VetScan® Thyroxine (T4) / Cholesterol Panel

CE

For Veterinary use only
Customer and Technical Service 1-800-822-2947

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1. Intended Use

The VetScan T4/Cholesterol reagent rotor used with the VetScan Whole Blood Analyzer utilizes dry and liquid reagents to provide *in vitro* quantitative determinations of thyroxine (T_4) and cholesterol in heparinized whole blood, heparinized plasma, or serum.

2. Summary and Explanation of Tests

Thyroxine (T_4)
Thyroxine is a hormone synthesized in and secreted by the thyroid gland. The primary secretory form of the thyroid hormone is tetraiodothyronine (T_4), although some triiodothyronine (T_3) is also secreted into the blood. The ratio of T_4 to T_3 is 25:1 in canine plasma. Once in the blood, T_4 and T_3 are bound by transport proteins. The primary binding protein is thyroxine-binding globulin (TBG) in the dog and albumin in the cat. Upon delivery to the target cell, T_4 is deionated to T_3 at the cell surface. T_3 is the biologically active form of the thyroid hormone and more readily enters the target cell.

Thyroid hormone has many effects on the body, including clinical, physiological, calorigenic, metabolic (carbohydrate, protein and lipid), developmental, reproductive and hematologic. T_4 determinations aid in the diagnosis of hypothyroidism and hyperthyroidism and in monitoring sodium levothyroxine and methimazole therapies.

Clinical signs of abnormal T_4 levels are often vague. The most common observable signs of canine hypothyroidism are skin and coat changes, such as alpecia or a dry, dull coat. Other signs in dogs include lethargy, exercise intolerance, weakness, muscle atrophy, corneal lipid deposits and diarrhea. Clinical signs of feline hypothyroidism include lethargy and obesity (especially in iatrogenic hypothyroidism), alopecia, epilation of hair and bradycardia.

The most prevalent clinical signs of feline hyperthyroidism are weight loss and polyphagia. Other common signs are restlessness, tachycardia, polyuria-polydipsia, alopecia and diarrhea.

Cholesterol
Cholesterol is a major precursor of cholesterol ester, bile acids and steroid hormones and is a component of plasma membranes. The rate of cholesterol biosynthesis in the liver is indirectly proportional to dietary intake. Levels of cholesterol in the body are indirectly controlled by thyroid hormone, which stimulates bile acid production. Since bile acids are synthesized from cholesterol, cholesterol concentrations vary inversely with thyroid hormone activity.

Cholesterol levels may be used to aid in detection of hyperlipidemia or as a screening test for hypothyroidism and hyperadrenocorticism. Cholesterol results are most useful when analyzed in conjunction with other clinical chemistry tests.

As with any diagnostic test procedure, all other test procedures including the clinical status of the patient should be considered prior to final diagnosis.

3. Principles of Procedure

Thyroxine (T_4)
The first clinically feasible direct method to measure thyroxine was a competitive protein-binding assay (CPBA) developedby Murphy & Pattee in the early 1960s.[1] Radioimmunoassay techniques, with higher sensitivity and specificity, largely replaced CPBA.[2] Concerns about radioactive waste and potential health hazards helped prompt the development of non-isotopic tests such as enzyme and fluorescence immunoassays. Enzyme immunoassays (EIAs) for thyroxine have been shown to have, at clinically important levels, accuracy and precision equivalent to automated RIA procedures.[3] An isotope dilution-mass spectrometric procedure has been proposed as a reference method, but is very complicated and labor intensive.[4]

Abaxis has adapted a comercially available EIA method for use in the VetScan Whole Blood Analyzer. In the reaction, 8-anilino-1-napthalene sulfonic acid (ANS) causes the release of endogenous T_4 from the binding proteins. The released endogenous T_4 competes for antibody (Ab) binding sites with T_4 labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH-T_4 conjugate). G6PDH-T_4 conjugate bound to antibody has lower activity than does unbound conjugate. As the binding of endogenous T_4 increases, the amount of the unbound enzyme conjugate increases. The active enzyme reduces nicotinamide adenine dinucleotide (NAD^+) to NADH.

$$T_4 + T_4\text{-G6PDH} + NAD^+ \xrightarrow{Ab} Ab{:}\ T_4 + Ab{:}T_4\text{-G6PDH} + NADH + H^+$$

The rate of change of the absorbance at 340 nm is due to the conversion of NAD^+ to NADH and is directly proportional to the amount of endogenous T_4 in the sample.

