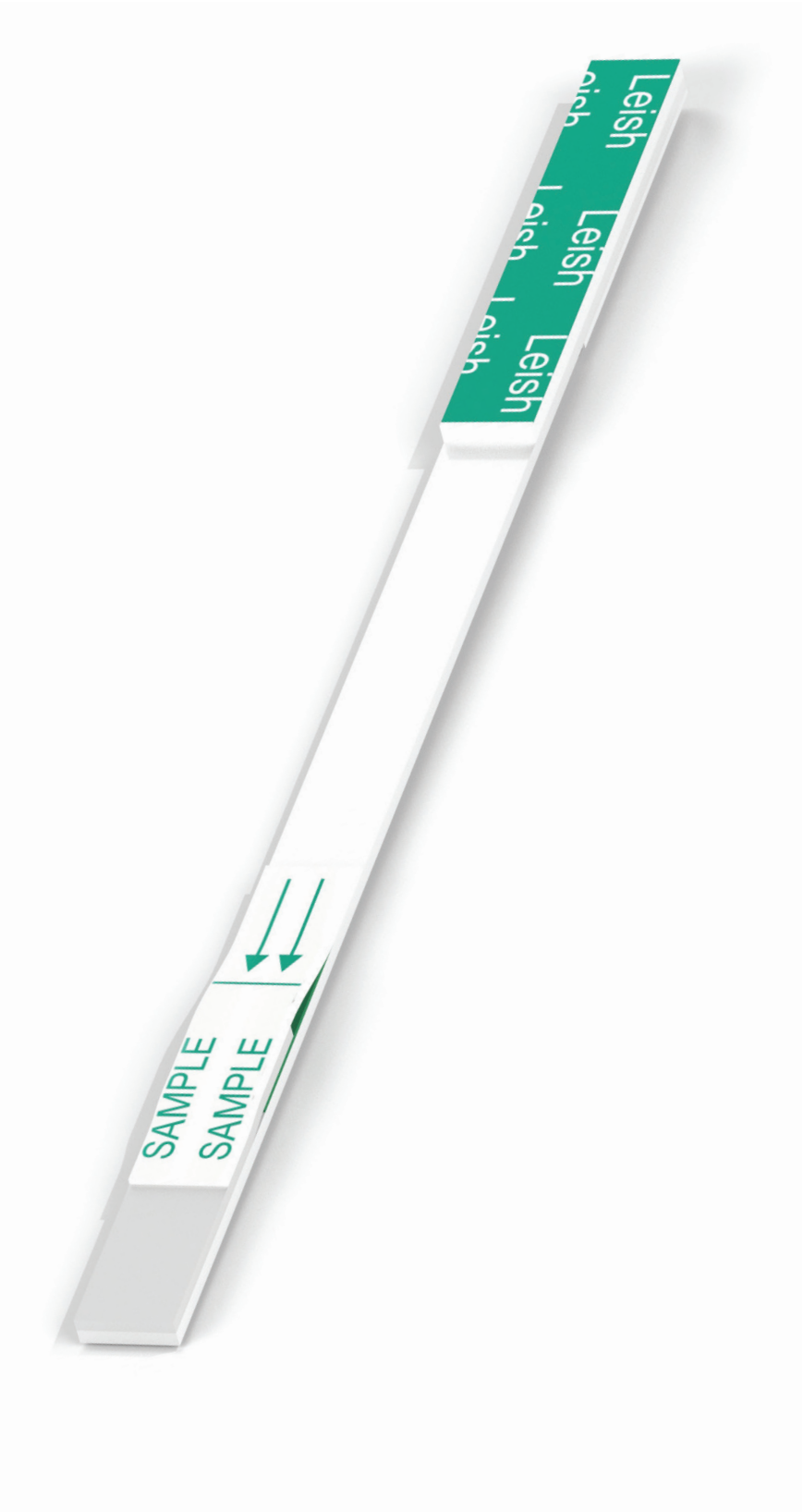
Rapydtest® FOR

A RAPID, ONE STEP TEST FOR THE QUALITATIVE DETECTION OF ANTIBODY TO VISCERAL LEISHMANIASIS IN SERUM



PARASITOLOGY

SINGLE USE IN VITRO DIAGNOSTIC DEVICE

APACOR

Leishmania Rapydtest®

Leishmania Rapydtest®

Performance Benefits

- Ten minutes one step test
- Rapid chromatographic immunoassay
- Detection in serum
- High sensitivity / specificity (rK39)

Summary

Leishmaniasis is a spectrum of disease caused by the Leishmania species. The Leishmania species is transmitted to humans and in the body it proliferates and disseminates throughout the reticuloendothelial system as obligate intracellular parasites.

The clinical manifestations of leishmaniasis vary depending on the Leishmania species and the T cell mediated immune responses of the host. There are three major forms of disease: cutaneous, mucocutaneous, and visceral leishmaniasis (VL). VL has been demonstrated as an important opportunistic infection associated with AIDS infection.

