INTRODUCTION:

*R. conorii* is the causal agent of the mediterranean spotted fever. The onset of the disease is characterized by fever, exanthema and black spot at the site of the tick bite. Indirect immunofluorescence is the most commonly used technique, but it can be replaced by ELISA with similar sensitivity and specificity results. This technique can also be used for the detection of IgM antibodies. IgM can be detected after one week of illness.

PRINCIPLE OF THE TEST:

The ELISA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution.

KIT FEATURES:

All reagents, except for the washing solution, are supplied ready to use. Serum dilution solution and conjugate are coloured to help in the performance of the technique. Sample predilution is not necessary. Break-apart individual wells are supplied, so that the same number of wells is consumed than the number of tests performed.

KIT CONTENTS:

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>STABILITY AND STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x washing solution</td>
<td>4 months at 2-8°C</td>
</tr>
<tr>
<td>Rest of reagents</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. Do not let the plate dry between washing and reagent addition. Substrate solution is light sensitive. Avoid light exposure and discard if blue colour develops during storage. Substrate solution should not get in contact with oxidizers such as bleach solutions or metals. Make sure that no metal components come in contact with the substrate. Use only the amount of washing, serum dilution, conjugate and TMB solutions required for the test. Do not return the excess solution into the bottles.

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 serum dilution, washing, stopping and substrate solutions are compatible with the equivalents in other VIRCELL ELISA references and lots.
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.

FOR IN VITRO DIAGNOSTIC USE

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6. Serum dilution solution, plate, 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 Hepatitis B Surface Antigen (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 R. conorii 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. Substrate solution may be irritant to skin and mucus. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.

8. Before incorporating this product onto an automatic processing system, we strongly recommend the performance of a pre-evaluation assay. To this purpose, VIRCELL counts with sets of samples reserved for evaluation in parallel with the manual technique. These sets of samples are available on request, as well as a list of commercial systems which have already been validated for use with the VIRCELL ELISA range.

9. During incubation times, an adequate sealing of the plates with the adhesive film included in the kit avoids the desiccation of the samples, and guarantees the repeatability of the results.

10. For IgM test, this product has been designed for exclusive use in conjunction with VIRCELL human IgG sorbent (Virell ref. S001).

**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 (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation.

**Do not use plasma.**

**PRELIMINARY PREPARATION OF THE REAGENTS:**

Only the washing solution must be prepared in advance. Fill 50 ml of 20x washing solution up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C.

**ASSAY PROCEDURE:**

1.-Set incubator/water bath to 37±1°C.
2.-Bring all reagents to room temperature before use (approximately 1 hour), without removing the plate from the bag.
3.-Shake all components.
4.-Remove the plate from the package. Determine the numbers of wells to be employed counting in four wells for the controls: two for the cut off serum and one each for the negative and positive sera. Wells not required for the test should be returned to the pouch, which should then be sealed.
5.-For IgG test, add 100 µl of serum diluent [3G] to all wells. Add 5 µl of each sample, 5 µl of positive control [5G], 5 µl of cut off control [5G] (in duplicate) and 5 µl of negative control [5G] into the corresponding wells. If the assay is performed manually, shake the plate in a plate shaker (2 min) in order to achieve a homogenous mixture of the reagents. If for some reason correct shaking cannot be guaranteed, a pre-dilution of the sample in a separate tube or plate should be made, using double volume of reagents and sample.
6.-For IgM test, add 25 µl of VIRCELL IgG sorbent (ref. S001) to each of the required wells, except for the wells where controls will be dispensed. Add 5 µl of sample and then 75 µl of the serum diluent [3G] to each well. Prepare the control wells by adding first 100 µl of the serum diluent [3G] to each well and then 5 µl of the positive control [5G], 5 µl of the cut off control [5G] (in duplicate) and 5 µl of the negative control [5G] to the corresponding wells. If the assay is performed manually, shake the plate in a plate shaker (2 min) in order to achieve a homogenous mixture of the reagents. If for some reason correct shaking cannot be guaranteed, a pre-dilution of the sample in a separate tube or plate should be made, using double volume of reagents and sample.

7.-Cover with a sealing sheet and incubate at 37±1°C for 45 min.
8.-Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution [8M] per well. Drain off any remaining liquid.
9.-Immediately add 100 µl of IgG conjugate solution [4G] or IgM conjugate [4M] solution into each well.
10.-Cover with a sealing sheet and incubate in incubator/water bath at 37±1°C for 30 min.
11.-Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution [8M] per well. Drain off any remaining liquid.
12.-Immediately add 100 µl of substrate solution [8M] into each well.
13.-Incubate at room temperature for 20 minutes protected from light.
14.-Add immediately 50 µl of stopping solution [8M] into all wells.
15.-Read with spectrophotometer at 450/620 nm within 1 hour of stopping.

