

Vcheck

T4 & TSH

Thyroid function tests

BIONOTE Marketing team

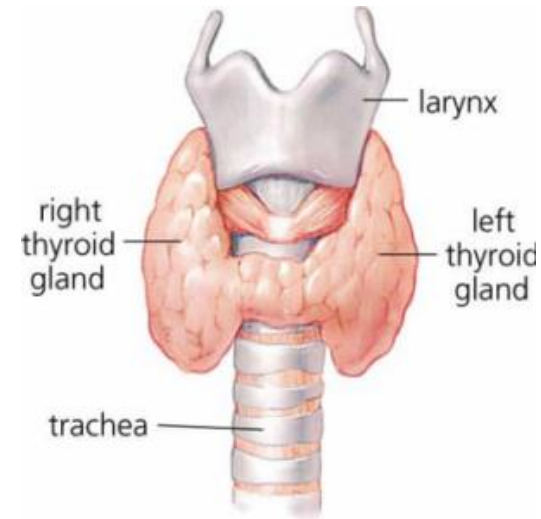
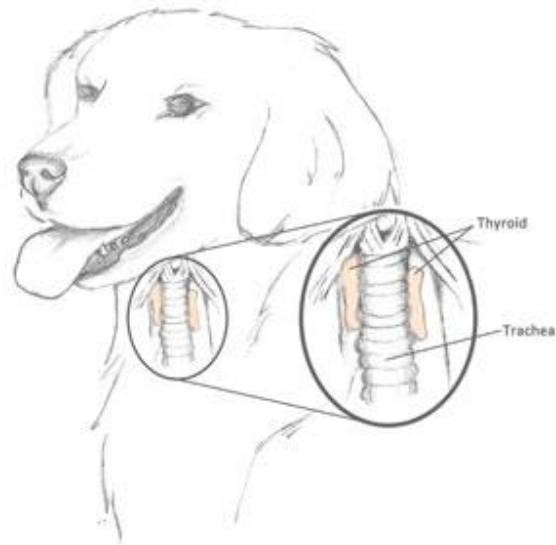
Mar. 2020



Vcheck T4 & TSH

- Thyroid Hormones (T4, T3, TSH)
- The Thyroid Feedback Mechanism

Vcheck T4 & TSH
Thyroid gland



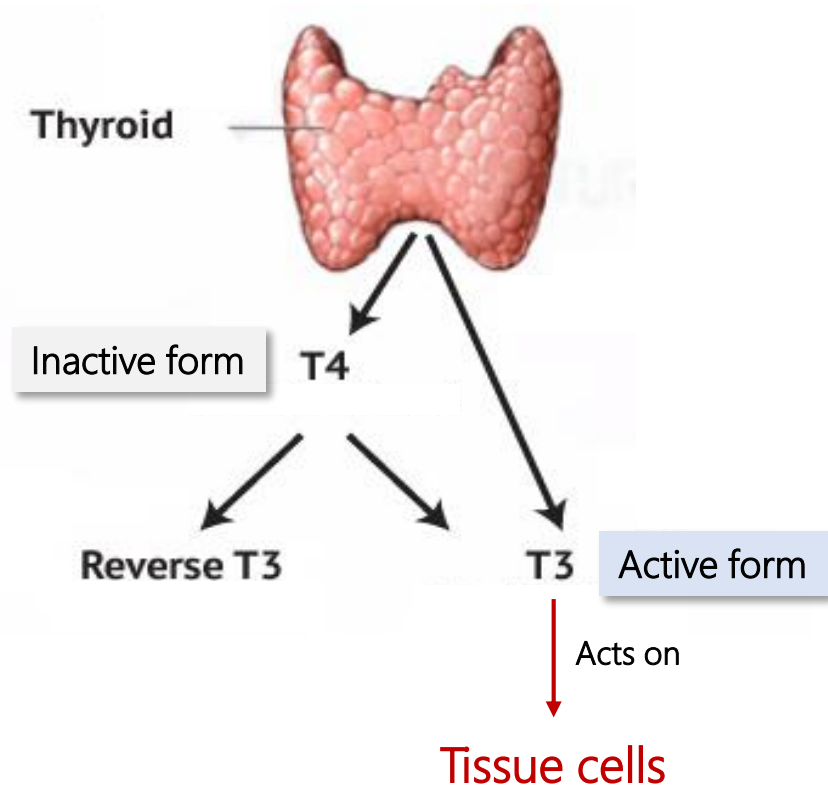
- The thyroid glands are paired structures located along the trachea, about halfway down the neck of dogs.
- These glands produce thyroxin, a hormone that regulates the body's metabolism.

Vcheck T4 & TSH

Thyroid Hormones

* Thyroid Hormones

- T3: Triiodothyronine
- T4: Thyroxine

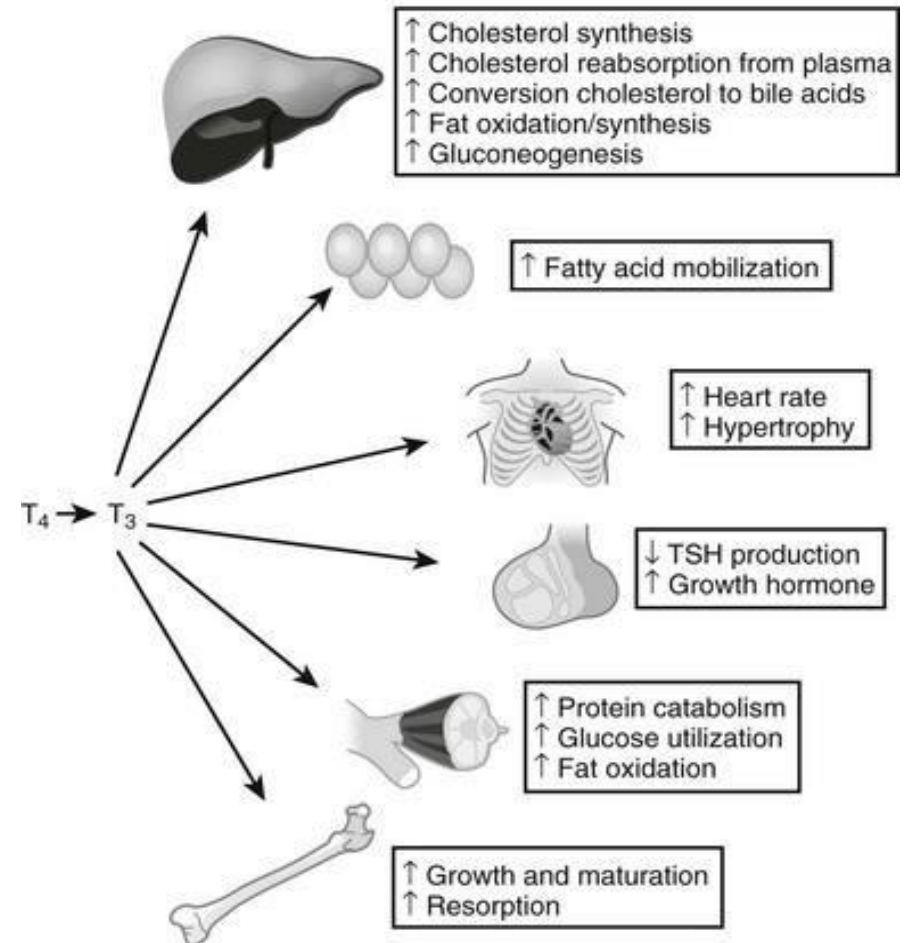


- Most of the thyroid hormone consists of T4 and only small quantities of T3 and rT3.
- **Conversion:** T4 (Inactive form) \Rightarrow T3 (Active form)
- Once released into circulation, only small amounts of T4 and T3 are unbound (Free T4, T3)
- In dogs, the amount of free hormones in plasma is low (less than 1% for T4, slightly more than 1% for T3)
 - ✓ **Total T4:** Measures the bound and free hormone
 - ✓ **Free T4:** Measures free hormone (what is not bound)

