PLATELIA™ RUBELLA IgG
96 TESTS 72850

QUANTITATIVE DETERMINATION OF IgG ANTIBODIES TO RUBELLA VIRUS IN HUMAN SERUM OR PLASMA BY ENZYME IMMUNOASSAY

IVD
1. **INTENDED USE**

Platelia™ Rubella IgG is an indirect ELISA immunoassay for quantitative determination of IgG antibodies to rubella virus in human serum or plasma.

2. **CLINICAL VALUE**

Rubella, commonly referred to as “German measles”, is a viral illness with worldwide distribution. Rubella infection is predominantly a mild disease in children and adults. Clinical manifestations include a low-grade fever, headache, sore throat, and a generalized skin rash. However, Rubella infection during pregnancy is more serious, with well documented multiple congenital complications, including deafness, cataracts, mental retardation, and fetal death.

Aggressive immunization of pre-school children has greatly reduced the incidence of Rubella epidemics, but a need still exists for accurate monitoring of immune status, especially for women of child-bearing age. Demonstration of Rubella IgG antibody in women prior to conception provides assurance of fetal protection from possible Rubella viral infection during pregnancy. Vaccination efficiency can also be determined by detection of Rubella IgG antibody in serum following immunization.

Since the isolation of Rubella virus in 1962, the detection of specific Rubella antibodies has been of great interest due to the teratogenic risk of the virus. Several test methods have been developed in the past including serum neutralization, complement binding, and immunofluorescence. These assays are either difficult to perform in a routine laboratory setting, or yield unstable or irregular results. Hemagglutination inhibition techniques allow a rapid diagnosis of both the acute infected state and patient immune status. Engvall and Perlmann described the first enzyme immunoassay procedures in 1971. The development of the enzyme immunoassay procedure has contributed to an improved specificity and sensitivity in the detection of a wide variety of antigens and antibodies.

Interpretations of successive serological tests, demonstrating the presence of IgM, the apparition or a significant increase in IgG antibody titer (doubling of the titer) in two serum samples obtained at a minimum interval of three weeks should be considered as evidence of exposition to Rubella virus even if clinical pathognomonic signs of this infection are not present.

3. **PRINCIPLE**

Platelia™ Rubella IgG is a test for detection and titration of IgG antibodies to rubella virus in human serum or plasma using an indirect ELISA immuno-enzymatic method.

Rubella antigen is used for coating the microplate. A monoclonal antibody labeled with peroxydase which is specific for human gamma chains (anti-IgG) is used as the conjugate. The test uses the following steps:
• **Step 1**  
Patients samples and calibrators are diluted 1/21 and then distributed in the wells of the microplate. During this incubation of one hour at 37°C, IgG antibodies to rubella present in the sample bind to the rubella antigen coated on microplate wells. After incubation, unbound non specific antibodies and other serum proteins are removed by washings.

• **Step 2**  
The conjugate (peroxydase labeled monoclonal antibody specific for human gamma chains) is added to the microplate wells. During this incubation of one hour at 37°C, the labeled antibody binds to the serum IgG captured by the rubella antigen. The unbound conjugate is removed by washings at the end of the incubation.

• **Step 3**  
The presence of immune-complexes (rubella antigen, IgG antibodies to rubella, anti-IgG conjugate) is demonstrated by the addition in each well of an enzymatic development solution.

• **Step 4**  
After incubation at room temperature (+18-30°C), the enzymatic reaction is stopped by addition of 1N sulfuric acid solution. The optical density reading obtained with a spectrophotometer set at 450/620 nm is proportional to the amount of IgG antibodies to Rubella antigen present in the sample and is converted into IU/ml using a standard curve calibrated against WHO International Standard RUBI 1-94.

### 4. PRODUCT INFORMATION

Supplied quantities of reagents have been calculated to allow 96 tests. All reagents are exclusively for *in vitro* diagnostic use.

