**RHEUMATOID FACTOR**

**LATEX-ENHANCED IMMUNOTURBIDIMETRIC ASSAY**

**FOR SERUM RHEUMATOID FACTOR**

**INTENDED USE**
The RF latex-enhanced immunoturbidimetric assay is for the quantitative determination of RF in serum. The test system is intended to be used as an aid in the diagnosis of rheumatoid arthritis. For in vitro diagnostic use only.

**Cat. No.**

RF 790 30 Tests 1. RF Assay Buffer 1 x 30 ml
2. RF Latex Reagent 1 x 10 ml

RF 2574 600 Tests 1. RF Assay Buffer 3 x 100 ml
2. RF Latex Reagent 2 x 100 ml

**CLINICAL SIGNIFICANCE**
The agglutination reaction between antibody coated red blood cells and sera from patients with Rheumatoid Arthritis (RA), was first demonstrated by Waaler and Rose in 1940. The reaction has since been shown to be caused by certain factors in the sera from patients with RA. These Rheumatoid Factors (RF) are a heterogeneous group of high molecular weight auto-antibodies directed against the body's own immunoglobulins. They are produced by plasma cells present at sites of tissue injury. The initiating antigen is thought to be one or more viruses or viral antigens that persist in the joint tissues. Research has shown that both environmental and genetic factors can affect the production of RF with various biological properties. RF have also been observed in the serum of patients with lupus erythematosus, hepatitis, liver cirrhosis, syphilis and various other conditions; however the RF titre in these conditions is much lower than that observed in patients with Rheumatoid Arthritis.

**PRINCIPLE**
Rheumatoid factors are antibodies directed against the Fc portion of IgG. The majority of rheumatoid factors are IgM antibodies, but may be IgG or IgA. Conditions giving rise to such factors include rheumatic conditions and chronic inflammatory processes. The Randox RF latex reagent is a suspension of polystyrene latex particles of uniform size coated with human IgG. When serum containing rheumatoid factor is mixed with the RF latex reagent an increase in turbidity can be measured at 550 nm. By constructing a standard curve from the absorbance of the standards, the rheumatoid factor concentration can be determined.

**SAMPLE COLLECTION AND STORAGE**
Fresh serum is recommended. The serum may be stored at +1 to +4°C for up to 48 hours after collection. If the test cannot be carried out within this period of time, the serum should be frozen at -20°C. Lipaemic or haemolytic samples or high levels of detergent may interfere in this assay.

**REAGENT COMPOSITION**

**Contents**

<table>
<thead>
<tr>
<th>RF Assay Buffer</th>
<th>Phosphate Buffer 150 mmol/l Triton X-100 maximum 2% (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF Latex Reagent</td>
<td>particle suspension containing latex coated with human IgG</td>
</tr>
</tbody>
</table>

**PRECAUTIONS AND WARNINGS**
For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

All solutions contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes, or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Each human-based component in the kit has been tested for the presence of HIV and HCV antibodies and the hepatitis B surface antigen, by FDA approved methods, and found to be non-reactive. However, as no method can offer complete assurance, these reagents should be handled as if capable of transmitting infection.

Health and Safety data sheets are available on request.

**PREPARATION OF REAGENTS AND STABILITY**

**RF Assay Buffer**
Supplied ready for use. Stable up to expiry date when stored at +2 to +8°C.

**RF Latex Reagent**
Supplied ready for use. Stable up to expiry date when stored at +2 to +8°C.

**PROCEDURE**

**Materials Provided**
RF Assay Buffer
RF Latex Reagent

**Materials Required But Not Provided**
Randox RF Standard Cat. No. RF2301
Randox RF Positive Control Cat. No. RF2302

**MANUAL TEST METHOD**

Application sheets for a range of automated analysers are available from Randox

| Wavelength: | 550 nm |
| Cuvette: | 1 cm light path |
| Temperature: | 37°C |
| Measurement: | Against Air |

Pipette into appropriate disposable microcuvette (or test tube):

