K-ASSAY® RF
For the Quantitative Determination of Human Rheumatoid Factor (RF) in Serum
Cat. No. KAI-031

INTENDED USE

For the quantitative determination of human rheumatoid factor in patient serum based on immunoturbidimetric assay. FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

The K-ASSAY® RF is intended for the quantitative determination of human rheumatoid factor (RF) by immunoturbidimetric assay. The rheumatoid factor, an autoantibody, in the patient's serum interacts with the aggregated human IgG in the reagent forming immune complexes. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum rheumatoid factor.

Autoantibodies of the IgG, IgM, or IgA isotype, which are reactive with the crystallizable fraction (Fc) of IgG, are called rheumatoid factors. Rheumatoid factor is found in 50-79% of adults with classical rheumatoid arthritis. Quantification of rheumatoid factor has been shown to be useful in the clinical diagnosis and prognosis of rheumatoid arthritis.

Rheumatoid factor has been measured using a variety of methods, including agglutination, latex fixation, nephelometric, and enzyme-linked immunosorbent assay. The K-ASSAY® RF uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST

The K-ASSAY® RF quantifies the rheumatoid factor in the patient's serum based on immunoturbidimetric assay. Calibrators, controls, and patient samples are pipetted into sample cups. Microvolumes of samples and reagent diluent are automatically pipetted into individual cuvettes.

Following an initial incubation and measurement of sample blank, gamma globulin containing aggregated human IgG is added to the cuvettes. The sample (autoantibodies), solution and gamma globulin reagent (antigen) are then mixed in the reaction cuvettes. Insoluble antigen-antibody (immune) complexes form. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum rheumatoid factor. Following an incubation period lasting approximately 5 minutes, the absorbance of the solution is measured at 340 and 700 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of proteins and using the instrument's data reduction capability or manually plotting the change in absorbance versus concentration. Concentration of the control and patient samples is interpolated from the calibration curve. Gamma globulin containing aggregated human IgG is used in the kit, which the human rheumatoid factor reacts with.

For a complete discussion of the methodology, see the following references:

1. Carter, R. W., & C. M. Smith. "Autoantibodies of the IgG, IgM, or IgA isotype, which are reactive with the crystallizable fraction (Fc) of IgG, are called rheumatoid factors. Rheumatoid factor is found in 50-79% of adults with classical rheumatoid arthritis."
2. "Rheumatoid factor, an autoantibody, in the patient's serum interacts with the aggregated human IgG in the reagent forming immune complexes. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum rheumatoid factor."

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum is required for this assay. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to plastic tubes within 2 hours. Freshly drawn serum is preferred. Serum should be stored refrigerated (2-8°C) and used within one week. Samples can also be stored frozen at -20°C and used within 2 months.

Use plastic tubes for storing the sample, do not use glass.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent 4 x 20 mL
Reagent 2 (R-2) RF Reagent 2 x 8 mL

Materials Required But Not Supplied

Calibrators: K-ASSAY® RF Calibrator, Cat. No. KAI-032C...

Purified water

Two Reagent Clinical Chemistry Analyzer:

Capable of accurately dispensing readings at 340 / 700 nm

Capable of maintaining 37°C

Assay Procedure

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 717):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 µL</td>
<td>250 µL</td>
</tr>
</tbody>
</table>

- R1 (Buffer Reagent) 37°C, 5 min
- R2 (RF Reagent) 37°C, 5 min

2-point endpoint, 340/700 nm

Reagents contain pooled human serum. Each serum donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for HBsAg and antibody to HIV. However, it is not possible to guarantee that any human source material is free of these infectious agents. Therefore, all products containing human serum should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories." 2nd ed., 1988, HHS Publication No. (CDC) 88-8395.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for one year from date of manufacture as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY

Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard.

Opened reagents can be used for 1 month if stored at 2-8°C.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 340 and 700 nm. Refer to the instrument manual from the manufacturer regarding the following:

- a) Use or function
- b) Installation procedures and requirements
- c) Principles of operation
- d) Performance characteristics, operating instructions
- e) Calibration procedures including materials and / or equipment to be used
- f) Operational precautions, limitations, and hazards
- g) Service and maintenance information

WARNING: This product can expose you to chemicals including thiourea which is known to the State of California to cause cancer/birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov. The R-2 reagent contains 2.28% of thiourea (CAS No. 62-56-6).
LIMITATIONS OF PROCEDURE

The measuring range for Rheumatoid Factor is between 5 to 320 IU/mL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1:2 with isotonic saline or filtered to decrease nonspecific light scattering. If rheumatoid factor concentration in a patient sample is greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and reassay. Multiply results by 5 to compensate for dilution.

