Size : 137 x 218 mm

CE

Leishcheck™

Rapid test for detection of Visceral Leishmaniasis antibody in human serum/plasma

DEVICE

INTENDED USE

Leishcheck™ is a rapid, self-performing two site qualitative immunoassay for the detection of antibodies to *L. donovani* in human serum/ plasma. The test is intended for the presumptive diagnosis of Visceral Leishmaniasis (VL). For professional *in vitro* diagnostic use only.

SUMMARY

Visceral Leishmaniasis (VL), also known as Kalazar, is a vector-borne parasitic disease which is nearly always fatal if left untreated. VL is one of the world's most neglected diseases, largely affecting the poorest people, mainly in developing countries. VL is endemic in 60 countries and some 500,000 new cases occur annually. The disease is transmitted through the bite of an infected phlebotomine sandfly. The host reservoir varies in different parts of the world, reflecting a difference in parasite, in Europe and South America the causative organism agent is *Leishmania infantum*, which has the domestic dog as its main reservoir, in the Indian subcontinent (ISC) (Bangladesh, India, Nepal) and in East Africa, the causative organism is *L. donovani* strains.

Earlier, accurate VL diagnosis necessitated parasitological confirmation by microscopy or culture of the blood, bone-marrow, lymph nodes or spleen. Microscopic detection of parasites in clinical material from the spleen is still considered the reference standard; however, splenic aspirates are associated with risks of serious bleeding and should only be carried out in settings with access to blood transfusion and surgical services. The invasiveness and potentially fatal complications associated with splenic aspiration has motivated the development of non-invasive serological tests lateral flow immuno-chromatographic tests (ICT), commonly referred to as rapid diagnostic tests (RDT). RDT is seen as a potential breakthrough allowing user and patient friendly, rapid diagnosis in peripheral health care settings.

Leishcheck™ qualitatively detects the presence of IgG antibodies to Visceral Leishmaniasis in human serum or plasma.

PRINCIPLE

Leishcheck™ utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. The test membrane is stripped with recombinant K39 antigen and a reagent control. The test specimen is added followed by sample running buffer and allowed to move along the membrane. The IgG present in the specimen binds to the Protein-A coated on the colloidal gold forming an IgG-Protein-A gold complex. This complex moves along the membrane where it is immobilized by the recombinant K39 antigen (VL specific) coated on the membrane forming a pink/purple colored band. The absence of this colored band in the test region indicates a negative test result.

The unreacted conjugate and unbound complex if any, moves further on the membrane and are subsequently immobilized by the reagent control coated on the membrane at the control region, forming a pink/purple band. This control band acts as a procedural control and serves to validate the test performance.

REAGENTS AND MATERIALS SUPPLIED

A. Each **Leishcheck™** kit contains individual pouches each containing a,
   1. DEVICE Membrane assembly predispensed with Protein A-colloidal gold conjugate, recombinant K39 antigen (VL specific) and reagent control coated at the respective regions.
   2. Desiccant pouch.
   3. PIPETTE Disposable sample applicator.

B. BUF Sample running buffer in a dropper bottle.

C. Package insert.

REF	503100010	503100005
Σ	10T	5T

OPTIONAL MATERIAL REQUIRED

Calibrated micropipette capable of delivering 5µl sample accurately.

STORAGE AND STABILITY

The sealed pouches in the test kit & the kit components may be stored between 4°C to 30°C till the duration of the shelf life as indicated on the pouch/ carton. DO NOT FREEZE. After first opening of the sample running buffer bottle, it can be stored between 4°C to 30°C for the remaining duration of its shelf life.

NOTES

1. Read the instructions carefully before performing the test.

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2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
3. Do not use the kit beyond expiry date and do not re-use the test device.
4. Do not intermix the reagents from different lots.
5. Handle all specimens as potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infective material.
6. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
7. Sample running buffer contains Sodium Azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build-up in the plumbing.

