ADENOVIRUS IFA IgG

INTRODUCTION:

Adenovirus is an important respiratory tract agent that can produce pneumonia and bronchiolitis in small children. Adenovirus infections can be diagnosed in the laboratory by three classical methods: direct antigen detection on clinical specimens; culture techniques to isolate and identify the virus and serological tests to measure rises in antibodies. Immunoglobulin G (IgG) is the predominant antibody class measured but sometimes the IgM detection is the only way to reach a diagnosis. The most widely accepted tests are complement fixation (CF) and ELISA. The IFA test is being increasingly used to detect IgG and IgM. Sometimes, there is no serological response, but the IgM detection is often the only way to reach a diagnosis.

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 CONTENTS:

- VIRCELL ADENOVIRUS SLIDE: 10 slides of 10 wells each, coated with HEp-2 cells infected with adenovirus, Adenoid 71 strain (ATCC VR-1), formaldehyde treated, acetone fixed and mixed with non-infected cells.
- VIRCELL PBS: 1 vial of PBS pH 7.2 powder to reconstitute with 1 l of distilled water.
- VIRCELL ADENOVIRUS IgG POSITIVE CONTROL: 200 µl of positive control serum, containing sodium azide.
- VIRCELL ADENOVIRUS NEGATIVE CONTROL: 200 µl of negative control serum, containing sodium azide.
- 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.

STORAGE REQUIREMENTS:

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.

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 adenovirus 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.

FOR IN VITRO DIAGNOSTIC USE

Manufacturer: VIRCELL, S.L. Pza. Domínguez Ortiz 1. Polígono Industrial Dos de Octubre.18320 Santa Fe *GRANADA* SPAIN* Tel:+34.958.441264* Fax:+34.958.510712
http://www.vircell.com
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 1/128 and 1/256 dilution of serum samples by adding 10 µl of sample to 1270 µl of PBS (1/128 dilution). Make twofold dilutions with 50 µl of PBS (1/256 dilution). The control sera and should not be diluted.
3. Apply 20 µl of 1/128 and 1/256 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 slide briefly in distilled water.
6. Allow the slide to air dry.
7. Add a small drop 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 1/2048 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:
Positive control: Apple green fluorescence on nucleus and cytoplasm.
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 apple green fluorescence on nucleus and cytoplasm can be observed.

The reaction is negative when a red cellular pattern can be observed. Occasionally, a serum may react giving a positive fluorescence with over 50% of the cell. This pattern corresponds to a non-evaluable inespecific reaction.

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 reinfecions. In a primoinfection IgG and IgM appear in almost all cases (IgM appears before than IgG). In reinfecions IgM antibodies do not appear in all cases, therefore IgG detection is the only method useful to perform the diagnosis. High titters 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.

Seroconversion should be demonstrated to confirm the diagnosis, since high titters can be found in healthy individuals. With a single sample of serum, it is very important to take into account the seasonal variations, the patient’s age and the prevalence of the illness in the geographical area, for an adequate evaluation of the results. Generally, titters higher or equal to 256, together with clinical manifestations, are strongly suggestive of a viral disease.

**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.
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 serum 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 fetus 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. Sera from patients with autoimmune diseases may give a non-specific reaction over cells when using IFA. Those sera cannot be evaluated with this method.

**PERFORMANCE**

**SENSITIVITY AND SPECIFICITY:**

96 serum samples were assayed with ADENOVIRUS IFA IgG against an commercial available ELISA kit.

The results were as follows:

<table>
<thead>
<tr>
<th>SAMPLE NR</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
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<tbody>
<tr>
<td>IgG</td>
<td>97%</td>
<td>95%</td>
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</table>

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:
10 samples known to be positive for other virus of the syndromic group (respiratory syncytial virus (RSV), influenza A and B, parainfluenza), 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>
</tr>
</thead>
<tbody>
<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>℃</td>
<td>Use by (expiration date)</td>
</tr>
<tr>
<td>2°C</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>▲</td>
<td>Contains sufficient for &lt;X&gt; tests</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>📜</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>&lt;X&gt; wells</td>
<td>&lt;X&gt; wells</td>
</tr>
</tbody>
</table>

SUMMARY OF THE ASSAY PROCEDURE:

1. Add sera dilution and controls to the slide wells
2. Humid chamber 30 minutes at 37°C
3. Wash twice with PBS and once with distilled water
4. Air dry
5. Add fluorescein conjugate
6. Humid chamber 30 minutes at 37°C
7. Wash twice with PBS and once with distilled water
8. Air dry
9. A small drop of mounting medium
10. Cover with a coverslip
11. Read the slide at fluorescence microscopy 400x
LITERATURE:


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

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