Canine TLI

DOUBLE ANTIBODY

DPC®

Double Antibody Canine TLI

Intended Use

Double Antibody Canine Trypsin-Like Immunoreactivity (TLI) is a ^{125}I radioimmunoassay designed for the quantitative measurement of trypsin-like immunoreactivity (TLI) in canine serum. It is intended strictly for *in vitro* veterinary use as an aid in the clinical assessment of exocrine pancreatic function in dogs.

Catalog number: **KTLD1** (100 tubes).

The 100-tube kit contains less than 3.5 microcuries (130 kilobecquerels) of radioactive ^{125}I TLI.

Summary and Explanation of the Test

Quantitation of serum trypsin-like immunoreactivity (TLI) in dogs can aid in the clinical assessment of exocrine pancreatic function and specifically in the diagnosis of exocrine pancreatic insufficiency (EPI).[1-3] The DPC TLI antisera recognize both trypsin and its zymogen, trypsinogen, hence the term "trypsin-like immunoreactivity".[1,2,4]

Pancreatic acinar cells produce digestive enzymes, which are secreted into the duodenum as inactive zymogens.[3] EPI is caused by the progressive loss of acinar cells leading to the inadequate production of digestive enzymes and consequent malabsorption.[1,3] Clinical signs of EPI – including weight loss and increased appetite, often accompanied by diarrhea – do not generally appear until as much as 90% of the acinar function is lost.[3,5] Furthermore, clinical signs of EPI do not distinguish EPI from other causes of malabsorption in dogs, such as small intestinal disease.[1]

Pancreatic zymogens, including tryspinogen, are also normal constituents of the blood, occurring there in trace amounts.[3] Measurement of TLI in serum samples provides a useful method for assessing pancreatic acinar function in dogs.[1-4] Further, because trypsinogen is produced and stored only by the acinar cells of the pancreas, serum TLI is an organ-specific marker.[2,4]

Normal dogs have been reported to have circulating TLI levels ranging from 5.2 to 35 ng/mL, with a mean of 14 ng/mL, as measured by radioimmunoassay. Serum TLI levels below 2.5 ng/mL are indicative of EPI in dogs.[1,2,4,5] Dogs with intestinal disorders, such as small intestinal disease, have essentially the same circulating TLI levels as normal dogs.[1,2] High concentrations of serum TLI in dogs have also been reported to correlate with acute pancreatitis.[7] Because TLI is an organ-specific marker, serum TLI may prove to be a more reliable indicator of acute pancreatitis than either amylase or lipase.

Although radioimmunoassay for TLI provides a highly sensitive and specific test for EPI in dogs,[1-3,5] TLI is species-specific; hence an assay suitable for canine samples is not suitable for other species. For example, a canine TLI radioimmunoassay is not suitable for assaying TLI levels in feline samples.

Principle of the Procedure

The Double Antibody procedure is a liquid-phase radioimmunoassay, wherein ^{125}I-labeled TLCK-inactivated trypsin competes with both trypsin and trypsinogen in the canine serum sample for antibody sites. After incubation for a fixed time, separation of bound from free is achieved by the PEG-accelerated double antibody method. The antibody bound fraction is precipitated and counted. Patient sample concentrations are read from a calibration curve.

Reagents to Pipet: 3.

Total Incubation Time: 2 hours.

Total Counts at Iodination: Approximately 50,000 cpm.

Separation: The ready-to-use Precipitating Solution combines second antibody and dilute PEG.

Warnings and Precautions

For *in vitro* veterinary use only.

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

Canine TLI Antiserum (TLD1)
Lyophilized TLI antiserum, with preservative. Reconstitute by adding **10 mL** distilled water. Mix by *gentle* inversion. Stable at 2–8°C for 30 days after reconstitution. *Color:* blue (after reconstitution).
KTLD1: 1 vial.

^{125}I Canine TLI (TLD2)
Lyophilized tracer, consisting of iodinated TLCK-inactivated trypsin, with preservative. Reconstitute by adding **10 mL** distilled water. Mix by *gentle* inversion. Stable at 2–8°C for 30 days after reconstitution, or until the expiration date marked on the vial.
KTLD1: 1 vial.