<table>
<thead>
<tr>
<th>Label</th>
<th>Nature of reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td><strong>Microplate</strong></td>
<td>1</td>
</tr>
</tbody>
</table>
|       | Microplate: (Ready-to-use):  
12 strips with 8 breakable wells, coated with inactivated Rubella antigen |
| R2    | **Concentrated Washing Solution (20x)** | 1 x 70 mL |
|       | Concentrated Washing Solution (20x):  
TRIS-NaCl buffer (pH 7.4), 2% Tween® 20  
Preservative : < 1.5% ProClin™ 300 |
| R3    | **Calibrator 0**   | 1 x 0.75 mL  |
|       | Calibrator 0:  
Negative serum for IgG antibodies to rubella,  
Preservative : < 1.5% ProClin™ 300 |
<table>
<thead>
<tr>
<th>Label</th>
<th>Nature of reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R4a</td>
<td><strong>Calibrator 15</strong></td>
<td>Calibrator 15 IU/ml: Human serum reactive for IgG antibodies to rubella, and negative for HBs antigen, anti-HIV1, anti- HIV2 and anti-HCV Preservative : &lt; 1.5% ProClin™ 300</td>
</tr>
<tr>
<td>R4b</td>
<td><strong>Calibrator 60</strong></td>
<td>Calibrator 60 IU/ml: Human serum reactive for IgG antibodies to rubella, and negative for HBs antigen, anti-HIV1, anti- HIV2 and anti-HCV Preservative : &lt; 1.5% ProClin™ 300</td>
</tr>
<tr>
<td>R4c</td>
<td><strong>Calibrator 200</strong></td>
<td>Calibrator 200 IU/ml: Human serum reactive for IgG antibodies to rubella, and negative for HBs antigen, anti-HIV1, anti- HIV2 and anti-HCV Preservative : &lt; 1.5% ProClin™ 300</td>
</tr>
<tr>
<td>R6</td>
<td><strong>Conjugate (51x)</strong></td>
<td>Conjugate (51x): Murine monoclonal antibody to human gamma-chains coupled to horseradish peroxydase Preservative : &lt; 1.5% ProClin™ 300</td>
</tr>
<tr>
<td>R7</td>
<td><strong>Diluent</strong></td>
<td>Diluent for samples and conjugate (Ready-to-use): Tris-NaCl (pH 7,7), glycerol, 0.1% Tween® 20, phenol red Preservative : &lt; 1.5% ProClin™ 300</td>
</tr>
<tr>
<td>R9</td>
<td><strong>Chromogen TMB</strong></td>
<td>Chromogen (Ready-to-use): 3.3’.5.5’ tetramethylbenzidine (&lt; 0.1%), ( \text{H}_2\text{O}_2 ) (&lt;1%)</td>
</tr>
<tr>
<td>R10</td>
<td><strong>Stopping Solution</strong></td>
<td>Stopping Solution (Ready-to-use): 1N sulfuric acid solution</td>
</tr>
</tbody>
</table>

For storage conditions and expiration date, please refer to the indications stated on the box.

**5. WARNINGS AND PRECAUTIONS**

The reliability of the results depends on correct implementation of the following Good Laboratory Practices:

- Do not use expired reagents.
- Do not mix or associate within a given run reagents from different lots.
REMARK: For Washing Solution (R2, label identification: 20x colored green), Chromogen (R9, label identification: TMB colored turquoise) and Stopping Solution (R10, label identification: 1N colored red), it is possible to use other lots than those contained in the kit, provided these reagents are strictly equivalent and the same lot is used within a given test run.

REMARK: It is not permissible to use Diluent (R7) from lots other than provided in the kit. It is also not permissible to use the Diluent (R7) provided with Platelia™ Rubella IgM kits (Ref. 72851).

REMARK: In addition, the Washing Solution (R2, label identification: 20x colored green) can be mixed with the 2 other washing solutions included in various Bio-Rad reagent kits (R2, label identifications: 10x colored blue or 10x colored orange) when properly reconstituted, provided only one mixture is used within a given test run.

- Before use, wait for 30 minutes to allow reagents to reach room temperature (+18-30°C).
- Carefully reconstitute or dilute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugate.
- Use glassware thoroughly washed and rinsed with deionized water or, preferably disposable material.
- Washing the microplate is a critical step in the procedure: follow the recommended number of washings cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washings may lead to inaccurate results.
- Do not allow the microplate to dry between the end of the washings operation and the reagent distribution.
- Never use the same container to distribute the conjugate and the development solution.
- The enzymatic reaction is very sensitive to metal or metal ions. Consequently, do not allow any metal element to come into contact with the various solutions containing the conjugate or the chromogen.
- Chromogen solution (R9) should be colorless. The appearance of a blue color indicates that the reagent cannot be used and must be replaced.
- Use a new pipette tip for each sample.
- Check the pipettes and other equipments for accuracy and correct operations.

