IgG Antibodies to Rubella Virus
ELISA Quantitation Kit

Manual

Catalog number: 40-052-115027

For the quantitative determination of IgG Antibodies to Rubella Virus in serum.

This kit is for research use only, and is not for use in diagnostic procedures.

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**INTENDED USE**

The GenWay Biotech Rubella IgG ELISA is intended for use in evaluating serologic status to the rubella virus infection. It is also used to evaluate paired sera for the presence of a significant increase in specific Rubella IgG as indication of a recent or current rubella virus infection.

**INTRODUCTION**

Rubella is a herpes virus. Generally rubella is considered a mild adolescent disease. However, a maternal infection can be transmitted through the placenta to the fetus, causing congenital rubella. Congenital rubella may result in chronic cardiac disease, growth retardation hepatosplenomegaly, malformations and other severe anomalies. Children born asymptomatic may develop these abnormalities later in life. To reduce the risk of such severe complications, accurate serological methods must be performed to determine the serologic status of childbearing-aged women. The presence of rubella-specific IgG in the bloodstream attests immunity to rubella. A woman tested to be non-immune can be educated on infection and differentiate rubella from other exanthematous diseases. Expecting women with current rubella infection should be counseled on the consequences of congenital infection.

**PRINCIPLE OF THE TEST**

Purified Rubella antigen is coated on the surface of microwells. Diluted serum is added to the wells, and the Rubella IgG-specific antibody, if present, binds to the antigen during incubation. After washing the wells to remove unbound sample, antibody to human IgG conjugated with horseradish peroxidase (HRP) is added and incubated at 37°C for 30 minutes. Unbound conjugate is removed by a subsequent washing step. A solution of TMB Reagent is then added to the microwells. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrators and controls.

**REAGENTS**

**Materials provided with the kit:**

- **Antigen-Coated Wells (1 plate, 96 wells, 12 x 8 strips)**  Microtiter Wells coated with purified Rubella antigen
- **Enzyme Conjugate Reagent (12 ml)**  Contains goat anti-human IgG conjugated to horseradish peroxidase with preservatives
- **Sample Diluent (22 ml)**  Contains phosphate buffer with preservatives
- **Negative Calibrator: 0 IU/ml (150 µl/vial)**  Contains Rubella IgG negative serum with preservatives
- **Cut-off Calibrator: 15 IU/ml (150 µl/vial)**  Contains Rubella IgG positive serum with preservatives
- **Positive Calibrator: 30 IU/ml (150 µl/vial)**  Contains Rubella IgG positive serum with preservatives
- **Positive Calibrator: 100 IU/ml (150 µl/vial)**  Contains Rubella IgG positive serum with preservatives
- **Negative Control (150 µl/vial)**  Contains Rubella IgG negative serum with preservatives; Range stated on the label.
• **Positive Control (150 µl/vial)**
  Contains Rubella IgG positive serum with preservatives; Range stated on the label.

• **Wash Buffer Concentrate (20x) (50 ml)**
  Contains phosphate buffer and Tween 20

• **TMB Reagent (11 ml)**
  Contains 3, 3’, 5, 5’ tetramethylbenzidine (TMB) stabilized in buffer solution

• **Stop Solution -1N HCl (11 ml)**
  Diluted hydrochloric acid

**Materials required but not provided with the kit:**

• Distilled or deionized water
• Precision pipettes: 5 µl, 100 µl, and 200 µl
• Disposable pipette tips
• Container: 1 liter
• Microtiter plate reader with an optical density range of 0 to 2 OD or greater at 450 nm wavelength
• Vortex mixer or equivalent
• Absorbent paper

**Storage of Test Kits and Instrumentation**

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

**Warning and Precautions**

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.4
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For research use only.
**SPECIMEN COLLECTION AND PREPARATION**

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

**PROCEDURAL NOTES**

1. Pipetting Recommendations (single and multi-channel): Pipetting of all calibrators, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. Avoid microbial contamination of reagents by using a clean, disposable pipette tip for each reagent, calibrator, control, or specimen.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

**REAGENT PREPARATION**

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

**ASSAY PROCEDURE**

1. Place the desired number of coated wells into the holder.
2. Prepare 1:40 dilution of test samples, negative control, positive control, and calibrator by adding 5 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 absorbent solution in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.
6. Dispense 100 μl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.
9. Dispense 100 μl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 μl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
Note: Make sure there are no air bubbles in each well before reading.

13. Read O.D. at 450 nm **within 15 minutes** with a microwell reader.

**CALCULATION OF RESULTS**

1. Calculate the mean of duplicate cut-off calibrator (15 IU/ml) value $x_c$.
2. Calculate the mean of duplicate positive control ($x_p$), negative control ($x_n$) and samples ($x_s$).
3. Calculate the Rubella IgG Index of each determination by dividing the mean values of each sample ($x$) by calibrator mean value, $x_c$.