Effects of thyroid hormone

- ✓ Thyroid hormones are the primary factors for the control of basal metabolism.
- ✓ Thyroid hormone is important for the normal regulation of metabolic rate and activity in many tissues.

- **Canine hypothyroidism** is the common disease related to thyroid function in dogs.
- **Feline hyperthyroidism** is the most common endocrine disease affecting old cats.



The Thyroid Feedback Mechanism

- **Negative feedback**

- ✓ As thyroid hormone (T₃, T₄) production drops, (due to destruction of the thyroid gland)
 - ⇒ The negative feedback decreases
 - ⇒ TSH level increases in response

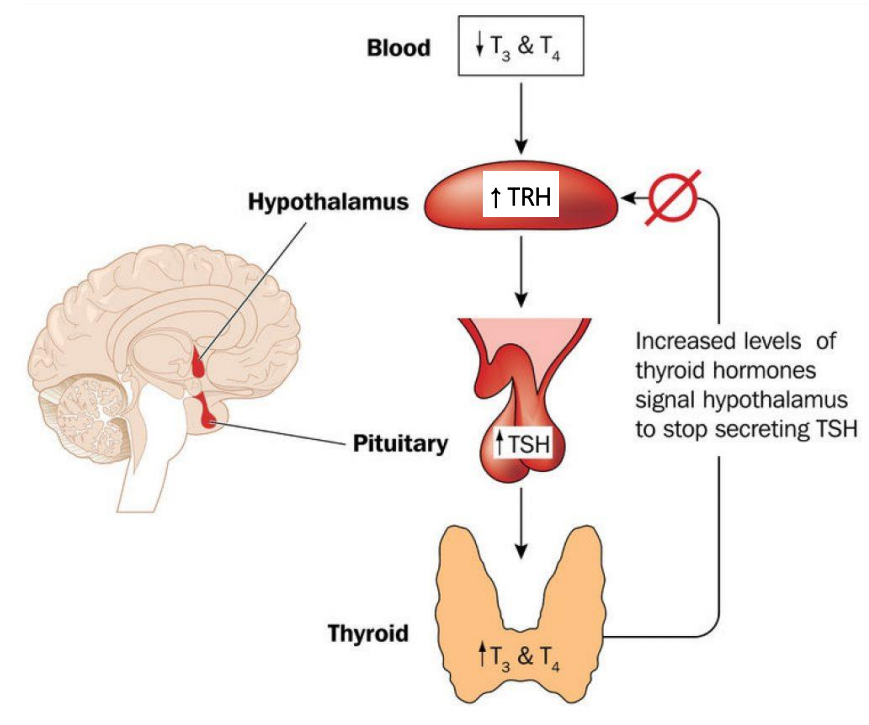
- **In hypothyroid dogs, (low T₄ & high TSH)**

- ✓ TSH increases in dogs due to a lack of thyroid hormone by negative feedback.
- ✓ TSH provides additional evidence for or against the diagnosis of hypothyroidism.

- **In hyperthyroid cats, (high T₄ & low TSH)**

- ✓ Hyperthyroid cats will generally have low levels of TSH by negative feedback.

The hypothalamic-pituitary-thyroid axis [Negative feedback mechanism]



Vcheck T4 & TSH

Canine hypothyroidism

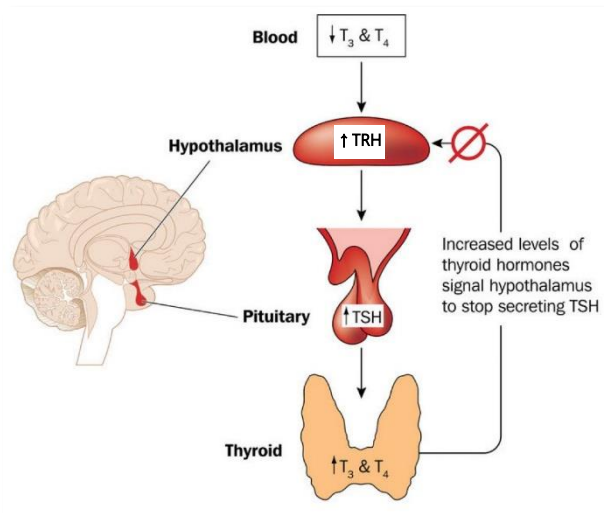
Vcheck T4 & TSH

Canine Hypothyroidism

Hypothyroidism in dogs is a disorder where the thyroid gland in the neck doesn't secrete enough thyroxine, a hormone that controls metabolism.

- One of the most common canine endocrine diseases
- Low concentrations of thyroid hormones (T4, T3) in the blood
- Results from impaired production and secretion of thyroid hormone

(Picture Credit: Getty Images)



Primary (Thyroidal) hypothyroidism (95%)

- ✓ Due to destruction of the thyroid gland itself
- ✓ Idiopathic atrophy of the thyroid, lymphocytic thyroiditis

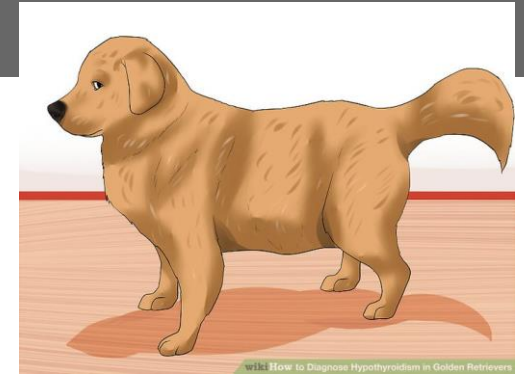
Secondary (Pituitary) hypothyroidism (<5%)

- ✓ Impaired ability of the pituitary gland to secrete TSH
- ✓ Anterior pituitary dysfunction, destruction from neoplasia

Canine Hypothyroidism

✓ Signalment

- **Age**
 - Middle-aged
 - Mean age 7 years, with a range of 4-10 years
- **Breed**
 - Large breed dogs (Golden Retrievers, Doberman Pinchers)
 - Rare in miniature and toy breeds
- **Sex**
 - Either sex at about the same rate
 - Neutered males and females have higher risk than intact ones.



