COAT-A-COUNT®

Canine T4

DPC®

Coat-A-Count Canine T4

Intended Use

Coat-A-Count Canine T4 is a solid-phase ^{125}I radioimmunoassay designed for the quantitative measurement of total thyroxine (T4) in canine serum. It is intended strictly for in *vitro* veterinary use as an aid in the clinical assessment of thyroid status.

Catalog numbers: **TKC41** (100 tubes), **TKC45** (500 tubes)

The 100-tube kit contains less than 5 microcuries (185 kilobecquerels) of radioactive ^{125}I T4, and the 500-tube kit contains less than 25 microcuries (925 kilobecquerels).

Summary and Explanation

Thyroid hormone assays have also proved of value in veterinary medicine.[1,5,14] However, most commercially available T4 RIAs have been designed for measurements in human serum. The reference range for dogs is much lower (approximately 0.73–2.9 µg/dL), with hyperthyroidism characterized by increased levels of circulating T4, hypothyroidism by decreased levels.[17] The differential diagnosis of hypothyroidism is of primary concern, since hyperthyroidism is a rare condition in dogs. The kit is supplied with calibrators prepared in T4-free *canine* serum, to avoid the systematic inaccuracies which can occur due to matrix differences.[3,8,16]

Principle of the Procedure

The Coat-A-Count Canine T4 procedure is a solid-phase radioimmunoassay, wherein ^{125}I-labeled T4 competes for a fixed time with T4 in the sample for antibody sites, in the presence of blocking agents for thyroid hormone-binding proteins. After the tubes are decanted and counted, the T4 concentration is read from a calibration curve.

Reagents to Pipet: 1

Total Incubation Time: 2 hours

Total Counts at Iodination: approximately 70,000 cpm

Warnings and Precautions

For *in vitro* veterinary use.

Reagents: Store at 2–8°C in a refrigerator designated for incoming radioactive materials. Dispose of in accordance with applicable laws.

Do not use reagents beyond their expiration dates.

Some components supplied in this kit may contain human source material and/or other potentially hazardous ingredients which necessitate certain precautions:

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Water: Use distilled or deionized water.

Radioactivity

A copy of any radioisotope license certificate (Specific or General) issued to a US customer must be on file with Diagnostic Products Corporation before kits or components containing radioactive material can be shipped. These radioactive materials may be acquired by any customer with the appropriate Specific license. Under a General license these radioactive materials may be acquired only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories and hospitals — and strictly for *in vitro* clinical or laboratory tests not involving external or internal administration of the radioactive material or its radiation to human beings or other animals. Its acquisition, receipt, storage, use, transfer and disposal are all subject to the regulations and a (General or Specific) license of the U.S. Nuclear Regulatory Commission or a State with which the NRC has entered into an

agreement for the exercise of regulatory control.

Handle radioactive materials according to the requirements of your General or Specific license. To minimize exposure to radiation, the user should adhere to guidelines set forth in the National Bureau of Standards publication on the *Safe Handling of Radioactive Materials* (Handbook No. 92, issued March 9, 1964) and in subsequent publications issued by State and Federal authorities.

Wipe up spills promptly and decontaminate affected surfaces. Avoid generation of aerosols. Dispose of solid radioactive waste according to license requirements. General licensees (holders of NRC Form 483) may dispose of solid radioactive waste as nonradioactive waste, after removing labeling. Specific licensees (NRC Form 313) should refer to Title 10, Code of Federal Regulations, Part 20. Licensees in Agreement States should refer to the appropriate regulations of their own state. General licensees may dispose of liquid radioactive waste of the type contained in this product through a laboratory sink drain. Licensees must remove or deface labels from empty containers of radioactive materials before disposal of solid waste. Specific licensees may dispose of small quantities of liquid radioactive waste of the type used in this product through a laboratory sink drain. Refer to the appropriate regulations applicable to your laboratory.

Materials Supplied: Initial Preparation

Total T4 Ab-Coated Tubes (TT41)
Polypropylene tubes coated with antibodies to T4 and packaged in zip-lock bags. Store refrigerated and protected from moisture, carefully resealing the bags after opening: stable at 2–8°C until the expiration date marked on the bag. Color: light green.
TKC41: 100 tubes. **TKC45:** 500 tubes.

^{125}I Total T4 (TT42)
Iodinated T4, ready to use, with blocking agents to thyroid hormone-binding proteins. Each vial contains 105 mL. Stable at 2–8°C for 30 days after opening, or until the expiration date marked on the vial. *Color:* red.
TKC41: 1 vial. **TKC45:** 5 vials.

Canine T4 Calibrators (CT43–8)
Six vials, labeled A through F, of lyophilized thyroxine calibrators, prepared in charcoal-absorbed canine serum. *At least 30 minutes before use*, reconstitute calibrator **A with 2.0 mL** distilled or deionized water and each of the remaining calibrators **B through F with 1.0 mL** of distilled or deionized water. Use volumetric pipets and mix by *gentle* swirling and inversion. Stable at 2–8°C for 30 days after reconstitution.
TKC41: 1 set. **TKC45:** 2 sets.

The reconstituted calibrators contain, respectively, 0, 0.5, 1.5, 3, 6 and 15 micrograms of thyroxine per deciliter (µg/dL) in processed canine serum; equivalently, 0, 6.4, 19.3, 38.6, 77.2 and 193 nanomoles per liter (nmol/L).

Materials Required But Not Provided

Gamma counter

Vortex mixer

Reagent Preparation
Distilled or deionized water

Volumetric pipets: 1.0 mL and 2.0 mL

Radioimmunoassay
Plain 12×75 mm polypropylene tubes – for use as Total Counts and NSB tubes, available from DPC

Micropipets – 25 µL and 1.0 mL.

Foam decanting racks – available from DPC

Logit-log graph paper – available from DPC (catalog number: ZP797)

A bi-level, canine serum-based control, containing canine T4 as one of multiple assayed constituents, is available from DPC (catalog number: K9CON).

Specimen Collection

The animal need not be fasting, and no special preparations are necessary. Collect blood by venipuncture into plain tubes (without anticoagulant), and separate the serum from the cells.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants. Coat-A-Count Canine T4 has not been tested with all possible variations of tube types.

Volume Required: 25 µL of serum per tube.

Storage: 7 days at 2–8°C or up to 2 months frozen at –20°C.

Before assay, allow the samples to come to room temperature (15–28°C) and mix by *gentle* swirling or inversion. Aliquot, if necessary, to avoid repeated thawing and freezing. Do *not* attempt to thaw frozen specimens by heating them in a waterbath.

Radioimmunoassay Procedure

All components must be at room temperature (15–28°C) before use.

1 **Plain Tubes:** Label four plain (uncoated) 12×75 mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

Because nonspecific binding in the Coat-A-Count procedure is characteristically low, the NSB tubes may be safely omitted without compromising accuracy or quality control.

Coated Tubes: Label twelve T4 Ab-Coated Tubes A (maximum binding) and B through F in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and samples.

Calibrators	µg/dL	nmol/L
A (MB)	0	0
B	0.5	6.4
C	1.5	19.3
D	3	38.6
E	6	77.2
F	15	193

2 Pipet **25 µL** of the zero calibrator A into the NSB and A tubes. Pipet **25 µL** of each remaining calibrator, control and sample into the tubes prepared. **Pipet directly to the bottom.**

Canine samples expected to contain T4 concentrations greater than the highest calibrator (15 µg/dL) should be diluted in the zero calibrator before assay.

3 Add **1.0 mL** of ^{125}I Total T4 to all tubes. Vortex.

Laboratories equipped with a reliable pipettor-diluter may handle steps 2 and 3 simultaneously. No more than 10 minutes should elapse during the dispensing of the tracer. Set the T tubes aside for counting at step 6; they require no further processing.