Cholesterol

The most common tests employ enzymatic endpoint reactions. These simple procedures typically use cholesterol esterase and cholesterol oxidase with a Trinder finish.[5,6] Abaxis has developed an enzymatic method that uses cholesterol dehydrogenase in place of cholesterol oxidase. The use of cholesterol dehydrogenase eliminates the Trinder reaction, thus avoiding interference from physiological analytes such as bilirubin and hemoglobin.

Cholesterol esterase hydrolyzes cholesterol esters and H_2O to form cholesterol and fatty acids. The cholesterol is oxidized by cholesterol dehyrdogenase to cholestenone and the nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH.

$$\text{Cholesterol Esters} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + NAD^+ \xrightarrow{CHDH} \text{Cholest-4-en-3-one} + NADH + H^+$$

Absorbance is measured bichromatically at 340 nm and 405 nm. A dedicated sample blank is also measured to ensure no extraneous reactions interfere with the calculations of cholesterol levels. The production of NADH in this endpoint reaction is directly proportional to the amount of cholesterol present in the sample.

4. Principle of Operation
See the VetScan Chemistry Analyzer Operator's Manual, for the Principles and Limitations of the Procedure.

5. Description of Reagents

Reagents
Each VetScan T4/Cholesterol reagent rotor contains dry test specific reagent beads. A dry sample blank reagent (comprised of buffer, surfactants, excipients and preservatives) is included in each reagent rotor for use in calculating sample indices. Each reagent rotor also contains a diluent consisting of surfactants, ANS, T_4 antibodies and preservatives.

Warnings and Precautions
- For *In vitro* Diagnostic Use
- The diluent container in the reagent rotor is automatically opened when the analyzer drawer closes. A rotor with an opened diluent container can not be re-used. Ensure that the sample or control has been placed into the rotor before closing the drawer.

- Reagent beads may contain acids or caustic substances. The operator does not come into contact with the reagent beads when following the recommended procedures. In the event that the beads are handled (e.g., cleaning up after dropping and cracking a reagent rotor), avoid ingestion, skin contact, or inhalation of the reagent beads.
- Reagent beads and diluent contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Reagents will not come into contact with lead and copper plumbing when following recommended procedures. However, if the reagents do come into contact with such plumbing, flush with a large volume of water to prevent azide buildup.

Instructions for Reagent Handling
Reagent rotors may be used directly from the refrigerator without warming. Open the sealed foil pouch and remove the rotor being careful not to touch the bar code ring located on the top of the reagent rotor. Use according to the instructions provided in the VetScan System Operator's Manual. A rotor not used within 20 minutes of opening the pouch should be discarded. Rotors in opened pouches can not be placed back in the refrigerator for use at a later time.

Storage
Store reagent rotors in their sealed pouches at 2-8˚C (36-46˚F). Do not expose opened or unopened rotors to direct sunlight or temperatures above 32˚C (90˚F). Do not allow the rotors sealed in their foil pouches to remain at room temperature longer than 24 hours prior to use. Open the pouch and remove the rotor just prior to use.

Indications of Reagent Rotor Instability or Deterioration
- All reagents contained in the reagent rotor, when stored as described above, are stable until the expiration date printed on the rotor pouch. Do **not** use a rotor after the expiration date. The expiration date is also encoded in the bar code printed on the bar code ring. An error message will appear on the VetScan Whole Blood Analyzer display if the reagents have expired.
- A torn or otherwise damaged pouch may allow moisture to reach the unused rotor and adversely affect reagent performance. Do not use a rotor from a damaged pouch.

6. Instrument
See the VetScan System Operator's Manual for complete information on using the analyzer.

7. Sample Collection and Preparation
The minimum required sample size is ~100 µL of heparinized whole blood, heparinized plasma, serum or serum control. The reagent rotor sample chamber can contain up to 120 µL of sample.

