IMMULITE®

Canine TLI

For use on the IMMULITE®
and IMMULITE® 1000 systems

DPC®

IMMULITE®/IMMULITE 1000 Canine TLI

Intended Use: For *in vitro* veterinary use with the IMMULITE and IMMULITE 1000 Analyzers — for the quantitative measurement of trypsin-like immunoreactivity (TLI) in canine serum, as an aid in the clinical assessment of exocrine pancreatic function in dogs.

Catalog Numbers: **LKLI1** (100 tests), **LKLI5** (500 tests)

Test Code: **cTL** Color: **Dark Blue**

CDC Test System Identifier Code: 10159
CLIA Complexity Category: Moderate

Summary and Explanation

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 dose determined by the assay reflects the concentration of 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 immunoassay 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 immunoassay is not suitable for assaying TLI levels in feline samples.

Principle of the Procedure

IMMULITE/IMMULITE 1000 Canine TLI is a solid-phase, enzyme-labeled, chemiluminescent immunometric assay.

Incubation Cycles: 1 × 30 minutes.

Specimen Collection

The animal should have fasted for at least 6 hours, and preferably overnight, before specimen collection. Collect blood by venipuncture into plain tubes, and separate the serum from the cells.

The use of an ultracentrifuge is recommended to clear lipemic samples.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Blood collection tubes from different manufactures may yield differing values, depending on tube materials and additives, including gel or physical barriers, clot activators and/or anticoagulants. IMMULITE/IMMULITE

1000 Canine TLI has not been tested with all possible variations of tube types.

Volume Required: 25 µL canine serum. (Sample cup must contain at least 100 µL more than the total volume required.)

Storage: 7 days at 2–8°C or 6 months (aliquotted) at –20°C.

Warnings and Precautions

For *in vitro* veterinary use.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

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.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

Canine TLI Test Units (LLI1)
Each barcode-labeled unit contains one bead coated with monoclonal murine anti-TLI. Stable at 2–8°C until expiration date. **LKLI1** 100 units. **LKLI5** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

Canine TLI Reagent Wedge (LLI2)
With barcode. 7.5 mL of an alkaline phosphatase (bovine calf intestine) conjugated to monoclonal murine anti-TLI antibody. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated. **LKLI1** 1 wedge. **LKLI5** 5 wedges.

Canine TLI Adjustors (LLIL, LLIH)
Two vials (Low and High) of lyophilized TLI in a protein/buffer matrix. Reconstitute each vial by adding **2.0 mL** distilled or deionized water. Mix by gentle swirling or inversion. Stable at 2–8°C for 2 weeks after reconstitution, or for 2 months (aliquotted) at –20°C.
LKLI1 1 set. **LKLI5** 2 sets.

Kit Components Supplied Separately

LSUBX: Chemiluminescent Substrate
LPWS2: Probe Wash Module
LKPM: Probe Cleaning Kit
LCHx-y: Sample Cup Holders (barcoded)
LSCP: Sample Cups (disposable)
LSCC: Sample Cup Caps (optional)

LLICM: A bi-level control module containing canine TLI in protein/buffer matrix.

Also Required
Sample transfer pipets, distilled or deionized water, controls.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE/IMMULITE 1000 Operator's Manual.

See the IMMULITE/IMMULITE 1000 Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

Visually inspect each Test Unit for the presence of a bead before loading it onto the system.

Recommended Adjustment Interval: 2 weeks.

Quality Control Samples: Use controls or sample pools with at least two levels (low and high) of TLI.

Expected Values

Based on its relationship to DPC's Double Antibody Canine TLI (see Method Comparison), the assay can be expected to have essentially the same reference ranges.

Results obtained with DPC's Double Antibody 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

A laboratory routinely performing the DPC Double Antibody Canine TLI assay, also evaluated the IMMULITE/IMMULITE 1000 Canine TLI assay. Their locally established ranges for exocrine pancreatic insufficiency (EPI) interpretation of results from the Double Antibody Canine TLI assay are:

- Values below 2.5 ng/mL are diagnostic for EPI.
- Dogs whose results fall between 2.5 and 5.0 ng/mL should be re-tested after one month, ensuring a 12- to 15- hour fast before the blood sample is collected.

When the above ranges were employed with both the Double Antibody and IMMULITE/IMMULITE 1000 Canine TLI assays on 243 canine sera samples submitted to the laboratory, the following was observed.

