Instruction Manual

Rubella IgM ELISA

Enzyme immunoassay based on microtiter plate for the detection and quantitative determination of human IgM antibodies against Rubellavirus in serum and plasma

Cat. No.: ILE-RUB03
Storage: 2-8°C
For in-vitro diagnostic use only

April 2008

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Symbols and Translations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>French</th>
<th>German</th>
<th>Italian</th>
<th>Spanish</th>
<th>Greek</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL</td>
<td>Calibrator</td>
<td>Étalon</td>
<td>Standard</td>
<td>Calibratore</td>
<td>Calibrador</td>
<td>Πρότυπο</td>
</tr>
<tr>
<td>CONJ</td>
<td>Conjugate</td>
<td>Conjugué</td>
<td>Konjugat</td>
<td>Coniugato</td>
<td>Conjugado</td>
<td>Διάλυμα</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate (&lt;n&gt;-fold)</td>
<td>Concentré (&lt;n&gt; fois)</td>
<td>Konzentrat (&lt;n&gt;-fach)</td>
<td>Concentrato</td>
<td>Concentrado (&lt;n&gt;-veces)</td>
<td>Συμπύκνωση (&lt;n&gt; φορές)</td>
</tr>
<tr>
<td>SAMP</td>
<td>Sample</td>
<td>Diluant échantillon</td>
<td>Proben verdünner</td>
<td>Diluente del campione</td>
<td>Diluyente de muestra</td>
<td>Διάλυμα Αμαρίωσης Δειγμάτων</td>
</tr>
<tr>
<td>DIL</td>
<td>Diluent</td>
<td>Solution d’arrêt</td>
<td>Stopp-Lösung</td>
<td>Soluzione d’arresto</td>
<td>Solución de parada</td>
<td>Διάλυμα Αναστολής</td>
</tr>
<tr>
<td>STOP</td>
<td>Stop</td>
<td>Solution</td>
<td>Stopp-Lösung</td>
<td>Soluzione d’arresto</td>
<td>Solución de parada</td>
<td>Διάλυμα Υποστρώματος</td>
</tr>
<tr>
<td>SUBS</td>
<td>Substrate</td>
<td>Substrat</td>
<td>Substrat</td>
<td>Substrato</td>
<td>Sustrato</td>
<td>Διάλυμα Υποστρώματος</td>
</tr>
<tr>
<td>MT PLATE</td>
<td>Microtiter plate</td>
<td>Microplaque</td>
<td>Mikrotiterplatte</td>
<td>Piastre</td>
<td>Placa microtiter</td>
<td>Μικρόπλακα</td>
</tr>
<tr>
<td>WASH BUF</td>
<td>Wash buffer</td>
<td>Tampon de lavage</td>
<td>Waschpuffer</td>
<td>Soluzione di lavaggio</td>
<td>Tampón de lavado</td>
<td>Γλυκόσιμο Διάλυμα</td>
</tr>
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</table>
1. Intended Use

The IMMUNOLAB Rubella IgM Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgM antibodies against Rubellavirus in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of IMMUNOLAB.

Monitoring of patients with engrafted organs as well as determination of antibodies in other body fluids were not validated and hence are within the responsibility of the user. This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

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.

Clinically the disease manifests itself like a light flu infection. The nucal and retroaurical 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 clinical 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 (HIT), 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 IMMUNOLAB Rubella IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Rubellavirus antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use calibrators are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Rubellavirus 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-IgM 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 IgM antibodies is directly proportional to the intensity of the color.
4. Limitations, Precautions and General Comments

- Only for in-vitro use! 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>MT PLATE</td>
<td>12</td>
</tr>
<tr>
<td>Rubellavirus antigen coated microtiter strips</td>
<td></td>
</tr>
<tr>
<td>CAL Calibrators with: 1, 10, 30, 90 U/mL</td>
<td>4 x 2 mL</td>
</tr>
<tr>
<td>CONJ Enzyme Conjugate</td>
<td>15 mL</td>
</tr>
<tr>
<td>SUBS Substrate</td>
<td>15 mL</td>
</tr>
<tr>
<td>STOP Stop Solution</td>
<td>15 mL</td>
</tr>
<tr>
<td>SAMPL DIL Sample Diluent</td>
<td>60 mL</td>
</tr>
<tr>
<td>WASH BUF CONC Washing Buffer (10×)</td>
<td>60 mL</td>
</tr>
<tr>
<td>Plastic foils</td>
<td>2</td>
</tr>
<tr>
<td>Plastic bag</td>
<td>1</td>
</tr>
</tbody>
</table>

