4 concentrations within the reference interval, and 2 had slightly low concentrations (Figure 1). Ten cats (83.3%) had TSH-CLIA concentrations within the reference interval, whereas 1 cat (8.3%) had undetectable concentrations (< 0.03 ng/mL) and 1 cat (8.3%) had a slightly high value (0.35 ng/mL; Figure 2). Conversely, all of the CKD cats had TSH-BAW concentrations within the reference interval (Figure 3).

Clinically normal cats—Normal cats had lower serum T_4 concentrations and higher TSH-CLIA and TSH-BAW concentrations ($P < .0001$) than the hyperthyroid cats (Supplementary Table S1). However, normal cats had higher serum T_4 concentrations and lower TSH-CLIA and TSH-BAW concentrations ($P < .0001$) than hypothyroid cats (Supplementary Table S1).

More clinically normal cats had undetectable serum TSH-CLIA concentrations (< 0.03 ng/mL) than low or undetectable TSH-BAW concentrations

(< 0.01 ng/mL) (20 [25.6%] vs 1 [1.3%]; $P < .0001$; Figures 2 and 3).

Sensitivity, specificity, PPV, and NPV of serum T_4 , TSH-CLIA, and TSH-BAW concentrations as diagnostic tests for hyperthyroidism and hypothyroidism

When evaluated as a diagnostic test for hyperthyroidism, serum TSH-BAW had higher sensitivity (91.1% vs 79.9%) and specificity (98.9% vs 76.7%) than serum TSH-CLIA (both $P < .001$; **Table 1**). Similarly, serum T_4 had higher sensitivity and specificity than serum TSH-CLIA ($P = .039$ and $P < .001$, respectively) but did not differ from that of serum TSH-BAW (88.2% and 97.8%; $P = .33$ and $P = 1.0$, respectively; **Table 1**). Serum TSH-BAW also had a higher PPV (86.9%) than did serum concentrations of TSH-CLIA (21.7%) or T_4 (76.3%). All assays had similar NPV (**Table 2**).

When evaluated as a diagnostic test for iatrogenic hypothyroidism, serum TSH-BAW and TSH-CLIA showed higher test sensitivity (100%) than did serum T_4 concentration (45.5%) ($P = .03$; **Table 3**). All 3 assays had similar values for specificity (**Table 3**). Serum TSH-BAW also had a higher PPV (100%) than did serum TSH-CLIA (88.2%) or serum

