LEISHMANIA IFA IgG

PLEDOG: Indirect immunofluorescent assay (IFA) kit for the diagnosis of Leishmania infantum IgG antibodies in human serum.

INTRODUCTION:

Leishmania infantum is a causal agent of kala-azar or visceral leishmaniosis and oriental Sore. The parasitism is endemic in the mediterranean area. Kala-azar is a serious disease characterized by fever, splenomegaly, anemia, weight loss and leukopenia; it can cause fever of unknown origin. The incidence of L. infantum infection in AIDS patients is very high. The IFA is generally accepted as suitable for the serological diagnosis of the systemic form of the disease, due to its high sensitivity and specificity. A low sensitivity is found in immunosuppressed people as in any antibody-detection based test carried out in these patients.

PRINCIPLE OF THE TEST:

The IFA method is based upon the reaction of antibodies in the sample, tested with the antigen adsorbed on the slide surface. The specific antibodies present in the sample react with the antigen, and the immunoglobulins not bound to the antigen are removed in the washing step. In the next step, the antigen-antibody complexes react with the fluorescein-labeled anti-human globulin. It can be examined using an immunofluorescence microscope.

KIT FEATURES:

All reagents, except for the PBS, are supplied ready to use. All the reagents have a number assigned for an easy identification. In the Assay Procedure, the numbers of the reagents to be used in each step are indicated.

KIT CONTENTS:

1. VIRCELL LEISHMANIA SLIDE: 10 slides of 10 wells each, coated with L. infantum promastigotes grown in RPMI-1640 medium, formaldehyde treated and acetone fixed.
2. VIRCELL PBS: 1 vial of PBS pH 7.2 powder to reconstitute with 1 l of distilled water.
3. VIRCELL LEISHMANIA IgG POSITIVE CONTROL: 200 µl of positive control serum, containing sodium azide.
4. VIRCELL LEISHMANIA NEGATIVE CONTROL: 200 µl of negative control serum, containing sodium azide.
5. VIRCELL ANTI-HUMAN IgG FITC CONJUGATE: 2 vials with 1.1 ml of fluorescein-labeled anti-human IgG fluorescein conjugate in a phosphate buffer containing Evan’s blue, sodium azide and a protein stabilizer.
6. VIRCELL MOUNTING MEDIUM: 3 ml of mounting medium: buffered glycerol, containing sodium azide.

Store at 2-8°C and check expiration date.

Materials required, but not supplied:

- Adequate precision micropipettes.
- Thermostatted incubator.
- Distilled water.
- 24x60 mm coverslips.
- Fluorescence microscope and suitable filters according to the manufacturer’s recommendations.
- Humid chamber.

STORAGE REQUIREMENTS:

Store at 2-8°C. Do not use the kit reagents beyond the expiration date printed on the label. Kits are stable through the end of the month indicated in the expiration date, when stored closed and at 2-8°C.

STORAGE OF REAGENTS ONCE OPENED:

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>STABILITY AND STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituted PBS</td>
<td>4 months at 2-8°C, never beyond its expiration date</td>
</tr>
<tr>
<td>Rest of the components</td>
<td>Refer to package label for expiration date</td>
</tr>
<tr>
<td></td>
<td>(at 2-8°C)</td>
</tr>
</tbody>
</table>

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations. Use only the amount of PBS, control serum and conjugate solutions required for the test. Do not return the excess solution into the bottles. After reconstitution, store PBS at 2-8°C and do not use if turbidity appears.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For in vitro diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only the PBS, mounting medium solutions and slides are compatible with the equivalents from other VIRCELL IFA references and lots. The rest of the components are compatible with other kits when the lot is the same.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Do not use in the event of damage to the package.
5. Never pipette by mouth.
6. Conjugates and controls in this kit include substances of animal origin. Controls include as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for HBsAg, Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. The wells are coated with inactivated L. infantum antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that these or other infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
7. Conjugate, mounting medium and controls contain sodium azide (concentration <0.1%). Avoid contact with acids and heavy metals.
8. Mounting medium contains glycerol. Avoid contact with acids and keep away from high temperatures.
9. Evan’s blue (concentration <0.1%) is a carcinogen. Avoid contact with skin or eyes. In case of contact with this solution, rinse thoroughly with water and seek medical attention.
10. Use only protocols described in this insert. Incubation times and temperatures other than specified may give erroneous results.
11. Cross-contamination of patient specimens on a slide can cause erroneous results. Take precautions to avoid it.
12. Microscope optics, light source condition and type will affect the fluorescence quality.
13. Do not leave the reagents at room temperature longer than absolutely necessary.
14. Each slide can be use only once. Do not break it, and do not reuse the wells not used.
15. The glass elements contained in kits could cause physical damage in the event of break. Handle with care.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8°C) upon collection or frozen (-20°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed, to avoid immunoglobulin titer decrease, specially IgM. Do not use hyperlipemic or contaminated sera. Samples containing particles should be clarified by centrifugation.