4 Incubate for **2 hours** at room temperature (15–28°C).

5 Decant thoroughly.

Removing all visible moisture will greatly enhance precision. Decant the contents of all tubes (except the T tubes) using a foam decanting rack, and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbant paper to shake off all residual droplets.

6 Count for **1 minute** in a gamma counter.

Calculation and Quality Control

To calculate T4 concentrations from a logit-log representation of the calibration curve, first calculate for each pair of tubes the average NSB-corrected counts per minute:

Net Counts = (Average CPM) minus (Average **NSB** CPM)

Then determine the binding of each pair of tubes as a percent of maximum binding (MB), with the NSB-corrected counts of the A tubes taken as 100%:

Percent Bound = (Net Counts / Net **MB** Counts) × 100

Using logit-log graph paper, plot Percent Bound on the vertical (probability) axis against Concentration on the horizontal (logarithmic) axis for each of the nonzero calibrators, and draw a straight line approximating the path of these points. Results for the unknowns may then be read from the line by interpolation.

Although other approaches are acceptable, data reduction by the logit-log method just described has certain advantages in this context – for example, in allowing easier recognition of deviant calibration points – since the Coat-A-Count Canine T4 procedure has been optimized for linearity in that representation.

Components: It is good laboratory practice to record for each assay the lot numbers and reconstitution dates of the components used. All components must be at room temperature before use.

Sample Handling: The instructions for storing samples and components, outlined in the sections on Specimen Collection and Materials Supplied, should be carefully observed. All samples, including the calibrators and controls, should be run in duplicate. Inspect the results for agreement within tube pairs.

Controls: Controls or serum pools with at least two T4 concentration levels (low and high) should be charted from day to day as described, for example, in Westgard JO, et al. A multi-rule chart for quality control. Clin Chem 1981;27:493-501. Repeat samples are a valuable additional tool for monitoring interassay precision.

Data Reduction: It is good practice to construct a graph of the calibration curve as a visual check on the appropriateness of the transformation used, even where the calculation of results is handled by computer. See further Davis SE, et al. Radioimmunoassay data processing with a small programmable calculator. J Immunoassay 1980;1:15-25; and Dudley RA, et al. Guidelines for immunoassay data reduction. Clin Chem 1985;31:1264-71.

QC Parameters: We recommend keeping track of these performance measures:

T = Total Counts (as counts per minute)

%NSB = 100 × (Average NSB Counts / Total Counts)

%MB = 100 × (Net Counts / Total Counts)

And the 20, 50 and 80 percent "intercepts," where

20% = Concentration at 20 Percent Bound, etc.

Example Run: For illustration only, not for calculating results from another run. (See “Example Run” table.)

Expected Values

A reference range study performed with the DPC Coat-A-Count Canine T4 kit on a total of 82 dogs (29 male and 53 female) yielded a median of 1.8 µg/dL (23 nmol/L) and a range of

0.73 to 2.9 µg/dL (9.4 to 37 nmol/L).

In addition, circulating T4 levels were evaluated in 12 young adult TSH-treated dogs, both before and after TSH stimulation. The results are summarized below in terms of T4 concentrations (median, absolute range), both pre- and post-TSH stimulation. For each of the 12 dogs, the post-stimulation/pre-stimulation *ratio* was computed: the results are also summarized below in terms of the median and absolute range for this statistic.

	Median (Absolute Range)		
	µg/dL	nmol/L	Ratio
Pre-TSH stimulation	2.5 (1.4–3.9)	33 (18–50)	
Post-TSH stimulation	7.3 (3.7–9.2)	94 (48–119)	
Post-TSH/ Pre-TSH			2.5 (1.8–4.1)

Performance Data

See Tables and Graphs for data *representative* of the assay's performance Canine T4 results in the sections below are expressed as µg/dL.

Conversion Factor:
µg/dL × 12.87 →nmol/L

Calibration Range: 0.5 to 15 µg/dL (6.4 to 193 nmol/L)

Analytical Sensitivity: 0.22 µg/dL (2.8 nmol/L).