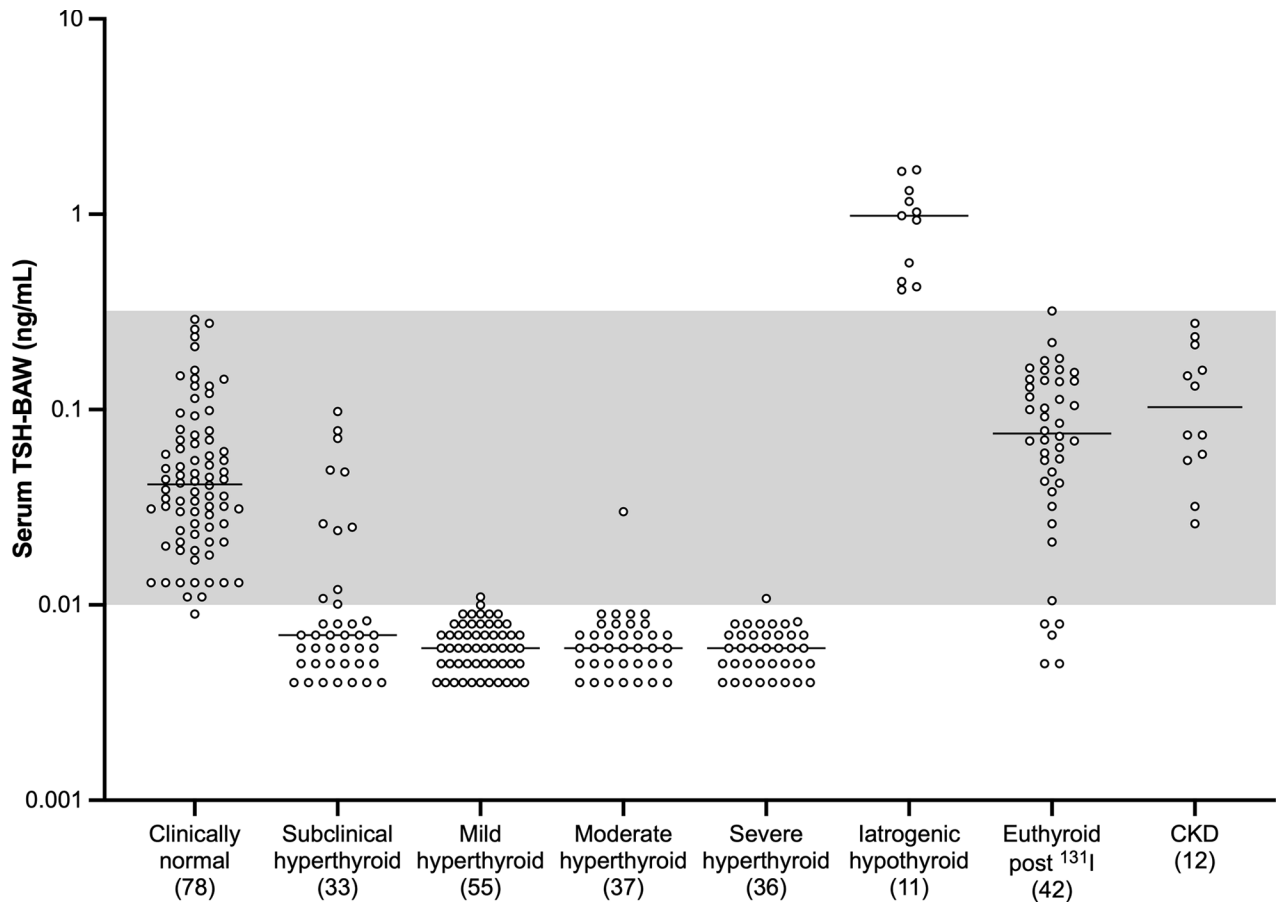


Figure 3—Serum thyroid-stimulating hormone bulk acoustic wave (TSH-BAW) concentrations in clinically normal cats, untreated hyperthyroid cats (divided into 4 severity groups), ¹³¹I-treated cats (divided into euthyroid and iatrogenic hypothyroid groups), and cats with chronic kidney disease (CKD). Horizontal lines represent the median for each group, and the shaded box represents the reference interval. Results below the detection limit of 0.008 ng/mL are plotted as low concentrations of 0.004 to 0.007 ng/mL.

Table 1—Calculation of diagnostic test sensitivity and specificity for serum concentrations of T₄, thyroid-stimulating hormone chemiluminescent immunoassay (TSH-CLIA), and thyroid-stimulating hormone bulk acoustic wave (TSH-BAW) in 169 cats with untreated hyperthyroidism (divided into 4 severity groups), 78 clinically normal cats, and 12 cats with chronic kidney disease (CKD).

Sensitivity for hyperthyroidism (95% CI)					
Serum hormone	Hyperthyroid (169)	Subclinical (40)	Mild (55)	Moderate (37)	Severe (37)
T ₄	88.2 (82.3–92.6)	50.0 (33.8–66.2)	100 (93.6–100)	100 (90.5–100)	100 (90.5–100)
TSH-CLIA	79.9 (73.0–85.7)	72.5 (56.1–85.4)	81.8 (69.1–90.9)	86.5 (71.2–95.5)	78.4 (61.8–90.2)
TSH-BAW	90.5 (85.1–94.5)	72.5 (56.1–85.4)	96.4 (87.5–99.6)	97.3 (85.9–99.9)	97.3 (85.9–99.9)
T ₄ plus TSH-CLIA	72.2 (64.8–78.8)	40.0 (24.9–56.7)	81.8 (69.1–90.9)	86.5 (71.2–95.5)	78.4 (61.8–90.2)
T ₄ plus TSH-BAW	84.6 (78.3–89.7)	45.0 (29.3–61.5)	96.4 (87.5–99.6)	97.3 (85.8–99.9)	97.3 (85.8–99.9)

Specificity for hyperthyroidism (95% CI)			
Serum hormone	Clinically normal (78)	CKD (12)	All euthyroid (90)
T ₄	97.4 (91.0–99.7)	100 (73.5–100)	97.8 (92.2–99.7)
TSH-CLIA	74.4 (63.2–83.6)	91.7 (61.5–99.8)	76.7 (66.6–84.9)
TSH-BAW	98.7 (93.1–100.0)	100 (73.5–100.0)	98.9 (94.0–100.0)
T ₄ plus TSH-CLIA	100 (95.4–100)	100 (73.5–100)	100 (96.0–100)
T ₄ plus TSH-BAW	100 (95.4–100)	100 (73.5–100)	100 (96.0–100)

Table 2—Calculation of diagnostic test positive and negative predictive values for serum concentrations of T₄, thyroid-stimulating hormone chemiluminescent immunoassay (TSH-CLIA), and thyroid-stimulating hormone bulk acoustic wave (TSH-BAW) in 169 cats with hyperthyroidism (divided into 4 severity groups), 78 clinically normal cats, and 12 cats with chronic kidney disease (CKD).

