Rubella IgG ELISA

Catalog No.: GWB-BQK13F (96 tests)

INTENDED USE
The Rubella IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG 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 percent 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 is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. Nonbound materials are washed away and the enzyme conjugate is added to bind to the antibody of interest. The test is read by determining the absorbance of reaction with TMB substrate. A positive result is indicated by an absorbance value above a predetermined cutoff point. The cutoff is determined from the absorbances of a calibrator with known antibody concentration. 

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microwell coated with Rubella antigen</td>
<td>12x8x1</td>
</tr>
<tr>
<td>2. Sample Diluent: 1 bottle (ready to use)</td>
<td>22 ml</td>
</tr>
<tr>
<td>3. Calibrator: 1 vial (ready to use)</td>
<td>1.5ml</td>
</tr>
<tr>
<td>4. Positive Control: 1 vial (ready to use)</td>
<td>1.5ml</td>
</tr>
<tr>
<td>5. Negative Control: 1 vial (ready to use)</td>
<td>1.5ml</td>
</tr>
<tr>
<td>6. Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>7. TMB Substrate: 1 bottle (ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>8. Stop Solution: 1 bottle (ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>9. Wash concentrate 20X: 1 bottle</td>
<td>25ml</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

STORAGE AND STABILITY

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

REFERENCES

4. Johnson CE; Kumar ML; Whitwell BO; Rome LP; Dinakar C; Humi W; Nalin DR. Antibody persistence after primary Rubella-mumps-rubella vaccine and response to a second dose given at four to six vs. eleven to thirteen years. Pediatr Infect Dis J 1996;15(8):687-92.
5. Matter L; Kogelschatz K; Germann D. Serum levels of rubella virus antibodies indicating immunity; response to vaccination of subjects with low or undetectable antibody concentrations. J Infect Dis 1997; 175(4):749-55.
WARNINGS AND PRECAUTIONS
1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found non-reactive for
   hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method
   that can offer complete assurance that Hepatitis B virus or other infectious agents are absent. These reagents
   should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes
   of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. This kit is designed for research use only.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the
   exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be
   mixed.
6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and
   copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING
1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8° C for up to seven days or frozen for up to six months. Avoid repetitive
   freezing and thawing.

REAGENT PREPARATION
Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store
at room temperature (18-26 °C).

ASSAY PROCEDURE
Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 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:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

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 IgG 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 IgG by ELISA.
0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1 Detectable antibody to Rubella IgG by ELISA.

LIMITATIONS OF THE TEST
1. 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.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS
1. Sensitivity and Specificity
   327 patient sera were tested by Rubella IgG ELISA and a reference ELISA method. 240 sera were positive and 74
   were negative by both methods (96% agreement). The results are summarized below:

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubella IgG ELISA</td>
<td>+</td>
<td>240</td>
<td>0.94</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Reference ELISA</td>
<td>+</td>
<td>240</td>
<td>0.94</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td>74</td>
<td>0.94</td>
<td>0.04</td>
</tr>
</tbody>
</table>

2. Precision

<table>
<thead>
<tr>
<th>Intra-Assay Study</th>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>16</td>
<td>0.88</td>
<td>0.02</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16</td>
<td>0.18</td>
<td>0.01</td>
<td>6.73</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16</td>
<td>0.18</td>
<td>0.01</td>
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