Rubella IgM ELISA

INTENDED USE
The Rubella IgM ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgM class antibodies to Rubella in human serum or plasma. For research use only.

SUMMARY AND EXPLANATION
Rubella is usually a mild disease with infrequent complication. In unvaccinated populations, rubella is primarily a childhood disease. Where children are well-immunized, adolescent and adult infections become more evident. Rubella is spread by direct contact with nasal or throat secretions of infected individuals. Symptoms may include a rash, slight fever, joint aches, headache, discomfort, runny nose and reddened eyes. The incubation period for rubella is 12-23 days; in most cases, symptoms appear within 16-18 days. If contracted during the first trimester of pregnancy, Rubella infection can lead to congenital rubella syndrome (CRS). Infection of a pregnant woman may result in a miscarriage, stillbirth or the birth of an infant with abnormalities, which may include deafness, cataracts, heart defects, liver and spleen damage and mental retardation. CRS occurs among at least 25% of infants born to women who have had rubella during the first trimester of pregnancy. The presence of IgG antibody to rubella virus is indicative of vaccination or previous exposure. In individuals with acute rubella infection, four-fold or greater increase in IgG antibody level is indicative of recent infection. Rubella IgM antibodies are detected by ELISA in 100% of patients between days 11-25 after onset of the exanthem, in 60-80% of individuals at days 15-25 after vaccination and in 90-97% of infants with congenital rubella between 2 weeks and 3 months after birth. Rubella IgM antibody often persists for 20-30 days after acute infection or vaccination.

PRINCIPLE OF THE TEST
Diluted patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with Rubella antigen</td>
<td>12x8x1</td>
</tr>
<tr>
<td>Sample Diluent: (ready to use)</td>
<td>22 ml</td>
</tr>
<tr>
<td>Calibrator: (ready to use)</td>
<td>1ml</td>
</tr>
<tr>
<td>Positive Control: (ready to use)</td>
<td>1ml</td>
</tr>
<tr>
<td>Negative Control: (ready to use)</td>
<td>1ml</td>
</tr>
<tr>
<td>Enzyme conjugate: (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>TMB Substrate: (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop Solution: (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Wash concentrate 20X: 1 bottle</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

REFERENCES

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Warning
All GenWay kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of GenWay customers to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

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ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:2 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>O.D.</th>
<th>Ab Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator</td>
<td>0.8</td>
<td>1.6 / 0.4</td>
</tr>
<tr>
<td>Positive</td>
<td>1.2</td>
<td>3</td>
</tr>
<tr>
<td>Patient</td>
<td>1.6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should be greater than 1.2.

INTERPRETATION

The following is intended as a guide to interpretation of Rubella IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

- <0.9: No detectable antibody to Rubella IgM by ELISA.
- 0.9-1.1: Borderline positive. Follow-up testing is recommended if clinically indicated.
- >1.1: Detectable antibody to Rubella IgM by ELISA.

LIMITATIONS OF THE TEST

1. To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and has shown no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. The test results obtained using this kit are for research use only and are not intended to be used as a part of any official diagnosis.
4. Lipemic or hemolyzed samples may cause erroneous results.