Serum hormone	Positive predictive value (95% CI*)				
	Hyperthyroid (169)	Subclinical (40)	Mild (55)	Moderate (37)	Severe (37)
T ₄	76.3 (44.9–92.7)	64.6 (30.9–88.2)	78.5 (48.1–93.5)	78.5 (48.1–93.5)	78.5 (48.1–93.5)
TSH-CLIA	21.7 (15.9–28.9)	20.1 (14.2–27.2)	22.1 (16.1–29.7)	23.1 (16.8–30.9)	21.4 (15.3–29.1)
TSH-BAW	86.9 (48.5–97.9)	84.1 (42.7–97.4)	87.6 (50.0–98.0)	87.7 (50.3–98.0)	87.7 (50.3–98.0)
T ₄ plus TSH-CLIA	100 (97.2–100)	100 (79.4–100)	100 (92.1–100)	100 (89.1–100)	100 (88.1–100)
T ₄ plus TSH-BAW	100 (97.5–100)	100 (81.5–100)	100 (93.3–100)	100 (90.3–100)	100 (90.3–100)

Serume hormone	Negative predictive value (95% CI*)		
	Clinically normal (78)	CKD (12)	All euthyroid (90)
T ₄	99.0 (98.5–99.4)	99.1 (96.4–99.4)	99.0 (98.5–99.4)
TSH-CLIA	98.0 (97.2–98.6)	98.4 (97.7–98.8)	97.9 (97.2–98.5)
TSH-BAW	99.2 (98.8–99.5)	99.2 (98.8–99.5)	99.2 (98.8–99.5)
T ₄ plus TSH-CLIA	97.8 (97.2–98.3)	97.8 (97.2–98.3)	97.8 (97.2–98.3)
T ₄ plus TSH-BAW	98.8 (98.3–99.1)	98.8 (98.3–99.1)	98.8 (98.3–99.1)

*Estimated population prevalence 7.5%.

Table 3—Calculation of diagnostic test sensitivity, specificity, and positive and negative predictive values for serum concentrations of T₄, thyroid-stimulating hormone chemiluminescent immunoassay (TSH-CLIA), and thyroid-stimulating hormone bulk acoustic wave (TSH-BAW) in 11 cats with ¹³¹I-induced iatrogenic hypothyroidism and 78 clinically normal and 12 cats with chronic kidney disease (CKD).

Serum hormone	Sensitivity for hypothyroidism (95% CI*)		Specificity for hypothyroidism (95% CI*)		
	Hypothyroid (11)		Clinically normal (78)	CKD (12)	All euthyroid (90)
T ₄	45.5 (16.8–76.6)		97.4 (91.0–99.7)	83.3 (51.6–97.9)	95.6 (89.0–98.8)
TSH-CLIA	100 (71.5–100)		97.4 (91.0–99.7)	91.7 (61.5–99.8)	96.7 (90.6–99.3)
TSH-BAW	100 (71.5–100)		100 (95.4–100)	100 (73.5–100)	100 (95.9–100)
T ₄ + TSH-CLIA	45.5 (16.8–76.6)		100 (95.4–100)	100 (73.5–100)	100 (96.0–100)
T ₄ + TSH-BAW	45.5 (16.8–76.6)		100 (95.4–100)	100 (73.5–100)	100 (96.0–100)

Serum hormone	Positive predictive value (95% CI*)		Negative predictive value (95% CI*)		
	Hypothyroid (11)		Clinically normal (78)	CKD (12)	All euthyroid (90)
T ₄	71.9 (44.6–89.1)		87.7 (80.6–92.5)	85.9 (77.1–91.7)	87.5 (80.3–92.3)
TSH-CLIA	88.2 (71.1–95.8)		100 (95.3–100)	100 (71.5–100)	100 (95.6–100)
TSH-BAW	100 (71.5–100)		100 (95.4–100)	100 (73.5–100)	100 (95.9–100)
T ₄ + TSH-CLIA	100 (47.8–100)		88.0 (81.1–92.6)	88.0 (81.1–92.6)	88.0 (81.1–92.6)
T ₄ + TSH-BAW	100 (47.8–100)		88.0 (81.1–92.6)	88.0 (81.1–92.6)	88.0 (81.1–92.6)

*Estimated prevalence 20%.

