Avidity Rubella Virus IgG
(ARUB7400)

Performance Characteristics
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1 Introduction

Rubella is an enveloped RNA virus belonging to the toga viruses. It has a spherical shape measuring about 50-70 nm in diameter. There appears to be only one antigenic type, and no cross-reactivity with alpha viruses or other members of the toga virus group has been found. Rubella viruses are pathogens of the respiratory tract and transmitted mainly by droplet infection. Rubella is a worldwide common contagious disease with mild constitutional symptoms and a generalized rush. In childhood, it is an inconsequential illness, but when it occurs during pregnancy, there is a significant risk of severe damage to the foetus.

The risk of congenital rubella depends primarily on the month of pregnancy in which infection is acquired: overall, app. 16% of infants have major defects at birth following maternal rubella in the first 3 months of pregnancy. Congenital rubella infection may lead to a syndrome with single or multiple organ involvements, known as embryopathia rubeolosa. In some cases infection is inapparent but results in consequential damages as eye defects, deafness, growth retardation, and others. Naturally acquired immunity usually is long-lasting, but reinfection is possible due to decreasing levels of circulating antibodies. For immunization a vaccine containing live virus is used.

Table 1: Rubella Virus - Symptoms and Mechanism of Infection

<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)</td>
<td>Generalized rush (fever, nausea)</td>
<td>Transmission by close person-to-person contact; spread most probably by droplets via the respiratory tract</td>
</tr>
<tr>
<td></td>
<td>Congenital rubella syndrome (Embryopathia rubeolosa)</td>
<td>Cardiovascular lesions, eye defects, hearing impairment, CNS involvement and others</td>
<td>Foetal infection: transmission by haematogenous spread during maternal viremia</td>
</tr>
</tbody>
</table>

The presence of pathogen resp. infection may be identified by:

- PCR (detection of viral RNA)
- Hemagglutination inhibition (HAI), Haemolysis-in-gel test (HiG)
- EIA, ELISA (detection of specific antibodies).

The presence of IgG antibodies to Rubella virus indicates the occurrence of the infection but does not distinguish between recent and past infection. Virus-specific IgM antibodies are first detected ten days and peak at about four weeks post infection. They may persist for more than seven months after acute infections. Based on the evidence that antibody avidity gradually increases after exposure to an immunogen, avidity of IgG antibodies can be used as a marker for distinguishing recent primary from long-term infections. Avidity describes the binding strength of a specific antibody to its antigen. Low-avidity IgG antibodies indicate a primary infection, whereas the presence of IgG antibodies with high avidity points to persistency or reactivation of infection.
2 Intended Use
The Avidity Rubella Virus IgG test is intended to indicate the rubella-specific IgG avidity in human serum or plasma (citrate, heparin) to differentiate between acute and past infection.

3 Principle of the Assay
The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microplates are coated with specific antigens to bind corresponding antibodies of the sample (dual pipetting). After washing the wells to remove all unbound sample material, one well is incubated with avidity reagent and the corresponding well with washing buffer. The avidity reagent removes the low-avidity antibodies from the antigens whereas the high-avidity ones are still bound to the specific antigens. After second washing step to remove the rest of avidity reagent and low-avidity antibodies, a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a third washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Material

<table>
<thead>
<tr>
<th>Avidity Rubella Virus IgG</th>
<th>Lot: ARUB-003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production date: 2014-08</td>
<td>Expiry date: 2015-08-31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Avidity Rubella Virus IgG</th>
<th>Lot: ARUB-004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production date: 2014-09</td>
<td>Expiry date: 2015-09-30</td>
</tr>
</tbody>
</table>

Test Description
The reproducibility of the Avidity Rubella Virus IgG test was determined by comparing 24 replicates of 3 different samples in one assay (within-run) and by comparing 3 different samples assayed in 10 different runs (between-run).

Acceptance Criterion: CV < 15 %
Results

Within-run and between-run precision were estimated by analysis of variance and are presented in tables 2 and 3.

Table 2: Within-Run Precision (ARUB--003)

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (Avidity [%])</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>24</td>
<td>87.4</td>
<td>4.1</td>
</tr>
<tr>
<td>#2</td>
<td>24</td>
<td>11.5</td>
<td>7.1</td>
</tr>
<tr>
<td>#3</td>
<td>24</td>
<td>84.0</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Table 3: Between-Run Precision (ARUB-004)

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (Avidity [%])</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>10</td>
<td>90.8</td>
<td>2.3</td>
</tr>
<tr>
<td>#2</td>
<td>10</td>
<td>10.8</td>
<td>13.9</td>
</tr>
<tr>
<td>#3</td>
<td>10</td>
<td>85.4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Conclusion

The acceptance criterion was met for all samples.

4.2 Diagnostic Performance

Introduction

The purpose of this study was to determine the efficiency of the assay to discriminate between high-avidity (past infection) and low-avidity (acute infection) clinical samples.

To evaluate the diagnostic performance of the Avidity Rubella Virus IgG test, internal studies were conducted by NovaTec with well defined samples from External Quality Control Schemes (INSTAND e.V., Labquality, UK NEQAS, RfB) and Boston Biomedica, Inc. (Anti-Rubella Mixed Titer Performance Panel), and in comparison to an immunoassay already established on the market (SERION ELISA classic Rubella Virus Avidity Test).

Samples from newborns and immunocompromised individuals were excluded from the study as in these patients serological data only have limited value.

Material

Avidity Rubella Virus IgG  Lot: ARUB-001
Production date: 2014-08  Expiry date: 2015-08-31
Avidity Rubella Virus IgG  Lot: ARUB-003
Production date: 2014-08  
Avidity Rubella Virus IgG  
Lot: ARUB-004  
Expiry date: 2015-08-31

Production date: 2014-09  
Avidity Rubella Virus IgG  
Lot: ARUB-008  
Expiry date: 2015-09-30

Production date: 2016-01  
Avidity Rubella Virus IgG  
Lot: ARUB-008  
Expiry date: 2017-01-31

SERION ELISA classic Rubella Virus  
Avidity Reagent (B129AVID)  
Lot: SID.CI  
Expiry date: 2023-09

SERION ELISA classic Rubella Virus IgG  
(ESR129G)  
Lot: SKD.DH  
Expiry date: 2015-10

78 samples (including 37 EQAS samples)

**Results**

Table 4: Diagnostic Performance

<table>
<thead>
<tr>
<th>Demand</th>
<th>low avidity</th>
<th>high avidity</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avidity Rubella Virus IgG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low avidity</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>high avidity</td>
<td>1</td>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td>Σ</td>
<td>15</td>
<td>59</td>
<td>74</td>
</tr>
</tbody>
</table>

(Equivocal results were not included in the calculations)

**Conclusion**

Agreement: 98.6 % (73/74)

The acceptance criterion for the diagnostic performance evaluation is met.

07.06.2015 Dr. I. Ovsiy