RF
Rheumatoid Factor
REF 447070 (300 tests)

For In Vitro Diagnostic Use

ANNUAL REVIEW

<table>
<thead>
<tr>
<th>Reviewed by:</th>
<th>Date</th>
<th>Reviewed by:</th>
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<tbody>
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</table>

PRINCIPLE

INTENDED USE

RF reagent, when used in conjunction with IMMAGE® Immunochemistry Systems and Calibrator 5 Plus, is intended for the quantitative determination of rheumatoid factor (RF) in human serum or plasma by rate nephelometry.

CLINICAL SIGNIFICANCE

Rheumatoid Factors are antibodies that react with the individual’s own immunoglobulin. The antibodies are usually directed against the Fc region of the IgG molecule. Rheumatoid Factor can be detected in the serum of the majority of patients with rheumatoid arthritis and is important for the diagnosis and prognosis of those patients with higher concentrations. These patients tend to suffer a more severe form of the illness and develop extra-joint complications more readily. Rheumatoid factors are not disease specific and can occur in lower frequencies in several other autoimmune disorders, chronic inflammation and normal individuals.

METHODOLOGY

The RF test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction.

CHEMICAL REACTION SCHEME

Rheumatoid Factor(sample) + Particle bound-human IgG(antigen) → [Rheumatoid Factor(sample)-Antigen Complex]

SPECIMEN

TYPE OF SPECIMEN

Serum samples are recommended. Plasma samples (Lithium Heparin and Sodium Heparin) can be used. EDTA is an unacceptable anticoagulant.

Serum or plasma samples should be collected in the manner routinely used for any clinical laboratory test. Freshly drawn serum or plasma from a fasting individual is preferred. Anticoagulants tested are listed in the PROCEDURAL NOTES section of this chemistry information sheet.
SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.\(^7\)

2. If serum samples are not assayed within 8 hours, samples may be stored at +2°C to +8°C for up to 7 days. Frozen samples are not recommended. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.\(^7\)

3. Plasma samples can be stored at +2°C to +8°C for up to 72 hours. Plasma samples should not be frozen.

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

For sample volumes refer to the Sampling Template.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:
REAGENTS

CONTENTS

Each kit contains the following items:

<table>
<thead>
<tr>
<th>KIT COMPONENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF Cartridge</td>
<td>1</td>
</tr>
<tr>
<td>Antigen</td>
<td></td>
</tr>
<tr>
<td>Evaporation Caps</td>
<td>2</td>
</tr>
<tr>
<td>RF Reagent Bar Code Card</td>
<td>1</td>
</tr>
</tbody>
</table>

INITIAL VOLUMES OF SAMPLE AND REAGENTS IN THE CUvette

| Sample Volume            | 5 µL     |
| Total Reagent Volume     | 346 µL   |
| Antigen                  | 21 µL    |
| Buffer 2                 | 300 µL   |
| Diluent 1                | 25 µL    |

REACTIVE INGREDIENTS

<table>
<thead>
<tr>
<th>REAGENT CARTRIDGE CONSTITUENTS</th>
<th>VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF Antigen (particle bound human immunoglobulin G)</td>
<td>7.5 mL</td>
</tr>
<tr>
<td>Sodium Azide (used as a preservative)</td>
<td>&lt; 0.1% (w/w)</td>
</tr>
</tbody>
</table>

Also bovine serum albumin and non-reactive chemicals necessary for optimal system performance.

⚠️ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

⚠️ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control’s Biosafety Level 2 guidelines.
MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

IMMAGE Immunochemistry Systems Wash Solution
IMMAGE Immunochemistry Systems Buffer 2
IMMAGE Immunochemistry Systems Diluent 1
Calibrator 5 Plus
At least two levels of control material

REAGENT PREPARATION

1. Invert cartridge gently before removing screw caps.
2. Remove screw caps from reagent cartridges. Check each cartridge for bubbles and remove any bubbles present.
3. Place evaporation caps on both reagent cartridge compartments before loading the cartridge on the instrument. See Appendices for evaporation cap directions.
4. Reagent cartridges should be stored upright and can be removed from the refrigerator and used immediately.
5. Mix all buffers and diluents thoroughly by inversion. Remove screw cap from container. Check each container for bubbles and remove any bubbles present. Place evaporation cap on container before loading the container on the instrument. See Appendices for evaporation cap directions.

