GLOBAL ACCESS DIAGNOSTICS

IT LEISH

REF 710124

Individual test for antibody detection in human visceral leishmaniasis

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UK CA

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INTRODUCTION

Leishmaniasis is caused by protozoan flagellates of the *Leishmania* genus. The disease is found worldwide and is transmitted by the bite of an infected female sandfly. Clinical manifestations vary from benign self-limiting sores to life-threatening, fulminant visceral disease [1, 3].

Human visceral leishmaniasis (VL) is due to the closely related species *L. donovani, L. chagasi,* and *L. infantum. L. donovani* is responsible for VL in East Africa and India. *L. infantum* is the agent of Mediterranean VL and *L. chagasi* of VL in Latin American countries. For *L. infantum* and *L. Chagasi*, transmission via blood transfusion has been documented but there is currently no mandatory mass donor screening in any country [3]. However, some data show a high frequency of infection by *Leishmania* parasites in blood donors [4, 5].

INTENDED PURPOSE

The **IT LEISH** assay is a manually performed, visually read, single-use rapid chromatographic *in vitro* diagnostic immunoassay for the qualitative detection of *Leishmania spp.* specific antibodies in serum or capillary whole blood during infection. The test is intended as an aid to diagnosis of visceral leishmaniasis in suspected cases. For professional use only. Not for self-testing or automation. Test results should be considered in conjunction with other diagnostic and clinical information.

PRINCIPLES OF THE TEST

IT LEISH is an immunochromatographic test, using the recombinant antigen K39, to detect the presence of antibodies against *Leishmania spp.* [2, 6].

The test can be conducted with whole blood (capillary) or with serum; the result is obtained within 20 minutes with whole blood and 10 minutes with serum.

PERFORMANCE DATA

Clinical Sensitivity/Specificity: A large study was conducted in the northeast of India in 2003 using the **IT LEISH** [6]. The summarised data were as follows:

1) **Sensitivity**: 99% with whole blood samples (in 100 cases of human VL)
2) **Specificity**: 100% with whole blood samples (in 167 individuals either healthy or suffering from other diseases and presenting with fever and/or splenomegaly).

Cross Reactivity: The following study [7] focused on the impact of Chagas disease and cutaneous leishmaniasis. The results are displayed below.

IT LEISH		
	Number of samples	**Specificity (95% CI)**
Healthy volunteers	30	100 (85-100)
Chagas disease (blood culture)	30	100 (85-100)
Chagas disease (serology)	30	96 (81-99)
Cutaneous leishmaniasis	20	80 (56-93)

The report concluded that *"The rapid test based on recombinant K39 is a useful diagnostic assay, although false-positive results can occur in patients with a serological diagnosis of Chagas disease and in patients with cutaneous leishmaniasis in rare circumstances."*

A study of three RDTs, including IT LEISH, were evaluated [8] *"One patient with malaria (who was negative for VL) was positive on all RDTs. Two patients with cutaneous leishmaniasis were positive by one or two RDTs."* The report does not state which device gave the incorrect result.

A study [9] of 150 individuals with diseases other than VL found a total of 5% of samples

produced a false positive result which included tuberculosis (four patients), malaria (two patients), and chronic myelogenous leukemia (one patient).

A study [10] evaluated 10 malaria-positive samples (four *P. falciparum*, three *P. vivax*, one *P. ovale*, one *P. malariae,* and one *P. falciparum* and *P. ovale* mixed infection). IT LEISH gave no false positive results.

Finally, a study [11] in which the coinfection rate was 29% (from 133 patients) with only one false positive stated, *"Sensitivity is not influenced by presence of malaria"*, in reference to the IT LEISH test.

In conclusion, although rare interactions with malaria, Chagas, and cutaneous leishmaniasis have been observed, the **IT LEISH** device has been shown to be effective.

Interference: The following interferents showed no observable effect on the device test designation; bilirubin (40mg/dL), haemoglobin (1000mg/dL) and triglycerides (1500mg/dL).

Repeatability: Overall precision of the IT Leish device.