▲ Hypothyroidism in Golden Retrievers

Several breeds are genetically predisposed to the disorder, including ...

Airedale Terriers	Golden Retrievers
Boxers	Greyhounds
Cocker Spaniels	Irish Setters
Dachshunds	Labrador Retrievers
Doberman Pinschers	Miniature Schnauzers

Canine Hypothyroidism

✓ Clinical signs

An underactive thyroid affects so many bodily functions that rely on thyroxine.

⇒ Symptoms of the disorder vary widely

- Lack of energy **Hallmark sign**
- Frequent napping
- Exercise intolerance
- Loss of interest in running and playing
- Weight gain without increase in appetite or calorie intake
- Low tolerance for the cold
- Dull, dry, brittle, thin or greasy coat
- Hair loss or failure to regrow clipped hair



▲ Hair loss in a dog with hypothyroidism



▲ Weight gain without increase in appetite in a dog with hypothyroidism

Canine Hypothyroidism

Never base a diagnosis on a single test result!

✓ Diagnosis ①

Based on a combination of clinical signs, physical examination, CBC, biochemistry, test of thyroid gland function.

- **Haematology**
 - Mild normocytic, normochromic, non-regenerative anemia (~4-50%)
- **Serum Biochemistry**
 - Hypercholesterolemia (75%)
 - Mild elevations in liver enzymes (ALP, ALT)

Canine Hypothyroidism

✓ Diagnosis ②

Never base a diagnosis on a single test result!

Based on a combination of clinical signs, physical examination, CBC, biochemistry, test of thyroid gland function.

Thyroid Function Tests

- Serum Total T4 (TT4)

- A good screening test (high sensitivity)
- Low specificity especially in the presence of concurrent disease
- ⇒ Increases markedly if used in conjunction with endogenous TSH analysis

- Serum TSH (Thyroid Stimulating Hormone)

- Primary hypothyroidism: Low T4 & High TSH
- Poor sensitivity
- ⇒ Approaches 100% in combination with a low fT4 or TT4

- Serum Free T4 (fT4)

- Measures unbound fraction of T4
- Influenced less by euthyroid sick syndrome

[Mechanism]

As thyroid hormone production drops,

⇒ Negative feedback

⇒ TSH levels secreted will be increased in response.

Vcheck T4 & TSH

Canine Hypothyroidism

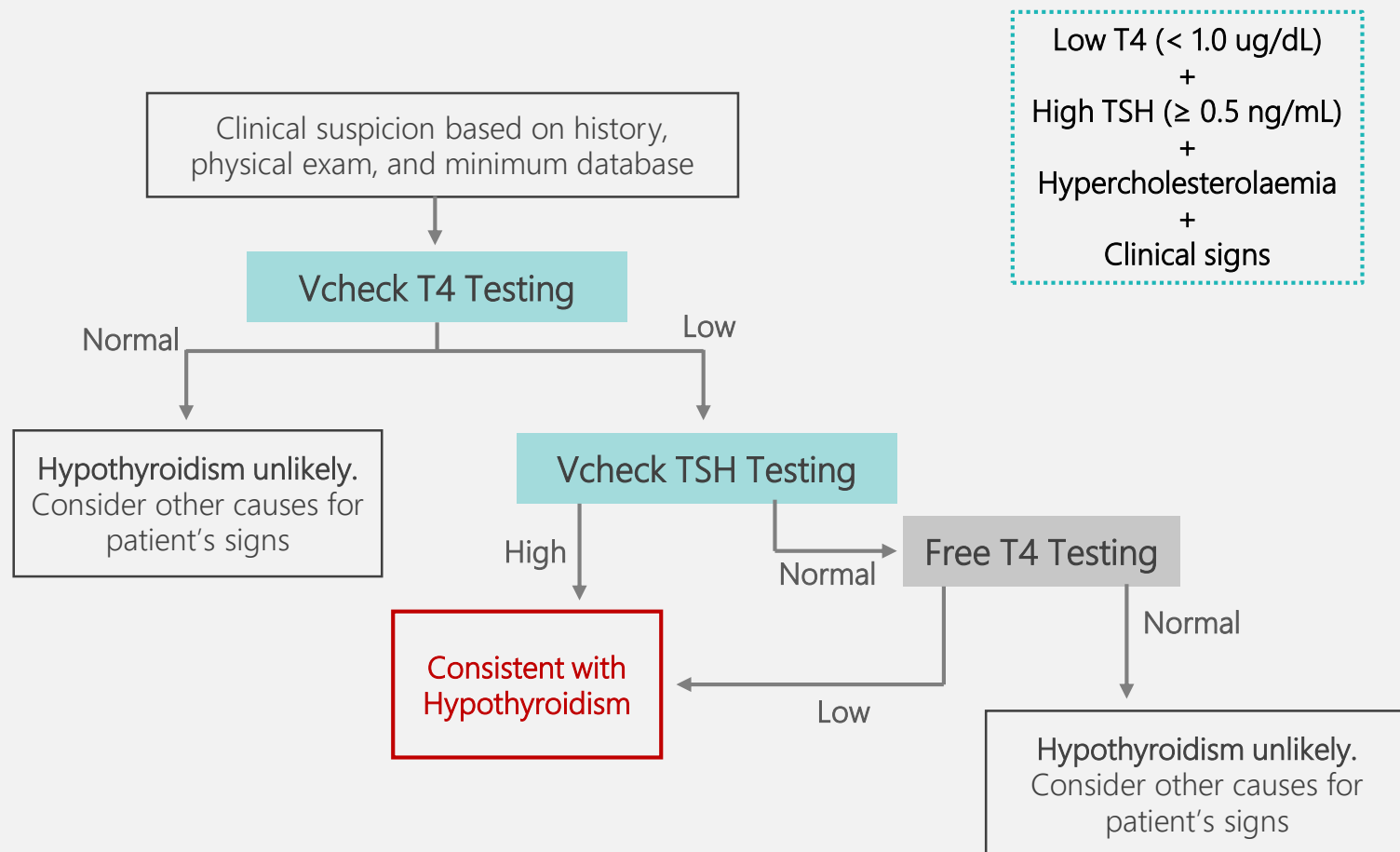
✓ Diagnosis ②

Thyroid Function Tests

- T4 + TSH combination test is mandatory for diagnosis of canine Hypothyroidism.



* A stepwise approach is helpful in accurately diagnosing canine hypothyroidism



Canine Hypothyroidism

✓ Diagnosis ②

Thyroid Function Tests

- T4 + TSH combination test is mandatory for diagnosis of canine Hypothyroidism.
- Combination of elevated serum TSH and decreased T4 or fT4 has a specificity of 98% for diagnosis of hypothyroidism

Thyroid Tests	T4 Normal	T4 Decreased	
cTSH Normal	<ul style="list-style-type: none"> • Euthyroid * End thyroid investigation 	<ul style="list-style-type: none"> • Non-thyroidal illness (NTI) • Drugs • 20% of hypothyroid dogs 	<p>Perform further tests (Ex. free T4) for accurate diagnosis!</p>
cTSH Increased	<ul style="list-style-type: none"> • Sulfonamide treatment • Recovery from NTI * Withdraw drug Tx and retest * Wait until recovery complete and retest 	<ul style="list-style-type: none"> • Hypothyroid * Treat with T4 therapy 	<p>Diagnosis of hypothyroidism in dogs</p>

Canine Hypothyroidism

✓ Diagnosis ②

Thyroid Function Tests

Additional test (Ex. Free T4) is warranted in the following scenarios:

- If serum T4 is <1.0 ug/dL, but hypercholesterolaemia and clinical signs are absent.
- If severe systemic illness is present and the potential for ESS is high.
- If drugs known to decrease serum T4 concentration are being administered (prednisolone, phenobarbitone, etc).

What Is Euthyroid Sick Syndrome (ESS) ?

- ✓ Thyroid gland is secondarily affected by disease in some other organ system
 - Other endocrine diseases
(Hyperadrenocorticism, Diabetes mellitus)
 - Liver, cardiac, renal, pancreatic, lung etc.
(Cardiomyopathy, demodicosis, hepatitis, infections, renal failure)
- ✓ So, the diagnosis of hypothyroidism should never be based on a hormone assay alone, but depends on a large range of findings

Canine Hypothyroidism

Considerations

- Greyhounds, Scottish deerhounds: have low T4 levels naturally
⇒ Diagnose based on clinical signs as well as test results; treat if clinically evident.
- Remember sick animals and animals on certain medications (anti-epileptics) may have depressed T4 levels.
(Euthyroid sick syndrome) ⇒ Wait and re-test after treatment of underlying cause if clinical signs persist.
- Several medications have been demonstrated to lower the serum T4 concentration of dogs.

Drugs That Alter Canine Thyroid Hormone Function or Test Results

- ✓ Prednisone (high dose)
- ✓ Phenobarbital
- ✓ Trimethoprim–sulfamethoxazole
- ✓ Aspirin (high dose)
- ✓ Clomipramine
- ✓ Thyroxine supplementation

Canine Hypothyroidism

Treatment

(Thyroid hormone replacement)

- Needs to be administered lifelong
- Thyroid supplement: **Synthetic sodium levothyroxine**
 - ✓ A starting dose of 20 µg/kg, Maximum dose is 800 µg (usually BID)
 - ✓ Daily administration (usually BID)
- **Improvement of Clinical signs**
 - ✓ General sense of well being will improve within a few weeks, but improvement in dermatological signs, myocardial function and weight loss may take 2–3 months.

- T4 levels can be measured 6–8 weeks after initiating treatment.
 - ✓ **4–6 hours after administration (peak)**: within high normal range (3.0~4.0 ug/dL)
 - ✓ **Just before tablet administration (trough)**: within low normal range (1.0~2.0 ug/dL)
- Regular rechecks are recommended including bloodwork.



T4 Monitoring During Treatment

Canine Hypothyroidism

Case Study-1

- **Signalment**

- **Age:** 11 year-old
- **Sex:** Castrated Male
- **Breed:** Cocker spaniel

- **Chief Complaint**

- Lethargy, Dull
- Dermatologic lesion (Alopecia)

- **Tests Performed**

- Physical examination
- CBC & Serum chemistry
- Thyroid hormones testing



▲ 11Y, Castrated Male

Canine Hypothyroidism

Case Study-1

*Test Results

- Thyroid function test
- CBC: mild anemia
- Serum Chemistry

Name	Reference	Result
Total T4	1.0-4.0 ug/dL	<0.5
ft4	0.6-3.7 ng/dL	<0.3
TSH	0-0.5 ng/ml	1.08

Name	Reference	Result	Name	Reference	Result
ALB	2.9-4.2	2.6	ALKP	15-127	791
ALT	19-100	293	AST	0-50	97
GGT	0-6	55	T-bil	0-0.4	0.4
TP	5.4-7.4	6.8	Ca	7.8-12.0	8.8
CRE	0.8-1.6	0.85	Chol	135-345	>520
GLU	70-118	99	TriG	10-100	178
BUN	8-31	14	Phos	3-6.2	3.2



▲ 11Y, Castrated Male

- Final Diagnosis
 - Canine Hypothyroidism
- Treatment
 - Levothyroxine 0.02 mg/kg bid PO

Canine Hypothyroidism

Case Study-1

*Test Results

- **Treatment**

- Drug therapy
- Levothyroxine 0.02 mg/kg bid PO

- **Monitoring** (8 weeks after treatment)

- Increased activity
- Condition recovery
- **Thyroid function testing: Normal**

Name	Reference	Result
Total T4	1.0-4.0 ug/dL	3.1
fT4	0.6-3.7 ng/dL	1.7
TSH	0-0.5 ng/ml	0.42

- Plan to monitor T4 concentrations every 6 months during drug therapy



▲ 11Y, Castrated Male

Canine Hypothyroidism

Case Study-2

- **Signalment**

- **Age:** 13 year-old
- **Sex:** Sprayed Female
- **Breed:** Cocker spaniel

- **Chief Complaint**

- Depression
- Weight gain these days
- Decreased vitality
- Skin pigmentation

- **Physical Examination**

- Exercise intolerance
- Alopecia
- Seborrehea sicca
- Both external ear- lichenification



▲ ddoli (13Y, Sprayed female)

Canine Hypothyroidism

Case Study-2

- Tests Performed

- Physical examination
- CBC & Serum chemistry
- Thyroid hormones testing

- Differential Diagnosis

- Alopecia from hyperadrenocorticism or follicular dysplasia
- Obesity from overfeeding or cushing's

- Tests Results

- Hypercholesterolemia
378 (110-320 mg/dL)
- Increased ALT 139 (10-125 U/L)
- **Thyroid function test**

Name	Reference	Result
Total T4	1.0-4.0 ug/dL	< 0.5
TSH	0-0.5 ng/ml	2.7

- Final Diagnosis

- Canine Hypothyroidism



▲ ddoli (13Y, Sprayed female)

Canine Hypothyroidism

Case Study-2

- **Treatment**

- Drug therapy
- Levothyroxine 0.02 mg/kg bid PO

- **Monitoring** (8 weeks after treatment)

- Improved clinical signs
- Weight gain
- Increased activity

Name	Reference	Result
Total T4	1.0-4.0 ug/dL	2.7
TSH	0-0.5 ng/ml	0.48

- Plan to monitor T4 concentrations every 6 months during drug therapy



▲ ddoli (13Y, Sprayed female)

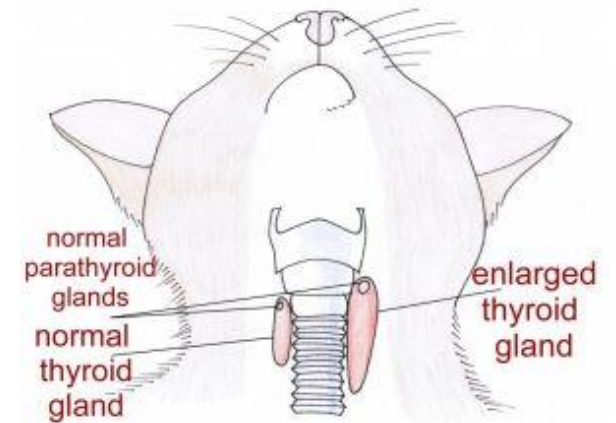
Vcheck T4 & TSH

Feline hyperthyroidism

Feline Hyperthyroidism

Hyperthyroidism is one of the most commonly diagnosed diseases of the older cat.

