

LEISHMANIA IFA SLIDE

For *in vitro* diagnostic use

SLEDO: Indirect immunofluorescent assay slides to test antibodies against *Leishmania infantum* in human serum/plasma.

INTRODUCTION:

Leishmania infantum is a causal agent of kala-azar or visceral leishmaniasis 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:

The slide has a number assigned for an easy use with the corresponding VIRCELL IFA kit.

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.

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

Materials required, but not supplied:

- Adequate precision micropipettes.
- Thermostatized incubator.
- Distilled water.
- 24x60 mm coverslips.
- Fluorescence microscope and suitable filters according to the manufacturer's recommendations.
- Humid chamber.
- VIRCELL IFA kit of the corresponding specificity.

STORAGE REQUIREMENTS:

Store at 2-8°C. Do not use beyond the expiration date printed on the label. Slides 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:

Use immediately once opened the package.

STABILITY AND HANDLING OF REAGENTS:

Handle in aseptic conditions to avoid microbial contaminations.

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. Only use with the corresponding VIRCELL IFA kits.
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. 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. Use only protocols described in this insert. Incubation times and temperatures other than specified may give erroneous results.
8. Cross-contamination of patient specimens on a slide can cause erroneous results. Take precautions to avoid it.
9. Microscope optics, light source condition and type will affect the fluorescence quality.
10. Do not leave at room temperature longer than absolutely necessary.
11. Each slide can be use only once. Do not break it, and do not reuse the wells not used.
12. 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/plasma 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 samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

ASSAY PROCEDURE:

Slides are aimed to be used with VIRCELL IFA kit reagents of the corresponding specificity. The numbers indicated in the assay procedure are the numbers assigned in the corresponding VIRCELL IFA kit.

IgG determination:

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 samples by adding 10 µl of sample to 390 µl of PBS **2** (1/40 dilution). Make twofold dilutions with 50 µl of PBS (1/80 dilution). The control sera **3** and **4** should not be diluted.
3. Apply 20 µl of 1/40 and 1/80 dilution in two slide wells **1**. Do the same with the positive **3** and negative **4** controls.
4. Incubate slide in a humid chamber for 30 minutes at 37°C.



5. Rinse slide **1** briefly with a gentle stream of PBS **2** (avoid directing PBS at wells) and immerse for ten minutes in PBS. Dip wash slide briefly in distilled water.
6. Allow the slide **1** to air dry.
7. Add 20 µl of anti-human IgG FITC conjugate solution **5G** to each well. (No dilution required).
8. Repeat steps 4, 5 and 6.
9. Add a small drop of mounting medium **6** 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 to no more than 24 hours, until observation.
11. If the testing dilutions, further analyze with up to 1/640 dilutions.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control (Q.C.) testing before batch release. Final Q.C. results for each particular lot are available.

VALIDATION PROTOCOL FOR USERS:

The validation protocol for users is the one indicated in the corresponding VIRCELL IFA kit:

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:

Positive control: Peripheral, cytoplasmic and flagellar fluorescence.

Negative control: Red cellular pattern.

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.

The reaction is negative when a red cellular pattern can be observed.

Results different from the specified in this insert should not be considered as positive.

IgG and IgM antibodies show a different behaviour during the primoinfections and reinfections. In a primoinfection IgG and IgM appear in almost all cases (IgM appears before than IgG). In reinfections IgM antibodies do not appear in all cases, therefore IgG detection is the only method useful to perform the diagnosis. High titers of IgG can exist in a lot of diseases during the whole patient life, while IgM, generally, only is measurable in sera during 2 or 3 months after the infection, and therefore is a suitable marker of recent infection.

Although a 1/40 titer suggests infection, it is advisable to titrate sera positive at this dilution. Confirmation of the diagnosis by direct demonstration of the parasite in the bone marrow either by staining or culture is advisable.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma. Slides are aimed to be used with VIRCELL IFA kits of the corresponding specificity. VIRCELL does not accept responsibility for the results obtained in case of use with reagents from other origins.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In

particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.

3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures.

4. This test will not indicate the site of infection. It is not intended to replace isolation.

5. Lack of significant rise in antibody level does not exclude the possibility of infection.

6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed, or a second sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.

7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.

8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.

9. In visceral leishmaniasis immune response of high intensity and can be detected by IFA. In endemic areas cross-reactions with *Trypanosoma cruzi* may be expected, therefore serological results must be confirmed by alternative techniques.

10. In patients with antinuclear antibodies a fluorescence pattern over the kinetoplast and nucleus can be found. It should not be considered as specific, neither a sign of disease.

11. The performance results showed correspond to comparative studies with commercial predictive devices in a defined population sample. Small differences can be found with different populations or different predictive devices.

PERFORMANCES:

The detailed performances were obtained with the corresponding VIRCELL IFA kit:

• SENSITIVITY AND SPECIFICITY:

141 serum/plasma samples were assayed with LEISHMANIA IFA IgG against another commercial available IFA kit.

The results were as follows:

	Samples No.	Sensitivity	Specificity
IgG	141	100%	100%

Samples 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.



• CROSS REACTIVITY AND INTERFERENCES:

3 samples known to be positive for *Toxoplasma gondii* (taxonomic relation), were assayed for IgG testing.

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:

	In vitro diagnostic medical device
	Use by (expiration date)
	Store at x-y°C
	Contains sufficient for <n> test
	Batch code
	Catalogue number
	Consult instructions for use
	<X> wells

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