ImmunoSpec Rickettsia Test is a rapid immunochromatographic assay for the simultaneous detection of IgG and IgM antibodies to Rickettsia in human whole blood, serum or plasma. The assay is used as a screening test for Rickettsia infection and as an aid for differential diagnosis of primary and secondary infections in conjunction with other criteria.

**SUMMARY**

Members of the genera Rickettsia and Orientia are morphologically and biochemically similar to other gram-negative bacteria. They are, however, fastidious bacterial organisms that are obligate intracellular parasites. Although rickettsial species are arthropod-associated bacteria, they are also frequently capable of infecting vertebrates, including human, usually as accidental hosts. Rickettsiae are transmitted to humans by infected arthropodites or feces. The main symptoms of infection consist of fever and headache. Cutaneous eruption, which is sometimes associated with inoculation eschar, is reported in most cases. The pathogenesis of these diseases is vasculitis caused by the proliferation of organisms in the endothelial lining of small arteries, veins, and capillaries.

Immunospec Rickettsia Test is a new generation rapid Immunochromatographic test using recombinant bacterial antigens to detect specific antibody response.

**TEST PRINCIPLE**

Immunospec Rickettsia Test utilizes the principle of Immunochromatography. Anti-human IgM and human IgG antibodies are immobilized on the nitrocellulose membrane respectively, as two individual test lines (IgM line and IgG line) in the test window of the test device. The IgG line in the test window is closer to the sample well and followed by IgM line. As the test sample flows through the membrane assembly within the test device, the colored Rickettsia specific recombinant antigen-colloidal gold conjugate complexes with specific antibodies (IgM or IgG) of Rickettsia, if present in the sample. This complex moves further on the membrane to the test region where it is immobilized by the anti-human IgM and/or human IgG binding proteins coated on the membrane leading to formation of a colored band, which confirms a positive test results. Absence of this colored band in the test window indicates a negative test result. A built-in control line will always appear in the test window when the test has performed properly, regardless of the presence or absence of anti-Rickettsia virus antibodies in the specimen.

**INTRODUCTION**

Immunospec Rickettsia Test is a rapid immunochromatographic assay for the simultaneous detection of IgG and IgM antibodies to Rickettsia in human whole blood, serum or plasma. The assay is used as a screening test for Rickettsia infection and as an aid for differential diagnosis of primary and secondary infections in conjunction with other criteria.

**SPECIMEN COLLECTION AND PREPARATION**

1. No prior special preparation of the patient is required before sample collection by approved techniques.
2. Fresh serum / plasma is preferable. Serum / plasma may be stored at 2-8°C up to 3 days in case of delay in testing. For long-term storage, freeze the specimen at -20°C for 3 months or -70°C for longer periods.
3. The test works best on fresh whole blood samples. If testing cannot be done immediately, Blood samples collected with a suitable anticoagulant such as EDTA or Heparin or Oxalate should be stored at 2-8°C up to 3 days. Blood samples should not be frozen.
4. Repeated freezing and thawing of the specimen should be avoided.
5. Do not use haemolysed, clotted, contaminated, lipemic and viscous/turbid specimen.
6. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
7. Do not heat inactivate the sample.
8. Shipment of samples should comply with local regulations for transport of etiologic agents.

**PROCEDURE**

1. Bring the kit components to room temperature before testing.
2. Open the pouch and remove the Card. Once opened, the test card must be used immediately.
3. Label the test card with patients identity.
4. Add 4 drops of sample buffer to the enclosed sample tube.
5. Apply 5 µL of serum, plasma or 10 µL of whole blood to the sample tube.
6. Place the cap of the sample tube back and gently tap or shake the tube to mix the sample with sample buffer well.
7. Hold the tube vertically with the tip towarding to the sample well marked as “S”, add 3 drops.

**STORAGE AND STABILITY**

1. The sealed pouches in the test kit may be stored between 2-30°C for the duration of the shelf life as indicated on the pouch.
2. The sample buffer should he stored at 2-8°C.
8. At the end of 15 minutes read the results. A strong positive sample may show result earlier.

SPECIFICITY = 50/50 = 100%

2. Interference
These compounds do not affect the test sensitivity and specificity at the indicated concentrations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>bilirubin</td>
<td>10 mg/dL</td>
</tr>
<tr>
<td>hemoglobin</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>triglycerides</td>
<td>300 mg/dL</td>
</tr>
</tbody>
</table>

REFERENCES


INTERPRETATION OF RESULTS

1. Negative
   Only control line appears.

2. IgM Positive
   Both control line and the second test line (the higher test line) appear. It indicates the possibility of primary infection.

3. IgG and IgM Positive
   Control line and both test lines appear. It indicates the possibility of acute secondary infection.

4. IgG Positive
   Both control line and the second test line (the lower test line which is closer to the sample well) appear. It indicates the possibility the secondary infection or past infection.

5. Invalid Result
   If after 15 minutes no line is visible within the test or control window, the result is invalid. The test should be repeated with a new test card.

QUALITY CONTROL

1. The control band is an internal reagent and procedural control. It will appear if the test has been performed correctly and the reagents are reactive.

2. Good Laboratory Practice recommends the daily use of control materials to validate the reliability of the device. Control materials which is not provided with this test kit may be commercially available.

LIMITATIONS

1. The test is for qualitative detection of anti-Rickettsia antibody in human serum, plasma or blood sample and dose not indicate the quantity of the antibodies.

2. The test is for in vitro diagnostic use only.

3. As in case of all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test but should rather be made after all the clinical findings have been evaluated.

PERFORMANCE CHARACTERISTICS

1. Accuracy
   Thirty six (36) confirmed rickettsia samples and fifty (50) normal samples were tested. Immunospec Rickettsia Test showed 100% accuracy.

<table>
<thead>
<tr>
<th>Immunospec Rickettsia Test</th>
<th>Confirmed Clinical Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>36</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
</tr>
</tbody>
</table>

Sensitivity = 36/36 = 100%