Quantitative determination of Rheumatoid Factor (RF) in Serum by means of particle-enhanced turbidimetric immunoassay.

Only for in vitro use in clinical laboratory (IVD).

Clinical Significance

The most consistent serological feature of rheumatoid arthritis is the increased concentration of auto antibodies directed against antigenic sites in the Fc region of human and animal IgG, namely rheumatoid factors (RFs) in the blood and joint fluid. The potential role of these factors in the pathogenesis of this disease has been studied extensively, with the finding that both environmental and genetic factors affect production of RF. RF determinations are clinically important for the diagnosis, prognosis, and assessment of therapeutic efficacy of rheumatoid arthritis. Although RFs may be found in all immunoglobulin classes, the RF most frequently detected in the laboratory is IgM type, present in about 75 - 80 % of adult patients with rheumatoid arthritis but in about 10 % of children with juvenile rheumatoid arthritis.

Principle

This RF test is based upon the reactions between IgM-anti-IgG (RF) in patient’s sample and latex-covalently bound human IgG. RF values are determined photometrically.

Reagents

Each RF kit contains:

A. Buffer - 45 ml of Phosphate buffer (0.05 M) pH: 7.0 containing NaCl (0.15 M), detergent and polyethylene glycol. Preservative: Sodium azide < 1g/L

B. Latex reagent - 5 ml of suspension of latex microparticles covalently bound human IgG in a glycin buffer (0.1 M, pH: 8.2), containing NaCl (0.15 M) and bovine serum albumin (0.5%). Preservative: Sodium azide 0.075%

C. Buffer Dil – 15 ml of buffer TRIS, pH: 7.0. Preservative: sodium azide < 1g/L

D. - Calibrator – 1 ml Human - based reference fluid. Preservative: sodium azide, 0.075 %. All raw materials of human origin used in the manufacture of this product showed no reactivity when tested for HBsAg, anti-HIV-1/2 and HCV with commercially available test methods. However, this product should be handled as though capable of transmitting infectious diseases.

Reagent Preparation

Working Reagent is prepared with 1 part of Latex Reagent and 9 parts of Buffer Reagent. Prepare a fresh WR based on its workload. (shake gently the reagents before pipetting).

It is recommended that each Laboratory prepare a fresh Working Reagent based on its workload.

Stability

Reagents in the original vial are stable to the expiration date on the vial label when capped and stored at +2 - +8°C. Immediately following the completion of an assay run, the reagent vial should be capped until the next use in order to maximize curve stability. Once opened the reagent can be used within 6 months if stored tightly closed at +2 - +8°C after use. Do not freeze reagents.

The RF latex reagent should have a white, turbid appearance free of granular particulate. Visible agglutination or precipitation may be a sign of deterioration, and the reagent should be discarded.

The RF buffer reagent should be clear and colorless. Any turbidity may be a sign of deterioration and reagent should be discarded.

Calibration Curve and Controls

One-point calibration by diluting high-level calibrator with Buffer Dil. by 1+1 Analytical Range up to 200 IU/ml.

Calibrator 1 100 µl of Biolatex RF Calibrator

Calibrator 2 100 µl of Calibrator 1 + 100 µl of Buffer Dil

Calibrator 3 100 µl of Calibrator 2 + 100 µl of Buffer Dil

Calibrator 4 100 µl of Calibrator 3 + 100 µl of Buffer Dil

Calibrator 5 100 µl of Buffer Dil

(*) See values on the label or on the insert. Multiply by the appropriate factor.

For quality control use a suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Precautions

For in vitro diagnostic use only. Do not pipette by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices. Disposal of all waste material should be in accordance with local guidelines. As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Specimens

Serum specimens should be collected by venipuncture following good laboratory practices. RF remain stable for 72 hours at +2...+8°C. If the test should be performed later, it is recommended to freeze the serum. Heavily lipemic specimens, or turbid frozen specimens after thawing, must be clarified before the assay with a delipidating agent or by a high-speed centrifugation. Delipidation of samples do not affect the results of RF in serum samples. The cleared patient serum sample must be used on the same day, as turbidity may reoccur. Heat inactivation of the sera is not necessary since C1q complement factor do not interfere in the assay.