SPECIMEN COLLECTION AND PREPARATION

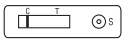
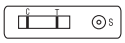
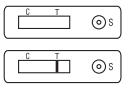
1. **Leishcheck**™ kit uses human serum/plasma as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. Though fresh specimen is preferable, in case of delay in testing, it may be stored at 2^{0}C to 8^{0}C for maximum upto 24 hours before testing.
4. Refrigerated specimens must be brought to room temperature prior to testing.
5. Do not use viscous/ turbid, lipaemic, hemolysed, clotted and contaminated serum/plasma specimens.
6. Specimens containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the **Leishcheck**™ kit components and specimen to room temperature before testing.
2. Open the pouch and retrieve the test device, sample applicator and desiccant pouch. Check the color of the desiccant. It should be blue, if it has turned colorless or pink discard the device and use another device. ***Once opened, the device must be used immediately.***
3. Label the test device with patient's identity.
4. Tighten the cap of the sample running buffer bottle provided with the kit in the clockwise direction to pierce the buffer bottle nozzle.
5. Dip the sample applicator into the serum/plasma sample. Ensuring that an applicator full of serum/plasma sample is retrieved, blot the collected sample in the sample port 'S' (This delivers approximately 5µl of the serum/plasma sample).

   OR

   Alternatively, using a calibrated micropipette add 5µl serum/plasma sample at the sample port 'S' of the test device.
   NOTE: Ensure that the serum/plasma sample from the sample applicator has been completely taken up by the sample port 'S'.
6. Immediately dispense two drops of sample running buffer in the same port. Allow the first drop to soak in, then add the second drop.
7. Read results at the end of **10 minutes** as follows:

	**Negative Result** Only one pink/purple band appears at the control region 'C'.
	**Positive Result** In addition to the control band, a pink/purple colored band also appears at the test region 'T'.
	**Invalid Result** The test should be considered invalid if no bands appear on the device. The test should also be considered invalid if only test band appears and no control band appear. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

PERFORMANCE CHARACTERISTICS

In an in-house study **Leishcheck**™ was evaluated with 50 positive serum samples and 200 negative serum/plasma samples tested with a commercially available similar product in parallel. The positive patient; from whom said serum samples were collected was also confirmed with splenic puncture microscopy LD body detection. The results obtained were as follows:

Sample	Total No. of Samples Tested	Competitor Brand	**Leishcheck**™
Negative	200	200	199
Positive	50	50	50
Total	250	250	249

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LIMITATIONS OF THE TEST

1. As with all diagnostic tests, the result must be correlated with clinical findings.
2. The test detects the presence of antibodies to *L. donovani* in the specimen and hence should not be used as a sole criterion for the diagnosis of Visceral Leishmaniasis infection. A false positive result may occur. It is advisable to confirm such cases with a confirmatory testing (such as culture).
3. A negative result at any time does not preclude the possibility of exposure to or infection with *L. donovani*. If the test result is negative and suspicion still exists, additional follow-up testing using other clinical methods is recommended.
4. **Leishcheck**™ should be used as a screening test in clinical suspected cases only and its results should be confirmed by confirmatory test methods (such as culture) before taking clinical decisions.
5. Most positive results develop within 10 minutes. However certain specimens may take a longer time to flow therefore, negative should be confirmed only at 15 minutes. Do not interpret the test results beyond 30 minutes.
6. Interference due to presence of heterophile antibodies in patient's sample can lead to erroneous analyte detection in immunoassay, has been reported in various studies. **Leishcheck**™ uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of these interferences.
7. The test may be false positive with specimens from patients having malaria and Chagas disease.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. WHO. 1990. Control of the Leishmaniases, World Health Organization, Technical Report Series No. 793.
2. Badaro, R., S.G. Reed, and E.M. Carvalho. 1996, The Journal of Infectious Diseases 173; 758-761.
3. Burns, J.M., W.G. Shreffler, D.R. Benson, et al. 1993. Proc. Natl. Acad. Sci. (90) 775-779.
4. Data on file: Zephyr Biomedicals.

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Do not reuse
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	This side up	PIPETTE — Disposable sample applicator
Use by	REF — Catalogue Number	DEVICE — Device	EC REP — Authorised Representative in the European Community
Σ — Contains sufficient for <n> tests	LOT — Batch Number / Lot Number	BUF — Sample Running Buffer	

Manufactured by:

Zephyr Biomedicals

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EC REP

CMC Medical Devices & Drugs S.L., Spain.

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