**INTRODUCTION:**

Invasive candidiasis is a disease of fungal etiology with an increasing incidence, specially in immunosuppressed patients (graft recipients, neutropenic and AIDS patients, etc), long-stay hospitalized and catheterized patients, as well as those subjected to extensive surgery or receiving broad spectrum antibiotic therapy. The diagnosis of invasive candidiasis is specially difficult due to the absence of pathognomonic symptoms specific of the disease and the low recovery of the microorganism in culture. The present commercial diagnostic techniques show a low specificity and sensitivity, being some of them too difficult to be carried out in a Clinical microbiology laboratory. In order to overcome these problems, a technique for the serologic diagnosis of invasive candidiasis has been developed. This test is based upon the detection of specific antibodies against antigens located on the cell wall surface of the micelium of *Candida albicans*. These antibodies are normally present in sera from patients with invasive candidiasis caused by *C. albicans* and other species of this genus. The assay is performed by indirect immunofluorescence after removal of other anti-candida antibodies usually found in most human serum, thus avoiding possible false positive results. This test can be performed with equipment available in a clinical laboratory and is completed in 3 hours.

**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 binded 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 CONTENTS:**

- VIRCELL CANDIDA ALBICANS SLIDE: 10 slides of 10 wells each, coated with *C. albicans*, strain NCPF-3153, grown in Sabouraud broth, heat inactivated and acetone fixed.
- VIRCELL PBS: 1 vial of PBS pH 7.2 powder to reconstitute with 1 l of distilled water.

**STORAGE OF REAGENTS ONCE OPENED:**

- Reconstituted PBS: 4 months at 2-8ºC, never beyond its expiration date.
- Rest of the components: Refer to package label for expiration date (at 2-8ºC).

**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.

**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 Immunodeficency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. The wells and the sorbent contain inactivated *C. albicans* 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 used 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 1/4 dilution of serum samples by adding 10 µl of sample to 30 µl of PBS (1/4 dilution). In vials apart prepare aliquots of yeast phase of C. albicans (VIRCCELL CANDIDA SORBENT) of 80 µl by vial. Add to these vials, 20 µl of serum dilution (1/4), shake the tube and label as 1/20 dilution. Incubate the tube at room temperature for 1 hour with gentle shaking. Centrifuge at 700 g for 5 minutes. Take out the supernatant. Make twofold dilutions with 50 µl of PBS up to 1/160 dilution. The control sera and controls should not be diluted nor adsorbed.

3. Apply 20 µl of the corresponding serum dilutions on the slide wells.

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 to 24 hours, until observation.

**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:

- Positive control: Apple-green fluorescence on the mycellium phase, leaving the yeast phase red.
- Negative control: Red cell pattern over both the mycellium and yeast phases.

**INTERPRETATION OF RESULTS:**

The serum titer is the highest dilution at which a positive reaction is observed.

The reaction is positive when apple-green fluorescence on the mycellium phase, leaving the yeast phase red can be observed.

The reaction is negative when red cell pattern over both the mycellium and yeast phases can be observed.

In high titer sera, lower dilutions may show weak fluorescence over the yeast phase, but the antibody titers must be determined taking into account only reaction on the mycellium phase. If anti-yeast antibodies are observed in all dilutions, serum must be absorbed again. A titer higher or equal to 1/160 is suggestive of invasive candidiasis.

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

**LIMITATIONS:**

1. This kit is intended to be used with human serum.

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. A negative result in an immunosuppressed patient does not exclude the presence of infection.

6. 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.

**PERFORMANCE**

**SENSITIVITY AND SPECIFICITY:**

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<th>SAMPLE</th>
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<td>Sensitivity</td>
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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.
CROSS REACTIVITY AND INTERFERENCES:
8 samples known to be positive for other microorganism (aspergillus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumanii, Streptococcus pneumoniae), 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:

SUMMARY OF THE ASSAY PROCEDURE:

1. Dilute sera 1/4
2. Add diluted sera to C. albicans sorbent aliquots
3. Make twofold dilutions
4. Humid chamber 30 minutes at 37°C
5. Wash twice with PBS and once with distilled water
6. Air dry
7. Add fluorescein conjugate 5G
8. Humid chamber 30 minutes at 37°C
9. Wash twice with PBS and once with distilled water
10. Air dry
11. A small drop of mounting medium
12. Cover with a coverslip
13. Read the slide at fluorescence microscopy 400x
LITERATURE:


For any question please contact customer service:

technicalservice@vircell.com

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