

OneStep Leishmania Serum RapiDip™ InstaTest

REF 176070-1-25



Sensitivity 90%

INTENDED USE

The *Cortez Diagnostics Inc.* Test for Visceral Leishmaniasis (VL) is a rapid immunochromatographic strip assay for the qualitative detection of antibodies to members of *L. donovani* in human serum. This kit is intended for professional in vitro diagnostic use only. It is not intended for use in blood donor centers or by blood component manufacturers.

SUMMARY AND EXPLANATION

VL is a severe disease with high mortality, endemic in 88 countries including 17 developed nations (1,2). A serious problem in much of the world including Brazil, China, East Africa, India and areas of the Middle East, leishmaniasis is also endemic in the Mediterranean region including southern France, Italy, Greece, Spain, Portugal and Northern Africa. In areas where leishmaniasis is endemic, recent migration patterns of people, vectors (sandfly) and reservoirs (dogs) have led to the urbanization of VL (3). In Southern Europe, VL has become the leading opportunistic infection in AIDS patients (4,13).

L is caused by members of the *Leishmania donovani* complex and canines have been identified as the major reservoir for transmission (5-8). Serodiagnosis has been widely utilized to establish infection because antileishmanial 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 (9-11). 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 specific for Visceral Leishmaniasis (12) caused by parasite members of the *L. donovani* complex.

TEST PRINCIPLE

The *Cortez Test* for VL is a qualitative, membrane based immunoassay for the detection of antibodies to Visceral Leishmaniasis in human serum. The membrane is pre-coated with rK39 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 rK39, 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.

SPECIMEN COLLECTION AND PREPARATION

- Human serum should be tested with this test strip. Whole blood should not be used with this test as it may affect ones ability to read the test line correctly due to excessive background. Dilutions of serum in buffer cannot be tested directly. Positive serum can be diluted with disease negative sera.
- 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 up to 3 days. Otherwise sera should be stored below 20°C.
- 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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

Cortez test strip's membrane is pre-coated with a recombinant rK39 on the test line region and chicken anti-protein A on the control line region.

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

ASSAY PROCEDURE

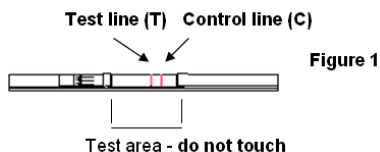
- Allow the sera to reach room temperature prior to testing.
- Remove the *Cortez Test* for VL from the foil pouch or vial.
- Place the test strip horizontally on a flat surface or vertically in a micro-well or test tube.
- Add 20 µl of sera to the test strip in the area beneath the arrow.
- If the serum was applied to a horizontal test strip on a flat surface, pick it up by the green label and place it vertically, arrow pointing downwards, into a test tube or micro-well containing 2-3 drops (150 µl) of Chase Buffer.
- If the serum was applied to a vertical test strip, then add 2-3 drops (150 µl) of the Chase Buffer solution provided with this test kit to the base of the well or test tube.
- Read the results in 10 minutes. It is crucial that the background is clear before reading the test, especially when samples have low titer of anti-Leishmanial antibody, and only a weak band appears in the test region (T). Results interpreted after 10 minutes can be misleading.

Note: Do not test this product with the Chase Buffer solution alone. 20 µl of human serum must be added first.

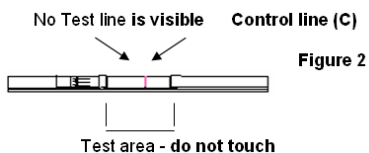
Note: If migration of the gold is not observed within 10-15 seconds after the addition of chase buffer, lightly press on sample tape region of dipstick until migration of gold is observed.

RESULTS

- **Positive:** The test is positive when a control line and test line appear in the test area as shown in Figure 1. A positive result indicates that the Cortez dipstick detected antibodies to members of *L. donovani* complex. 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 results between a weak positive red line to a faintly red, almost white background. ("Weakly positive" samples are those with low affinity or low titer antibodies against the recombinant test antigen.)