Intraassay Precision (Within-Run): Statistics were calculated for each of three samples from the results of 10 tubes in a single assay. (See “Intraassay Precision” table.)

Linearity: Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Samples spiked 1 to 19 with three canine T4 solutions (20, 40, and 80 µg/dL) were assayed. (See "Recovery" table.)

Specificity: The Coat-A-Count Canine T4 antiserum is highly specific for L-thyroxine, with a particularly low crossreactivity to other naturally occurring compounds that may be present in the samples. Although some compounds do crossreact, they are not normally present in concentrations that would significantly interfere with the Coat-A-Count Canine T4 assay. The crossreactivities tabulated were calculated at approximately 50% B/B_0. (See “Specificity” table.)

Method Comparison: The Coat-A-Count Canine T4 procedure was compared to DPC's IMMULITE Canine Total T4 assay on 46 canine samples, with T4 concentrations ranging from 0.8 to 11.5 µg/dL. By linear regression:

(CAC) = 1.01 (IML) – 0.11 µg/dL
r = 0.958

Means:
2.4 µg/dL (Coat-A-Count)
2.5 µg/dL (IMMULITE)

References

1) Belshaw BE, Rijnberk A. Radioimmunoassay of plasma T4 and T3 in the diagnosis of primary hypothyroidism in dogs. J Am Animal Hosp Assoc 1979;15:17-23. 2) Chastain CB. Canine pseudohypothyroidism and covert hypothyroidism. Probl Vet Med 1993;2:693-716. 3) Ekins RP. Remarks on radioimmunoassay specificity. In: Margoulies M, editor. Proteins and polypeptide hormones. Amsterdam: Excerpta Medica, 1969: 575-81. 4) Gaschen F, Thompson J, Beale K, Keisling K. Recognition of triiodothyronine-containing epitopes in canine thyroglobulin by circulating thyroglobulin autoantibodies. Am J Vet Res 1993;54(2):244-7. 5) Kaneko JJ. Thyroid Function. In: Kaneko JJ, editor. Clinical biochemistry of domestic animals. 3rd ed. New York: Academic Press, 1980: 491-512. 6) Kaptein EM, Moore GE, Ferguson DC, Hoenig M. Thyroxine and triiodothyronine distribution and metabolism in thyroxine-replaced athyreotic dogs and normal humans. Am J Physiol 1993;264:E90-E100. 7) Kubasik NP, et al. Neonatal hypothyroidism: assessment of a commercial kit for thyroxine radioimmunoassay. Clin Chem 1977;23:2174-5. 8) Kubasik NP, et al. Clinical evaluation of two thyrotropin radioimmunoassay kits: human serum matrix calibrators and bovine serum matrix calibrators. Clin Chem 1981;27:504-5. 9) Lee DE, Lamb SV, Reimers TJ. Effects of hyperlipemia on radioimmunoassays for progesterone, testosterone, thyroxine and cortisol in serum and plasma samples from dogs. Am J Vet Res 1991;52(9):1489-91. 10) Peterson M, Swerdloff RS. Separation of bound from free hormone in radioimmunoassay of lutropin and follitropin. Clin Chem 1979;25:1239-41. 11) Refetoff S. Thyroid function tests. In: DeGroot LJ, editor. Endocrinology. New York: Grune & Stratton, 1979;1:387-428. 12) Reimers TJ, Lamb SV, Bartlett SA, Matamoros RA, Cowan RG, Engle JS. Effects of hemolysis and storage on quantification of hormones in blood samples from dogs, cattle and horses. Am J Vet Res 1991;52(7):1075-80. 13) Reimers TJ, Lawler DF, Sutaria PM, Correa MT, Erb HN. Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. Am J Vet Res 1991;51(3):1489-91. 14) Siegel ET. Endocrine diseases of the dog. Philadelphia: Lea & Febiger, 1977: 54-80. 15) Thacker EL, Refsal KR, Bull RW. Prevalence of autoantibodies to thyroglobulin, thyroxine, or triiodothyronine and relationship of autoantibodies and serum concentrations of iodothyronines in dogs. Am J Vet Res 1992;53(4):449-53. 16) Wood WG, et al. A second external quality control survey from serum triiodothyronine (T3) and thyronine (T4) assays. J Clin Chem Clin Biochem 1980;18:511-9. 17) Clinical endocrinology: update on thyroid testing. Vet Ref Lab Newsletter 1983 March/Apr;7:2.

Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782, 310.645.8200
Fax: 310.645.9999. To place an order:
Tel: 800.372.1782. Fax: 800.234.4372.
Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 13485:2003.

Tables and Graphs

Example Run

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	T4 µg/dL
T	65,099 65,015	65,557			
NSB	192 154	173			
A (MB)	30,551 30,779	30,665	30,492	**100%**	**0**
B	27,503 28,144	27,824	27,651	**91%**	**0.5**
C	23,654 22,735	23,195	23,022	**76%**	**1.5**
D	18,803 18,580	18,692	18,519	**61%**	**3**
E	13,646 13,996	13,821	13,648	**45%**	**6**
F	7,456 7,992	7,724	7,551	**25%**	**15**
Unknowns					
X1	26,400 26,486	26,443	26,270	86%	0.74
X2	21,173 21,404	21,289	21,116	69%	2.1
X3	15,783 16,129	15,956	15,783	52%	4.5

Quality Control Parameters:

T = 65,557 cpm
%NSB = 0.26%
%MB = 47%
20% Intercept = 20 µg/dL
50% Intercept = 4.8 µg/dL
80% Intercept = 1.2 µg/dL

Intraassay Precision (µg/dL)

	Mean	SD	CV
1	0.43	0.04	9.3%
2	1.4	0.06	4.3%
3	3.2	0.10	3.1%

Recovery (µg/dL)

Sample	Spiking Solution	Observed	Expected	%O/E
1	—	1.4	—	—
	A	2.2	2.3	96%
	B	3.3	3.3	100%
	C	4.7	5.3	89%
2	—	1.6	—	—
	A	2.2	2.5	88%
	B	3.2	3.5	91%
	C	5.0	5.5	91%
3	—	1.7	—	—
	A	2.4	2.6	92%
	B	3.1	3.6	86%
	C	5.4	5.6	96%

Linearity (µg/dL)

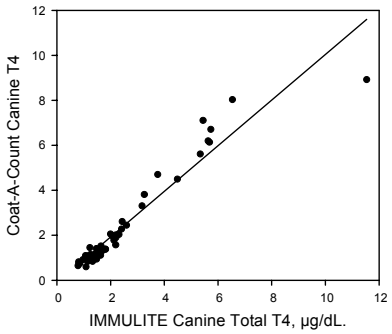
Sample	Dilution	Observed	Expected	%O/E
1	8 in 8	3.4	—	—
	4 in 8	1.7	1.7	100%
	2 in 8	0.86	0.85	101%
2	8 in 8	5.9	—	—
	4 in 8	3.1	3.0	103%
	2 in 8	1.5	1.5	100%

Specificity

Compound	Added (µg/dL)	Percent Cross-reactivity
L-Thyroxine	25	100%
	10	100%
D-Thyroxine	10	64%
Tetraiodothyroacetic acid	10	104%
Triiodo-L-thyronine	100	2%
	25	ND
Triiodo-D-thyronine	1	ND
Triiodothyroacetic acid	1,000	2%
	10	ND
Monoiodotyrosine	1,000	ND
	10	ND
Diiodo-L-tyrosine	1,000	ND
Methimazole	1,000	ND
5,5′-Diphenylhydantoin	1,000	ND
Phenylbutazone	1,000	ND
6-*n*-Propyl-2-thiouracil	1,000	ND

ND: nondetectable

Method Comparison



(CAC) = 1.01 (IML) – 0.11 µg/dL
r = 0.958

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