Repeatability			
	Sample Designation		
User Interpretation	**POS**	**NEG**	**Total**
POS	240	0	240
NEG	0	80	80
Total	240	80	320
Overall Percent Agreement	100.0%	95% CI: 98.8%-100.0%	
Positive Percent Agreement	100.0%	95% CI: 98.4%-100.0%	
Negative Percent Agreement	100.0%	95% CI: 95.4%-100.0%	

Reproducibility: Overall precision of the IT Leish device across three sites.

Overall Precision			
	Sample designation		
User Interpretation	**POS**	**NEG**	**Total**
POS	674	0	674
NEG	1	225	226
Total	675	225	900
Overall Percent Agreement	99.9%	95% CI: 99.4%-100%	
Positive Percent Agreement	99.9%	95% CI: 99.2%-100%	
Negative Percent Agreement	100.0%	95% CI: 98.3%-100%	

Summary of the precision of the IT Leish device assessed by site and batch.

Percentage agreement		
Batch 1 (3A0114L)	**Batch 2 (3A0115L)**	**Batch 3 (3A0116L)**
99.7% (95%CI 98.1%-99.9%)	100.0% (95% CI: 98.7%-100.0%)	100.0% (95% CI: 98.7%-100.0%)
Site 1 (GADx)	**Site 2 (LSTM)**	**Site 3 (SGUL)**
100.00% (95%CI 98.7%-100.0%)	100.00% (95%CI 98.7%-100.0%)	99.7% (95%CI 98.1%-99.9%)

In use stability: The device is stable when in use after opening device foil pouch for up to 60 minutes with the result being stable for up to 90 minutes (at 25°C, 70% RH).

High Dose Hook: There is no evidence of a hook effect across DAT titers (1:3200 to 102400).

MATERIALS PROVIDED

Each single test package contains:

- 1 device with dipstick, conjugate well, wash well
- 1 well cover
- 1 dropper ampoule with buffer
- 1 lancet
- 1 disinfecting swab
- 1 pipette (printed mark for 10 µL)
- 1 schematic test procedure
- 1 device with dipstick, conjugate

MATERIALS REQUIRED BUT NOT PROVIDED

- 1 timer

STORAGE AND HANDLING OF THE REAGENTS

Stability: see expiry date on aluminium foil. Do not freeze.
Important: When transporting or storing the packages, avoid exposure to high temperature (over 40°C) for a period longer than 1 day. Avoid any exposure to 60°C or higher (the reagents may be damaged).

SAMPLE MATERIAL

- Capillary blood collected from fingertip.
- Serum, follow local venipuncture procedures.

TEST PROCEDURE

Tear open the aluminium package and take out all the material.

Important: *Do not leave the material exposed to humidity and high temperature. In tropical conditions, use the test within 15 minutes after opening the aluminium package. In laboratory conditions at 25ºC/75%RH, use the device within 60 minutes after opening.*

1. Take the device, place it horizontally on a flat surface, write the patient's name or number on the label.
2. Tear open the ampoule of buffer, add **1 drop** of buffer to the first well (conjugate well, marked with a coloured line), and **4 drops** to the second well (wash well). Allow to stand for **1 minute**.
3. For finger prick blood: clean the fingertip with the swab, let dry, remove the lancet from its envelope, prick the finger. Take the pipette, squeeze it, place the open tip into the blood drop, release pressure and draw up blood to the black line. Discard used swab and lancet into a suitable waste container. When using venous blood, draw blood from the tube into the pipette in the same manner. When using centrifuged blood, draw the serum from the tube into the pipette in the same manner.
4. Add the entire volume of blood, serum (8-12 µL), by squeezing the pipette gently, to the first well (conjugate well, marked with a coloured line).
5. Stir gently with the upper end of the pipette and allow to stand for **1 minute**. Discard the pipette into a suitable waste container.
6. Pull the IT device apart. Hold the device with the wells between thumb and forefinger and, with the other hand, pull out the dipstick holder (with the label). Place the wells

on a flat surface, insert the legs of the dipstick holder into the holes beside the conjugate well (with coloured line) so that the dipstick end reaches the bottom of the conjugate well. Allow to stand for **10 minutes (when using serum, allow to stand for 5 - 10 minutes)**. The blood/conjugate (serum/conjugate) mixture should then be completely soaked up.