*Storage and limitation of usage* (expiry dates are printed on the labels)
Store the components of the kit at 2-8°C. After usage put the plate in the plastic bag, close the bottles with their screw caps, and again store the kit 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. **MT PLATE** Microtiter Strips

12 strips with 8 breakable wells each, coated with a Rubellavirus antigen (strain HPV-77, cultivated in monkey kidney cells). Ready-to-use.
5.2. **CAL** Calibrators A-D
4 x 2 mL, human serum diluted with PBS, with 1, 10, 30, 90 U/mL of IgM antibodies against Rubellavirus. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.3. **CONJ** Enzyme Conjugate
15 mL, anti-human-IgM-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. **SUBS** Substrate
15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.5. **STOP** Stop Solution
15 mL, 0.5 M sulfuric acid. Ready-to-use.

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

5.7. **WASH BUF CONC** 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 Foils
2 pieces to cover the microtiter strips during the incubation.

5.9. 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. Specimen 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).

**Important note:** To avoid false positive results, positively determined samples should be confirmed generally by the use of an appropriate RF-absorbent (e.g. cat. no. ILE-MJS01).

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 enclosed foil 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 enclosed foil 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 enclosed foil 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%.

Example

<table>
<thead>
<tr>
<th></th>
<th>OD Value</th>
<th>corrected OD</th>
<th>Mean OD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate Blank</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator A (1 U/mL)</td>
<td>0.026 / 0.024</td>
<td>0.006 / 0.004</td>
<td>0.005</td>
</tr>
<tr>
<td>Calibrator B (10 U/mL)</td>
<td>0.504 / 0.544</td>
<td>0.484 / 0.524</td>
<td>0.504</td>
</tr>
<tr>
<td>Calibrator C (30 U/mL)</td>
<td>1.168 / 1.202</td>
<td>1.148 / 1.182</td>
<td>1.165</td>
</tr>
<tr>
<td>Calibrator D (90 U/mL)</td>
<td>2.288 / 2.308</td>
<td>2.268 / 2.288</td>
<td>2.278</td>
</tr>
</tbody>
</table>

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently no reference values which have to be found in other laboratories in the same way.

9.1. Qualitative Evaluation
The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off calibrator (10 U/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 patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

9.2. Quantitative Evaluation

The ready-to-use calibrators of the Rubellavirus antibody kit are defined and expressed in Units per millilitre (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for the calibrators in International Units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the calibrators are graphically drawn against their concentrations. From the resulting calibration curve the concentration values for each patient 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>Rubellavirus ELISA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay-Precision</td>
<td>6.5 - 9.2 %</td>
</tr>
<tr>
<td>Inter-Assay-Precision</td>
<td>1.8 - 33.4 %</td>
</tr>
<tr>
<td>Inter-Lot-Precision</td>
<td>0.8 - 30.9 %</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>1.24 U/mL</td>
</tr>
<tr>
<td>Recovery</td>
<td>75 - 86 %</td>
</tr>
<tr>
<td>Linearity</td>
<td>78 - 118 %</td>
</tr>
<tr>
<td>Cross-Reactivity</td>
<td>No cross-reactivity to cytomegaly, toxoplasma, measles, mumps, varicella, parainfluenza and EBV-VCA. Interferences of parvovirus positive samples or samples of donors suffering from an acute EBV infection cannot totally be excluded.</td>
</tr>
<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>
</tr>
<tr>
<td>Clinical Specificity</td>
<td>100 %</td>
</tr>
<tr>
<td>Clinical Sensitivity</td>
<td>100 %</td>
</tr>
</tbody>
</table>
11. References