Canine TLI Calibrators (TLD3-9)
Seven vials, labeled A through G, of lyophilized TLI calibrators, with preservative. *At least 30 minutes before use,* reconstitute the zero calibrator **A with 5.0 mL** distilled water, and each of the remaining calibrators **B through G with**

2.0 mL distilled water. Use volumetric pipets and mix by *gentle* inversion. Stable at –20°C for 30 days after reconstitution. Aliquot, if necessary, to avoid repeated freezing and thawing.
KTLD1: 1 set.

The calibrators have been prepared in a buffered protein matrix. They represent 0, 1, 2, 5, 10, 20 and 50 nanograms of TLCK-inactivated trypsin per milliliter (ng/mL). Intermediate calibration points can be obtained by mixing calibrators in suitable proportions.

Canine TLI Controls (TLCO1-3)
Three vials, labeled Canine TLI Control 1, 2 and 3, of lyophilized controls containing TLI in a buffered protein matrix, with preservative. *At least 30 minutes before use,* reconstitute each vial with **2.0 mL** distilled water. Use volumetric pipets and mix by *gentle* inversion. Stable at –20°C for 30 days after reconstitution. Aliquot, if necessary, to avoid repeated freezing and thawing. *Note:* refer to the control package insert for *lot-specific* control values.
KTLD1: 1 set.

Precipitating Solution (N6)
110 mL of Precipitating Solution, consisting of goat anti-rabbit gamma globulin (second antibody) and dilute PEG in saline, with preservative. The Precipitating Solution is supplied in liquid form, ready to use. Stable at 2–8°C for 30 days after opening. This reagent should be thoroughly mixed before use, as a fine, visible precipitate may form during storage. *Color:* blue.
KTLD1: 1 vial.

Materials Required But Not Provided

Gamma counter — compatible with standard 12×75 mm tubes

Centrifuge — preferably refrigerated and capable of at least 3000×g

Vortex mixer

Reagent Preparation:
Distilled or deionized water

Volumetric pipets: 2.0, 5.0 and 10.0 mL

Radioimmunoassay:
Plain 12×75mm polypropylene tubes – available from DPC

Micropipets: 100 µL

Repeating dispenser: 1.0 mL

Foam decanting rack – available from DPC

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

Specimen Collection

The animal should have fasted for at least 6 hours, and preferably overnight, before specimen collection. No other special preparations are necessary. Collect blood by venipuncture into plain tubes, and separate the serum from the cells.

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 water bath.

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. Double Antibody Canine TLI has not been tested with all possible variations of tube types.

Volume Required: 100 µL of serum per tube.

Storage: 2–8°C for up to 7 days, or 6 months frozen at –20°C.

Radioimmunoassay Procedure

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

1 Label eighteen tubes in duplicate: T (total counts), NSB (nonspecific binding), A (maximum binding) and B through G. Label additional tubes, also in duplicate, for canine serum samples and controls.

Calibrators	ng/mL
A (MB)	0
B	1.0
C	2.0
D	5.0
E	10
F	20
G	50

2 Pipet **100 µL** of the zero calibrator A into the NSB and A tubes, and **100 µL** of each of the remaining calibrators B through G into correspondingly labeled tubes. Pipet **100 µL** of each canine sample (undiluted) and each control into the tubes prepared.

3 Add **100 µL** of ^{125}I Canine TLI to all tubes. Shake the rack gently for a brief period of time.

A repeating dispenser is recommended for this step and for the addition of antiserum in the following step. Remove the T tubes for counting at step 9; they require no further processing.

4 Add **100 µL** of Canine TLI Antiserum (Blue) to all tubes except the NSB (and T) tubes. Vortex.

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

6 Add **1.0 mL** of *cold,* well-mixed Precipitating Solution (Blue) to all tubes. Vortex.

For the 1.0 mL reagent addition a repeating dispenser may be employed.

7 Centrifuge for **15 minutes** at 3000×g – or for a suitably longer period of time at a lower acceleration, e.g. 30 minutes at 1500×g.