PERFORMANCE

Precision

The precision for the K-ASSAY® RF was determined using packaged reagents, pooled human serum, and a Hitachi model 717 chemistry analyzer.

Precision Assay: Within Run

### Sample I Sample II Sample III

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.3</td>
<td>110.15</td>
</tr>
<tr>
<td>SD</td>
<td>0.86</td>
<td>1.5</td>
</tr>
<tr>
<td>CV</td>
<td>26.197%</td>
<td>1.359%</td>
</tr>
</tbody>
</table>

Precision Assay: Between Runs

### Sample I Sample II Sample III

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7.9</td>
<td>106.3</td>
</tr>
<tr>
<td>SD</td>
<td>2.13</td>
<td>2.06</td>
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<tr>
<td>CV</td>
<td>26.98%</td>
<td>4.04%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.94%</td>
</tr>
</tbody>
</table>

Accuracy / Correlation

\[
y = 0.876x - 20.268 \\
r = 0.984 \\
n = 43 \\
x = company A's ITA \\
y = K-ASSAY® RF
\]

Assay Range

5 - 320 IU/mL

INTERFERENCE

- Bilirubin C: No interference up to 20 mg/dL
- Bilirubin F: No interference up to 20 mg/dL
- Hemoglobin: No interference up to 500 mg/dL
- IntraLipid: No interference up to 300 mg/dL

EXPECTED VALUE

The expected value as reported is below 10 U/mL or below 11 IU/mL. Each laboratory should establish its own expected values using this kit.

REFERENCES

2. Center for Disease Control, "Reference preparation of rheumatoid factor." Catalog #ISO949, Atlanta, GA, Lot 87-008.

LABELING SYMBOLS

- **LOT**: Lot Number
- **RGT**: Reagent
- **EXP**: Expiration or "Use By" Date
- **CAT**: Catalog Number
- **VIT**: For In Vitro Diagnostics Use
- **TE**: Temperature Limitation
- **MMF**: Manufacturer
- **P/I**: Consult Package Insert for Instructions for Use
- **EC**: Authorized Representative in the European Community

K-ASSAY® RF

Chemistry Parameters for Automatic Analyzer

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSTRUMENT</td>
<td>Roche / Hitachi 717</td>
</tr>
<tr>
<td>TEMPERATURE</td>
<td>37°C</td>
</tr>
<tr>
<td>TEST</td>
<td>(RF)</td>
</tr>
<tr>
<td>ASSAY CODE</td>
<td>(2 POINT) : (24) - (50)</td>
</tr>
<tr>
<td>SAMPLE VOLUME</td>
<td>(115) µL (NO)</td>
</tr>
<tr>
<td>R-1 VOLUME</td>
<td>(250) µL (NO)</td>
</tr>
<tr>
<td>R-2 VOLUME</td>
<td>(50) µL (NO)</td>
</tr>
<tr>
<td>WAVELENGTH</td>
<td>(700) - (340)</td>
</tr>
<tr>
<td>CALIB. METHOD</td>
<td>(NONLINEAR) (4) (4)</td>
</tr>
<tr>
<td>STD.(1) Conc.-POS.</td>
<td>0.0 - (1)</td>
</tr>
<tr>
<td>STD.(2) Conc.-POS.</td>
<td>(2) - (2)</td>
</tr>
<tr>
<td>STD.(3) Conc.-POS.</td>
<td>(3) - (3)</td>
</tr>
<tr>
<td>STD.(4) Conc.-POS.</td>
<td>(4) - (4)</td>
</tr>
<tr>
<td>STD.(5) Conc.-POS.</td>
<td>0.0 - (0)</td>
</tr>
<tr>
<td>STD.(6) Conc.-POS.</td>
<td>0.0 - (0)</td>
</tr>
<tr>
<td>SD LIMIT</td>
<td>(9999)</td>
</tr>
<tr>
<td>DUPLICATE LIMIT</td>
<td>(10000)</td>
</tr>
<tr>
<td>SENSITIVITY LIMIT</td>
<td>(0)</td>
</tr>
<tr>
<td>ABS. LIMIT (SLOPE)</td>
<td>(32000) (INCREASE)</td>
</tr>
<tr>
<td>PROZONE LIMIT</td>
<td>(-32000) (LOWER)</td>
</tr>
<tr>
<td>EXPECTED VALUE</td>
<td>(-9999) (9999)</td>
</tr>
<tr>
<td>PANIC VALUE</td>
<td>(-9999) (9999)</td>
</tr>
<tr>
<td>INSTRUMENT FACTOR</td>
<td>(1.00)</td>
</tr>
</tbody>
</table>

Use isotonic saline for calibrator #1. RF Calibrators are used for calibrators #2-4.

*2:4 - Input concentration of RF Calibrators.

Parameters for other automated analyzers are available.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls falls outside the stated recovery range.