RICKETTSIA TYPHI IFA IgG

For *in vitro* diagnostic use

PRITYG: Indirect immunofluorescent assay kit to test IgG antibodies against *Rickettsia typhi* in human serum/plasma.

INTRODUCTION:

The rickettsiae that are pathogens of humans are subdivided into three major groups based on clinical characteristics of disease: spotted fever group (*Rickettsia rickettsii*, *R. akari*, *R. conorii*, *R. sibirica*, *R. australis*, *R. japonica*); typhus group *(R. prowazekii*, *R. typhi*); and scrub typhus group (*Orientia tsutsugamushi*). Most rickettsial pathogens are transmitted by ectoparasites such as fleas, lice, mites, and ticks.

Most symptoms associated with acute rickettsial infections are nonspecific (fever, headache, malaise) and require further tests to make an accurate diagnosis. Most tick-transmitted rickettsioses are accompanied by a maculopapular, vesicular, or petechial rash or an eschar at the site of the tick bite. Murine typhus is a zoonotic disease caused by *R. typhi,* which is maintained in nature by a rodent-flea cycle. Although it is a self-limiting febrile illness, life-threatening disease and death can occur.

Microimmunofluorescence is the most commonly used technique for the diagnosis of rickettsioses, allowing a simple and early diagnosis. But it does not allow differentiation of infection among rickettsiae of the same clinical group. Due to the difficulties of culturing these intracellular microorganisms, nucleic acid tests are used for the direct diagnosis of rickettioses.

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:

All reagents, except for the PBS, are supplied ready to use. All the reagents have a number assigned for an easy identification. In the Assay Procedure, the numbers of the reagents to be used in each step are indicated.

KIT CONTENTS:

1 VIRCELL RICKETTSIA TYPHI SLIDE: 10 slides of 10 wells each, coated with *Rickettsia typhi*, strain 18, grown in Vero cells, formaldehyde treated and acetone fixed. Suspended in 0.5% normal chicken yolk sac to improve the antigen adhesion and avoid the bacterial aggregation.

2 VIRCELL PBS: 1 vial of PBS pH 7.2 powder to reconstitute with 1 l of distilled water.

3 VIRCELL RICKETTSIA TYPHI IgG POSITIVE CONTROL: 200 µl of positive control serum, containing sodium azide.

4 VIRCELL RICKETTSIA TYPHI NEGATIVE CONTROL: 200 µl of negative control serum, containing sodium azide.

5G 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.

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

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

STORAGE REQUIREMENTS:

Store at the recommended temperature indicated. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are capped and stored at the indicated temperature.

STORAGE OF REAGENTS ONCE OPENED:

Reagents	Stability
Reconstituted PBS	4 months at 2-8ºC, never beyond its expiration date
VIRCELL SLIDE	Once opened, use it in the same day
Rest of the components	Refer to package label for expiration date (at 2-8º C)

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 2-8ºC 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 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 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/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.

PRELIMINARY PREPARATION OF THE REAGENTS:
Only the PBS must be prepared in advance. Add the contents of the vial 2 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 1/40 and 1/80 dilution of samples by adding 10 µl of sample to 390 µl of PBS 2 (1/40 dilution). Make twofold dilutions with 50 µl of PBS (1/80 dilution). The control sera 3 and 4 should not be diluted.
3. Apply 20 µl of 1/40 and 1/80 dilutions 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 these screening testing dilutions are positive, further annalize with up to 1/640 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.
Serum antibodies can be detected early by IFA (since the 7 th day after the onset of the symptoms). Titers 1/40 are usually considered positive.

LIMITATIONS:
1. This kit is intended to be used with human serum/plasma.
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:

- **SENSITIVITY AND SPECIFICITY:**

118 serum/plasma samples were assayed with RICKETTSIA TYPHI IFA IgG against another commercial available IFA kit.
The results were as follows:

	Samples No.	Sensitivity	Specificity
IgG	118	95%	100%

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:**

12 samples known to be positive for other bacteria of the syndromic group (Brucella, Salmonella), other bacteria with taxonomic relation (*Coxiella burnetii*) 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:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
Y°C / X°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

BIBLIOGRAPHY:

1. Blanco, J.R. and J.A. Oteo. 2006. Rickettsiosis in Europe. Ann. N. Y. Sci. 1078: 26-33

2. Bolaños-Rivero, Margarita, Évora Santana-Rodríguez, Alfonso Ángel-Moreno, Michele Hernández-Cabrera, José-María Limiñana-Canal, Cristina Carranza-Rodríguez, Antonio-Manuel Martín-Sanchez, Jose-Luis Pérez-Arrelano. 2011 Seroprevalence of *Rickettsia typhi* and *Rickettsia conorii* infections in the Canary Islands (Spain). Int. J. Infect. Dis. 15, e481-e485.

3. Civen, Rachel and Van Ngo. 2008 Murine Typhus: An unrecognized Suburban Vectorborne Disease. Clinical practice, CID 2008:46 913-918.

4. Gideon, Endemic Typhus Group: Global Status. 2011 Typhus endemic. 2011 edition, 1-50.

5. Hebert, G. Ann, T. Tzianabos, W.C. Gamble and W. A. Chappell. 1980. Development and characterization of high-titered, group-specific Fluorescent-Antibody Reagents for Direct Identification of Rickettsiae in clinical specimens. J. Clin. Microbiol. MAy, p. 503-507.

6. Lledó L, González R, Gegúndez MI, Beltrán, M, Saz JV. Epidemiological study of rickettsial infections in patients with hypertransaminemia in Madrid (Spain). Int. J. Environ. Res. Public Health, 2009, 6, 2526-2533.

7. Jensenius, Mogens, Pierre-Edouard Fournier, and Didier Raoult. 2004. Rickettsioses and the International Traveler. Travel Medicine, CID 204:39, 15 Nov, 1493-1499.

8. Phetsouvanh, Rattanaphone, Stuart D. Blacksell, Kemajittra Jenjaroen, Nicholas P.J. Day, and Paul N. Newton. 2009. Comparison of Indirect Immunofluorescence Assays for Diagnosis of Scrub Typhus and Murine Typhus Using Venous Blood and Finger Prick Filter paper Blood Spots. Ann. J. Trop. Med. Hyg. 80(5), 837-840.

9. Raoult, D, Dasch, GA. Immunoblot cross-reactions among *Rickettsia*, *Proteus* spp. and *Legionella* spp. in patients with Mediterranean spotted fever. FEMS Imm and Med Microbiol 11 (1995) 13-18.

10. Teysseire, N, Raoult, D. Comparison of Western immunoblotting and microimmunofluorescence for diagnosis of mediterranean spotted fever. J Clin Microbiol, Feb. 1992, p. 455-460.

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REVISED: 2017/04