Rubella Virus IgG Immunoblot

GWB-521231

www.genwaybio.com

FOR RESEARCH USE ONLY

The GenWay Rubella Virus IgG Immunoblot is a qualitative assay for the detection of rubella virus specific IgG antibodies in human serum

1. INTRODUCTION

Rubella virus is an enveloped, positive-sense, single-stranded RNA virus and the only member of the genus rubivirus within the togavirus family. It has a spherical shape measuring about 50-70 nm in diameter. The rubella virion is composed of an inner icosahedral nucleocapsid that is surrounded by a lipid-containing envelope with glycoproteins E1 and E2. Epitopes involved in hemagglutination and neutralization have been identified on E1. In assembled virions E1 forms heterodimers with E2. Rubella virus is of a single antigenic type and does not crossreact with other members of the togavirus group.

Rubella virus causes the children’s disease Rubella or ‘German Measles’ and is spread via respiratory transmission from human to human. The incubation period for rubella is about 14 to 21 days. Rubella virus infects cells of the respiratory tract and spreads via the lymph nodes to the blood. The primary symptom of rubella virus infection is the appearance of a rash (exanthem) on the face which spreads to the trunk and limbs and usually fades after three days. Other symptoms include headache, low-grade fever, lymphadenopathy, upper respiratory symptoms and conjunctivitis. Virus is excreted in nasopharyngeal secretions and in the urine. Persons infected with rubella shed the virus for 7 days before and after the exanthem.

Rubella virus occurs worldwide, and humans are its only known reservoir. In industrial countries natural infection rates in school children are about 50 %. Naturally occurring Rubella is a relatively mild and harmless disease in children and adolescents.

However, if a pregnant woman contracts rubella, the virus can cause serious birth defects or even be fatal to the fetus. The younger the fetus is at the time of infection, the more likely the so-called congenital rubella syndrome (CRS) is to occur and the more severe the effects are likely to be. There appears to be up to a 90 % chance of fetal damage after infection in the first 8 weeks of gestation, this decreases to about 25 % to 35 % for the second trimester. Defects are rare when infection occurs after 16 weeks gestation.

The virus affects the developing organs. Rubella embryopathy (Gregg-Syndrome) is characterized by the classical trias of cardiac defects (patent ductus arteriosus), eye defects (cataracts) and deafness. In addition, a low birth weight, thrombocytopenic purpura, hepatosplenomegaly, encephalitis, hepatitis or mental retardation is possible.

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Symptoms</th>
<th>Mechanism of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubella Virus</td>
<td>acquired rubella (German Measles) Congenital Rubella Syndrome (Embryopathia rubelosia)</td>
<td>generalized rash (fever, nausea) cardiovascular lesions, eye defects, hearing impairment, CNS involvement and others</td>
<td>Transmission by close person-to-person contact, spread most probably by droplets via the respiratory tract fetal infection; transplacental transmission during maternal viremia</td>
</tr>
</tbody>
</table>

Infection may be identified by:
- Detection of virus by RT-PCR (prenatal)
- Hemagglutination inhibition (HAI), Haemolysis-in-gel test (HIG)
- Detection of antibodies by EIA, ELISA, immunoblot

Measurement of serum antibodies is important for the determination of the immune status. Even a previous infection though rather overt may not yield a long-lasting immunity, but may result in an antibody titer too low to prevent reinfection. Especially the screening of adolescents and young women should be a mandatory routine in prenatal care.

2. INTENDED USE

The GenWay Rubella Virus IgG Immunoblot is a qualitative assay for the detection of rubella virus specific IgG antibodies in human serum. In addition it constitutes a useful tool to discriminate between acute and past infections, since the immune reaction to the E2-glycoprotein is delayed. In nonreducing immunoblot E2-specific bands are detectable at the earliest 3 months past infection or vaccination.
3. PRINCIPLE OF THE ASSAY

Proteins derived from rubella virus were separated electrophoretically according to their molecular weight using nonreducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoresis, the separated protein bands were transferred to a PVDF membrane. The antigen bearing membrane was blocked and cut into ready-to-use strips. Diluted human sera are incubated with the Antigen Strips. Rubella-specific antibodies, if present, will bind to their target antigens. After washing, anti-human IgG conjugated with horseradish peroxidase is added. At the end of a second incubation, unbound conjugate is removed by washing and aspiration. The bound conjugate is visualized by the addition of a chromogenic substrate. Strips are dried and analysed. Using the kit-specific Template supplied with the kit, the position of the stained bands can be correlated with defined rubella virus antigens.

4. MATERIALS

4.1. Reagents supplied

- **Rubella Virus Antigen Strips (IgG)**: 1 tube containing 10 consecutively numbered western blot strips coated with rubella virus antigens.
  - **Template**: 1 predeveloped control strip, kit-specific.
  - **IgG Sample Diluent***: 1 bottle containing 50 ml of buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
  - **Washing Solution (20x conc.)***: 1 bottle containing 50 ml of a 20-fold concentrated buffer, pH 7.2 ± 0.2 for washing the Antigen Strips; white cap.
  - **Rubella Virus anti-IgG Conjugate**: 1 bottle containing 12 ml of peroxidase labelled anti-human IgG; coloured red, ready to use; black cap.
  - **TMB Substrate-Solution Membrane**: 1 bottle containing 12 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; blue cap.
  - **Rubella IgG Cut-off Control***: 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.