- The most **common endocrinopathy** of older cats.
- Increase in production of thyroid hormones (T3, T4) from an enlarged thyroid gland
- Bilateral (70%) / Unilateral (30%)
- **Cause:** unknown
 - **Possible contributing factors:** deficiencies or excesses of certain compounds in the diet and chronic exposure to thyroid-disrupting chemicals in food or the environment
- **Overall prevalence:** 2-4%
 - > 9 years of age: more than 6% (nearly 10% of geriatric cats)



Feline Hyperthyroidism



▲ Feline Hyperthyroidism

✓ Signalment

- Age

- Older than 8 years (Most)
- Mean age: 12–13 years

- Breed

- Siamese and Himalayan breeds are at decreased risk

- Sex

- Either sex at about the same rate
- Neutered males and females have higher risk than intact ones.

- Risk Factor

- Consumption of tinned cat food (especially fish or liver and giblet flavour)
- The use of cat litter

Vcheck T4 & TSH

Feline Hyperthyroidism

✓ Clinical signs

Develops a variety of signs that may be subtle at first but that become more severe as the disease progresses.

- Weight loss **Hallmark sign**
- Increased appetite
- Hyperactivity and Aggression
- Vomiting (up to 30% of cases)
- Diarrhea
- Increased thirst and urination (PU/PD)
- Unkempt, matted, or greasy coat



▲ Weight loss in a cat with hyperthyroidism



◀ The thin, unkempt appearance of a cat with hyperthyroidism.

Vcheck T4 & TSH

Feline Hyperthyroidism

✓ Diagnosis ①

Never base a diagnosis on a single test result!

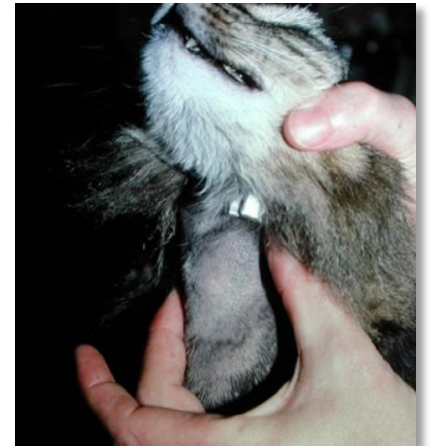
Based on a combination of clinical signs, physical examination, CBC, biochemistry, test of thyroid gland function.

- **Physical examination**

- Palpation of the cat's neck area to check for an enlarged thyroid gland
- Thyroid gland enlargement is palpable in 90 % of cases.

- **Ophthalmoscopic examination**

- Reveals evidence of hypertension with retinal vessel engorgement and rarely retinal detachment.



▲ Palpation for an enlarged thyroid gland

Feline Hyperthyroidism

✓ Diagnosis ②

Never base a diagnosis on a single test result!

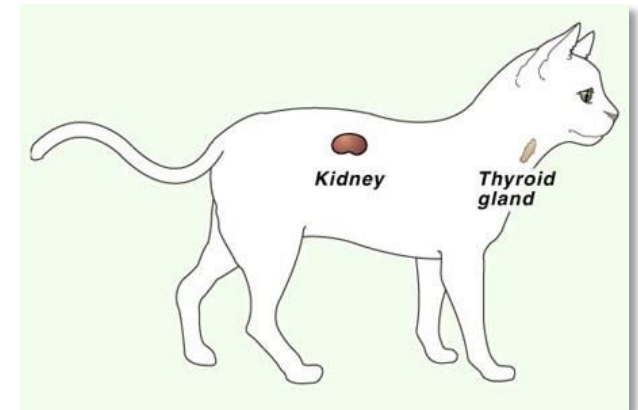
Based on a combination of clinical signs, physical examination, CBC, biochemistry, test of thyroid gland function.

- **Haematology**

- Increased haematocrit (40–50% of cases)
- Some might show a stress leukogram.

- **Serum Biochemistry**

- Increased ALT, ALP (more than 75% of cases)
- Hyperphosphataemia (20% of cases)
- Increased BUN, Creatinine
- * Concomitant renal disease is common in older cats.



▲ Concurrent Hyperthyroidism and Chronic Renal Failure in Older Cats

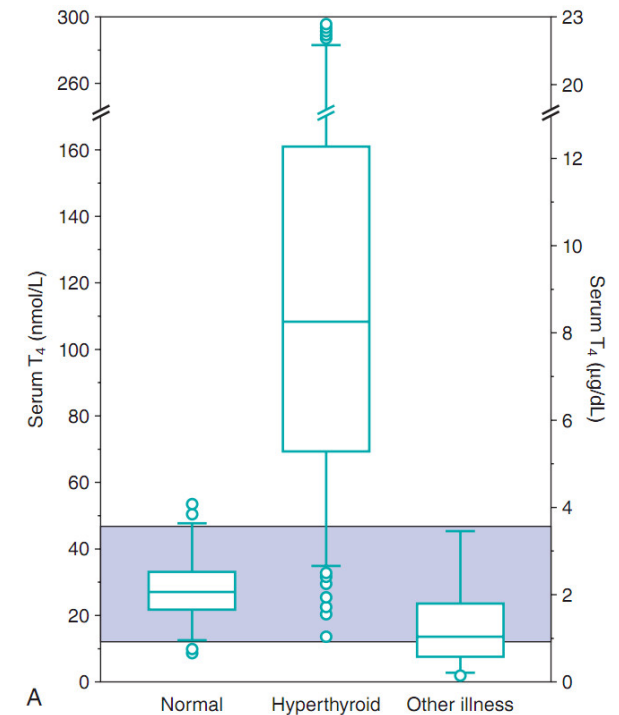
Feline Hyperthyroidism

✓ Diagnosis ③

Thyroid Function Tests

The **best screening test** for the diagnosis of hyperthyroidism is the total T4 (TT4) concentration.

- **Serum Total T4 (TT4)**
 - The best screening test (high sensitivity)
 - An increased Total T4 is specific for hyperthyroidism
 - However, false negative results may occur with non-thyroidal illness (e.g., CKD). ⇒ **Repeat the test in two weeks**
- **Serum free T4 (fT4)**
 - More sensitive compared to total T4
 - However, More false positive results



Vcheck T4 & TSH

Feline Hyperthyroidism

✓ Diagnosis ③

Thyroid Function Tests



Clinical signs?
(Weight loss despite good
appetite, palpable thyroid nodule)

Vcheck T4
Measures serum
total T4

T4 clearly high
(> 4.7 ug/dL)

**Diagnosis of
Hyperthyroidism**

T4 high-normal
($3.4 - 4.7$ ug/dL)

- Identify and treat any non-thyroidal illness
- **Recheck total T4 in 2 weeks**

Free T4 or TSH testing

T4 normal
($0.8 - 4.7$ ug/dL)

- Recheck total T4
in 6 months

** Diagnostic approach to a cat
With suspected hyperthyroidism*

Feline Hyperthyroidism

✓ Diagnosis ③

Thyroid Function Tests

What if the results are border line?

