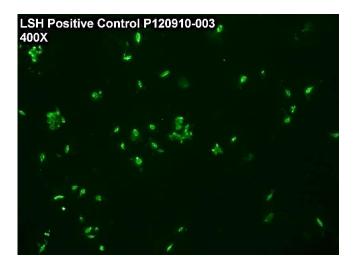
CERTIFICATE OF ANALYSIS

LEISHMANIA INFANTUM

IFA Positive Control Serum

Catalog No.:	PC-IFA-LSH
Volume:	1 ml
Lot:	P120910-003
Expiration:	13 November 2016
Agent:	Leishmania infantum



Description:

Canine serum diluted in PBS, 10% DBS, 0.09% sodium azide.

Quality Control Method:

IFA using VMRD, Inc. Leishmania 12-well slide (catalog no. SLD-IFA-LSH), Leishmania Negative Control (catalog no. NC-IFA-LSH), and Anti-Canine IgG FITC Conjugate (catalog no. CJ-F-CANG-1ML or 10ML).

Specific Reaction: 4+ fluorescence with the positive control at neat and an endpoint titer greater than 1/16. **Other Comments:** NA

Pattern Of Fluorescence:

Diffuse membranous fluorescence of organisms varying in shape from globus to trypanosome-like, some with clearly defined flagella. Single organisms and large clumps. High titer test sera will fluoresce brighter than this positive control serum.

Intended Use:

As a positive control serum in detection of antibody to *Leishmania infantum* by indirect FA technique. This serum should be used undiluted to demonstrate positive fluorescence.

Storage:

Store at 2-7°C. DO NOT FREEZE! If control becomes cloudy it should be discarded.

References: NA

Recommended Staining Procedure for Indirect FA:

- 1. Warm slide to room temperature before removing from foil pouch.
- 2. Place diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. FASDB-100ML) however if high background due to anti-bovine IgG activity is present it may be advisable to use SSDB-100ML.
- 3. Incubate slide in humid chamber at 37°C for 30 minutes.
- 4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
- 5. Drain slide and dry around wells by pressing blotter (included in pouch) to front surface. Place labeled anti-IgG or IgM on the wells.
- 6. Incubate as in step 3.
- 7. Rinse as in step 4.
- 8. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
- 9. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be made at 400X.

Recommended Staining Procedure for Direct FA:

- 1. Warm slide to room temperature before removing from foil pouch.
- 2. Place direct FA conjugate on the designated wells.
- 3. Incubate slide in humid chamber at 37°C for 30 minutes.
- 4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
- 5. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
- 6. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

Serum Diluting Buffer (pH 7.2):*

- Na₂HPO₄......1.19 gm
- NaH₂PO₄.....0.22 gm
- NaCl......8.55 gm
- BSA.....10.0 gm
- DI/dH2O.....Q.S. to 1 liter

*This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 2-7 C. Add 0.09% NaN₃ if diluted serum is not going to be used within one week.

4X FA Rinse Buffer (pH 9.0):

- Na₂CO₃.....11.4 gm

- DI/dH₂O.....Q.S. to 1 liter

Final pH should be 9.0-9.5. This is a 4X concentrate and should be diluted 1/4 with DI/distilled water for use as a working buffer. Keep in a tightly stoppered container at room temperature. MOUNTING FLUID is made by mixing glycerol and FA rinse buffer, pH 9.0, in equal proportions.

FOR IN VITRO LABORATORY USE ONLY.