* contains 0.1 % Bronidox L after dilution ** contains 0.2 % Bronidox L *** contains 0.1 % Kathon

4.2. Materials supplied

- 1 test protocol
- 1 result sheet
- 2 incubation trays

4.3. Materials and Equipment needed

- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Shaking platform
- Micropipettes, 10 and 1000 µl
- Plastic tweezers for handling Antigen Strips
- Vacuum apparatus
- Timer
- Filter paper

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at +2...+8 °C.

6. REAGENT PREPARATION

*It is very important to bring all reagents, samples and controls to room temperature (+20...+25 °C) before starting the test run!*

6.1. Rubella Virus Antigen Strips

The ready to use western blot strips are coated with rubella virus antigens. Store at +2...+8 °C. **After first opening stability until the expiry date when stored at +2...+8 °C. Do not interchange or mix Antigen Strips from different kits!**

6.2. Rubella Virus anti-IgG Conjugate

The bottle contains 12 ml of a solution with anti-IgG conjugated to horseradish peroxidase, buffer, stabilizers, preservatives and an inert red dye. The solution is ready to use. Store at +2...+8 °C. **After first opening stability until the expiry date when stored at +2...+8 °C.**

6.3. Control

The bottle labelled with Cut-off Control contains a ready to use control solution. It contains 0.1 % Kathon and has to be stored at +2...+8 °C. **After first opening stability until the expiry date when stored at +2...+8 °C.**
6.4. IgG Sample Diluent
The bottle contains 50 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at +2...+8 °C. After first opening stability until the expiry date when stored at +2...+8 °C.

6.5. Washing Solution (20x conc.)
The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute washing solution 1+19; e.g. 10 ml Washing Solution + 190 ml fresh and germ free redistilled water. The diluted buffer is stable for 5 days if stored at room temperature. Crystals in the solution disappear by warming up to +37 °C in a water bath. After first opening the concentrate is stable until the expiry date when stored at +2...+8 °C.

6.6. TMB Substrate-Solution Membrane
The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at +2...+8 °C, away from the light. The solution should be colourless to pale yellow. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening stability until the expiry date when stored at +2...+8 °C.

7. SPECIMEN COLLECTION AND PREPARATION
Use human serum samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at +2...+8 °C; otherwise they should be aliquoted and stored deep-frozen (-20...-70 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing! Controls are ready to use and must not be diluted.

7.1. Sample Dilution
Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE
Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Perform all assay steps in the correct order and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each sample.

1. Using tweezers, carefully remove the number of required Antigen Strips from the tube and place them in the channels of the incubation tray.
2. Transfer 1 ml controls and diluted serum to the appropriate channels of the incubation tray. A new pipette tip must be used with each separate sample. Check that all Antigen Strips are completely immersed and, if needed, gently shake the tray or push delicately the strips into the solution with a clean pipette tip. The numbered side of the strip must face up; antigens are bound to this side of the membrane.
3. Incubate the strips at room temperature for 60 min on a shaking platform.
4. Wash procedure
   Carefully aspirate the contents of each channel, add 1 ml of Washing Solution. Place the incubation tray on the shaking platform for 5 min at room temperature. Aspirate the contents of each channel and repeat the wash procedure two more times.
5. Add 1 ml of anti-IgG Conjugate to the appropriate channels and incubate at room temperature for 30 min on the shaking platform.
7. Add 1 ml of chromogenic TMB Substrate-Solution Membrane to the appropriate channels and incubate for 10 min at room temperature and under observation on the shaking platform.CAUTION: DO NOT OVERDEVELOP. Some sera show excessive background staining of the strips. To avoidbackground, stop the reaction earlier by washing with deionised water.
8. Stop the reaction by aspirating the contents of each channel and rinse each strip with 1 ml of deionised water. Place back the incubation tray on the shaking platform for 5 min. Repeat the 1 ml -5 min rinse.
9. Remove the strips from the incubation tray and place them with the number face up on filter paper to dry (approximately 30 min at room temperature).

IMPORTANT: to prevent fading, the strips should be protected from exposure to light.

9. RESULTS

9.1. Interpretation of Results
Once dried, attach the strips to the result sheet using clear tape.

The Rubella Virus IgG Immunoblot utilizes a kit-specific Template consisting of a developed Antigen Strip cut from the membrane used to prepare the Antigen Strips. This strip has been exposed to a positive control serum in order to exhibit bands representative of a rubella virus infection. The corresponding protein bands are listed on the right side of the template. Identification of the reactive bands is based upon comparison with the exposed bands on the Template. The horizontal line at the bottom of the strip must be aligned with the index line near the bottom of the control strip.
For band scoring, determine the reactivity of the following bands: E1-E1 (116 kDa), E1-E2 (100-105 kDa), C Dimer (66 kDa), E1 (58 kDa), E2 (42-47 kDa)

