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Kalazar Canine Rapid Test For the Detection of Visceral Leishmaniasis Antibody in Canine Serum

Catalog No.V6-902

Intended Use

The Immunospec Kal*azar* Canine Rapid Test for Visceral Leishmaniasis (VL) is a rapid immunochromatographic strip assay for the qualitative detection of antibodies to VL in canine serum. This test is intended for research use only. Not for use in diagnosis of disease in animals. Analytical and performance characteristics have not been established.

Summary and Explanation

VL is caused by members of the *Leishmania donovani* complex and canines have been identified as the major reservoir for transmission (1-4). Serodiagnosis has been widely utilized to establish infection because anti-Leishmanial antibody titers are high during acute disease. The preferred method of diagnosis in a laboratory situation is by ELISA, although fluorescent antibody (IFAT) or direct agglutination tests (DAT), both utilizing whole parasites, are still widely used (5-7). These tests are highly cross-reactive with trypanosomes and mycobacteria. In addition, the whole parasite preparations used are unstable and variable in quality. This rapid assay is for the qualitative determination of antibodies to a recombinant antigen is specific for Visceral Leishmaniasis (8) caused by parasite members of the *L. donovani* complex.

Principle

The Kalazar Canine Rapid Test for Visceral Leishmaniasis is a qualitative, membrane based immunoassay for the detection of antibodies to Visceral Leishmaniasis (VL) in canine serum. The membrane is pre-coated with a novel recombinant VL antigen on the test line region and chicken anti-protein A on the control line region. During testing, the serum sample reacts with the dye conjugate (protein A-colloidal gold conjugate) which has been pre-coated in the test device. The mixture then migrates upward on the membrane chromatographically by capillary action to react with recombinant VL antigen on the membrane and generates a red line. Presence of this red line indicates a positive result, while its absence indicates a negative result. Regardless of the presence of antibody to VL, as the mixture continues to migrate across the membrane to the immobilized chicken anti-protein A region, a red line at the control line region will always appear. The presence of this red line serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

Precautions

- For research use only. Do not use after expiration date.
- Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of sera and used kits.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly when finished.
- Avoid all contact between hands and eyes or mucous membranes during testing.
- Do not eat, drink or smoke in the area where the sera and kits are handled.
- Chase Buffer contains a preservative; avoid all possible contact with skin and mucous membranes.

Storage

The sealed pouch or vial containing the test strip is designed to be stored at room temperature (20°C-28°C) for the duration of its shelf life. The bottle containing the Chase Buffer is designed to be stored at room temperature for the duration of its shelf life. Exposure to temperatures over 30°C can impact the performance of the test and should be minimized. The strips should not be frozen. The test should be used within 1 hour after removal from the pouch or vial to prevent exposure to humidity.

Sera Collection

- Canine serum should be tested with this test strip. Whole blood or dilutions of serum cannot be tested directly.
- Remove the serum from the clot of red cells as soon as possible to avoid hemolysis.
- Test should be performed as soon as possible after sera collection. Do not leave sera at room temperature for prolonged periods. Sera can be refrigerated at 2-8°C for up to 3 days. Otherwise sera should be stored frozen.
- Bring sera to room temperature prior to testing. The frozen sera must be completely thawed prior to testing. Sera should not be repeatedly frozen and thawed.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

Kit Contents

1. Twenty-five (25) test strips, individually pouched or 25 test strips in a vial with desiccant in the cap.
2. One (1) vial of Chase Buffer solution.

Test Procedure

1. Allow the sera to reach room temperature prior to testing.
2. Remove the *Kalazar* Canine Rapid Test for VL from the foil pouch or vial.
3. Add 20 µl of sera to the test strip in the area beneath the arrow.
4. Place the test strip into a test tube or well of a 96 well tissue culture plate so that the end of the strip is facing downward as indicated by the arrows on the strip.
5. Add 2-3 drops (100-150 µl) of the Chase Buffer solution provided with this test kit.
6. Read the results in 10 minutes. It is significant that the background is clear before reading the test, especially when sera have low titer of anti-Leishmanial antibody. In this case, only a weak, but unequivocal band may appear in the test region. Results interpreted after 10 minutes can be misleading.

Note: Do not test this product with the Chase Buffer solution alone. **20** µl of canine serum must be added first. If gold migration is not observed within 10-15 seconds of the addition of chase buffer, lightly press on sample tape region of dipstick until gold migration is observed.

Interpretation of Results

A Positive Result

The test is positive when a control line (C) and test line (T) appear in the test area as shown in Figure 1. A faint line is considered a positive result. As a guide for interpretation, the red color in the test region will vary depending on the concentration of anti-Leishmanial antibodies present. The test line for "weakly positive" sera samples may show a weak positive but distinctly red line. ("Weakly positive" samples are those with low affinity antibodies.)

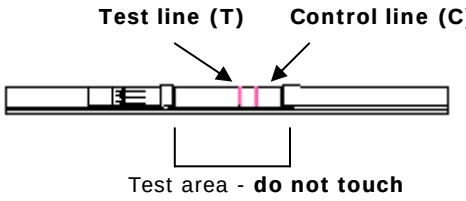

Figure 1

A Negative Result

The test is negative when only the control line appears. No test line is present as in Figure 2.

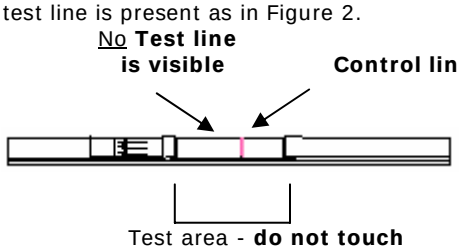

Figure 2

An Invalid Result

No lines appear at either the control or test line areas. The test is also invalid if no control line appears, but a test line is seen. It is recommended to retest using a new *Kalazar* Canine Rapid Test for VL and fresh serum.

Note: The red color in the test region will vary depending on the concentration of anti-Leishmanial antibodies present. However, neither the quantitative value nor the rate of increase in antibodies can be determined by this qualitative test.

Limitations

This test is intended for research use only. Not for use in diagnosis of disease in animals. Analytical and performance characteristics have not been established.

- This test will only indicate the presence of antibodies to Visceral Leishmaniasis in the canine serum and should not be used as the sole criterion for the diagnosis of Leishmaniasis. (As with all diagnostic tests, all results must be considered with other clinical information available to the veterinarian.)
- If the result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of Leishmaniasis.
- Do not use serum or plasma samples containing any glycerol or other viscous materials. This will compromise the sensitivity of the assay dramatically.

References

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