PRELIMINARY PREPARATION OF THE REAGENTS:
Only the PBS must be prepared in advance. Add the contents of the vial to 1 litre of distilled water. Shake it until the complete dissolution. Once diluted, store at 2-8°C.

ASSAY PROCEDURE:
1.-Bring all reagents to room temperature before use. Allow the slides to reach room temperature before opening.
2.-Prepare a 1/40 and 1/80 dilution of serum samples by adding 10 µl of sample to 390 µl of PBS (1/40 dilution). Make twofold dilutions with 50 µl of PBS (1/80 dilution). The control sera should not be diluted.
3.-Apply 20 µl of 1/40 and 1/80 dilution in two slide wells. Do the same with the positive and negative controls.
4.-Incubate slide in a humid chamber for 30 minutes at 37°C.
5.-Rinse slide briefly with a gentle stream of PBS (avoid directing PBS at wells) and immerse for ten minutes in PBS. Dip wash slide briefly in distilled water.
6.-Allow the slide to air dry.
7.-Add 20 µl of anti-human IgG FITC conjugate solution to each well. (No dilution required).
8.-Repeat steps 4, 5 and 6.
9.-Add a small drop of mounting medium to each well and carefully cover with a coverslip.
10.-Read the slide as soon as possible in a fluorescence microscope at 400x magnification. If this is not possible, store in the dark at 2-8°C up no more than 24 hours, until observation.
11.-If the testing dilutions, further annalize with up to 1/640 dilutions.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available. The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:
Positive and negative controls should be included into each test run. It allows the validation of the assay and kit.

The observed fluorescence pattern should be:

INTERPRETATION OF RESULTS:
The serum titer is the highest dilution at which a positive reaction is observed. The reaction is positive when peripheral, cytoplasmic and flagellar fluorescence can be observed.

Sera with non-specific reactivity were excluded from final calculations.

INTRA-ASSAY PRECISION:
3 sera (2 positive and 1 negative) were individually pipetted in groups of 5 in a single assay performed by the same operator in essentially unchanged conditions. Titer shifts of no more than one dilution were observed.

INTER-ASSAY PRECISION:
3 sera (2 positive and 1 negative) were individually pipetted on 5 different conditions in which the operator or the test day were different. Titer shifts of no more than one dilution were observed.

FOR IN VITRO DIAGNOSTIC USE
Manufacturer: VIRCELL, S.L. Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre: 18320 Santa Fe *GRANADA* SPAIN* Tel.+34.958.441264* Fax+34.958.510712 http://www.vircell.com
CROSS REACTIVITY AND INTERFERENCES:

3 samples known to be positive for *Toxoplasma gondii* (taxonomic relation), were assayed.
The negative results of the test demonstrate the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>IVD</td>
<td><em>In vitro diagnostic medical device</em></td>
</tr>
<tr>
<td>2ºC</td>
<td>Store at 2-8ºC</td>
</tr>
<tr>
<td>Lot</td>
<td>Batch code</td>
</tr>
<tr>
<td>Ref</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>Wells</td>
<td>&lt;X&gt; wells</td>
</tr>
<tr>
<td>USE</td>
<td>Use by (expiration date)</td>
</tr>
<tr>
<td>X</td>
<td>Contains sufficient for &lt;X&gt; tests</td>
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SUMMARY OF THE ASSAY PROCEDURE:

1. Add sera dilution and controls to the slide wells.
2. Humid chamber at 37ºC for 30 minutes.
3. Wash twice with PBS and once with distilled water.
4. Air dry.
5. Add fluorescein conjugate.
6. Humid chamber at 37ºC for 30 minutes.
7. Wash twice with PBS and once with distilled water.
8. Air dry.
10. Cover with a coverslip.
11. Read the slide at fluorescence microscopy 400x.

LITERATURE:


For any question please contact customer service: technicalservice@vircell.com

REVISED: October-05