T₄ concentration (71.9%). All assays had similar NPV (Table 3).

Discussion

In this study, a novel, sensitive TSH-BAW assay distinguished euthyroid cats with low-normal TSH concentrations from hyperthyroid cats with truly low TSH concentrations better than the current TSH-CLIA assay, displaying higher test sensitivity, specificity, and PPV. Additionally, the TSH-BAW assay displayed similar test performance as the TSH-CLIA assay for measuring high serum TSH concentrations and diagnosing ¹³¹I-induced hypothyroidism.

In human patients, measurement of circulating TSH concentration represents the first-line test for assessment of thyroid function in most clinical

situations, including hyperthyroidism and hypothyroidism.^{7–10,39} The pituitary gland constantly monitors circulating concentrations of T₄ and T₃ and decreases or increases the secretion of TSH, respectively, in response to even the slightest increase or decrease in thyroid hormone concentrations.⁴⁰ Therefore, finding a low serum TSH concentration is diagnostic for hyperthyroidism, even if total or free T₄ concentrations remain in the high-normal range (a situation defined as subclinical hyperthyroidism).^{7–9} In contrast, finding a high serum TSH concentration is considered diagnostic for hypothyroidism, even if serum total or free T₄ concentrations remain low-normal (a situation defined as subclinical hypothyroidism).^{9,41,42} If thyroid disease is suspected but serum TSH concentration remains within its reference interval, then a diagnosis of hyperthyroidism or hypothyroidism is

effectively ruled out.^{9,41} It should be noted that the terms subclinical hyper- and hypothyroidism are something of a misnomer, as these terms refer to a laboratory diagnosis but do not actually imply that the patients are completely asymptomatic (although symptoms, when present, are generally mild).^{39,42} Likewise, cats diagnosed with “subclinical” hyperthyroidism or hypothyroidism variably show clinical signs of disease, but when present, the clinical signs are generally mild and warrant continued monitoring, rather than immediate treatment.^{11,43}

Historically, clinicians have used canine TSH-CLIA assays most commonly to measure TSH concentrations in cats. Although this assay has proven very useful as a diagnostic test for thyroid disease in cats,⁴⁴ it is relative insensitive, with a detection limit of 0.03 ng/mL, a concentration not low enough to distinguish low-normal values from truly low TSH concentrations.^{6,11,15} We found that 25.6% of our clinically normal cats had serum TSH-CLIA concentrations below < 0.03 ng/mL, consistent with findings in a previous study⁶ in which 44/131 (33.5%) older euthyroid cats had undetectable serum TSH-CLIA concentrations. When used as a diagnostic test for hyperthyroidism, the undetectable TSH-CLIA concentrations found in many euthyroid cats become a false-positive result, which greatly hampers the use of this assay for accurately identifying hyperthyroid cats.

In contrast to the TSH-CLIA assay, researchers optimized the TSH-BAW assay to detect lower concentrations of feline TSH. The resultant lower detection limit allows one to measure serum TSH concentrations down to 0.008 ng/mL, slightly below the lower end of the test’s reference interval (0.01 ng/mL). Consequently, of 20 clinically normal cats that had an undetectable (< 0.03 ng/mL) TSH-CLIA concentration, only 1 had a truly low TSH concentration (0.009 ng/mL) when measured by the TSH-BAW assay. Conversely, most hyperthyroid cats (especially those with mild to severe disease) had low TSH-BAW concentrations (< 0.01 ng/mL), as expected with hyperthyroid-induced negative feedback inhibition on pituitary TSH secretion.⁴⁰ Therefore, measuring serum TSH-BAW provides a more clinically useful diagnostic test for hyperthyroidism in cats than does serum TSH-CLIA concentrations, as evidenced by its higher diagnostic test sensitivity, specificity, and PPV (Tables 1 and 2). Put another way, with a PPV of only 21.7% for TSH-CLIA, finding a suppressed serum TSH-CLIA concentration (< 0.03 ng/mL) equates to only a 1 in 5 chance of being hyperthyroid. In contrast, with a PPV of 87% for TSH-BAW, finding a low or suppressed TSH-BAW concentration indicates that the individual cat has almost a 9 in 10 chance of being hyperthyroid.