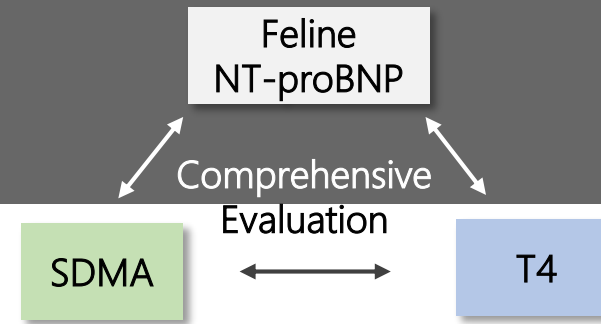
- **The simplest approach**
 - ① Wait 2 weeks
 - ② Retest the T4 concentrations again
- **With some chronic concomitant diseases,**
 - T4 concentration depressed into the upper normal range (by the principal of the sick euthyroid syndrome)
⇒ Elevated free T4 + other classic clinical findings

Euthyroid sick syndrome

- ✓ Geriatric cats with hyperthyroidism may also have concurrent chronic kidney disease (CKD).
- ✓ Concurrent chronic kidney disease (CKD)
 - Depresses thyroid hormone concentrations
 - ⇒ making it more difficult to diagnose hyperthyroidism

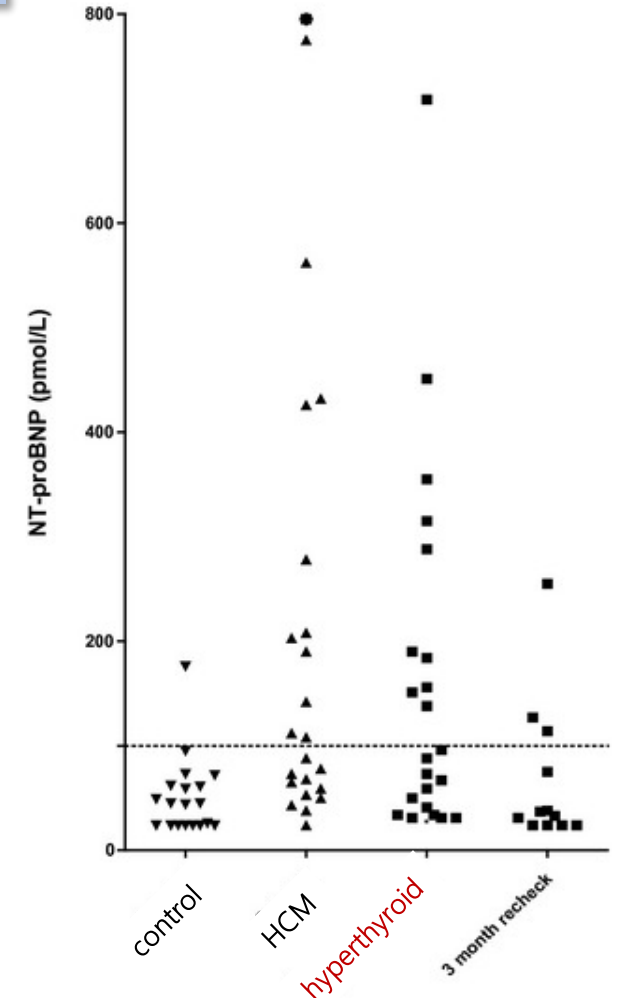


Feline Hyperthyroidism



Considerations

- **SDMA:** Hyperthyroidism can mask the diagnosis of concurrent chronic kidney disease (CKD)
 - ✓ Increases glomerular filtration rate (GFR) in cats with CKD
 - ⇒ Reductions in BUN Creatinine, **SDMA** levels ⇒ Makes it more difficult to detect concurrent CKD
 - ✓ CKD will become apparent after treatment of the hyperthyroidism. ⇒ Retesting for CKD should be performed in 2-3 months.
- **NT-proBNP:** No differences between hyperthyroid cats and cats with HCM
 - ✓ Cardiovascular abnormalities in hyperthyroid cats:
 - Diverse and comprise subtle, clinically inconsequential myocardial changes
 - (+) Severe changes that can be associated with development of heart failure.
 - ⇒ Myocardial abnormalities resolve after treatment in many cats, but persist in others.
 - ✓ Hyperthyroid cats with increased **NT-proBNP** with echocardiographic abnormalities should be re-evaluated ≥3 months after resolution of hyperthyroidism.



Feline Hyperthyroidism

Treatment

(Methimazole trial treatment)

- **Methimazole**
 - ✓ Blocks incorporation of iodine into thyroglobulin
 - ✓ 2.5 mg (BID) for the first 2 weeks, 2.5 mg (TID) for a further 2 weeks
- Most hyperthyroid cats are euthyroid within 2–3 weeks of commencing treatment with antithyroid drugs ⇒ **T4 should be monitored after that time period**
- **Monitor serum T4 concentrations every 3–6 months if on drug therapy**

* Renal disease/failure unveiled when thyroid levels controlled (2-3 months after medication started)

* Blood pressure and kidney values should be checked routinely



Feline Hyperthyroidism

Case Study

- Signalment

- Age: 18 year-old
- Sex: Sprayed female
- Breed: Korean shorthair cat

- Chief Complaint

- Decreased appetite
- Weight loss for 1 year
- Chronic vomiting
- Diarrhea



▲ 18 Y, Sprayed female, Korean shorthair cat

Feline Hyperthyroidism

Case Study

- Tests performed

- Physical examination: Palpated enlarged thyroid gland
- CBC: mildly increased HCT
- Serum chemistry: Increased ALT 272 (10-100)
- Auscultation: No murmur
- NT-ProBNP: Negative result

- Ultrasonography: No enlarged thyroid gland (3mm)
- Thyroid function testing
 - Total T4: 9.6 (0.8-4.7 ug/dL)
 - Free T4: >6.0 (0.7 – 2.6 ng/dL)



▲ 18 Y, Sprayed female, Korean shorthair cat

Total T4	Free T4	Fructosamine
0.8 – 4.7 ug/dL	0.7 – 2.6 ng/dL	191 – 349 umol/L
9.6	>6.0	219

▲ Thyroid function testing

✓ Final diagnosis: **Feline Hyperthyroidism**

Vcheck T4 & TSH

Feline Hyperthyroidism

Case Study

- **Treatment**
 - Drug therapy considering the cat's age
 - Methimazole: 5mg q 12hr
- **Monitoring** (4 weeks after treatment)
 - Improved clinical signs (vomiting, diarrhea)
 - Decreased Thyroid test results (Total T4, Free T4)