HEALTH AND SAFETY INSTRUCTIONS

Human origin material used in the preparation of reagents has been tested and found non-reactive for hepatitis B surface antigen (HBs Ag), antibodies for hepatitis C virus (anti-HCV), and to human immunodeficiency virus (anti-HIV1
and anti-HIV2). Because no method can absolutely guarantee the absence of infectious agents, handle reagents of human origin and patient samples as potentially capable of transmitting infectious diseases:

- Any material, including washings solutions, that comes directly in contact with samples and reagents containing materials of human origin should be considered capable of transmitting infectious diseases.
- Wear disposable gloves when handling samples and reagents.
- Do not pipette by mouth.
- Avoid spilling samples or solutions containing samples. Spills must be rinsed with bleach diluted to 10 %. In the event of a spill with an acid, it must be first neutralized with sodium bicarbonate, and then cleaned with bleach diluted to 10% and dried with adsorbent paper. The material used for cleaning must be discarded in a contaminated residue container.
- Patient samples, reagents containing human origin material, as well as contaminated material and products should be discarded after decontamination only:
  - either by immersion in bleach at the final concentration of 5 % of sodium hypochloride during 30 minutes,
  - or by autoclaving at 121°C for 2 hours at the minimum.

**CAUTION: Do not introduce solutions containing sodium hypochloride into the autoclave**

- Avoid any contact of reagents, including those considered as not dangerous, with skin and mucosa.
- Chemical and biological residues must be handled and disposed off in accordance with Good Laboratories Practices.
- All reagents in the kit are exclusively for *in vitro* diagnostic use.

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**Caution: Some of the reagents contain ProClin™ 300 < 1.5%**

R43: May cause sensitisation by skin contact

S28-37: After contact with skin, wash immediately with plenty of water and soap. Wear suitable gloves

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### 6. SAMPLES

1. Serum and plasma (EDTA, heparin or citrate) are the recommended sample types.
2. Observe the following recommendations for handling, processing and storage of blood samples:
   - Collect all blood samples observing routine precaution for venipuncture.
   - For serum, allow samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - After centrifugation, separate the serum or plasma from the clot or red cells in a tightly stoppered storage tube.
• The specimens can be stored at +2-8°C if test is performed within 7 days.
• If test will not be completed within 7 days, or for shipment, freeze the samples at -20°C or colder.
• Do not use samples that have been thawed more than 5 times. Previously frozen specimens should be thoroughly mixed (Vortex) after thawing prior to testing.

3. Samples containing 90 g/l of albumin or 100 mg/l of unconjugated bilirubin, lipemic samples containing the equivalent of 36 g/l of triolein (triglyceride), and hemolysed samples containing up to 10 g/l of hemoglobin do not affect the results.

4. Do not heat the samples.

7. ASSAY PROCEDURE

7.1 Materials required but not provided
• Vortex mixer.
• Microplate reader equipped with 450 nm and 620 nm filters (*).
• Microplate incubator thermostatically set at 37±1°C (*).
• Automatic, semi-automatic or manual microplate washer (*).
• Sterile distilled or deionized water.
• Disposable gloves.
• Goggles or safety glasses.
• Adsorbent paper.
• Automatic or semi-automatic, adjustable or preset, pipettes or multi-pipettes, to measure and dispense 10 µl to 1000 µl, and 1 ml, 2 ml and 10 ml.
• Graduated cylinders of 25 ml, 50 ml, 100 ml and 1000 ml capacity.
• Sodium hypochloride (bleach) and sodium bicarbonate.
• Container for biohazard waste.
• Disposable tubes.
(*) Consult our technical department for detailed information about the recommended equipment.

7.2 Reagents reconstitution
• **R1:** Allow 30 minutes at room temperature (+18-30°C) before opening the bag. Take out the carrier tray, return unused strips in the bag immediately and check the presence of desiccant. Carefully reseal the bag and store it at +2-8°C.

• **R2:** Dilute 1/20 the washing solution R2 in distilled water: for example 50 ml of R2 and 950 ml of distilled water to get the ready-to-use washing solution. Prepare 350 ml of diluted washing solution for one plate of 12 strips if washing manually.
• **R3, R4a, R4b, R4c:** Dilute 1/21 in Diluent (R7) (example: 300 µl of R7 + 15 µL of Calibrator).

• **R6+R7:** Conjugate (R6) is concentrated 51x and must be homogenized before use. Dilute 1/51 in Diluent (R7). For one plate, dilute 0.5 ml of Conjugate (R6) in 25 ml of Diluent (R7). Divide these volumes by 10 to obtain the volume needed for one strip.