ACCEPTABLE REAGENT PERFORMANCE

Acceptability of a reagent is determined from the successful performance of quality control testing, as defined in the QUALITY CONTROL section of this chemistry information sheet.

REAGENT STORAGE AND STABILITY

Storage conditions other than those recommended may cause erroneous results.

Reagent Cartridges

1. Return all reagent cartridges to the refrigerator (+2°C to +8°C) upon completion of the daily workload.
2. The RF reagents are stable for 30 days with the evaporation caps in place. Alternatively, reagent life can be maximized by replacing evaporation caps with screw caps and storing at +2°C to +8°C upon completion of the daily workload.
3. The RF reagents are stable until the expiration date on the label if the reagents are stored at +2°C to +8°C with the screw caps in place.

Diluent 1 and Buffer 2

1. Diluent 1 and Buffer 2 are stable on the system for 30 days with the evaporation caps in place.
2. Diluent 1 and Buffer 2 are stable until the expiration date on the label if they are stored at room temperature with the screw caps in place.

Reagent storage location:
CALIBRATION

CALIBRATOR REQUIRED

Calibrator 5 Plus

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

The calibrator is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C.

Calibrator storage location:

⚠️ CAUTION

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IMMAGE IMMUNOCHEMISTRY SYSTEM CALIBRATION INFORMATION

1. The IMMAGE® Immunochemistry Systems calibration is reagent lot specific.
2. The RF reagent lot should be recalibrated when changing the Buffer 2 lot or following specific part replacements or maintenance procedures as defined in the IMMAGE Operations Manual.
3. The IMMAGE Immunochemistry System is designed for minimum calibration. Calibrations retained in system memory should be monitored by the performance of quality control procedures on each day of testing.
4. Calibration for RF is stable for 30 days.
5. The system will automatically perform a verification check during calibration and produce a calibration report. The system will alert the operator of a failed calibration. An explanation of any accompanying error message can be found in the TROUBLESHOOTING Section of the IMMAGE® Immunochemistry Systems Operations Manual.
6. Calibration verification information can be found in the CALIBRATION VERIFICATION section of the IMMAGE® Immunochemistry Systems Chemistry Reference Manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.
QUALITY CONTROL

It is recommended that at least two levels of control material, normal and abnormal, be analyzed daily. Refer to the CALIBRATORS AND CONTROLS section of the IMMAGE® Immunochemistry Systems Chemistry Reference Manual, for a list of Beckman Coulter controls. Controls should also be run with each new calibration, with a new lot of reagent or buffer, and after specific maintenance or troubleshooting as detailed in the IMMAGE® Immunochemistry Systems Operations Manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on work load and work flow.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

<table>
<thead>
<tr>
<th>CONTROL NAME</th>
<th>SAMPLE TYPE</th>
<th>STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

**TESTING PROCEDURE(S)**

1. After setup, load reagents onto the system as directed in the IMMAGE Operations Manual.
2. Select chemistries to be calibrated, if necessary. Load bar coded calibrators, controls, and samples or program and load non-bar coded controls and samples for analysis as directed in the IMMAGE Operations Manual.
3. Follow the protocols for system operation as directed in the IMMAGE Operations Manual.

**CALCULATIONS**

The IMMAGE Immunochemistry System will automatically calculate results.