### 9.2. Band Specificity

<table>
<thead>
<tr>
<th>Band</th>
<th>Antigen Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-E1 (116 kDa)</td>
<td>E1 envelope glycoprotein; homodimer</td>
</tr>
<tr>
<td>E1-E2 (100-105 kDa)</td>
<td>Heterodimer of envelope glycoproteins E1 and E2</td>
</tr>
<tr>
<td>C Dimer (66 kDa)</td>
<td>Capsid protein; disulfide linked dimer; associated with the viral RNA</td>
</tr>
<tr>
<td>E1 (58 kDa)</td>
<td>Envelope protein; contains several hemagglutinating and neutralizing epitopes</td>
</tr>
<tr>
<td>E2 (42-47 kDa)</td>
<td>Envelope protein; exists in two forms, E2a (47 kDa) and E2b (42 kDa), which differ in their glycosylation</td>
</tr>
</tbody>
</table>

Human sera may exhibit other bands than those mentioned above. Such bands should not be considered when interpreting the results.

### 9.3. Evaluation of Antigen Bands

- The humoral antibody response to rubella virus antigens shows different kinetics: antibodies to structural proteins E1 and C are detectable 2-3 weeks post infection or vaccination. In contrast, reactivity to the E2 protein is considerably delayed. **In nonreducing immunoblot E2-specific antibodies are detectable no earlier than 3 months after infection or vaccination.** If the E2 antigen band is present, an infection within the last 3 months can be ruled out.
- Positive blots are very similar to the control strip on the kit Template.

**IMPORTANT:** The band intensities are assessed in comparison to the Cut-off Control. Only bands with the same or stronger colour intensities than the corresponding band on the Cut-off Control strip are considered for evaluation.

#### Antigen bands in Rubella IgG Virus Immunoblot and probable infection status

<table>
<thead>
<tr>
<th></th>
<th>E1-E1</th>
<th>E1-E2</th>
<th>C Dimer</th>
<th>E1</th>
<th>E2</th>
<th>Probable status of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>no positive bands</td>
<td>positive</td>
<td>one or more bands positive</td>
<td>one or more bands positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no Rubella virus specific antibodies present fresh infection within the last days can not be excluded</td>
<td>delayed or failed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fresh rubella virus infection within the last 3 months can be excluded</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 10. SPECIFIC PERFORMANCE CHARACTERISTICS

#### 10.1. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is >98 %.

#### 10.2. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is >98 %.
10.3. Interferences
Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 30 mg/ml triglycerides and 1 mg/ml bilirubin.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

11. LIMITATIONS OF THE PROCEDURE
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. The absence of antibodies reactive with rubella virus antigens on the Rubella Virus IgG Immunoblot cannot exclude a rubella virus infection in all cases. In the early phase of infection, detectable amounts of antibodies may not or not yet be present. In immunocompromized patients and newborns serological data only have restricted value. Prenatal diagnosis is not possible with Rubella Virus IgG Immunoblot.

12. PRECAUTIONS AND WARNINGS
Only for research use.
All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
Do not interchange reagents from different production lots or strips from different kits.
No reagents of other manufacturers should be used along with reagents of this test kit.
Do not use reagents after the expiry date stated on the label.
Use only clean pipette tips, dispensers, and lab ware.
Do not interchange screw caps of reagent vials to avoid cross-contamination.
Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use. Always use tweezers to handle Antigen Strips.
During incubations and washing, the Antigen Strips must remain completely covered with fluid and the numbered side of the strips must face up.
To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
The GenWay Immunoblot is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING: In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes.

12.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

SCHEME OF THE ASSAY
Rubella Virus IgG Immunoblot

Test Preparation

Prepare samples as described. Note sample and strip numbers as well as lot number and antibody isotype. Put the required number of antigen strips into the wells of the incubation tray. Prepare the required volume of Washing Solution.

Assay Procedure

<table>
<thead>
<tr>
<th>Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>pipette 1 ml Control or diluted sample</td>
</tr>
<tr>
<td>↓</td>
</tr>
<tr>
<td>incubate for 60 min at room temperature while shaking gently</td>
</tr>
<tr>
<td>↓</td>
</tr>
<tr>
<td>wash three times for 5 min with 1 ml Washing Solution while shaking</td>
</tr>
<tr>
<td>↓</td>
</tr>
<tr>
<td>add 1 ml Conjugate</td>
</tr>
<tr>
<td>↓</td>
</tr>
<tr>
<td>incubate for 30 min at room temperature while shaking gently</td>
</tr>
<tr>
<td>↓</td>
</tr>
<tr>
<td>wash three times for 5 min with 1 ml Washing Solution while shaking</td>
</tr>
<tr>
<td>↓</td>
</tr>
<tr>
<td>add 1 ml Substrate-Solution</td>
</tr>
<tr>
<td>↓</td>
</tr>
<tr>
<td>incubate for 10 min at room temperature while shaking gently</td>
</tr>
<tr>
<td>↓</td>
</tr>
<tr>
<td>wash two times for 5 min with 1 ml deionised water</td>
</tr>
<tr>
<td>dry and read</td>
</tr>
</tbody>
</table>