Kalon Biological Ltd

A latex agglutination test for the diagnosis of visceral leishmaniasis

IVD 100 tests. Catalogue number L3-040 L3-041 CE

INTENDED USE

For the detection of *Leishmania spp* antigens in urine as an aid to the diagnosis of visceral leishmaniasis.

INTRODUCTION

Visceral leishmaniasis (VL) or kala-azar is a disease caused by infection with various species of the intracellular protozoan parasite *Leishmania*. The disease is transmitted by sandflies from reservoirs of infection which include domestic dogs, cats and wild animals. The disease is endemic on the Indian subcontinent, as well as parts of East Africa, South America and around the Mediterranean basin. It is characterised by a widespread infection of the monocytes, particularly in the liver and spleen. The symptoms of VL are not specific and include a prolonged, unexplained fever, hepatosplenomegaly, pancytopaenia and weight loss. In co-infections with HIV many of these clinical signs are missing and over 40% are VL antibody negative, yet co-infected patients have been found to have high urinary concentrations of the parasite antigen. Identification of the parasite in bone marrow or spleen aspirates by microscopy is the gold standard of diagnosis.

PRINCIPLE OF THE ASSAY

A urine sample, taken from a patient suspected of having VL, is pre-treated to inactivate heat-labile material capable of causing a false-positive reaction. Test latex - sensitised with antibodies raised against *L.donovani* antigen - is next mixed with the sample on the glass slide. The reaction can be read after mixing for two minutes. Antigen present in the sample causes cross-linking (agglutination) of the sensitised latex and is indicative of VL.

KIT PRESENTATION

- Test latex in dropper bottle, contains sodium azide preservative (0.1%). 5 mL
- Positive control in dropper bottle, contains sodium azide preservative (0.1%). 5 mL
- Negative control in dropper bottle, contains sodium azide preservative (0.1%). 5 mL
- Empty sample tubes (*not included in kit L3-041*) 100
- Wooden mixing sticks 100
- Reusable glass test slide 1

Store the reagents refrigerated (2° to 8°C).

DO NOT ALLOW TO FREEZE.

ADDITIONAL REQUIREMENTS

- Boiling water bath
- Micropipettes to deliver 50 µL. and disposable tips.
- Clock or watch (to read 2 minutes accurately).
- Disposable paper towels.
- For kit L3-041 only: sample tubes. 2 mL screw capped microcentrifuge tubes are recommended.

SPECIMEN COLLECTION AND STORAGE

1) Treat all specimens as potentially infectious.
2) Collect a urine sample into a clean, preferably sterile, container.
3) Store for up to 7 days refrigerated (2 to 8°C)
4) Specimens can be stored for longer periods either frozen (-20°C) or by the addition of sodium azide to 1 g/L.

TEST PROCEDURE

Sample pre-treatment

Pre treatment of all urine samples is **essential** to eliminate false positive results.

1) Label sufficient sample tubes for each patient to be tested.
2) Transfer from 0.25 to 1 mL of each urine sample into the sample tubes.
3) Place the sample tubes in a rack immersed in **boiling** water and heat for **5 minutes**.
4) Allow the samples to cool to the ambient temperature before performing the test.

Latex test

1) Bring all reagents to the ambient temperature.
2) Shake the test latex well immediately before use.
3) Add 50 µL of the treated urine sample to a reaction zone on the glass slide.
4) Add one drop of test latex
5) Stir both liquids to a completely homogenous mixture that covers the whole surface of the reaction zone.
6) Tilt the glass slide with a rotating action - clockwise and anticlockwise - continuously for two minutes.
7) After 2 minutes, read the degree of agglutination obtained.

Procedural Notes

For every assay, run the negative control in a reaction zone next to the test sample(s) to distinguish between a weak positive and negative result.

It is very important to read the negative control and all test samples after exactly the same reaction time (of two minutes).

Use the positive control to monitor the performance of the Test latex. It is recommended to run the Positive Control the first time the kit is used and periodically when removed from storage.