**REPORTING RESULTS**

**REFERENCE INTERVALS**

Greater than 95% of a group of 108 apparently healthy adults in Southern California showed no detectable RF (<20 IU/mL) with this assay. Published "upper limits of normal" vary widely among different sources.9,10,11 Each laboratory must establish its own reference interval based on its patient population. The presence of RF in patient serum should be considered as one criterion of rheumatoid disease, and not as a definitive diagnosis.12

**Table 2.0 Reference intervals**

<table>
<thead>
<tr>
<th></th>
<th>REFERENCE INTERVALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Coulter</td>
<td>&lt; 20 IU/mL (in 95% of the population tested)</td>
</tr>
</tbody>
</table>
Refer to References (13,14,15,16) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

<table>
<thead>
<tr>
<th>UNITS AND CONVERSION FACTOR</th>
</tr>
</thead>
</table>
Results for the RF test are reported in default units of IU/mL. Metric conversion within the same unit category will occur automatically if a new unit is selected. A conversion factor must be entered when selecting a unit category different from the default.

Refer to the System Setup section of the IMMAE Operations Manual for more detailed information on units and conversion factors.

**PROCEDURAL NOTES**

**ANTICOAGULANT TEST RESULTS**

The following anticoagulants were tested. The results of 20 serum and 20 plasma samples, containing the same level of RF, were tested and averaged.

**Table 3.0 Anticoagulant Test Results**

<table>
<thead>
<tr>
<th>ANTICOAGULANT</th>
<th>LEVEL OF ANTICOAGULANT TESTED(^a)</th>
<th>AVERAGE SERUM RESULT (IU/mL)</th>
<th>AVERAGE PLASMA RESULT (IU/mL)</th>
<th>% DIFFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA(^b)</td>
<td>1.5 mg/mL</td>
<td>149</td>
<td>238</td>
<td>59.7</td>
</tr>
<tr>
<td>Lithium Heparin</td>
<td>14 Units/mL</td>
<td>164</td>
<td>160</td>
<td>-2.44</td>
</tr>
<tr>
<td>Sodium Heparin</td>
<td>14 Units/mL</td>
<td>154</td>
<td>152</td>
<td>-1.30</td>
</tr>
</tbody>
</table>

\(^a\) Based on full tube collection.
\(^b\) EDTA is an unacceptable anticoagulant.

**LIMITATIONS**

1. Rheumatoid factor is not a finding isolated to rheumatoid arthritis and may be present in a number of connective tissue and inflammatory diseases including infectious mononucleosis, systemic lupus erythematosus, scleroderma, and hepatitis.\(^17\)

2. Extremely high RF concentrations (> 6,000 IU/mL) in serum may result in an antigen excess condition producing a low RF value on the IMMAE System. If the patient's clinical condition does not correlate with the reported RF concentration, dilute the sample and repeat the analysis.

**INTERFERENCES**

1. The following substances were tested in serum for interference with this methodology at the initial dilution:
Table 4.0 Interferences

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>SOURCE</th>
<th>LEVEL TESTED</th>
<th>OBSERVED EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>Porcine</td>
<td>5 – 30 mg/dL</td>
<td>None</td>
</tr>
<tr>
<td>Lipid</td>
<td>Human Triglyceride</td>
<td>50 – 300 mg/dL</td>
<td>None^a</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Human</td>
<td>200 – 1,000 mg/dL</td>
<td>None</td>
</tr>
</tbody>
</table>

^a Quantitation of specific proteins by nephelometry may not be possible in lipemic samples due to the extreme light scattering properties of the sample.

2. Samples stored refrigerated or frozen for extended periods may exhibit aberrant results.

3. Serum samples may yield different concentration values for rheumatoid factor when analyzed at various dilutions. This phenomenon may be caused by the presence of rheumatoid factor complexes with inhibitors or interfering substances present in the serum. Sample dilution will reduce the effective concentration of the interfering substances so that the final result obtained for rheumatoid factor in the diluted sample will be increased over the result obtained for the undiluted (neat) or lesser diluted sample.