8 Decant (or aspirate) the supernatant, retaining the precipitate for counting.

Using a foam decanting rack, tap the tubes on absorbent paper, and blot the rims, to remove residual droplets.

9 Count each tube for **1 minute** in a gamma counter.

Calculation and Quality Control

To calculate TLI 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 Canine TLI procedure has been optimized for linearity in that representation.

Record Keeping: It is good laboratory practice to record for each assay the lot numbers and reconstitution dates of the components used, as well as control results and QC parameters.

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 assayed in duplicate. Inspect the results for agreement within tube pairs.

Controls: Controls or serum pools with at least two TLI concentration levels (low and high) in a canine serum matrix should routinely be assayed as unknowns, and the results charted from assay to assay 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. Radio-immunoassay 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.

Centrifugation: The procedure calls for centrifuging at 3000×g for 15 minutes. Lower accelerations have also proven satisfactory when the centrifugation time is increased appropriately – for example, 30 minutes at 1500×g. A high-speed, refrigerated centrifuge is desirable but not essential. Use the formulas below to calculate the acceleration of your centrifuge at a given speed, or the speed (in revolutions per minute) required to achieve a desired g force.

g force = $28.38 \times (\text{rpm} / 1{,}000)^2 \times$ radius [inches]

rpm = $187.7 \times \sqrt{(\text{g force} / \text{radius [inches]})}$

Q.C. 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

Results obtained with DPC's Canine TLI kit are tabulated below. They are consistent with the literature, which indicates that canine TLI values exceed 5.2 ng/mL in healthy dogs and that values less than 1.9 ng/mL are indicative of exocrine pancreatic insufficiency.[5]

TLI (ng/mL)

Canine Samples	Median	Absolute Range	*n*
Normal	11	5.4 – 32	30
Exocrine Pancreatic Insufficiency	ND	ND – 1.3	11

ND: nondetectable

Assay of trypsin-like immunoreactivity (TLI), as the name suggests, is a measure of a heterogeneous mixture of related analytes with varying immunoreactivities, including reactivity to trypsin, trysinogen and trypsin-antitrypsin complexes.[8] Serial dilutions of certain specimens may therefore deviate from strict dilutional parallelism. Reference ranges have been established by assaying undiluted specimens. Accordingly, we recommend that assays be performed on *undiluted* samples only, for the evaluation of pancreatic insufficiency.

Laboratories should consider these results *as guidelines only*. Each laboratory should establish its own reference ranges.

Performance Data

See Tables and Graphs for data *representative* of the Double Antibody Canine TLI kit's performance. Except as noted, all results in the sections below were obtained on serum samples. Results are expressed as ng/mL.

Calibration Range: 1 to 50 ng/mL (0.52 to 35 nmol/L).

Analytical Sensitivity: 0.44 ng/mL

Intraassay Precision (Within-Run): Statistics were calculated for each of five samples from the results of 20 pairs of tubes in a single assay. See also the precision profile below. (See “Intraassay Precision” table and Precision Profile.)

Interassay Precision (Run-to-Run): Statistics were calculated for each of five samples from the results of pairs of tubes in 20 different assays. (See “Interassay Precision” table.)

End-of-Run Effect: none up to approximately 300 tubes. (See "End-of-Run Effect" table.)

Recovery: Samples spiked 1 to 19 with three canine TLI solutions (155, 480 and

740 ng/mL) were assayed. (See "Recovery" table for representative data.)