7. Transfer the dipstick to the second well (wash well) and allow to stand for **10 minutes (when using serum, allow to stand for 5 minutes only)**. The reaction field should then be completely cleared of blood or serum. The control band must be clearly visible.
8. Remove the dipstick from the wash well and click it back into the clear plastic piece. Close the wells with the well cover, break them off, and break the two legs off from the clear plastic piece. Discard them into a suitable waste container.
9. Read the reaction and interpret the results (see: Interpretation). The dipstick slide should be kept for future reference and for comparison in case of further tests for monitoring the efficiency of treatment.

For routine use, you may follow the schematic test procedure enclosed in each test package

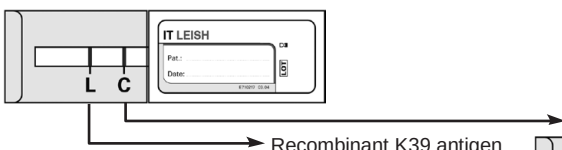
INTERPRETATION OF THE RESULTS

A) Principle

IT LEISH is a rapid detection test for antibodies against *Leishmania spp.* using a dipstick coated with recombinant K39 antigen (rK39, a repetitive epitope closely conserved among members of the *L. donovani* complex).

In case of the presence of such antibodies in the sample, the antibodies captured by the conjugate react with the specific coated rK39 antigen.

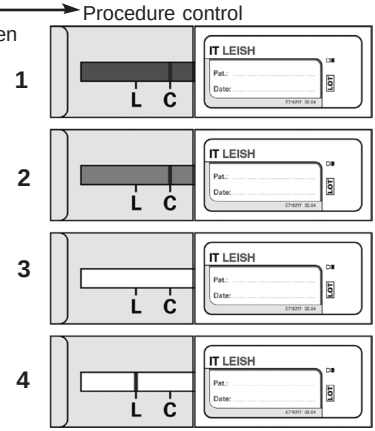
The reactions are demonstrated by the appearance of dark purple bands on the dipstick. The procedure control band must always be present to validate the result. Where the control band is absent, the test is not valid. In the event of repeated invalid results contact local distributer or Technical Support



B) Validation of the results

Results are valid if:

- the control band is clearly visible
- the reaction field is cleared of blood or serum



Results are NOT valid if:

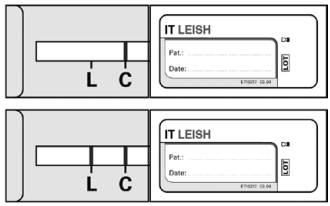
- the dipstick is not sufficiently cleared, reaction field remains red [1], or purple if use of serum[2]
- the control band is not present ([3] and [4]: even if there is one “reactive” band for L)

In such cases, repeat the test, precisely following the test procedure!

C) Interpretation of the reaction pattern

Negative reaction: a negative reaction indicates absence of detectable specific antibodies.

Positive reaction: a positive reaction indicates the presence of antibodies to *Leishmania spp.*



WARNING AND PRECAUTIONS

- For use by healthcare professionals only.
- Do not use if packaging or contents are damaged.
- Keep dry, do not use devices that have become wet.
- Do not use sharp objects to open the pouch.
- Careful handling the device, do not drop or damage. Do not take apart the lateral flow device.
- Only use materials provided in conjunction with this test.
- Do not re-use the test device or components.
- This product contains human or animal components. Handle with care.
- For hazard and precaution recommendations related to some chemical components in the kit buffer ampule, please refer to the pictogram(s) mentioned on the labels and the information supplied below:

