SMYPN: Slides for the diagnosis of Mycoplasma pneumoniae antibodies in human serum by indirect immunofluorescent assay (IFA).

INTRODUCTION:

Atypical pneumonia produced by Mycoplasma pneumoniae is most frequently found in children and adolescents. The isolation in culture is tedious and consequently serological diagnosis is most frequently performed. Due to the difficult, to fix the microorganism to slides, M. pneumoniae antigen is fixed previously to cells. The most traditional serological method has been the Complement Fixation (CF) whereas enzyme-linked immunosorbent assay (ELISA) and IFA are now more often used. IgG IFA titers of 64 suggest recent infection, since the 97% of the healthy adult controls present lower titers. The determination of IgM by IFA has a sensitivity of 87% with respect to CF, together with a specificity of 100%.

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:

- VIRCELL MYCOPLASMA PNEUMONIAE SLIDE: 10 slides of 10 wells each, coated with M. pneumoniae, FH strain (ATCC 15531), grown in McCoy cells, formaldehyde inactivated, acetone fixed and mixed with non-infected cells.

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 M. pneumoniae 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.
13. Check that a visible precipitate appears after the addition of the sorbent to the sample.

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.

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 1/64 and 1/128 dilution of serum samples by adding 10 µl of sample to 630 µl of PBS (1/64 dilution). Make twofold dilutions with 50 µl of PBS (1/128 dilution). The control sera and should not be diluted.
3. Apply 20 µl of 1/64 and 1/128 dilution to 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.

FOR IN VITRO DIAGNOSTIC USE

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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 2-8ºC 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. 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: 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. Results different from the specified in this insert should not be considered as positive.

IgG and IgM antibodies show a different behaviour during the primoinfection 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 titer 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 it is a suitable marker 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.

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

SENSITIVITY AND SPECIFICITY:
71 serum samples were assayed with MYCOPLASMA PNEUMONIAE IFA IgG against an ELISA kit.

The results were as follows:

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<tr>
<th>SAMPLE NR</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
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<tbody>
<tr>
<td>IgG</td>
<td>94.6%</td>
<td>97.0%</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:
12 samples known to be positive for other bacteria of the syndromic group (Legionella pneumophila, Chlamydophila pneumoniae, Coxiella burnetti), 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:

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SUMMARY OF THE ASSAY PROCEDURE:

1. Add sera dilution and controls to the slide wells
2. Humid chamber 30 minutes at 37°C for IgG testing
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. Air dry
12. Read the slide at fluorescence microscopy 400x

LITERATURE:


For any question please contact customer service:
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