**Example of typical results:**  Note: The O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.

<table>
<thead>
<tr>
<th>Cut-off Calibrator</th>
<th>Rubella IgG Index = 1.0</th>
</tr>
</thead>
</table>

1. Calibrator O.D. = 0.855, 0.816  \[ x_c = 0.835 \]
2. Negative Control O.D. = 0.199, 0.194  \[ x_n = 0.197 \]
   \[ \text{Rubella IgG Index} = \frac{x_n}{x_c} = \frac{0.197}{0.835} = 0.23 \]
3. Positive Control O.D. = 1.570, 1.558  \[ x_p = 1.564 \]
   \[ \text{Rubella IgG Index} = \frac{x_p}{x_c} = \frac{1.564}{0.835} = 1.87 \]
4. Experimental Sample O.D. = 2.941, 2.884  \[ x_s = 2.913 \]
   \[ \text{Rubella IgG Index} = \frac{x_s}{x_c} = \frac{2.913}{0.835} = 3.49 \]

**QUANTITATIVE DETERMINATION OF RUBELLA IgG**

For a quantitative determination of anti-Rubella IgG levels of positive specimens in IU/ml, OD of cut-off and positive calibrators are plotted on the Y-axis of a graph versus their corresponding anti-Rubella IgG concentrations of 0, 15, 30, and 100 IU/ml on the X-axis. The estimates of levels in sera are read off the graph using their individual OD values. For example:

<table>
<thead>
<tr>
<th>Rubella IgG (IU/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.061</td>
</tr>
<tr>
<td>15</td>
<td>0.914</td>
</tr>
<tr>
<td>30</td>
<td>1.327</td>
</tr>
<tr>
<td>100</td>
<td>1.843</td>
</tr>
</tbody>
</table>
Note: The standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

**QUALITY CONTROL**

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

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The Rubella G Index or IU/ml unit for Negative and Positive Control should be in the range stated on the Certificate of Analysis Controls.

**INTERPRETATION**

Negative: Rubella IgG Index less than 0.90 is negative for IgG antibody to Rubella virus. (<13.5 IU/ml)

Equivocal: Rubella IgG Index between 0.91-0.99 is equivocal. Sample should be retested.

Positive: Rubella IgG Index of 1.00 or greater, or IU value greater than 15 is seropositive. It indicates prior exposure to the rubella virus (>15 IU/ml).

**PERFORMANCE CHARACTERISTICS**

I. Specificity and Sensitivity:
A total of 185 samples were used to evaluate specificity and sensitivity of the test. The GenWay Biotech's Rubella IgG ELISA test results were compared to a commercial ELISA kit results:
Sensitivity = A / (A+B) = 113 / 124 = 91.1%
Specificity = D / (C+D) = 40 / 40 = 100.0%
Accuracy = (A+D) / (A+B+C+D) = 153 / 164 = 93.3%

II. Precision:
A study was performed to document typical assay precision with the GenWay Biotech Rubella IgG EIA product. The mean, SD, and %CV were calculated for Intra- and Inter-Assay in concentration value (IU/mL).

Intra-Assay Precision
The following table presents the summary of the results of five (5) samples individually pipetted in groups of twenty-four (24) in a single assay.

<table>
<thead>
<tr>
<th>SERUM SAMPLE</th>
<th>MEAN (IU/mL)</th>
<th>S.D.</th>
<th>% C.V.</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95</td>
<td>4</td>
<td>4.0</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>4</td>
<td>6.9</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>3</td>
<td>8.8</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>1</td>
<td>6.8</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0.2</td>
<td>7.1</td>
<td>24</td>
</tr>
</tbody>
</table>

Inter-Assay Precision
The following table presents the summary of the Inter-assay precision data determined by replicate testing of five (5) samples individually pipetted in groups of five (5).

<table>
<thead>
<tr>
<th>SERUM SAMPLE</th>
<th>MEAN (IU/mL)</th>
<th>S.D.</th>
<th>% C.V.</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>97</td>
<td>3</td>
<td>3.3</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>2</td>
<td>3.3</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>3</td>
<td>6.9</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>0.5</td>
<td>3.2</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0.3</td>
<td>9.4</td>
<td>20</td>
</tr>
</tbody>
</table>

**LIMITATIONS OF THE PROCEDURE**

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

REFERENCES


Troubleshooting

The following are some common problems encountered with the use of ELISA kits, and some of the causes of these problems.

1. **Problem: Low absorbance**
   - Incorrect dilutions or pipetting errors.
   - Improper incubation times
   - Improper mixing of the TMB substrate. Each component is mixed in equal parts.
   - Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS.
   - Kit materials or reagents are contaminated or expired.
   - Incorrect reagents used.

2. **Problem: High Absorbance**
   - Cross contamination from other samples or positive control.
   - Incorrect dilutions or pipetting errors.
   - Improper washing.
   - Wrong filter on microtiter reader.
   - Contaminated buffers or enzyme substrate.
   - Improper incubation times.
   - Kit materials or reagents are contaminated or expired.

3. **Problem: Poor Duplicates**
   - Poor mixing of specimens.
   - Incorrect dilutions or pipetting errors.
   - Technical error.
   - Inconsistency in following ELISA protocol.
   - Inefficient washing.

4. **Problem: All wells are positive**
   - Contaminated buffers or enzyme substrate.
   - Incorrect dilutions or pipetting errors.
   - Kit materials or reagents are contaminated or expired.
   - Inefficient washing.
5. **Problem: All wells are negative**
   - [ ] Procedure not followed correctly.
   - [ ] Contaminated buffers or enzyme substrate.
   - [ ] Contaminated conjugate.
   - [ ] Kit materials or reagents are contaminated or expired.