### 7.3 Storage and validity of opened and / or reconstituted reagents

The kit must be stored at +2-8°C. When the kit is stored at +2-8°C before opening, each component can be used until the expiration date indicated on the outer label of the kit.

- **R1:** Once opened, the strips remain stable for up to 8 weeks if stored at +2-8°C in the same carefully closed bag (check the presence of desiccant).

- **R2:** Once diluted, the Washing Solution can be kept for 2 weeks at +2-30°C. Once opened, the concentrated Washing Solution stored at +2-30°C, in absence of contamination, is stable until the expiration date indicated on the label.

- **R3, R4a, R4b, R4c, R6, R7:** Once opened and without any contamination, the reagents stored at +2-8°C are stable for up to 8 weeks.

- **R6+R7:** Once diluted, the conjugate working solution is stable for 8 hours at room temperature (+18-30°C) or 2 weeks at +2-8°C.

- **R9:** Once opened and without any contamination, the reagent stored at +2-8°C is stable for up to 8 weeks.

- **R10:** Once opened and without any contamination, the reagent stored at +2-8°C is stable until the expiration date indicated on the label.

### 7.4 Procedure

Strictly follow the assay procedure and Good Laboratory Practices.

Before use, allow reagents to reach room temperature (+18-30°C).

The use of breakable wells requires a special attention during handling.

Use all calibrators with each run to validate the assay results.

1. Carefully establish the distribution and identification plan for calibrators and patients samples.
2. Prepare the diluted Washing Solution (R2) [Refer to Section 7.2].
3. Take the carrier tray and the strips (R1) out of the protective pouch [Refer to Section 7.2].
4. In individually identified tubes, dilute Calibrators R3, R4a, R4b, R4c and patients samples (S1, S2...) in Diluent (R7) to give a 1/21 dilution: 300 µl of Diluent (R7) and 15 µl of sample [Refer to Section 7.2]. Vortex diluted samples.
5. Strictly following the indicated sequence below, distribute in each well with 200µl of diluted calibrators and patient samples:
6. Cover the microplate with an adhesive plate sealer, then press firmly onto the plate to ensure a tight seal. Incubate the microplate immediately in a thermostat controlled water bath or in a dry incubator for 1 hour ± 5 minutes at 37°C ± 1°C.

7. Before the end of the first incubation period, prepare the conjugate working solution (R6+R7) [Refer to Section 7.2].

8. At the end of the first incubation period, remove the adhesive plate sealer. Aspirate the content of all wells into a container for biohazard waste (containing sodium hypochloride). Wash microplate 4 times with 350 µl of the Washing Solution (R2). Invert the microplate and gently tap on adsorbent paper to remove remaining liquid.

9. Distribute immediately 200 µl of the conjugate working solution (R6+R7) in all wells. The solution must be shaken gently before use.

10. Cover the microplate with an adhesive plate sealer, then press firmly onto the plate to ensure a tight seal. Incubate the microplate immediately in a thermostat controlled water bath or in a dry incubator for 1 hour ± 5 minutes at 37°C ± 1°C.

11. At the end of the second incubation period, remove the adhesive plate sealer. Aspirate the content of all wells into a container for biohazard waste (containing sodium hypochloride). Wash microplate 4 times with 350 µl of the Washing Solution (R2). Invert the microplate and gently tap on adsorbent paper to remove remaining liquid.

12. Quickly distribute into each well and away from light 200 µl of Chromogen solution (R9). Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (+18-30°C). Do no use adhesive plate sealer during this incubation.
13. Stop the enzymatic reaction by adding 100 µl of Stopping Solution (R10) in each well. Use the same sequence and rate of distribution as for the development solution.

14. Carefully wipe the plate bottom. Read the optical density at 450/620 nm using a plate reader within 30 minutes after stopping the reaction. The strips must always be kept away from light before reading.

15. Before reporting results, check for agreement between the reading and the distribution plan of plate and samples.

8. INTERPRETATION OF RESULTS

8.1 Establishing the standard curve

Presence and concentration of IgG antibodies to Rubella in a sample will be determined by comparison of optical density (OD) of this sample to the concentration in International Units per milliliter (IU/ml) of the calibrators of the standard curve. The Platelia™ Rubella IgG assay is standardized to WHO International Standard RUBI 1-94.

Draw the standard curve [OD = function (IU/ml)] by plotting OD readings of calibrators R3, R4a, R4b, R4c on the vertical (Y) axis, then by plotting their respective concentration in IU/ml on the horizontal (X) axis. For each tested sample, calculate the anti-Rubella IgG antibody titer by determining from the drawn standard curve the corresponding concentration to the measured OD.

NB: If the OD reading of a test sample diluted 1/21 is > OD R4c, this test sample should be diluted 1/210 in R7 diluent and re-run in the assay. The related IU/ml values must then be multiplied by a factor of 10.

8.2 Quality Control

Include all calibrators for each microplate and for each run, and analyze the obtained results. For validation of the assay, the following criteria must be met:

- Optical density values:
  - OD R4a ≥ 0.200
  - OD R4b ≥ 0.400

- Optical density ratios:
  - OD R4a / OD R3 ≥ 5.00
  - OD R4b / OD R4a ≥ 1.50
  - OD R4c / OD R4b ≥ 1.20

If those quality control criteria are not met, the test run should be repeated.
8.3 Interpretation of results

<table>
<thead>
<tr>
<th>Anti-rubella antibodies titer (International Units/ml)</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer &lt; 10 IU/ml</td>
<td>Negative</td>
<td>A negative or equivocal result is indicative of absence of acquired immunity, but cannot exclude a recent infection. If a recent infection of the patient is suspected, a second sample should be run about two weeks later.</td>
</tr>
<tr>
<td>10 IU/ml ≤ Titer &lt; 15 IU/ml</td>
<td>Equivocal</td>
<td></td>
</tr>
<tr>
<td>Titer ≥ 15 IU/ml</td>
<td>Positive</td>
<td>A positive result is usually indicative of a past-infection. However, a recent infection cannot be excluded, especially if anti-rubella IgM antibodies are present.</td>
</tr>
</tbody>
</table>

A higher variation of titers can be observed above concentrations of 150 IU/ml.

8.4 Trouble Shooting Guide

Non validated or non repeatable reactions are often caused by:

- Inadequate microplate washings.
- Contamination of negative samples by serum or plasma with a high antibody titer.
- Contamination of the development solution by chemical oxidizing agents (bleach, metal ions...).
- Contamination of the Stopping Solution.

9. PERFORMANCES

Performances of Platelia™ Rubella IgG were evaluated at 2 sites using a total of 952 samples from pregnant women and blood donors. On one site, a comparative study was performed using Platelia™ Rubella IgG TMB (72912). At a second site, the performance of Platelia™ Rubella IgG was evaluated against another commercially available EIA test.

9.1 Prevalence

Prevalence of anti-rubella IgG antibodies using Platelia™ Rubella IgG was estimated on a panel of 322 samples from pregnant women monitored during their pregnancy and from the Northern part of France. 289 samples were positive for anti-rubella IgG antibodies. Prevalence measured with Platelia™ Rubella IgG assay is established at 89.8% (289/322).
9.2 Comparative study (Site 1)

The performance of Platelia™ Rubella IgG was evaluated using a panel of 534 samples grouped as follows:

- 414 sera from blood donors
- 120 sera from pregnant women

Results were compared with those obtained using Platelia™ Rubella IgG TMB (72912) used as a reference.

<table>
<thead>
<tr>
<th>Platelia™ Rubella IgG TMB (72912)</th>
<th>Negative</th>
<th>Doubtful*</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelia™ Rubella IgG (72850)</td>
<td>149</td>
<td>0</td>
<td>0</td>
<td>149</td>
</tr>
<tr>
<td>Doubtful*</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>2</td>
<td>377</td>
<td>379</td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>4</td>
<td>377</td>
<td>534</td>
</tr>
</tbody>
</table>

- Global agreement: 526/526 100.0% [IC 95% = 99.3% - 100.0%]
- Relative specificity: 149/149 100.0% [IC 95% = 97.5% - 100.0%]
- Relative sensitivity: 377/377 100.0% [IC 95% = 99.0% - 100.0%]

*Doubtful samples were not included for sensitivity, specificity and agreement calculations.

[IC95%] = 95% confidence interval.

In addition, 50 sera from 7 vaccination follow-ups (2 with MERUVAX II vaccine, 5 with RUDIVAX vaccine) were evaluated with the two kits:

- The appearance of IgG anti-Rubella followed the same scheme for 6 of the 7 follow-ups.
- The appearance of IgG anti-Rubella was delayed of 5 days with Platelia™ Rubella IgG in one follow-up.