Danger
Hazard statements
H319 - Causes serious eye irritation
H360FD - May damage fertility. May damage the unborn child
H412 - Harmful to aquatic life with long lasting effects
Precautionary Statements - EU (§28, 1272/2008)
P201 - Obtain special instructions before use
P273 - Avoid release to the environment
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P501 - Dispose of contents/ container in accordance with national regulations
P337 + P313 - If eye irritation persists: Get medical advice/attention
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P308 + P313 - IF exposed or concerned: Get medical advice/attention

CONTRAINDICATIONS

- Samples from patients with HIV infection and with suspected VL may not have detectable antibodies and therefore the test may be negative. Where the reaction is negative, cutaneous Leishmaniasis cannot be excluded as the test is 60% sensitive in this group of patients. Rare interactions with malaria, Chagas and cutaneous leishmaniasis have been observed, although the device has been shown to be effective.

LIMITATIONS

- Any modification of the described test procedure or use of other reagents may

modify the reaction pattern and invalidate the test.

- Remember that the result of **IT LEISH** is to be interpreted within the epidemiological, clinical and therapeutic context.
- Depending on the amount of antibody present in the sample, the strength of the reaction line varies. A very faint line must be considered positive for the presence of specific antibodies.
- The unused dipsticks present faint lines at the position of the 2 antibodies. These faint lines are present for in process control and quality control during the manufacturing process. They disappear completely as soon as the reaction field of the dipstick is in contact with the sample and conjugate.

BIBLIOGRAPHY

1. Evans TG (1993). Leishmaniasis. Infect Dis ClinNorth Am. 7(3), 527-546.
2. Burns JM Jr, Shreffler WG, Benson DR, Ghalib HW, Badaro R, Reed SG. (1993) Molecular characterization of a kinesin-related antigen of Leishmania chagasi that detects specific antibody in African and American visceral leishmaniasis. Proc Natl Acad Sci U S A. Jan 15;90(2):775-9.
3. Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, Wasunna MK, Bryceson AD. (2002) Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. Lancet Infect Dis. Aug;2(8): 494-501
4. Le Fichoux Y, Quaranta JF, Aufeuvre JP, Lelievre A, Marty P, Suffia I, Rousseau D, Kubar J (1999). Occurence of Leishmania Infantum parasitemia in asymptomatic blood donors living in area of endemicity in southern France. J Microbiol. 37(6): 1953-1957.
5. Shulman IA (2002). Parasitic infections and their impact on blood donor selection and testing. Arch Pathol lab Med. 118(4), 366-370.
6. Sundar S. et al. (2003): Evaluation of a new rapid immunochromatographic diagnostic test (**IT LEISH**) for indian visceral leishmaniasis and PKDL. Abstract - ASTMH 52nd Annual Meeting, Philadelphia (USA).
7. Amato Neto, V., et al., False-positive results of a rapid K39-based strip test and Chagas disease. Int J Infect Dis, 2009. 13(2): p. 182-5.
8. Rezaei, Z., et al., Diagnostic Performance of Three rK39 Rapid Diagnostic Tests and Two Direct Agglutination Tests for the Diagnosis of Visceral Leishmaniasis in Southern Iran. J Trop Med, 2022. 2022: p. 3569704.
9. Sundar, S., et al., Rapid, noninvasive diagnosis of visceral leishmaniasis in India: comparison of two immunochromatographic strip tests for detection of anti-K39 antibody. J Clin Microbiol, 2006. 44(1): p. 251-3.
10. Kassa, M., et al., Diagnostic accuracy of direct agglutination test, rK39 ELISA and six rapid diagnostic tests among visceral leishmaniasis patients with and without HIV coinfection in Ethiopia. PLoS Negl Trop Dis, 2020. 14(12): p. e0008963.
11. Kumar, D., et al., Comparative evaluation of blood and serum samples in rapid immunochromatographic tests for visceral leishmaniasis. J Clin Microbiol, 2013. 51(12): p. 3955-9.

GLOSSARY OF SYMBOLS

 Manufacturer

 Use-by-date

 Batch code

 Catalogue number

 Temperature limit

 Do not re-use

 Consult instructions for use

 *In vitro* diagnostic medical device

 Contains sufficient for <n> tests

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