	IMMULITE/IMMULITE 1000 Canine TLI		
Double Antibody Canine TLI	Pos	Ind	Neg
Positive	31	1	0
Indeterminate	7	17	6
Negative	0	5	176

Total Agreement 92.2% 224/243
Relative Sensitivity 96.9% 31/32
Relative Specificity 97.2% 176/181

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, trypsinogen 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.

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

Performance Data

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in ng/mL. (Unless otherwise noted, all were generated on canine serum samples collected in tubes without gel barriers or clot-promoting additives.)

Calibration Range: Up to 50 ng/mL

Analytical Sensitivity: dose at 2 standard deviations away from zero: 0.3 ng/mL

High-dose Hook Effect:
None up to 250,000 ng/mL

Precision: Samples were assayed in duplicate over the course of 10 days, four runs per day, for a total of 40 runs and 80 replicates. (See “Precision” table.)

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

Recovery: Samples spiked 1 to 19 with three TLI solutions (50, 200 and 400 ng/mL) were assayed. (See "Recovery" table for representative data.)

Specificity: The antibody is highly specific for canine TLI. (See "Specificity" table.)

Method Comparison: The assay was compared to DPC's Double Antibody Canine TLI on 218 canine samples. (Concentration range: up to approximately 47 ng/mL. See graph.) By linear regression:

(IML) = 1.08 (DAb) – 1.35 ng/mL
r = 0.877

Means:
19.0 ng/mL (IMMULITE)
18.9 ng/mL (Double Antibody)

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 or 973.927.2828
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

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

Tables and Graphs

Precision (ng/mL)

		Within-Run		Total	
	Mean	SD	CV	SD	CV
1	2.10	0.13	6.2%	0.16	7.6%
2	11.9	0.32	2.7%	0.74	6.2%
3	26.9	0.59	2.2%	1.34	5.0%
4	29.4	0.70	2.4%	2.07	7.0%
5	46.4	1.23	2.7%	3.29	7.1%

Linearity (ng/mL)

	Dilution	Observed	Expected	%O/E
1	8 in 8	8.45	—	—
	4 in 8	3.88	4.23	92%
	2 in 8	2.05	2.11	97%
	1 in 8	1.15	1.06	108%
2	8 in 8	14.7	—	—
	4 in 8	7.24	7.35	99%
	2 in 8	3.90	3.68	106%
	1 in 8	2.19	1.84	119%
3	8 in 8	19.3	—	—
	4 in 8	9.60	9.65	99%
	2 in 8	4.85	4.83	100%
	1 in 8	2.66	2.41	110%
4	8 in 8	30.4	—	—
	4 in 8	14.0	15.2	92%
	2 in 8	6.59	7.60	87%
	1 in 8	3.44	3.80	91%
5	8 in 8	37.3	—	—
	4 in 8	17.2	18.7	92%
	2 in 8	8.27	9.33	89%
	1 in 8	4.27	4.66	92%
6	8 in 8	43.6	—	—
	4 in 8	20.1	21.8	92%
	2 in 8	10.1	10.9	93%
	1 in 8	5.08	5.45	93%

Recovery (ng/mL)

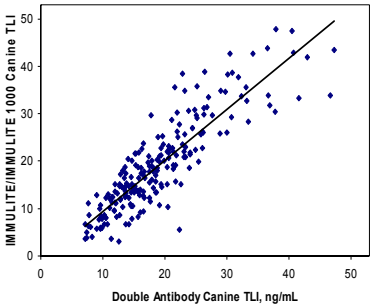
	Solution	Observed	Expected	%O/E
1	—	3.04	—	—
	A	5.30	5.39	98%
	B	12.2	12.9	95%
	C	21.8	22.9	95%
2	—	6.53	—	—
	A	8.95	8.70	103%
	B	16.3	16.2	101%
	C	26.6	26.2	102%
3	—	9.25	—	—
	A	11.8	11.3	104%
	B	19.5	18.8	104%
	C	30.0	28.8	104%
4	—	11.0	—	—
	A	12.6	13.0	97%
	B	20.5	20.5	100%
	C	30.9	30.5	101%
5	—	15.6	—	—
	A	17.1	17.3	99%
	B	25.2	24.8	102%
	C	35.3	34.8	101%
6	—	19.0	—	—
	A	20.5	20.6	100%
	B	29.4	28.1	105%
	C	39.8	38.1	104%

Specificity

Compound	ng/mL Added	Apparent ng/mL	% Cross-reactivity
Chymotrypsin	100	ND	ND

ND: not detectable.

Method Comparison



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