COXIELLA BURNETII IFA SLIDE

For *in vitro* diagnostic use

SCOBU: Indirect immunofluorescent assay slides to test antibodies against *Coxiella burnetii* in human serum/plasma.

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 specifical IgM responses. In the acute phase, IgM antigen 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 ìs 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 higr 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 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 COXIELLA BURNETII SLIDE: 10 slides of 10 wells each, coated with purified *C. burnetii* phase II, Nine Mile strain (ATCC 616-VR), grown in MRC-5 cells. The bacteria are formaldehyde inactivated, acetone fixed and suspended in 0.5% normal chicken yolk sac, to improve the adhesion and avoid bacterial aggregation.

Store at 2-8ºC 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 2-8ºC. 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 2-8ºC.

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 *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. 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/plasma 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 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 1/64 and 1/128 dilution of samples by adding 10 µl of sample to 630 µl of PBS 2 (1/64 dilution). Make twofold dilutions with 50 µl of PBS (1/128 dilution). The control sera 3 and 4 should not be diluted.
3. Apply 20 µl of 1/64 and 1/128 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 37ºC.
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 2-8ºC up no more than 24 hours, until observation.
11. If the testing dilutions, further annalize with up to 1/2048 dilutions.

IgM determination:

1. Bring all reagents to room temperature before use. Allow the slides to reach room temperature before opening.
2. Prepare a 1/2 dilution of samples by adding 25 µl of sample to 25 µl of PBS 2. The control sera 3 and 4 should not be diluted.
3. Treat diluted samples with anti-human IgG sorbent 7, by adding 5 µl of diluted samples to 25 µl of sorbent and thoroughly mix. Control sera 3 y 4 must not be diluted nor sorbent treated. The treated samples can be used directly, or centrifuged to remove the precipitate, which does not interfere with the test.
4. Add 20 µl of sorbent-treated sample in every slide well 1. Do the same with positive 3 and negative 4 control.
5. Place the slide in a humid chamber and incubate at 37ºC for 90 minutes.
6. 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.
7. Allow the slide 1 to air dry.
8. Add 20 µl of anti-human IgM FITC conjugate solution 5M to each well. (No dilution required).
9. Incubate slide in a humid chamber for 30 minutes at 37ºC.
10. Repeat steps 6 and 7.
11. Add a small drop of mounting medium 6 to each well and carefully cover with a coverslip.
12. 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.

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 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, 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/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. Occasionally a sample may contain antibodies reacting with egg antigens, that give unespecific fluorescence with yolk sac, used to fix the antigen to the slide. When this occurs, the sample should not be annalyzed by IFA.
10. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

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

- **SENSITIVITY AND SPECIFICITY:**

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

	Samples No.	Sensitivity	Specificity
IgG	86	100%	98.1%

74 serum/plasma samples were assayed with COXIELLA BURNETII IFA IgM against another commercial available IFA kit.
The results were as follows:

	Samples No.	Sensitivity	Specificity
IgM	74	100%	97.6%

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:**

20 samples known to be positive for other bacteria of the syndromic group (*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila*), other bacteria with phylogenetic relation (*Rickettsia conorii*) and antinuclear antibodies, were assayed for IgG testing.
20 samples known to be positive for other bacteria of the syndromic group (*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila*), other bacteria with phylogenetic relation (*Rickettsia conorii*) and antinuclear antibodies, were assayed for IgM testing.

The negative results of the test demonstrate the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

OTHER INTERFERENCES STUDIES:
An IFA assay was performed to 25 samples, known to be positive for rheumatoid factor, to determine IgG and IgM antibodies against 2 viral and 2 bacterial antigens. It also was performed an IFA assay for IgG and IgM testing to another 2 samples determined for each one antigen. For IgM testing the samples were treated with anti-IgG sorbent. The results showed the efficacy of the sorbent to avoid interferences in IgM testing caused by rheumatoid factor.
The recommended sorbent has been tested and found effective to prevent false negative results due to an excess of IgG antibodies.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
X°C Y°C	Store at x-yºC
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

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