- Specimen collected in a heparinized micropipette should be dispensed into the reagent rotor **immediately** following sample collection.
- Use only lithium heparin (green stopper) evacuated specimen collection tubes for whole blood or plasma samples. Use no-additive (red stopper) evacuated specimen collection tubes or serum separator tubes (red or red/black stopper) for serum samples.
- Whole blood samples obtained by venipuncture must be homogenous before transferring a sample to the reagent rotor. Gently invert the collection tubes several times just prior to sample transfer. Do **not** shake the collection tube. Shaking can cause hemolysis.
- The test must be started within 10 minutes of transferring the sample into the reagent rotor.
- Whole blood venipuncture samples should be run within 60 minutes of collection; if this is not possible, separate the sample and transfer it into a clean test tube.[7] Run the separated plasma or serum sample within 5 hours of centrifugation. If this is not possible, refrigerate the sample in a stoppered test tube at 2-8°C (36-46°F) for no longer than 48 hours. A plasma or serum sample can be stored at -10°C (14°F) for up to 5 weeks in a freezer that does not have a self-defrost cycle.

Known Interfering Substances
- The only anticoagulant recommended for use with the VetScan Whole Blood Analyzer is lithium heparin.
- Physical interferents (hemolysis, icterus, and lipemia) may cause changes in the reported concentrations of some analytes. The sample indices are printed on the bottom of each result card to inform the operator about the levels of interferents present in each sample. The VetScan Whole Blood Analyzer suppresses any results that are affected by >10% interference from hemolysis, lipemia, or icterus. "HEM", "LIP", "ICT" is printed on the result card in place of the result.

8. Procedure

Materials Provided
- One VetScan T4/Cholesterol Reagent Rotor

Materials Required but not Provided
- VetScan Whole Blood Chemistry Analyzer

Test Parameters
The VetScan System operates at ambient temperatures between 15°C and 32°C (59-90°F). The analysis time for each VetScan T4/Cholesterol Reagent Rotor is less than 14 minutes. The analyzer maintains the reagent rotor at a temperature of 37°C (98.6°F) over the measurement interval.

Test Procedure
The complete sample collection and step-by-step operating procedures are detailed in the VetScan System Operator's Manual.

Calibration
The VetScan Whole Blood Analyzer is calibrated by the manufacturer before shipment. The barcode printed on the barcode ring provides the analyzer with rotor-specific calibration data. Please see the VetScan System Operator's Manual**.**

Quality Control
Controls may be run periodically on the VetScan Whole Blood Analyzer to verify the accuracy of the analyzer. Abaxis recommends that a serum-based commercially available control be run. Run controls on the reagent rotor in the same manner as for patient samples. See the VetScan System Operator's Manual to run controls.

9. Results

The VetScan Whole Blood Analyzer automatically calculates and prints the analyte concentrations in the sample. Details of the endpoint and rate reaction calculations are found in the VetScan System Operator's Manual.

Interpretation of Results

Increased T_4
- T_4 concentrations tend to be higher in dogs less than one year old and decrease as the dog ages.
- An increased T_4 level in a feline is a reliable indicator of hyperthyroidism. Hyperthyroidism is the most common cause of elevated T_4 and is one of the most frequently diagnosed diseases in small animals. The typical cause of spontaneous hyperthyroidism in cats is functional thyroid adenoma. Hyperthyroidism is rarely observed in dogs but, when seen, is usually indicative of neoplasia or the administration of too much sodium levothyroxine to a hypothyroid dog. Approximately 66% of the canine neoplasmas are adenocarcinomas.
- Feline thyroid testing is usually conducted to diagnose hyperthyroidism, to monitor the effects of antithyroid treatment, or to monitor thyroid replacement treatment following destruction of neoplastic thyroid glands. When evaluating total T_4 in cats, considerations must be allowed for age and concurrent diseases. T_4 values are higher in younger cats and normally decrease with age. In older cats with suspected hyperthyroidism, concurrent diseases such as renal failure cause a condition known as euthyroid sick syndrome that may depress total T_4 values. In these cases a free T_4 by equilibrium dialysis test (fT4ED) is used to confirm a diagnosis of hyperthyroidism.
- Three common conditions require confirmation with the fT4ED test. High normal T_4 values (3-5 mg/dL) in a young cat without marked weight loss is normal. High values (>5 mg/dL) in an old cat showing signs of weight loss is usually diagnostic for hyperthyroidism. High normal values (3-5 mg/dL) in an old cat may indicate hyperthyroidism. Since these values can be suppressed by concurrent disease, a test for active hormone (fT4ED) is necessary to diagnose this occult hyperthyroidism.

