In vitro diagnostic reagents for the quantitative determination of Rheumatoid Factor (RF) in human serum by means of particle-enhanced turbidimetric immunoassay.

**Background**

The most consistent serological feature of rheumatoid arthritis is the increased concentration of autoantibodies 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.

**Test 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**

**Buffer**
- Phosphate buffer (0.05 M) pH: 7.0 containing NaCl (0.15M), detergent and polyethylene glycol.
- Preservative: sodium azide < 1g/L

**Latex reagent**
- A suspension of latex particles covalently bound human IgG, in a glycine buffer (0.1 M, pH: 8.2), containing NaCl (0.15 M) and bovine serum albumin (0.5%).
- Preservative: Sodium azide 0.075%

**Buffer Dil**
- Buffer TRIS, pH: 7.0. Preservative: sodium azide < 1g/L

**Calibrator**
- 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. 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 activation of the sera is not necessary since C1q complement factor do not interfere in the assay.

**Precautions and Warnings**

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.

**Material Required**
- Spectrophotometric analyzer.
- Controls.

**Storage and Stability**

Reagents in the original vial is stable to the expiration date on the vial label when capped and stored at (2-8 ºC). Immediately following the completion of an assay run, the reagent vial should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 1 month if stored tightly closed at (2-8 ºC) 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 colourless. Any turbidity may be sign of deterioration and reagent should be discarded.

**Specimen Collection and Preparation**

Serum specimens should be collected by venipuncture following good laboratory practices. RF remain stable for 72 hours at (2-8 ºC). 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 activation of the sera is not necessary since C1q complement factor do not interfere in the assay.

**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.

**Procedure**

<table>
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<tr>
<th>Substance</th>
<th>Volume</th>
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<tbody>
<tr>
<td>Calibrator</td>
<td>15 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>15 µl</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>500 µl</td>
</tr>
<tr>
<td>Work. Reagent</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

Mix and measure absorbance immediately (A1) incubate 2 min (37 ºC), 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 an one point calibration:

\[
\frac{(A2-A1)_{\text{Sample}} - (A2-A1)_{\text{blank}}}{(A2-A1)_{\text{Calibrator}} - (A2-A1)_{\text{blank}}} \times \text{Calibrator concentration}
\]
Linearity

Up to 140 IU/mL

Calibration and Quality Control

| Calibrator 1 | 100 µl of Spectrum RF Calibrator* |
| Calibrator 2 | 100 µl of Calibrator 1 + 100 µl of Saline Solution |
| Calibrator 3 | 100 µl of Calibrator 2 + 100 µl of Saline Solution |
| Calibrator 4 | 100 µl of Calibrator 3 + 100 µl of Saline Solution |
| 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 Spectrum Control or other 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.

Expected 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.

References


Sonderdruck aus DG Klinische Chemie Mitteilungen 1995; 26: 207 – 224

ORDERING INFORMATION

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