INTERPRETATION OF RESULTS

Record the degree of agglutination as follows

Appearance	Result
The latex has agglutinated and much has collected around the edge of the reaction zone.	positive +++
Agglutinated particles can clearly be seen against a background of granular latex.	positive ++
Agglutination can just be discerned when compared to the negative control.	positive +
No agglutination compared to negative control	negative

LIMITATIONS

A false positive rate in excess of 20% is seen in samples which have not been pre-treated according to protocol.

This test diagnoses only current and active VL infections.

To date, the indications are that "latent" leishmania infections are not detected.

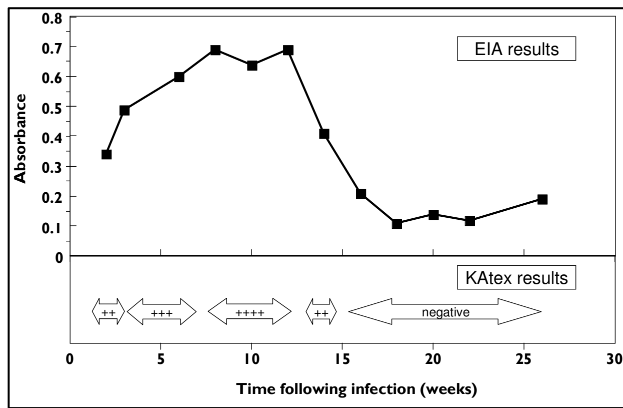
The results from this test are intended to be an aid to diagnosis only. Each clinician must interpret the results in light of the patient's clinical history, symptoms and other diagnostic procedures.

EXPECTED RESULTS

Performance (1)

Cotton rats were experimentally infected with *L.donovanii*. Treatment was commenced on week 12 and urine samples were taken at two weekly intervals. The presence of leishmania antigen was monitored using an enzyme immunometric assay (EIA) and *KA*tex. A typical result is shown in the figure 1.

Figure 1.



Performance (2)

*KA*tex was evaluated in a field trial conducted in the Gedarif State of Sudan for which spleenic aspirates, urine and blood samples were taken from 62 patients suspected of having VL. The *KA*tex and microscopy results are summarised in table 1.

Table 1.

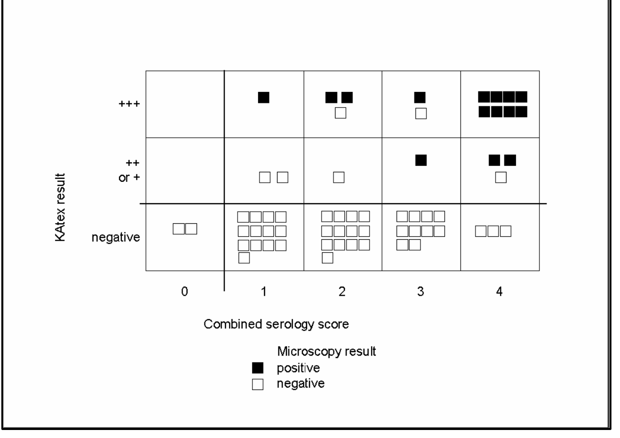
	Microscopy	*KA*tex +	*KA*tex -
Parasites observed	15	15	0
No parasites observed	47	6	41
Relative sensitivity (%)		100%	
Relative specificity (%)		87%	

*KA*tex was evaluated alongside four serological tests. See table 2. As there was only poor agreement between these serological tests, a combined serological score was made (4, all tests positive; 0 no tests positive). The combined score compared with the *KA*tex result for urinary antigen is shown in figure 2.

Table 2.

Serology test	Definition of positive result
DAT (direct agglutination test)	titre > 1:1600
ELISA (Novum, Germany)	result > 2 × cut-off OD
IFAT. Positive	titre > 80
K39 dipstick test	red line at upper end of dipstick

Figure 2.



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