References

1) Williams DA, Batt RM. Diagnosis of canine exocrine pancreatic insufficiency by the assay of serum trypsin-like immunoreactivity. J Small Anim Practice 1983;24:582-8. 2) Williams DA. New tests of pancreatic and small intestinal function. Compendium on Continuing Education for the Practicing Veterinarian 1987;9:1167-74. 3) Williams DA. Exocrine pancreatic disease. In: Ettinger SJ, editor. Textbook of veterinary internal medicine: Diseases of the dog and cat. 2nd ed. Philadelphia: W.B. Saunders, 1989: 1528-54. 4) Williams DA, Batt RM. Exocrine pancreatic insufficiency diagnosed by radioimmunoassay of serum trypsin-like immunoreactivity in a dog with a normal BT-PABA test result. J Am Anim Hosp Assoc 1986;22:671-74. 5) Williams DA, Batt RM. Sensitivity and specificity of radioimmunoasay of serum trypsin-like immunoreactivity for the diagnosis of canine exocrine pancreatic insufficiency. J Am Anim Hosp Assoc 1988;192:195-201. 6) Williams DA. Kansas State University, personal communications. 7) Simpson KW, et al. Circulating concentrations of trypsin-like immunoreactivity and activities of lipase and amylase after pancreatic duct ligation in dogs. Am J Vet Res 1989;50:629-32. 8) Borgström A, Ohlsson K. Immunoreactive trypsins in sera from dogs before and after induction of experimental pancreatitis. Hoppe-Seyler's Z Physiol Chem 1980;361:625-31.

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.

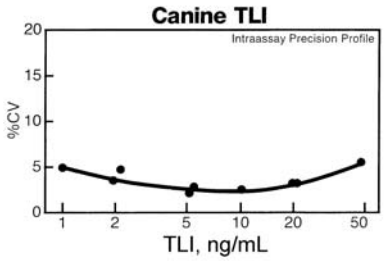
Tables and Graphs

Example Run

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	TLI ng/mL
T	44,561	44,756			
	44,950				
NSB	848	814	0		
	779				
A(MB)	21,644	21,398	20,585	**100%**	**0**
	21,152				
B	18,690	18,688	17,874	**87%**	**1.0**
	18,685				
C	16,184	16,545	15,731	**76%**	**2.0**
	16,905				
D	11,727	11,786	10,972	**53%**	**5.0**
	11,844				
E	8,317	8,277	7,463	**36%**	**10**
	8,236				
F	5,620	5,505	4,691	**23%**	**20**
	5,389				
G	3,097	3,061	2,248	**11%**	**50**
	3,025				
Unknowns					
X1	18,527	18,529	17,716	86%	1.02
	18,531				
X2	10,465	10,440	9,626	47%	6.7
	10,414				
X3	4,112	4,188	3,375	16%	29
	4,264				

Quality Control Parameters:
T = 44,756 cpm
%NSB = 1.8%
%MB = 46%
20% Intercept = 23 ng/mL
50% Intercept = 5.9 ng/mL
80% Intercept = 1.6 ng/mL

Precision Profile



Intraassay Precision (ng/mL)

	Mean	SD	CV
1	1.2	0.05	4.2%
2	3.1	0.12	3.9%
3	6.5	0.14	2.2%
4	13	0.34	2.6%
5	30	0.81	2.7%

Interassay Precision (ng/mL)

	Mean	SD	CV
1	1.2	0.08	6.7%
2	3.1	0.19	6.1%
3	6.7	0.34	5.1%
4	13	0.47	3.6%
5	30	1.4	4.7%

End-of-Run Effect (ng/mL)

	Tubes 31–46	Tubes 87–102	Tubes 143–158	Tubes 199–214	Tubes 255–270	Tubes 311–326
1	1.1	1.2	1.2	1.2	1.1	1.1
2	1.8	1.8	2.0	1.9	1.8	1.8
3	2.9	3.1	3.0	3.0	3.0	3.1
4	5.1	5.0	5.1	5.1	4.9	5.0
5	6.4	6.3	6.4	6.6	6.5	6.6
6	13	12	12	13	12	13
7	20	19	20	19	19	20
8	29	29	29	30	30	30

Recovery (ng/mL)

	Spiking Solution	Observed	Expected	O/E
1	—	4.8	—	—
	A	12	12	100%
	B	26	29	90%
	C	36	42	86%
2	—	5.0	—	—
	A	12	13	92%
	B	27	29	93%
	C	35	42	83%
3	—	5.1	—	—
	A	12	13	92%
	B	26	29	90%
	C	38	42	90%
4	—	9.6	—	—
	A	15	17	88%
	B	31	33	94%
	C	39	46	85%

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