▲ 18 Y, Sprayed female, Korean shorthair cat

Total T4	Free T4	Fructosamine
0.8 – 4.7 ug/dL	0.7 – 2.6 ng/dL	191 – 349 umol/L
6	5.3	233

▲ Thyroid function testing (4 weeks after treatment)

- Plan to monitor T4 concentrations every 6 months during drug therapy

Product Introduction

- Vcheck T4
- Vcheck TSH

Vcheck T4

Specifications

- **Species:** Dog, Cat
- **Sample:** Serum 50 μ l
- **Testing Time:** 20 min.
- **Measuring Range:** 0.5~8 μ g/dL (6.44~102.96 nmol/L)

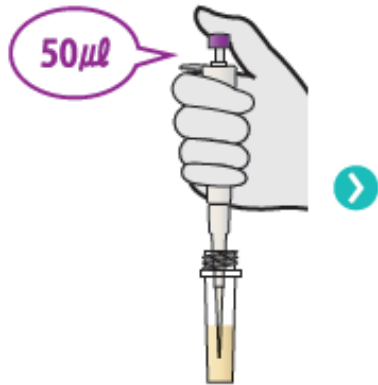


Product Introduction

Vcheck T4



Test procedure



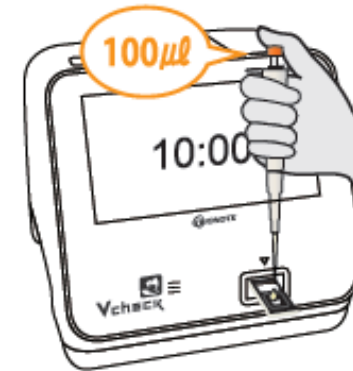
Draw 50 µl of serum and add it into an assay diluent tube



Mix over 8 times until the tablet is completely dissolved



Wait 10 minutes for incubation



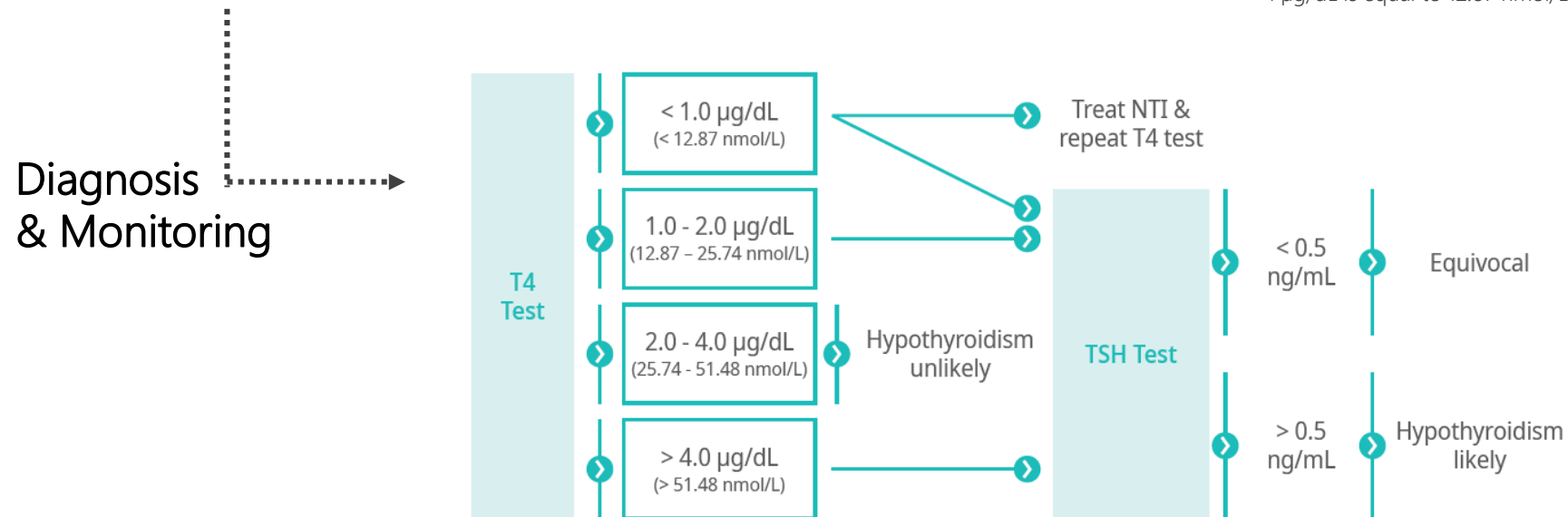
Add 100 µl of mixture

Result Interpretation (Dog)

Diagnosis of Canine hypothyroidism and treatment monitoring

< 1.0 µg/dL (< 12.87 nmol/L)	1.0~2.0 µg/dL (12.87 – 25.74 nmol/L)	1.0~4.0 µg/dL (12.87 - 51.48 nmol/L)	> 4 µg/dL (> 51.48 nmol/L)	2.1~5.4 µg/dL (27.03 – 69.50 nmol/L)
Low	Low normal	Normal	High	Therapeutic

* 1 µg/dL is equal to 12.87 nmol/L



Result Interpretation (Cat)

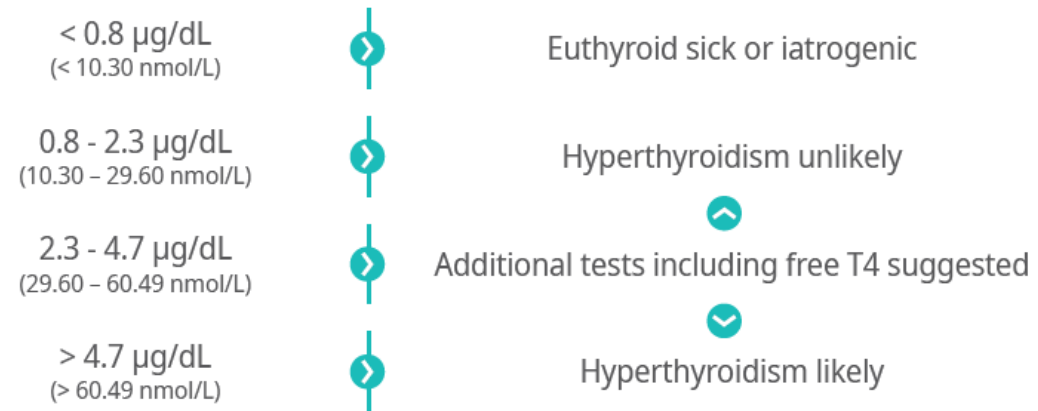
Diagnosis of Feline hyperthyroidism and treatment monitoring

< 0.8 µg/dL (< 10.30 nmol/L)	0.8~4.7 µg/dL (10.30 – 60.49 nmol/L)	2.3~4.7 µg/dL (29.60 – 60.49 nmol/L)	> 4.7 µg/dL (> 60.49 nmol/L)
Low	Normal	Gray zone	Consistent with hyperthyroidism