Although most hyperthyroid cats had low, suppressed TSH-BAW concentrations, some had serum TSH-BAW concentrations that remained within the reference interval. Such incomplete TSH suppression has also been rarely reported in human patients with Graves disease^{7,45} and in cats with mild hyperthyroidism, in which a small percentage (2% to 4%) had

measurable TSH-CLIA concentrations.^{6,43} As might be expected, 11/15 of our cats with normal TSH-BAW concentrations had subclinical hyperthyroidism, and 10/11 of these also had serum T₄ concentration that remained within the reference interval (Figure 1). Of the remaining 4/15 cats with a normal serum TSH-BAW concentration, 3/4 cats had low-normal concentrations (0.01 to 0.011 ng/mL), very close to the lower reference limit cutoff (< 0.01 ng/mL).

Interestingly, we found no difference in median serum TSH-BAW concentrations between cats with subclinical hyperthyroidism and those with mild ($P > .99$), moderate ($P = .48$), or severe ($P = .30$) disease, suggesting that serum TSH suppression occurs very early in the course of hyperthyroidism in most cats, similar to humans with subclinical hyperthyroidism.^{9,10} This premise is also supported by a previous study¹¹ that showed euthyroid cats with TSH-CLIA concentrations < 0.03 ng/mL were more likely to develop overt hyperthyroidism within 14 months than cats with TSH-CLIA concentrations > 0.03 ng/mL. However, T₄ concentrations differed between cats with subclinical hyperthyroidism and those with mild ($P < .0001$), moderate ($P < .0001$), and severe ($P < .0001$) disease. This suggests that while measurement of TSH-BAW can help differentiate euthyroid cats from cats with subclinical hyperthyroidism (which should be monitored closely for disease progression), serum T₄ concentrations should also be monitored to help distinguish subclinical hyperthyroid cats from cats with more advanced disease that require treatment.

In agreement with previous studies,^{21,23} determination of serum TSH concentration (measured either by CLIA^{21,23} or BAW methods) showed a much higher diagnostic test sensitivity and PPV than serum T₄ concentration for identifying hypothyroid cats after ¹³¹I treatment (100.0% vs 45.5%). Both serum T₄ and TSH (both CLIA and BAW methods) also showed very high diagnostic test specificity and NPV for identifying cats with ¹³¹I-induced hypothyroidism. Therefore, in addition to its use in diagnosis of feline hyperthyroidism, serum TSH-CLIA or TSH-BAW concentrations should also be monitored after ¹³¹I treatment to help diagnose iatrogenic hypothyroidism and determine the need for thyroid hormone supplementation.^{21,46}

One limitation of this study is that we did not investigate a large group of cats suffering from non-thyroidal illness syndrome (NTIS), but rather, only included a small group of cats with CKD. We previously reported⁴⁷ the results of serum T₄ and TSH-CLIA concentrations in 222 cats with NTIS and found that these sick cats commonly develop low to undetectable serum concentrations of T₄ and TSH-CLIA. It certainly would be of interest to repeat that study⁴⁷ to determine if cats suffering NTIS really do develop truly low TSH-BAW concentrations, as our previous study looking at TSH-CLIA suggested. Another limitation is that not all cats underwent “gold-standard” diagnostic testing with thyroid scintigraphy. Despite not having signs of hyperthyroidism or palpable thyroid nodules, it is possible the healthy or CKD cats could have had mild hyperthyroidism. That said, none

of the 12 CKD cats and only 1/78 clinically healthy cats had a low TSH-BAW concentration, making a missed diagnosis of hyperthyroidism highly unlikely. In addition, none of the CKD cats or clinically healthy cats had a high TSH-BAW concentration, thus helping to rule out the possibility of hypothyroidism.

In conclusion, our study demonstrates that this new TSH-BAW assay more accurately identifies hyperthyroid cats than the current TSH-CLIA assay and would improve the early diagnosis of hyperthyroidism, especially in cats with subclinical and mild disease in which the serum T_4 is normal or only slightly high. Our results also suggest that measuring serum TSH-BAW concentration be considered as a first-line screening test (together with serum T_4) to assess thyroid function in cats.

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Supplementary Materials

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