<table>
<thead>
<tr>
<th>Reagent Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>500 µl</td>
<td>500 µl</td>
</tr>
<tr>
<td>Latex Reagent</td>
<td>325 µl</td>
<td>325 µl</td>
</tr>
</tbody>
</table>

Mix well and incubate for 5 mins at 37°C. Then add:

| DDH2O | 20 µl |
| Standard | 20 µl |
| Sample | 20 µl |

Mix well, incubate for 30 seconds and measure absorbance A1. Incubate for a further 3 minutes exactly, then measure absorbance A2.
A set of standards should be run each time the assay is carried out. A calibration curve can be generated using the calculation:

$$ \Delta A_{\text{sample}} = (A_2 - A_1) - \Delta A_{\text{blank}} $$

Plot standard concentrations against the corresponding $\Delta A$ values using graph paper. [An appropriate computer programme curve fit to use, if available, is a logit/log transformation]. The sample RF concentration is obtained by reading off the $\Delta A$ value from the calibration curve. Do not attempt to extrapolate above or below the range of standards [nominally, 10 - 60 IU/ml] see Randox Cat. No. RF 2301.

An appropriate control should also be run with each assay.

### QUALITY CONTROL

Randox RF positive control is recommended for Quality Control. One control should be assayed after every 10 samples. Values obtained for this assay should fall within a specified range. If these values fall outside this range and repetition with fresh calibration excludes error, the following steps should be taken:

1. Check wavelength setting and light source.
2. Check cleanliness of glassware and pipettes.
3. Check water, contaminants, ie. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Check accuracy and precision of pipettes.
7. Contact Randox Laboratories Technical Support, Northern Ireland (01849) 422413.

### ASSAY RANGE

This method is linear between approximately 10 and 60 IU/ml. Samples with RF concentration greater than the top standard should be diluted (1+5) with 0.9% (w/v) NaCl and reassayed. The result should be multiplied by 6.

### SPECIFICITY

The antiserum is monospecific for human RF and has not been shown to cross-react with other serum proteins under the conditions of the assay.

### NORMAL RANGES

The cut off value which constitutes the upper limit of normal is subject to dispute. However, values less than 10 IU/ml are considered to be within the normal range. The World Health Organisation has suggested a level approaching 12.5 IU/ml as being the upper limit of normal. It is recommended that each laboratory should establish an expected range to reflect the geographical location and the age, sex and diet of the population.

### EXPECTED VALUES

RF as detected by serological techniques are not necessarily specific for rheumatoid arthritis. An elevated result should always be confirmed by performing parallel tests. Increased levels of rheumatoid factor may be observed in infectious mononucleosis and in patients suffering from systemic lupus erythematosus, sarcoidosis and various other conditions. The clinical significance of a positive result should be interpreted cautiously. However, an elevated rheumatoid factor concentration is associated with rheumatoid arthritis rather than rheumatic fever.

### SPECIAL PERFORMANCE CHARACTERISTICS

### PRECISION (COBAS FARA APPLICATION)

#### Intra-Assay Precision

Within run precision was determined by replicate determinations of 3 different patient samples in one assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Replicates</th>
<th>Mean [IU/ml]</th>
<th>SD</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>15.11</td>
<td>0.719</td>
<td>4.76</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>38.07</td>
<td>0.556</td>
<td>1.46</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>53.77</td>
<td>1.33</td>
<td>2.48</td>
</tr>
</tbody>
</table>

#### Inter-Assay Precision

Between run precision was determined by replicate determination of 3 different patient samples in 10 assays.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Replicates</th>
<th>Mean [IU/ml]</th>
<th>SD</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>16.22</td>
<td>0.96</td>
<td>5.9</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>37.78</td>
<td>1.19</td>
<td>4.3</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>54.88</td>
<td>2.34</td>
<td>3.2</td>
</tr>
</tbody>
</table>

### METHOD COMPARISON (COBAS FARA APPLICATION)

A comparison of Randox test method ($y$) was made against another commercially available method ($x$). Forty six samples (7.6 to 413 IU/mL) were tested. Linear regression analysis resulted in the following equation:

$$ Y = 1.07x + 1.14, \text{ with a correlation coefficient } r = 0.984. $$

### REFERENCES