PCOBUG: Indirect immunofluorescent assay (IFA) kit for the diagnosis of *Coxiella burnetii* IgG antibodies in human serum.

INTRODUCTION:

Q fever is a systemic disease caused by *Coxiella burnetii* that can produce fever, atypical pneumonia, hepatitis or endocarditis. The diagnosis is based on serological methods since isolation from clinical samples is difficult. *C. burnetii* expressed phase I antigen when isolated from humans and animals and phase II antigen when isolated from cell culture. Antibodies against phase II antigen are predominant in the acute phase of the disease. The tests normally employed are Complement Fixation (CF) and IFA. The IFA test is the best method to detect specific IgM responses. In the acute phase, IgM antibodies are detected by IFA between the second week and the third month of the disease, proving to be the most useful test for endemic areas. The highest level of IgG antibody is detected by IFA after 4-8 weeks and by CF after 12 weeks. A titer of 256 is generally considered a sign of recent disease. In those cases of acute infection where IgG antibodies are detected at higher titers, it is highly probable to find IgM as well.

PRINCIPLE OF THE TEST:

The IFA method is based upon the reaction of antibodies in the sample, tested with the antigen absorbed 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.

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 (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 *C. burnetii* 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.

VIRCELL COXIELLA 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.

VIRCELL MOUNTING MEDIUM: 3 ml of mounting medium: buffered glycerol, containing sodium azide.

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

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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 (2-8ºC) upon collection or frozen (-20ºC) 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 2-8ºC.

ASSAY PROCEDURE:

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 in two slide wells. Do the same with the positive and negative controls.
4.-Incubate slide in a humid chamber for 30 minutes at 37ºC.
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 annealize 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 of coco-bacillar morphology.
Negative control: No fluorescence.

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 of coco-bacillar morphology can be observed. The reaction is negative when no fluorescence 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.

Seroconversion should be demonstrated to confirm the diagnosis, since high titers 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. The limit is variable for bacterial respiratory diseases depending on the prevalence, and can be establish in 256 for Legionella and 64 for Coxiella and Mycoplasma.

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 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.-Occasionally a sera may contain antibodies reacting with egg antigens, that give unspecific fluorescence with yolk sac, used to fix the antigen to the slide. When this occurs, the sera should not be analyzed by IFA.

PERFORMANCE

SENSITIVITY AND SPECIFICITY:

86 serum samples were assayed with COXIELLA BURNETII IFA IgG against another commercial available IFA kit. The results were as follows:

<table>
<thead>
<tr>
<th>SAMPLE NR</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>100.0%</td>
<td>98.1%</td>
</tr>
</tbody>
</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.

FOR IN VITRO DIAGNOSTIC USE

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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:

20 samples known to be positive for other bacteria of the syndromic group (Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila), other bacteria with phylogenetic relation (Rickettsia conorii) and antinuclear antibodies, 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>8ºC</td>
<td>Use by (expiration date)</td>
</tr>
<tr>
<td>2ºC</td>
<td>Store at 2-8ºC</td>
</tr>
<tr>
<td>LOT</td>
<td>Contains sufficient for &lt;X&gt; tests</td>
</tr>
<tr>
<td>REF</td>
<td>Batch code</td>
</tr>
<tr>
<td>i</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>WELLS</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>x</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 37ºC
3. Wash twice with PBS and once with distilled water
4. Air dry
5. Add fluorescein conjugate 5G
6. Humid chamber 30 minutes at 37ºC
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:

technicalservice@vircell.com

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