4. Dust particles or other particulate matter (i.e. debris and bacteria) in the reaction solution may result in extraneous light-scattering signals, resulting in variable sample analysis.

5. Quality control materials prepared from serum pools are not recommended.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

The RF test is designed to detect concentrations of this analyte using an initial 1:6 serum sample dilution.

Table 5.0 Analytical Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>BECKMAN COULTER ANALYTICAL RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Initial: 20 – 800 IU/mL</td>
</tr>
<tr>
<td></td>
<td>Extended: 20 – 28,800 IU/mL</td>
</tr>
</tbody>
</table>

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 6.0 Reportable Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>LABORATORY REPORTABLE RANGE</th>
</tr>
</thead>
</table>

Refer to the IMMAGE® Immunochemistry Systems Chemistry Reference Manual section on CALIBRATION VERIFICATION, for more details on laboratory reportable range.

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for RF determination is 20 IU/mL.

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of samples to an accepted clinical method. Values obtained for human rheumatoid factor using the IMMAGE RF Test were compared to the values obtained using a commercially
available automated nephelometric assay (NIA) method. Both normal and abnormal rheumatoid factor serum samples were included in the analysis.

Table 7.0 Equivalency Values

<table>
<thead>
<tr>
<th></th>
<th>NIA METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>143</td>
</tr>
<tr>
<td>Slope</td>
<td>1.055</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean (IMMAGE)</td>
<td>154</td>
</tr>
<tr>
<td>Mean (NIA)</td>
<td>146</td>
</tr>
<tr>
<td>Correlation Coefficient (r)</td>
<td>0.940</td>
</tr>
</tbody>
</table>

The equivalency values were determined using patient samples ranging from 20.0 to 758 IU/mL. Refer to References (17,18) at the end of this chemistry information sheet for guidelines on performing equivalency testing.

PRECISION

A properly operating IMMAGE® Immunochemistry Systems should exhibit imprecision values less than or equal to the maximum performance limits listed below. Maximum performance limits were derived by an examination of the precision of various methods, proficiency test summaries, and literature sources.

Table 8.0 Maximum Performance Limits

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE TYPE</th>
<th>SD (IU/mL)</th>
<th>% CV</th>
<th>CHANGEOVER VALUE (IU/mL)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>Serum</td>
<td>5.0</td>
<td>4.0</td>
<td>125</td>
</tr>
<tr>
<td>Total</td>
<td>Serum</td>
<td>7.5</td>
<td>6.0</td>
<td>125</td>
</tr>
</tbody>
</table>

  a  When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance data for the IMMAGE® Immunochemistry Systems evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below. Each laboratory should characterize their own instrument performance for comparison purposes.

Table 9.0 Typical Imprecision Values

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE</th>
<th>Data Pointsa</th>
<th>Test Mean Value (IU/mL)</th>
<th>SD (IU/mL)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>Serum Level 1</td>
<td>80</td>
<td>124</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Serum Level 2</td>
<td>80</td>
<td>299</td>
<td>4.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Serum Level 3</td>
<td>80</td>
<td>637</td>
<td>12.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Total</td>
<td>Serum Level 1</td>
<td>80</td>
<td>124</td>
<td>3.5</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Serum Level 2</td>
<td>80</td>
<td>299</td>
<td>8.8</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>Serum Level 3</td>
<td>80</td>
<td>637</td>
<td>20.7</td>
<td>3.2</td>
</tr>
</tbody>
</table>

  a  The point estimate is based on the data from 1 system, run for 20 days, 2 runs per day, 2 observations per run on an instrument operated and maintained according to the manufacturer's instructions.

Refer to References (17,19) for guidelines on performing precision testing.
NOTICE

These degrees of precision were obtained in typical testing procedures and are not intended to represent performance specifications for this test procedure.

ADDITIONAL INFORMATION

For more information, refer to the IMMAGE Immunochemistry Systems *Operations Manual.*

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.
REFERENCES


