Waaler Rose: A slide passive haemagglutination test, without dilution of the sample

**PRINCIPLE OF THE METHOD**
The Waaler-Rose reagent is a suspension of stabilized sheep red cells sensitized with anti-sheep rabbit IgG.
The test reagent sensitivity has been adjusted to detect a minimum of 8 IU/ml of rheumatoid factors according to the WHO International Standard without previous sample dilution.

**REAGENTS**
- **Waaler Rose**: Stabilized sheep erythrocytes sensitized with rabbit IgG anti-sheep erythrocyte, pH 8.2. Sodium azide 0.95 g/L.
- **Control +**: Human serum with a RF concentration ≥ 30 IU/mL. Sodium azide 0.95 g/L.
- **Control -**: Animal serum. Sodium azide 0.95 g/L.

**PRECAUTIONS**
Reagents containing sodium azide may combine with copper and lead plumbing to form highly explosive metal azides. Dispose of reagent by flushing with large amounts of water to prevent azide build-up.
The positive controls were prepared from human sera, which have been tested using FDA licensed methods and are found to be non-reactive for the presence of HbsAg, HCV and HIV(1/2) antibodies. However, no test method can offer complete assurance that infectious agents are absent. Therefore all human specimens should be considered potentially infectious.

**CALIBRATION**
The Waaler Rose sensitivity is calibrated against the international RF Reference WHO 64/1 Rheumatoid Arthritis Serum.

**PREPARATION AND STABILITY**
Shake the Reagent before use. After that it must be uniform and without visible clumping. The test sensitivity depends on the drop volume (50 µl). Do not use droppers other than those provided and place the dropper perpendicular to the slide surface.
The reagent and control sera are stable up to the expiry date when stored at +2 to 8°C. Do not freeze!

**SAMPLES**
Use fresh clear serum specimens obtained by centrifugation of clotted blood. Lipemic, haematic or contaminated serum may cause erroneous results. If the test cannot be performed immediately, store the specimen at +2 to +8°C for up to 7 days. For longer storage up to 3 months, freeze the serum at –20°C. Samples with the presence of fibrin should be centrifuged before testing.

**PROCEDURE**
**Qualitative Test**
1. Bring reagents and specimens to room temperature before use.
2. Gently shake the reagent to disperse the particles
3. Place a drop (50 µl) of undiluted serum onto a circle of the slide.
4. Place a drop (50 µl) of each Positive and Negative controls on separate circles of the slide.
5. Add a drop (50 µl) of the Waaler Rose Reagent next to the drop of serum.
6. Mix both drops spreading them over the full surface of the circle.
7. Put the slide on a flat surface for 2 minutes.
8. After this time twist the slide 45 degrees once and let again rest for one minute.
9. Read the presence or absence of visible agglutination immediately avoiding any movement or lifting the slide during observation.

**Non specific agglutination could occur if the test is read later.**

**Semi - Quantitative Test**
The semi quantitative test will be performed in the same was as the qualitative test but using previous dilution of the serum sample in saline (NaCl 9g/l).

**Dilutions**

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>1/2</th>
<th>1/2</th>
<th>1/8</th>
<th>1/16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample serum</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Saline</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

**Volume of sample**

| 50 µl | 50 µl | 50 µl | 50 µl |

**READING AND INTERPRETATION**
Reading: Presence of agglutination indicates a contend of RF in the sample equal or greater than 8 IU/ml. The lack of agglutination indicates a RF level lower than 8 IU/ml in the sample.
The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.
Concentration will be the reciprocal of positive reading dilution:

<table>
<thead>
<tr>
<th>8 x n° of dilution</th>
<th>8 x 2</th>
<th>8 x 4</th>
<th>8 x 8</th>
<th>8 x 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU/ml</td>
<td>16</td>
<td>32</td>
<td>64</td>
<td>128</td>
</tr>
</tbody>
</table>

**Normal Levels**: Adults < 8 IU/ml

**QUALITY CONTROL**
Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.

**PERFORMANCE CHARACTERISTICS**
1. **Analytical Sensitivity**: 8 (6-16) IU/mL, under the described assay conditions.
2. **Prozone effect**: No prozone effect was detected up to 800 IU/mL.
3. **Diagnostic sensitivity**: 100 %.
4. **Diagnostic specificity**: 93.6 %.

**INTERFERENCES**
Hemoglobin (10 g/L), bilirubin (20 mg/dL) and lipemia (10 g/L), do not interfere. Other substances may interfere.

**LIMITATIONS**
The Rheumatoid Factors are immunoglobulins (most of the IgM) with antibody activity. These factors are present in most patients suffering Rheumatoid Arthritis. There are different rheumatoid factors and there is no test capable of detecting them all, due to the fact that some act against human IgG, other against animal IgG, and others against both IgG. We recommend the use of the RF latex test, specific for detection of rheumatoid factors acting against human IgG.
The incidence of false positive results is about 3-5 %. Individuals suffering from infectious mononucleosis, hepatitis, syphilis as well as elderly people may give positive results.

Langdorp, September 2007