**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, negative and cut off controls must be run with each test run. It allows the validation of the assay and kit. Optical densities (O.D.) must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

<table>
<thead>
<tr>
<th>CONTROL</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE CONTROL</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>NEGATIVE CONTROL</td>
<td>&lt;0.55</td>
</tr>
<tr>
<td>CUT OFF CONTROL</td>
<td>&gt;0.7 x(O.D. POSITIVE CONTROL)</td>
</tr>
<tr>
<td></td>
<td>&gt;1.5 x(O.D. NEGATIVE CONTROL)</td>
</tr>
</tbody>
</table>

**INTERPRETATION OF RESULTS:**

Calculate the mean O.D. for cut off serum.

Antibody index=(sample O.D./ cut off serum mean O.D.) x 10

<table>
<thead>
<tr>
<th>INDEX</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9</td>
<td>Negative</td>
</tr>
<tr>
<td>9-11</td>
<td>Equivocal</td>
</tr>
<tr>
<td>&gt;11</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Samples with equivocal results must be retested and/or a new sample obtained for confirmation. Samples with indexes below 9 are considered as not having IgG or IgM (depending on procedure) specific antibodies against R. conorii. Samples with indexes above 11 are considered as having IgG or IgM (depending on procedure) specific antibodies against R. conorii.

**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. A definitive diagnosis should be made by isolation techniques.

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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.-For IgM testing, human IgG sorbent must be used. Otherwise, false positive results may be obtained due to presence of rheumatoid factor or false negative results may be obtained due to an excess of IgG antibodies.

PERFORMANCE

SENSITIVITY AND SPECIFICITY:
153 serum samples were assayed with RICKETTSIA CONORII ELISA IgG/IgM against an immunofluorescence kit for IgG testing.
118 serum samples were assayed with RICKETTSIA CONORII ELISA IgG/IgM against an immunofluorescence kit for IgM testing.
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>153</td>
<td>85%</td>
</tr>
<tr>
<td>IgM</td>
<td>118</td>
<td>94%</td>
</tr>
</tbody>
</table>

Indeterminate values were omitted from the final calculations

INTRA-ASSAY PRECISION:

IgG TESTING
3 sera were individually pipetted 10 times each serum in a single assay performed by the same operator in essentially unchanged conditions. The results were as follows:

<table>
<thead>
<tr>
<th>SERUM</th>
<th>N</th>
<th>%C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>1.83</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>9.12</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>5.46</td>
</tr>
</tbody>
</table>

C.V. Coefficient of variation

IgM TESTING
3 sera were individually pipetted 10 times each serum in a single assay performed by the same operator in essentially unchanged conditions. The results were as follows:

<table>
<thead>
<tr>
<th>SERUM</th>
<th>N</th>
<th>%C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>2.12</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>13.79</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>6.62</td>
</tr>
</tbody>
</table>

C.V. Coefficient of variation

INTER-ASSAY PRECISION:

IgG TESTING
3 sera were individually pipetted on 5 consecutive days by 2 different operators. The results were as follows:

<table>
<thead>
<tr>
<th>SERUM</th>
<th>N</th>
<th>% C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>2.87</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>10.80</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>4.27</td>
</tr>
</tbody>
</table>

C.V. Coefficient of variation

IgM TESTING
3 sera were individually pipetted on 5 consecutive days by 2 different operators. The results were as follows:

<table>
<thead>
<tr>
<th>SERUM</th>
<th>N</th>
<th>% C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>2.16</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>12.33</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>5.39</td>
</tr>
</tbody>
</table>

C.V. Coefficient of variation

CROSS REACTIVITY AND INTERFERENCES:
8 samples known to be positive for Coxiella burnetii and Legionella pneumophila were assayed for IgG and IgM testing. 4 samples known to be positive for antinuclear antibodies were assayed for IgG testing. 4 samples known to be positive for rheumatoid factor were assayed for IgM testing.
The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

OTHER INTERFERENCE ASSAYS:
An ELISA assay was performed to 15 samples previously determined positive against antinuclear antibodies (ANA) and 25 samples previously determined positive against rheumatoid factor for IgG and IgM testing using 4 different ELISA kits (3 viral and 1 bacterial). For IgM testing the samples were treated with anti-IgG sorbent. The results of the test showed a lack of interferences in 96% of antinuclear antibodies sera and 100% of rheumatoid factor sera.
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:

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>USE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>PC</td>
<td>Use by (expiration date)</td>
</tr>
<tr>
<td>2ºC</td>
<td>Store at 2-8ºC</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>REF</td>
<td>Catalogue code</td>
</tr>
<tr>
<td>MOLS</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>&lt;X&gt;</td>
<td>&lt;X&gt; wells</td>
</tr>
</tbody>
</table>

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**SUMMARY OF THE ASSAY PROCEDURE**

**DILUTION FOR IgG**

- 100 μl serum diluent
- 5 μl serum and controls

**DILUTION FOR IgM**

- 25 μl sorbent
- 5 μl serum
- 75 μl serum diluent

**SAMPLES**

- 100 μl serum diluent
- 5 μl controls

**WASH**

- 5x (washing solution)

**45 min. at 37°C**

**100 μl conjugate**

**30 min. at 37°C**

**WASH**

- 5x (washing solution)

**100 μl substrate**

**20 min. at room temperature**

**50 μl stopping solution**

**Read at 450/620 nm**

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**LITERATURE:**


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For any question please contact: technicalservice@vircell.com

**REVISED: October-06**