Rubella IgG ELISA
Enzyme immunoassay for the determination of human IgG antibodies against Rubella in serum and plasma

REF  IB79800

For Research Use Only – Not for Use in Diagnostic Procedures
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1. INTENDED USE

The Rubella IgG Antibody ELISA Test Kit has been designed for the determination of specific IgG antibodies against Rubella in serum and plasma. **For Research Use Only – Not for Use in Diagnostic Procedures.**

2. GENERAL INFORMATION

Rubella infection belongs to the classical children’s diseases with a life-long immunity, and the virus is spread worldwide endemically. In non-vaccinated populations, 80-90% of the infections occur during the childhood. In spite of the rubella vaccination, introduced in 1974, in Germany there continue to appear connatal diseases.

The causative agent is a genetically stable RNA virus, which belongs to the genus rubivirus within the family of togaviridae. Human beings are the only known natural hosts for the rubella virus. The transmission occurs via droplet infection, with an incubation time of 14-23 days.

The disease manifests itself like a light flu infection. The nucal and retroaural lymph nodes are swollen, and a moderate enlargement of the spleen is observed. A short and medium raise of temperature appears together with a rather slight sensation of illness. Rubella is overcome easily with insignificant and light symptoms during the childhood, however more attention is required in the case of the infection of non-immunized pregnant women, because of the possible malformations of the foetus, which can be generated. As the infection can be transmitted via the placenta, the developing foetus can suffer severe damages, the frequency and gravity of which is dependent on the moment of infection during pregnancy. A rubella infection during the 1st till 4th month can lead to a spontaneous abortion or premature birth.

Since a specific causal therapy does not exist, the secondary signs like fever, arthritis or arthralgies are treated symptomatically.

The differential diagnosis is problematic, because similar exanthems and feverish illnesses appear also in the course of other children’s diseases like measles, scarlet and parvovirusitis. The following laboratory methods are available: hemagglutination inhibition test (HI), hemolysis-in-gel test or ELISA. The detection of virus-specific IgM antibodies is important for the assessment of fresh infections, and the IgG test is used for the determination of immunity. In the case of severe connatal infections, the isolation of the rubella virus from pharyngeal lavage, urine and other secretions can also be performed.

3. PRINCIPLE OF THE TEST

The Rubella IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Rubella antigen is bound on the surface of the microtiter strips. Diluted serum or ready-to-use calibrators are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Rubella antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.
4. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

- For Research Use Only – Not for use in diagnostic procedures! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HbsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18-25°C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, and they should not be mixed with one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to micropipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

5. REAGENTS PROVIDED

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume / Qty.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SORB MT</td>
<td>Rubella antigen coated microtiter strips</td>
</tr>
<tr>
<td>CAL</td>
<td>Calibrators with: 0, 10, 50, 200, 500 IU/mL</td>
</tr>
<tr>
<td>CONJ</td>
<td>Enzyme Conjugate</td>
</tr>
<tr>
<td>SUB TMB</td>
<td>Substrate</td>
</tr>
<tr>
<td>STOP SOLN</td>
<td>Stop Solution</td>
</tr>
<tr>
<td>DIL</td>
<td>Sample Diluent</td>
</tr>
<tr>
<td>BUF WASH</td>
<td>Washing Buffer (10×)</td>
</tr>
<tr>
<td>Plastic bag</td>
<td>1</td>
</tr>
</tbody>
</table>

Storage and Stability (refer to the expiry date on the outer box label)
Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.
5.1. SORB MT Microtiter Strips
12 strips with 8 breakable wells each, coated with a Rubella antigen (strain HPV-77, cultivated in kidney cells of monkeys). Ready-to-use.

5.2. CAL Calibrators A-E
5 x 2 mL, human serum diluted with PBS, with 0, 10, 50, 200, 500 IU/mL of IgG antibodies against Rubella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.3. CON Enzyme Conjugate
15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone, 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

5.4. SUB TMB Substrate
15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.5. STOP SOLN Stop Solution
15 mL, 1 N acidic solution. Ready-to-use.

5.6. DIL Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.7. BUF WASH 10x Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.8. Plastic Bag
Resealable, for the dry storage of non-used strips.

6. MATERIALS REQUIRED BUT NOT PROVIDED
- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Bidistilled water

7. SAMPLE COLLECTION AND HANDLING
Principally serum or plasma (EDTA, citrate) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.
For the performance of the test the samples (not the calibrators) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).
8. ASSAY PROCEDURE

8.1. Preparation of Reagents

**Washing Solution:** dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Calibrators and samples should be assayed in duplicates.
- A calibration curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them with desiccant at 2-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the calibrators and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** calibrators respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the re-usable plate cover and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the re-usable plate cover and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the re-usable plate cover and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.
9. EVALUATION

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

9.1. Evaluation (Cut-Off)

The calculated absorptions for the sera, as mentioned above, are compared with the value for the cut-off calibrator (10 IU/mL). If the value of the sample is higher, there is a positive result. For a value below the cut-off calibrator, there is a negative result. It seems reasonable to define a range of +/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same subject, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

9.2. Evaluation (IU/mL)

The ready-to-use calibrators of the Rubella antibody kit are defined and expressed in International Units (IU/mL) based on the 1st Intl. Standard RUBI-1-94. The values for the calibrators in International Units are printed on the QC data sheet.

For evaluation the absorptions of the calibrators are graphically drawn against their concentrations. From the resulting calibration curve the concentration values for each unknown sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

10. ASSAY CHARACTERISTICS

<table>
<thead>
<tr>
<th>Rubella ELISA</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay-Precision</td>
<td>4.3 – 7.2 %</td>
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<tr>
<td>Inter-Assay-Precision</td>
<td>2.6 – 17.0 %</td>
</tr>
<tr>
<td>Inter-Lot-Precision</td>
<td>5.3 – 23.2 %</td>
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<tr>
<td>Analytical Sensitivity</td>
<td>0.29 IU/mL</td>
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<tr>
<td>Recovery</td>
<td>102 – 118 %</td>
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<tr>
<td>Linearity</td>
<td>75 – 110 %</td>
</tr>
<tr>
<td>Cross-Reactivity</td>
<td>No cross-reactivity to herpes 1, cytomegaly, toxoplasma, dsDNA, measles, mumps, varicella and EBV-VCA. Interferences of parainfluenza and parvovirus positive samples cannot totally be excluded.</td>
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<tr>
<td>Interferences</td>
<td>No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL</td>
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<tr>
<td>Specificity</td>
<td>100 %</td>
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<tr>
<td>Sensitivity</td>
<td>100 %</td>
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<tr>
<td>Measuring Range</td>
<td>10 – 500 IU/mL</td>
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</table>

Manufactured for:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
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Email: ibl@ibl-america.com Web: www.ibl-america.com
### SYMBOLS USED WITH IBL-AMERICA ASSAYS

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<td>Contenu suffisant pour &quot;n&quot; tests</td>
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