Decreased T_4

- In dogs, the total T_4 can be used to rule out a diagnosis of hypothyroidism. If total T_4 is within the normal range it is highly unlikely that the dog is hypothyroid. A low or low-normal T_4 value may be suggestive of but does not confirm hypothyroidism because non-thyroidal factors such as drugs and illness affect T_4. A diagnosis of hypothyroidism in dogs can be confirmed with a free T_4 by equilibrium dialysis (fT4ED).
- Other causes of decreased T_4 levels may be associated with drug therapy and euthyroid sick syndrome. The glucocorticoids are the most clinically relevant of the drugs affecting T_4 levels. In euthyroid sick syndrome, decreased T_4 levels are seen with such nonthyroidal illnesses as acute and chronic renal failure, diabetes mellitus, hepatic insufficiency and obesity.
- After eliminating drug therapy and euthyroid sick syndrome, the most common cause of decreased T_4 levels is primary hypothyroidism. Hypothyroidism in dogs is most often a result of lymphocytic thyroiditis or idiopathic atrophy. Thyroid tumors that have destroyed >75% of the thyroid gland may cause clinical signs of hypothyroidism. Congenital defects of the pituitary gland, pituitary destruction and pituitary suppression can cause secondary hypothyroidism in dogs.
- Spontaneous hypothyroidism is rarely reported in cats. The common causes of feline hypothyroidism are bilateral thyroidectomy and overdoses of radioactive iodine or anti-thyroid drugs in hyper thyroid cats
- Hypothyroid patients may also have elevated cholesterol concentrations.
- To obtain an accurate basal T_4 concentration, medications should be withheld from the patient for several days.

Hypercholesterolemia

- A high fat diet or a blood sample collected shortly after the patient has eaten may cause hypercholesterolemia. Hypercholesterolemia is not apparent upon visual examination of the sample since it does not cause lipemia.
- A reduction in thyroid activity causes a decrease in the catabolism of cholesterol, resulting in elevated cholesterol levels. Observing a high cholesterol level on a screening profile may be the first indicator of hypothyroidism. Cholesterol, when used in conjunction with free T_4 levels, is a good indicator of canine hypothyroidism.
- A preliminary diagnosis of hyperlipidermia may be made using cholesterol levels and the lipemic index printed on the VetScan result card. Cholesterol concentrations > 300 mg/dL in conjunction with a 2+ or 3+ lipemic index can indicate hyperlipidemia in fasted dogs. Feline hyperlipidemia may be diagnosed when a cholesterol concentrations >200 mg/dL and an index of 1+ or greater is observed in fasted cats.
- Low levels of cholesterol are not usually a problem. Hypocholesterolemia has been observed with protein-losing enteropathy, some liver diseases, certain malignancies, and severe malnutrition. alanine aminotransferse (ALT), albumin, alkaline phosphatase (ALP), globulin, total bilirubin and total protein test results should be examined if liver disease is suspected. Low levels of protein, albumin and globulin can be observed in cases of protein-losing enteropathies and malnutrition.

10. Limitations of Procedure

General procedural limitations are discussed in the VetScan Systems Operator's Manual**.**

- **If a result for a particular test exceeds the assay range, the sample should be analyzed by another approved test method or sent to a referral laboratory**. **Do not dilute the sample and run it again on the VetScan Whole Blood Analyzer.**
- Samples with hematocrits in excess of 60% packed red cell volume may give inaccurate results. Samples with high hematocrits may be reported as hemolyzed. These samples may be spun down and the plasma then re-run in a new reagent rotor.
- The Abaxis T_4 method is susceptible to interference by T_4 autoantibody. In rare cases where T_4 autoantibody is present in a sample T_4 results will be low.

Warning: Extensive testing of the VetScan has shown that in very rare instances, sample dispensed into the reagent rotor may not flow smoothly into the sample chamber. Due to the uneven flow, an inadequate quantity of sample may be analyzed and results may fall outside your established reference ranges. The sample may be re-run using a new reagent rotor.

11. Expected Values

The most definitive normal ranges are those established for your patient population. Test results should be interpreted in conjunction with the patient's clinical signs

Animals should be fasted for 12 hours before sample is drawn so that cholesterol concentrations are not influenced by a recently consumed meal.