* 1 µg/dL is equal to 12.87 nmol/L

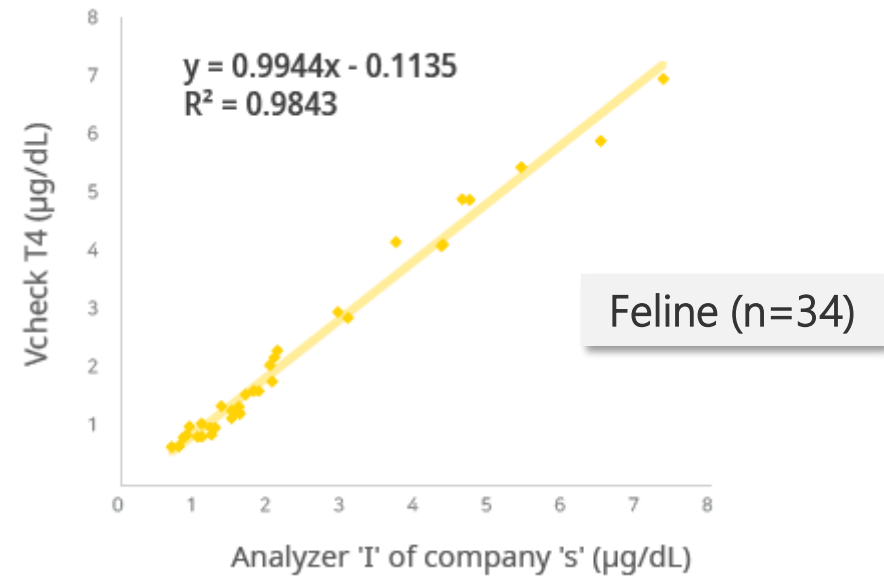
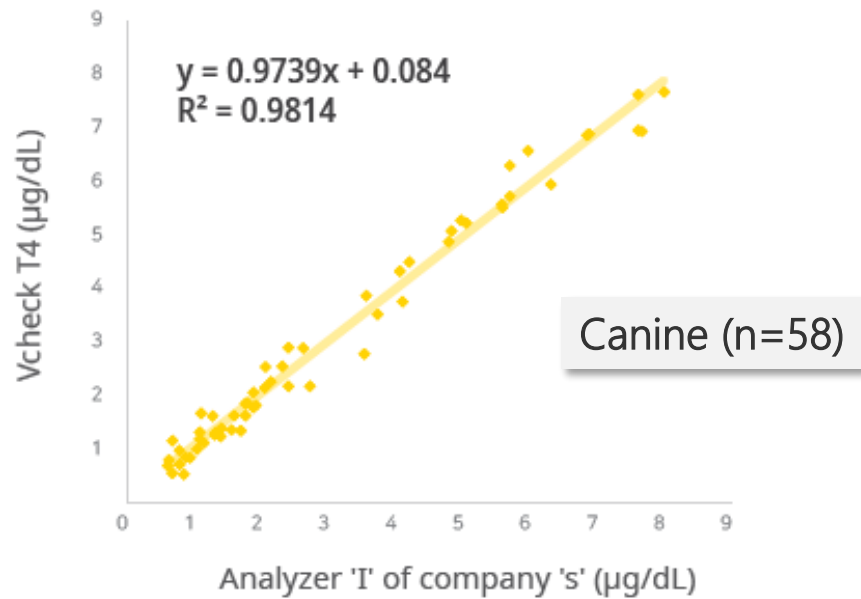


Diagnosis
& Monitoring



Evaluation Data

- Correlation with analyzer 'I' of company 'S'



Vcheck TSH

- **Species:** Dog
- **Sample:** Serum 100 μ l
- **Testing Time:** 15 min.
- **Measuring Range:** 0.25~5.00 ng/ml



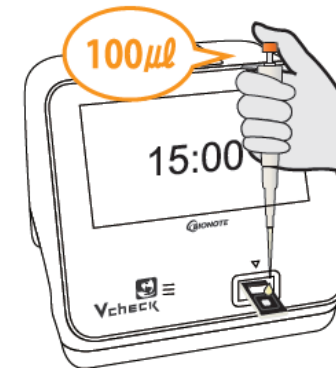
Test procedure



Draw 100 µl of serum and add it into an assay diluent tube



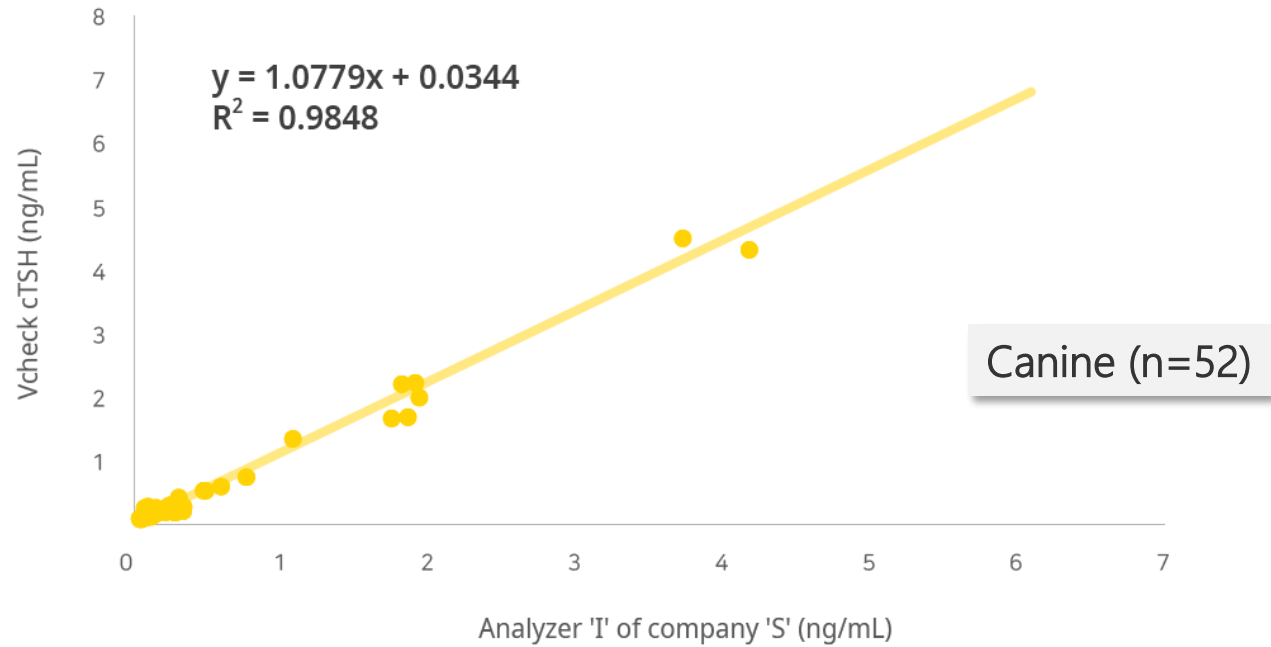
Mix well 5-6 times by using a 100 µl pipetting



Add 100 µl of mixture

Evaluation Data

- Correlation with analyzer 'I' of company 'S'



Thank you for your attention
Any Questions?

BIONOTE Marketing team

Mar. 2020