9.3 Performances (Site 2)

368 samples requesting serology for Rubella were tested using Platelia™ Rubella IgG (72850) and were split as follows:

- 44 samples from children below 18 years old (27 girls, 17 boys)
- 323 samples from adults (1 man, 322 women). Adult women samples were from pregnant women or women seen in post-natal consultations within weeks after delivery.
- One serum from the French National Quality Control survey.

Results were compared with those obtained from another commercially available EIA test used as a reference.
9.4 Cross Reactivity

A panel of 177 samples including 147 positive samples for Toxoplasmosis, CMV, EBV, HSV, VZV, mumps, measles and HIV, and 30 positive samples for rheumatoid factor, auto-antibodies and heterophile antibodies were tested with Platelia™ Rubella IgG (72850) and Platelia™ Rubella IgG TMB (72912). Among 10 samples found negative with Platelia™ Rubella IgG TMB (72912), 8 were confirmed negative and 2 were found doubtful with Platelia™ Rubella IgG.

9.5 Precision

- Within-run precision (repeatability):

In order to evaluate intra-assay repeatability, one negative and three positive samples were tested 32 times during the same run. The concentration (IU/ml) was determined for each sample. Mean of concentrations (IU/ml), Standard Deviation (SD) and Coefficient of Variation (%CV) for each specimen are listed in the table below:

<table>
<thead>
<tr>
<th></th>
<th>N=32</th>
<th>Negative Sample</th>
<th>Low Positive sample</th>
<th>Positive sample</th>
<th>High Positive Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration (IU/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>2.8</td>
<td>26.4</td>
<td>56.1</td>
<td>171.4</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td></td>
<td>0.1</td>
<td>1.4</td>
<td>2.8</td>
<td>14.0</td>
</tr>
<tr>
<td><strong>% CV</strong></td>
<td></td>
<td>3.8%</td>
<td>5.2%</td>
<td>5.0%</td>
<td>8.2%</td>
</tr>
</tbody>
</table>
Between-run precision (reproducibility):
In order to evaluate inter-assay reproducibility, the four samples (one negative and three positives) were tested in duplicate in two runs per day over a 20 day period. The concentration (IU/ml) was determined for each sample. Mean of concentrations (IU/ml), Standard Deviation (SD) and Coefficient of Variation (%CV) for each specimen are listed in the table below:

<table>
<thead>
<tr>
<th></th>
<th>N=80</th>
<th>Concentration (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative Sample</td>
<td>Low Positive sample</td>
</tr>
<tr>
<td>Mean</td>
<td>2.4</td>
<td>24.0</td>
</tr>
<tr>
<td>SD</td>
<td>0.8</td>
<td>2.7</td>
</tr>
<tr>
<td>% CV</td>
<td>35.6%</td>
<td>11.2%</td>
</tr>
</tbody>
</table>

9.6 Accuracy and linearity
In order to evaluate the accuracy of calibration, several dilutions of the WHO International Standard RUBI 1-94 were tested. Results obtained demonstrate that Platelia™ Rubella IgG assay is accurately calibrated against the WHO International Standard RUBI 1-94 for values up to 60 IU/ml. Above 60 IU/ml, obtained results are over-estimated about 20%.

Using serial dilutions on 5 positive samples, assay range of Platelia™ Rubella IgG was established between 10 and 150 IU/ml.

10. LIMITATIONS OF THE PROCEDURE
Diagnosis of rubella infection can only be established on the basis of a combination of clinical and biological data. The result of a single test of titration of anti-rubella IgG antibodies does not constitute sufficient proof for the diagnosis of a recent infection by rubella virus.

- Diagnosis of a recent infection can only be made with complete patient information including clinical and biological data (significant increase of anti-Rubella IgG antibodies on 2 patient sera drawn at 3 weeks interval and tested in the same run, presence of anti-Rubella IgM at a significant level, demonstration of low anti-Rubella IgG avidity).
- Presence of anti-Rubella IgM antibodies does not constitute a sufficient proof to confirm a recent infection because IgM can persist several months or even years after infection. When IgM are detected, a quantitative determination of anti-Rubella IgG antibodies should be performed, as well as a follow-up of the evolution of anti-Rubella antibodies at least on a second serum sampled three weeks later.
• If a sample is tested too early during a recent primo-infection, anti-Rubella IgM antibodies could be not yet present. If a suspicion exists, a second sample should be drawn about 3 weeks later on which IgM testing will be performed again.

11. QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to commercialization of the final product. Each lot is submitted to quality control assessments and is released to the market only after conforming to pre-defined acceptance criteria. The records related to production and controls of each single lot are kept within Bio-Rad.

12. REFERENCES


(US) - CE marking [European directive 98/79/CE on in vitro diagnostic medical devices]
(F) - Marquage CE [Directive européenne 98/79/CE relative aux dispositifs médicaux de diagnostic in vitro]
(E) - Marcado CE [Directiva europea 98/79/CE sobre productos sanitarios para diagnóstico in vitro]
(I) - Marchiatura CE [Direttiva europea 98/79/CE relativa ai dispositivi medico-diagnostici in vitro]
(D) - CE Konformitätskennzeichnung [Europäische Richtlinie 98/79/EG über in-vitro-Diagnostika]
(P) - Marcação CE [Directiva europeia 98/79/CE relativa aos dispositivos médicos de diagnóstico in vitro]
(S) - CE-märkning [Europa direktiv 98/79/EG om medicintekniska produkter lör in vitro-diagnostik]
(DK) - CE-mærkningen [Europa direktiv 98/79/EF om medicinsk udstyr til in vitro-diagnostik]
(Gr) - Χαρακτηρισμός CE (ευρωπαϊκή οδηγία 98/79/CE περί in vitro διαγνωστικών εργαλείων)
(PL) - CE oznaczenie [Direktyma unijna 98/79/CE dotycząca produktów medycznych do badań in vitro]
(LT) - CE ženklas [Europos sąjungos direktyva 98/79/CE dėl in vitro diagnostikos medicinos prietaisų]
(H) - CE jels [98/79/CE Európai irányelv az in vitro orvosi diagnosztikai eszközök]
(ES) - CE marcas [Diretiva europea 98/79/CE de productos médico-diagnósticos]
(SK) - CE značka [Evropská direktiva 98/79/CE o diagnostických zdravotníckych prostriedkoch]
(CZ) - CE značka [Evropská direktiva 98/79/CE o diagnostických zdravotníckych prostriedkoch]

(US) - For in vitro diagnostic use
(F) - Pour diagnostic in vitro
(E) - Para diagnóstico in vitro
(I) - Per uso diagnostico in vitro
(D) - In-vitro-Diagnostikum
(P) - Para uso diagnostico in vitro
(S) - In vitro diagnostik
(DK) - In vitro diagnose
(Gr) - Για in vitro διαγνωστική χρήση
(PL) - Do stosowania in vitro
(LT) - in vitro diagnostikai
(H) - Csak in vitro diagnosztikai alkalmazásra
(EST) - In vitro diagnostiliseks kasutamiseks
(SK) - Na diagnostiku in vitro
(CZ) - Pro diagnostiku in vitro

(US) - Manufactur
(F) - Fabricant
(E) - Fabricante
(I) - Produttore
(D) - Hersteller
(P) - Fabricante
(S) - Tillverkade
(DK) - Fremstilte af
(Gr) - Κατασκευαστής
(PL) - Producent
(LT) - Gamintojas
(H) - Gyáró
(EST) - Tootja
(SK) - Výrobca
(CZ) - Výrobce

(US) - Catalogue number
(F) - Référence catalogue
(E) - Número de catálogo
(I) - Numero di catalogo
(D) - Bestellnummer
(P) - Número de catálogo
(S) - Katalognummer
(DK) - Katalognummer
(Gr) - Αριθμός καταλόγου
(PL) - Numer katalogu
(LT) - Katalogo numeris
(H) - Cikkszám
(EST) - Katalooginumber
(SK) - Katalógové číslo
(CZ) - Katalogové číslo

(US) - Authorised Representative
(F) - Représentant agréé
(E) - Representante autorizado
(I) - Distributore autorizzato
(D) - Bevollmächtinger
(P) - Representante Autorizado
(S) - Auktoriserad representant
(DK) - Autoriseret repræsentant
(Gr) - Εξουσιοδοτημένος αντιπρόσωπος
(PL) - Upoważniony przedstawiciel
(LT) - Galiotasis atstovas
(H) - Meghatalmazott Képviselő
(EST) - Valitutud esindaja
(SK) - Autorizovaný zástupca
(CZ) - Zplnomocněný zástupce