Table 1: Canine and Feline Reference Intervals

Analyte	Canine	Feline
Thyroxine (T_4)	1.1-4.0 ug/dL (14.2-52.0 nmol/L)	1.5-4.8 ug/dL (19.4-61.9 nmol/L)
Cholesterol	125-270 mg/dL (3.24-6.99 mmol/L)	90-205 mg/dL (2.33-5.31 mmol/L)

12. Performance Characteristics

Linearity
The chemistry for each analyte is linear over the dynamic range listed below when the VetScan System is operated according to the recommended procedure (see the VetScan System Operator's Manual). The Dynamic Range table referenced below represents the spectrum that the VetScan System can detect**.**

Table 2: VetScan Dynamic Ranges

Analyte	Dynamic Range Common Units	SI Units
Thyroxine (T_4)	0.5-8.0 ug/dL	6.5-103.2 nmol/L
Cholesterol	20-520 mg/dL	0.5-8.4 mmol/L

Precision
Precision studies were conducted using the NCCLS EP5-A[8] Guidelines with modifications based on NCCLS EP18-P[9] for unit-use devices. Results for within-run and total precision were determined by testing bi-level controls. Controls were run in duplicate twice each day over a one-week period.

Table 3: Precision

Analyte	Sample Size	Within-Run	Total
Thyroxine (T_4) (ug/dL)	n=40		
Control 1			
Mean		1.5	1.5
SD		0.15	0.19
%CV		9.6	12.5
Control 2			
Mean		6.0	6.0
SD		0.32	0.33
%CV		5.3	5.4
Cholesterol (mg/dL)	n=40		
Control 1			
Mean		155.5	155.5
SD		3.96	4.0
%CV		2.5	2.6
Control 2			
Mean		313.4	313.4
SD		9.7	9.7
%CV		3.10	3.10

Correlation

Field studies were conducted at a veterinary medicine teaching hospital. The VetScan Whole Blood Analyzer and a comparative method analyzed serum samples for the thyroxine assay. Representative correlation statistics are displayed in Table 4.

Table 4: Correlation of the VetScan System with Comparative Method(s)

Thyroxine (ug/dL)	**Canine**	**Feline**
Correlation Coefficient (r)	0.96	0.96
Slope	0.82	0.94
Intercept	0.16	0.10
Sample Range	0.5-7.1	1.2-8.3
n	40	42
Cholesterol (mg/dL)	**Canine**	**Feline**
Correlation Coefficient (r)	0.99	0.99
Slope	0.99	1.06
Intercept	6	-3
Sample Range	103-450	63-257
n	159	34

13. Bibliography

1. Murphy BE, et al. Determinations of thyroxine utilizing the property of protein-binding. J Clin Endocrinol Metab. 1964;24:187-196.
2. Chen I-W, et al. Thyroxine: In:LA Kaplan and AJ Pesce, eds., Clinical Chemistry: Theory, Analysis and Correlation, 2nd ed. St. Louis: The C.V. Mosby Company; 1989:956-959.
3. Kaplan LA, et al. Evaluation and comparison of radio-flourescence and enzyme-linked immunoassays for serum thyroxine. Clin Biochem. 1981;14:182-186.
4. Moller, et al. Isotope dilution-mass spectrometry of thyroxin proposed as a reference method. Clin Chem. 1983;29:2106-2110.
5. Norma, et al. Polarographic method for rapid micodetermination of cholesterol with cholesterol esterase and cholesterol oxidase. 1976; 22:336-340.
6. Allain, et al. Enzymatic determination of total serum cholesterol. 1974; 20:472-475.
7. National Committee for Clinical Laboratory Standards (NCCLS). Procedures for Handling and Processing of Blood Specimens; tentative standard. NCCLS document H18-T. Villanova, PA: NCCLS, 1984.
8. National Committee for Clinical Laboratory Standards (NCCLS). Evaluation of precision performance of clinical chemistry devices; approved guideline NCCLS Document EP5-A. Wayne, PA: NCCLS, 1999.
9. National Committee for Clinical Laboratory Standards(NCCLS). Quality management for unit-use testing; proposed guideline. NCCLS Document EP18-P. Wayne, PA: NCCLS, 1999.