Procedure

Wavelength: 600 nm (600 - 650nm)

Temperature: 37°C

Cuvette: 1cm light path

Measure against distilled water blank.

Bring the reagents at 37°C and pipette:

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 µl</td>
<td>---</td>
</tr>
<tr>
<td>15 µl</td>
<td>15 µl</td>
</tr>
</tbody>
</table>

Mix and measure absorbance immediately (A1) incubate 2.5 min (37°C), after incubation read absorbance (A2).

Calculation

Plot the calibration curve and the sample concentration is obtained by interpolation the sample absorbance (A2-A1) in the calibration curve. If is a one point calculation:

\[
(A2 - A1)_{\text{sample}} = \frac{(A2 - A1)_{\text{blank}} X \text{Calibrator Concentration}}{(A2 - A1)_{\text{Calibrator}} - (A2 - A1)_{\text{blank}}}
\]

Reference Values

Values <20 IU/ml are within the normal range.

This data has to be interpreted as a guide. Each laboratory should establish its own reference intervals.

Reagent Performance

Linearity

The range interval for the multipoint calibration method is from 0 to 200 IU/ml. With this method you can use the one point calibration procedure using a calibrator with dilutions. One point calibration is linear up to 100 IU/ml. When values exceed this range the samples should be diluted with Dilution Buffer and the result should be multiplied by the appropriated factor.

Sensitivity

Calculating the mean plus 3SD of twenty replicates of zero standards resulted in a lower limit of detection less than 10 IU/ml.

Specificity

The assay is specific for RF determination. Interference from bilirubin (to 427 µmol/L), haemoglobin (to 15 g/L) and intralipid (to 0.5 %) has not been observed. Other substances can interfere. For a comprehensive review of interfering substances, refer to the publication by Young.
Prozone Effect
The system did not show prozone phenomenon at least up to 1500 IU/ml

Assay Precision
Intra-assay coefficients of variation (CV) for three samples (RF values ranging from 24 to 170 IU/ml) were between 2.1 and 4.7 %. Inter-assay CVs were between 2.5 and 3.2 %.

Method comparison
14 samples were correlated with a nephelometric commercial procedure. When comparing the results by Passing-Bablok regression the result was:

\[ y = 0.87x + 6.2 \]

Analytical characteristics have been obtained in a single experiment in a conventional spectrophotometer. Therefore, the data expressed in the present document should be interpreted as a guide example.

Test Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>Fixed Time Kinetic</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>600</td>
</tr>
<tr>
<td>Sample Volume (µl)</td>
<td>15</td>
</tr>
<tr>
<td>Working Reagent Volume (µl)</td>
<td>500</td>
</tr>
<tr>
<td>Lag Time (A1) (Sec.)</td>
<td>5</td>
</tr>
<tr>
<td>Measuring Time (A2) (Sec.)</td>
<td>150</td>
</tr>
<tr>
<td>Calibrator Conc. (IU/mL)</td>
<td>As on vial</td>
</tr>
<tr>
<td>Reaction temperature (°C)</td>
<td>37</td>
</tr>
<tr>
<td>Reaction Direction</td>
<td>Increasing</td>
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<tr>
<td>Normal Low</td>
<td>0</td>
</tr>
<tr>
<td>Normal High</td>
<td>20</td>
</tr>
<tr>
<td>Blank with</td>
<td>Distilled Water</td>
</tr>
<tr>
<td>Units</td>
<td>IU/ml</td>
</tr>
<tr>
<td>Linearity Limit</td>
<td>Upto 200 (Multipoint calibration)</td>
</tr>
<tr>
<td></td>
<td>Upto 100 (One point calibration)</td>
</tr>
</tbody>
</table>

Literatures
6. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two analytical methods.

Manufactured in Spain for

[Synergy logo]

A Division of

Euro Diagnostic Systems Pvt. Ltd.

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