(US) - Batch code
(F) - Code du lot
(E) - Código de lote
(I) - Codice del lotto
(D) - Chorgen-Bezeichnung
(P) - Código del lote
(S) - Batch nr.
(DK) - Batchkoden
(Gr) - Κωδικός παρτίδας
(PL) - Numer serii
(LT) - Serijos numeris
(H) - Gyártási szám
(EST) - Partiki kood
(SK) - Číslo šarže
(CZ) - Číslo šarže

(US) - Expiry date YYYY/MM/DD
(F) - Date de péremption AAAA/MM/JJ
(E) - Estable hasta AAAA/MM/DD
(I) - Da utilizzare prima del AAAA/MM/GG
(D) - Verwendbar bis MMM/MM/TT
(P) - Data de expiração AAAA/MM/DD
(S) - Utdrivningstid År/Månad/Dag
(DK) - Anvendes far AAAA/MM/DD
(Gr) - Ημερομηνία έξαπλωσης YYYY/MM/DD
(PL) - Data ważności YYYY/MM/DD
(LT) - Galioja iki YYYY/MM/DD
(H) - Szavatasság iói EEE/HH/NN
(EST) - Aegumistah tähteg AAAA/KK/PP
(SK) - Použitelné do RRRR/MM/DD
(CZ) - Datum expirace RRRR/MM/DD
<table>
<thead>
<tr>
<th>Language</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>The other languages which are required in conformity to the European Directive can be obtained from your local Bio-Rad agent.</td>
</tr>
<tr>
<td>F</td>
<td>Les autres langues requises par la Directive Européenne sont disponibles auprès de votre représentant Bio-Rad local.</td>
</tr>
<tr>
<td>E</td>
<td>Los otros idiomas que se requieren para la conformidad de la Directiva Europea puede ser obtenida en su oficina local Bio-Rad.</td>
</tr>
<tr>
<td>I</td>
<td>Le altre lingue che sono richieste in conformità con le Direttive Europee possono essere ottenute dal locale agente Bio-Rad.</td>
</tr>
<tr>
<td>D</td>
<td>Die anderen Sprachen, die in Übereinstimmung mit der europäischen IVD Direktive benötigt werden, erhalten Sie über Ihre lokale Bio-Rad Niederlassung.</td>
</tr>
<tr>
<td>P</td>
<td>As restantes linguas, obrigatórias em conformidade com a Directiva Europeia, podem ser obtidas através da subsidiária Bio-Rad mais próxima de si.</td>
</tr>
<tr>
<td>S</td>
<td>Övriga språk som krävs i enlighet med EG-direktivet kan erhållas från din lokala Bio-Rad-representant.</td>
</tr>
<tr>
<td>DK</td>
<td>De øvrige sprog som kræves i henhold til EU direktiv kan fås ved henvendelse til den lokale Bio-Rad leverandør.</td>
</tr>
<tr>
<td>GR</td>
<td>Τις υπόλοιπες γλώσσες που απαιτούνται για την συμμόρφωση στην Ευρωπαϊκή διάταξη μπορείτε να τις προμηθεύσετε από τον τοπικό σας αντιπρότοπο Bio-Rad.</td>
</tr>
<tr>
<td>PL</td>
<td>Tłumaczenie w innych językach które są wymagane w Dyrektywie Unijnej może być otrzymane od lokalnego przedstawiciela firmy Bio-Rad.</td>
</tr>
<tr>
<td>LT</td>
<td>Vertimus, reikalius pagal Europos sajungos direktyvos reikalavimus, j kitas kalbas galite gauti iš vietinio Bio-Rad atstovo.</td>
</tr>
<tr>
<td>H</td>
<td>A leírás az Európai Irányelv által előírt egyéb nyelveken hozzáférhető a Bio-Rad helyi kirendeltségéinél.</td>
</tr>
<tr>
<td>EST</td>
<td>Teised vastavalt Euroopa Direktivile nõutavad keeled on saadavat kohaliku Bio-Rad edasmüüja käest.</td>
</tr>
<tr>
<td>SK</td>
<td>Ostatné jazykové verzie, které sú vyžadované v zhode s Európskou direktivou, možno obdržať od všetkého lokálneho zástupcu Bio-Rad.</td>
</tr>
<tr>
<td>CZ</td>
<td>Další jazykové verze vyžadované ve shodě s evropskou direktivou jsou k dispozici u lokálního zástupce firmy Bio-Rad.</td>
</tr>
</tbody>
</table>