QUANTITATIVE DETERMINATION OF RHEUMATOID FACTOR (RF)

ORDER INFORMATION

REF: RF TURBILATEX  
Cont. R1: Diluent 1 x 45 ml  
R2: Latex 1 x 5 ml  
Calibrator 1 x 1 ml

CLINICAL SIGNIFICANCE

Rheumatoid Factors are a group of antibodies directed to determinants in the Fc portion of the immunoglobulin G molecule. Although rheumatoid factors are found in a number of rheumatoid disorders, such as systemic lupus erythematosus (SLE) and Sjogren’s syndrome, as well as in no rheumatic conditions its central role in clinic lies in its utility as an aid in the diagnosis of rheumatoid arthritis (RA). A study of the ‘American College of Rheumatology’ shows that 80.4% of RA patients were RF positive.

PRINCIPLE OF THE METHOD

The RF-Turbilatex is a quantitative turbidimetric test for the measurement of RF in human serum or plasma. Latex particles coated with human gammaglobulin are agglutinated when mixed with samples containing RF. The agglutination causes an absorbance change, dependent upon the RF content of the sample that can be quantified by comparison from a calibrator of known RF concentration.

REAGENTS

Diluent (R1)  
Calibrator Tris buffer 20 mmol/L, pH 8.2 sodium azide 0.95 g/L

Latex (R2)  
Latex particle coated with human gammaglobulin, Ph 8.2, sodium azide 0.95 g/L.

Calibrator  
Human serum. RF concentration is stated on the vial label

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8 °C and contaminations prevented during their use. Do not use reagent over the expiration date.

PREPARATION

RF Calibrator: Reconstitute with 2.0 ml. of distilled water. Mix gently and incubate at room temperature for 10 minutes before use.

CALIBRATION CURVE (RANGE FROM 20 TO 160 UL / ML)

Prepare the following RF calibrator dilutions in NaCl 9 G/Dl, Multiply stated in table below to obtain the RF concentration of each dilution.

<table>
<thead>
<tr>
<th>Calibrator dilution (μl)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator RF (μl)</td>
<td>-</td>
<td>10</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>NaCl 9 g/dl (μl)</td>
<td>100</td>
<td>90</td>
<td>75</td>
<td>50</td>
<td>75</td>
<td>-</td>
</tr>
<tr>
<td>Factor</td>
<td>0</td>
<td>0.1</td>
<td>0.25</td>
<td>0.50</td>
<td>0.75</td>
<td>1.0</td>
</tr>
</tbody>
</table>

ONE POINT CALIBRATOR (LINEA RANGE UP TO 100 IU / ML): Prepare a RF Calibrator dilution : - 30 ul RF Calibrator +70 ul NaCl 9 g/dl multiply the RF calibrator concentration of the diluted concentration by 0.33 to obtain the RF concentration of the diluted calibrator.

REAGENT DETERIORAION: Presence of particles and turbidity.

RECONSTITUTED CALIBRATOR: Stable for 1 month at 2-8 °C or 3 months at-20° C.
Do not freeze; frozen latex or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37 °C
- Spectrophotometer or photometer thermostable at 37 °C. with a 650 nm filter (600-650nm).

PROCEDURE

1. Mix the reagents and the photometer (cuvette holder) to 37 °C.
2. Assay conditions:
   - Wavelength : 650 nm (600-650 nm)
   - Temperature : 37 °C
   - Cuvette light path : 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

<table>
<thead>
<tr>
<th>Calibrator / Sample</th>
<th>Calibrator or sample (μl)</th>
<th>R1: Diluent (ml)</th>
<th>R2: Latex (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0</td>
<td>0.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

5. Mix and read the absorbance after 10 Secs. (A) and after 2 minutes (A) of the sample addition.

PRECUATION

Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV and antibody to HIV (1/2). However handle cautiously as potentially infectious.

CALIBRATOR

The sensitivity of the assay and the target value of the calibrator have been standardized against the ASO international reference WHO 64/1 (Rheumatoid Arthritis Serum). It is not recommended to use other commercially available RF Calibrator.

CALCULATIONS

Calibration curve (Note 1): Calculate the absorbance difference (A2-A1) of each calibrator and plot the values obtained against the RF concentration in the sample is calculated by interpolation of its (A2-A1) in the calibration curve.

One point calibration:

\[
\frac{(A2-A1) \text{ sample}}{(A2-A1) \text{ calibrator}} \times \text{ Diluted calibrator concentration} = \text{IU/ml. RF (A2-A1) calibrator}
\]

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures.

Each laboratory should establish its own quality control scheme and corrective actions if control do not meet the acceptable tolerances.

REFERENCE VALUES

1. Linearity (One point calibration: Up to 100 IU/ml, under the described assay conditions.
2. Limit detection: Values less than 3 IU/ML non-reproducible results.
3. Measurement range (Calibration CURve ) : 20-160 IU/ML, under the described assay conditions. THE Linearity limit and the measurement range depends on the sample volume, although the sensitivity of the test will proportionally decrease.
4. Prozone effect: No prozone effect was detected upto 800 IU/ML.
5. Sensitivity: 3.34 M aIU /MIL.

6. Precision:

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay (n=10)</th>
<th>Inter-assay (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (IU/mL)</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>236</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td>372</td>
<td>372</td>
</tr>
<tr>
<td>SD</td>
<td>3.4</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>16.2</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>5.9</td>
<td>17.8</td>
</tr>
<tr>
<td>CV</td>
<td>2.5</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>4.8</td>
</tr>
</tbody>
</table>

7. Accuracy: Results obtained using this reagent (Y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 86 samples ranging from 1 to 160 IU/ mL. of RF were assayed. The correction coefficient (r) was 0.95 and the regression equation y = 0.797x – 1.075.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
Hemoglobin (10g/Dl), Bilirubin (20mg/dl) and lipaemia (10g/dl) do not interfere. Other substances may interfere.

NOTES
1. Multipoint calibration gives more accurate results than one point calibration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY