INTRODUCTION

Rheumatoid factors are antibodies which are directed against the changed tertiary structure of the IgG Fc fragment. They occur in all immunoglobulin classes; the RF isotypes are designated IgM, IgG, and IgA rheumatoid factors. Numerous test system have been described for the determination of rheumatoid factors including agglutination of sheep erythrocytes sensitized with rabbit IgG, agglutination of polystyrene latex particles coated with human IgG, as well as radioimmunoassays (RIA) and enzyme immunoassays (ELISA).

Rheumatoid factors (antiglobulins of the IgG, IgA, and IgM class) have been shown to form immune complexes (ICs) in serum and joint fluid either by self-association or by reaction with native IgG. These ICs can be readily detected in biopsies by indirect immunofluorescence in a wide range of diseases affecting many tissues of the body. Complex deposition is most common in tissues where blood flow is restricted, resulting in dermatitis (skin), nephritis (kidney), synovitis and arthritis (joints), vasculitis (vessels in many tissues).

Testing for RF has a high diagnostic value as their exclusion or detection can support or place doubt on a tentative diagnosis made on the basis of case history data and clinical findings. A seropositive rheumatoid arthritis generally has a more unfavourable prognosis than the seronegative. Elevated RF titers often indicate a severe course and a generalized pathological process. The laboratory finding “seronegative RF” should be interpreted to mean that rheumatoid factors are detectable neither in the latex agglutination nor in the Waaler-Rose test. Results of the determination of rheumatoid factors should, however, always be assessed in connection with clinical and other laboratory findings as comparatively low RF levels do not necessarily indicate the absence of rheumatoid arthritis and as RH concentrations are not restricted only to rheumatoid diseases; additional test for the presence of a disease may be necessary.
PRINCIPLE

Latex particles (Polystyrene particles) coated with human gamma-globulin (IgG) are agglutinated when mixed with samples containing rheumatoid factors.

REAGENTS

A. An aqueous suspension of approximately 1% latex particles (polystyrene particles) which are sensitized (coated) with human gammaglobulin.
B. RF Control Serum, positive, (human), liquid human serum containing the labeled content of rheumatoid factors in I.U./ml, calibrated using the “International Reference C. Preparation of Rheumatoid Arthritis Serum.
Control Serum, negative, (human), fluid human serum with a rheumatoid factor activity of maximum 1 I.U/ml.

SPECIMENS

Use serum (clotted blood) or plasma which should be as fresh as possible (stored for max. 8 days at +2 to +8 C) or stored in the deep freeze. Deep frozen samples (at –25 C or below) must have been frozen within 24 hours after collection and must be used within 3 months; repeated thawing and freezing is to be avoided. Serum samples should be completely coagulated and should contain no particles or traces of fibrin after centrifugation. Highly lipaemic samples or frozen samples which become turbid after thawing should be clarified by centrifugation (10 min, at approx. 15,000 x g) before use in the test.

METHOD

A. Qualitative determination on the test plate

1. Bring the patient specimens and reagents to room temperature.
2. Apply 40 μl of the patient specimen (undiluted), one drop (approx. 40 μl) of positive and one drop of negative control serum onto separate fields of a test plate.
3. Place one drop of the aqueous suspension of the sensitized latex particles (approx. 40 μl) (shake well before use) next to each patient specimen and control serum; mix well with a stirring rod and then agitate the test plate with a rotating action.
4. After 2 minutes examine for agglutination.

- Distinct agglutination indicates the presence of rheumatoid factors (RF); patients specimens that do not react with the latex particles contain either NO rheumatoid factors or concentration below 20 I.U/ml.
B. Semi-quantitative determination

1. **Semi-quantitative Test Plate:** A semi-quantitative result can be obtained by preparing serial dilutions of the *patient specimen* and *positive control* with isotonic saline e.g., as follows:

   Then proceed as described under “A”.

   Without the use of the positive control, the RF concentration can be estimated by reference to the following table:

<table>
<thead>
<tr>
<th>Agglutination of the serum dilution</th>
<th>FR Conc. (I.U/m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 2</td>
<td>approx. 40</td>
</tr>
<tr>
<td>1: 4</td>
<td>approx. 80</td>
</tr>
<tr>
<td>1: 8</td>
<td>approx. 160</td>
</tr>
<tr>
<td>1: 16</td>
<td>approx. 320</td>
</tr>
</tbody>
</table>
2. **Semi-quantitative “Tube” Test.**

A. Label 12 test tubes (diameter approx. 9mm) and pipette 2 ml of isotonic saline into the 1st tube, 1 ml /tube into tubes 2 to 10 and 0.9 ml/tube into tubes 11 and 12.

B. Add 0.5 ml of patient sample to tube 1. Mix and serially transfer 1ml from tube 1 to 10. Discard 0.5 ml from tube 1 and 1.0 ml from tube 10.

C. Pipette 0.1 ml of the positive control serum into tube 11 and 0.1 ml of the negative control serum into tube 12.

D. For the tube test, 2 volumes of isotonic saline must be added to 1 volume of the latex suspension (polystyrene particles coated with human IgG) and mixed thoroughly (including the contents of the pipette). The diluted reagent has limited stability and should be prepared fresh each day.

E. Add 1 drop of the 1 + 2 diluted rheumatoid reagent to each sample dilution (tubes 1 to 12), mix and incubate for 15 minutes at + 37 C in the water bath.

F. Centrifuge the tubes for 5 minutes at 1000 x g (3000 r.p.m).

G. Gently shake the tubes in order to read the result.

H. Examine the tubes for visible agglutination against a dark background.

**Internal quality control**

RF control serum (human), positive, for IgG-latex coated particles, and control serum (human), negative, should be included in each test series. RF control serum, positive, must exhibit agglutination (DISTINCT AGGLUTINATION) and the negative control serum must exhibit a homogeneous suspension (NO AGGLUTINATION).

**EVALUATION**

A. Qualitative test

Distinct agglutination is exhibited by samples with an RF content of >20 I.U./ml. Samples with an RF content of below 20 I.U/ml exhibit no agglutination.

B. Semi-quantitative tube test

The titer is given by the last sample dilution in which there is visible agglutination. With the aid of the Behringwerke Rheumatoid Factor Reference Serum, values obtained from various methods can be standardized in international units and thus made comparable. For this purpose, the Rheumatoid Factor Reference Serum is run in parallel in the test and the titers obtained are used in the following formula:

\[
\text{I.U./ml} = \frac{\text{Titer (RF Reference serum)}}{\text{Titer (RF concentration in the patient sample)} \times \text{X (patient sample)}}
\]
LIMITATIONS & INTERFERENCES

Reading the results after more than 2 minutes can lead to False-positive result. Highly lipaemic sera/plasma may cause unspecific reactions. When citrated plasma are determined, the dilution factor hat to be taken into account in the evaluation. After use, close the dropper vials securely to avoid drying due to evaporation.

The strength of agglutination is not a measure of the RF activity in the sample. Do Not freeze the latex reagents.
Due to the difference in sensitivity between the slide and tube test, different results may be obtained on the test preparations at values below the detection limit of the slide test (i.e. below 20 I.U./ml).

REFERENCE INTERVALS

As reference ranges are subjected to many influencing variables which may differ for each population, each laboratory should establish its own reference range.

SENSITIVITY

In the qualitative RF test distinct agglutination is indicative of rheumatoid factors of approximately 20 I.U./ml. The sensitivity of the semi-quantitative tube test is approximately 1 I.U./ml.

SPECIFICITY

The test is specific for rheumatoid factors. The latex particles reacts preferentially with IgM rheumatoid factors.

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