Visceral leishmaniasis (kala-azar) is typically caused by several species belonging to the L. donovani complex, involving the liver, spleen, and other parts of the reticuloendothelial system. Symptoms may be insidious or of sudden onset, including fever, malaise, anorexia, and weight loss. Hepatosplenomegaly is a hallmark of visceral leishmaniasis. Laboratory findings commonly show anaemia, neutropenia, and hypergammaglobulinemia. A presumptive diagnosis of visceral leishmaniasis is made by the classic clinical presentation in an endemic area. The diagnosis is confirmed by identifying intracellular Leishmania amastigotes in tissue, usually by splenic or bone marrow aspiration. Circulating antibody to a novel antigen conserved in amastigotes of visceral leishmaniasis-inducing Leishmania strains, appears to be sensitive and specific for active visceral infection.

The Apacor Leishmania Dipstick is a rapid test to qualitatively detect the presence of antibody to visceral leishmania in serum specimens. The test utilises a combination of protein A-colloidal gold conjugate and recombinant Leishmania antigen to selectively detect antibody to Leishmania in serum.

Principle

The Apacor Leishmania Dipstick is a qualitative, membrane based immunoassay for the detection of antibodies to visceral leishmaniasis in human serum or plasma. The membrane is pre-coated with recombinant visceral leishmania antigen on the test line region and anti-protein A antibody 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 visceral leishmania antigen on the membrane and generate 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 visceral leishmania, as the mixture continues to migrate across the membrane to the immobilized 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.

Storage and Stability

The sealed pouch containing the dipstick and the bottle containing the Chase Buffer are designed to be stored at room temperature (20°C - 30°C) for the duration of the kit's shelf life. Exposure to temperatures over 30 °C can impact the performance of the test and should be minimized (5 days maximum). The strips and buffer should not be frozen. The test should be used within 1 hour after removal from the pouch to prevent exposure to humidity.

Precautions

- For professional in vitro diagnostic 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 kits used.
- 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.

Materials Provided

Apacor Leishmania Dipsticks and Chase Buffer

Materials Required but not Provided

Tubes for serum collection procedure, centrifuge, timer.

Specimen Collection

- Human serum must be used with this test strip. Whole blood should not be used with this test as it may affect one's 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 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 at or below -20°C.
- Bring sera to room temperature prior to testing. 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.

Test Procedure

- Allow the sera and Chase Buffer to reach room temperature prior to testing.
- Remove the dipstick from the foil pouch.
- Add 20µl of serum to the dipstick in the area below the arrow.
- Place the dipstick 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.
- 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 significant that the background is clear before reading the test, especially when sera 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. 20µl 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, apply light pressure on the sample tape region of test strip until gold migration is observed.

Interpretation of Results

Positive

The test is positive when a control line and test line appear in the test area as shown. A positive result indicates that the dipstick detected antibodies to members of L. donovani complex. A faint line is considered a positive result. As a guide for interpretation, the red colour 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 Leishmania dipstick did not detect antibodies to members of L. donovani complex. No test line is present.

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 dipstick and fresh serum.

Note: The red colour 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 will only indicate the presence of antibodies to rK39 in patients with visceral leishmaniasis 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 doctor.)
- 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.
- A false positive result may occur. Confirmatory testing (such as by culture) is advised especially in cases where no symptoms exist.
- Do not use serum or plasma samples containing any glycerol or other viscous materials. This will decrease the sensitivity of the assay.
- Persons with advanced HIV infection or other immunocompromised diseases frequently have low or undetectable anti-Leishmanial antibodies.
- This test may yield false positive results with samples from patients having malaria.
- The performance of this test has not been evaluated with L. infantum.
- Certain Rheumatoid Factor (RF)-positive sera may produce false positive results when this test is used.

Performance Characteristics

Reproducibility Study

The reproducibility of the Leishmania Dipstick (Kalazar Detect) 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 readings were in line with the ELISA titer. This data indicates that the reproducibility of the Leishmania Dipstick (Kalazar Detect) is excellent.

Cross-reactivity 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 Leishmania Dipstick (Kalazar Detect) 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 Leishmania Dipstick (Kalazar Detect) for the presence of visceral leishmaniasis. All patient sera tested negative for Leishmania.

Field Studies

The Kalazar Detect™ test for VL.

Site 1: Brazillian Study: Kalazar Detect Test Compared to Microscopy

		+	-	
Kalazar Detect™	+	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: Kalazar Detect Test Compared to Microscopy

		+	-	
Kalazar Detect™	+	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.

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Ordering Information

PRODUCT	PACK SIZE	CODE
Apacor Leishmania Dipstick Rapydtest®	40	1601

Products can be ordered direct from Apacor or from an appointed distributor

Visit our website for all the latest information www.apacor.com or e-mail on: orders@apacor.com

APACOR

UNIT 5, SAPPHIRE CENTRE,
FISHPONDS ROAD, WOKINGHAM,
BERKSHIRE, RG41 2QL, UNITED KINGDOM

TEL: +44 (0)118 979 5566
FAX: +44 (0)118 979 5186

EC REP MDSS GmbH
Schiffaraben 41
30175 Hanover
Germany

IVD