- **Negative:** The test is negative when only the control line appears. A negative result indicates that the Cortez dipstick did not detect antibodies to members of *L. donovani* complex. No test line is present as in Figure 2.



- **Invalid :** 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 *Cortez 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.

Expected Value:

In endemic areas, the sensitivity of the Cortez test is 90% or better. The specificity of the test may vary with geographic location. For example, in India thirteen out of 104 healthy controls showed positive reactivity with the Cortez Test.

PERFORMANCE CHARACTERISTICS

Reproducibility Study:

The reproducibility of the Cortez test strip was evaluated at 3 sites using a panel of confirmed VL sera. Positive, low/weak and normal serum samples were used. The samples were coded and tested at each site in triplicate for 3 consecutive days. The results indicate that for each day, the technician scored the test the same. Once the samples were decoded, the reading was in line with the ELISA titer. This data indicates that the reproducibility of the Cortez Test strip is excellent.

Interference Studies:

Indian Study: Patients with neoplastic disease, viral infection, chronic bronchitis, amebic liver abscess, idiopathic thrombocytopenic purpura, rheumatic heart disease, myelodysplastic syndrome, myoclonus, leprosy, tuberculosis, syphilis and malaria were tested with the Cortez test strip for the presence of Leishmania. Only one patient with malaria produced a false positive result. All other patients tested negative.

Brazilian Study: Sera from patients with malaria, chagas, tuberculosis, cutaneous leishmaniasis and Hansen disease were tested with the Cortez test strip for the presence of visceral leishmaniasis. All patient sera tested negative for Leishmania.

Field Studies:

The *Cortez* test for VL was field tested at 2 sites. The table below summarizes the results of these studies.

Site 1: Brazilian Study: Cortez Test Compared to Microscopy

		+	-		
Cortez	+	115	0		
	-	13	59		
		128	59	187	
Sensitivity		89.844		Specificity	100
Std. Error		2.67			0
95% CI		(82.936, 94.263)			(92.384, 100)

Site 2: Indian Study: Cortez Test Compared to Microscopy

		+	-		
Cortez	+	225	14		
	-	0	190		
		225	204	429	
Sensitivity		100		Specificity	93.137
Std. Error		0			1.77
95% CI		(97.908, 100)			(88.517, 96.054)





Note: Site 2 had a high prevalence of VL patients.

LIMITATIONS OF PROCEDURE

1. This test will only indicate the presence of antibodies to the recombinant test antigen rK39 in patients with Visceral Leishmaniasis and should not be used as the sole criterion for the diagnosis of Leishmaniasis. This test alone must not be used for any clinical treatment decision. As with all diagnostic tests, all results must be considered with other clinical information available to the doctor.
2. 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.
3. A false positive result may occur. Confirmatory testing (such as by culture) is advised especially in cases where no symptoms exist.
4. Do not use serum samples containing any glycerol or other viscous materials. This will decrease the sensitivity of the assay.
5. Persons with advanced HIV infection or other immunocompromised diseases frequently have low or undetectable anti-Leishmanial antibodies.
6. This test may yield false positive results with samples from patients having malaria.
7. The performance of this test has not been evaluated with *L. infantum*.
8. Certain Rheumatoid Factor (RF) sera may produce false positive results when Cortez is used.

PRECAUTIONS

1. For professional in vitro diagnostic use only. Do not use after expiration date.
2. 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.
3. Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly when finished.
4. Avoid all contact between hands and eyes or mucous membranes during testing.
5. Do not eat, drink or smoke in the area where the sera and kits are handled.
6. Chase Buffer contains a preservative; avoid all possible contact with skin and mucous membranes.
7. 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.

<p>ISO 13485 ISO 9001</p>  <p> Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116 Woodland Hills, California 91367 USA</p>	
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