**Alinity c Rheumatoid Factor (RF)-29**

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**BIENNIAL REVIEW:**

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Rheumatoid Factor (RF)

# INTENDED USE

The Rheumatoid Factor assay is used for the quantitation of rheumatoid factor in human serum on the Alinity c system.

# SUMMARY AND EXPLANATION OF THE TEST

Rheumatoid factor (RF) is an autoantibody against human immunoglobulin G (IgG) commonly present at a high concentration in sera of patients with certain conditions, particularly in patients with rheumatoid arthritis.

The measurement of rheumatoid factor may aid in the diagnosis of rheumatoid arthritis. Rheumatoid factor may also be observed in systemic lupus erythematosus, Sjögren's syndrome, cryoglobulinemia, chronic hepatic diseases and other autoimmune and inflammatory diseases.[1] This assay is designed to accurately and reproducibly measure serum rheumatoid factor using latex agglutination.

# PRINCIPLES OF THE PROCEDURE

The Rheumatoid Factor assay is an automated clinical chemistry assay.

Rheumatoid Factor is an *in vitro* diagnostic assay for the quantitative determination of rheumatoid factor in human serum. **The Rheumatoid Factor assay is a latex enhanced immunoturbidimetric assay** that involves an antigen-antibody reaction between rheumatoid factor in the sample and denatured human IgG, which has been adsorbed to latex particles. The resulting agglutination is detected as an absorbance change (572 nm), with the magnitude of the change being proportional to the quantity of rheumatoid factor in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentration.

**Methodology: Immunoturbidimetric**

For additional information on system and assay technology, refer to the **Alinity ci-series Operations Manual, Section 3.**

# REAGENTS

## Kit Contents

Rheumatoid Factor Reagent Kit 04R99

Volumes (mL) listed in the following table indicate the volume per cartridge.

| REF | **04R9924** |
|---|---|
| Tests per cartridge | 100 |
| Number of cartridges per kit | 4 |
| Tests per kit | 400 |
| R1 | 20.5 mL |
| R2 | 9.3 mL |
| R1 Active ingredient: ethylenediaminetetraacetic acid disodium salt dihydrate (1.84%). Preservative: sodium azide (0.09%). | |
| R2 Active ingredient: latex particle adsorbed human IgG (0.16%). Preservative: sodium azide (0.08%). | |

## Warnings and Precautions

- IVD

- For *In Vitro* Diagnostic Use
- Rx ONLY

**Safety Precautions**

**CAUTION**: This product contains human-sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents, human specimens, and all consumables contaminated with potentially infectious materials be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents. *2*, *3*, *4*, *5*

The human-sourced material used in R2 is nonreactive for HBsAg, anti-HIV-1/HIV-2, anti-HCV, and HIV-1 RNA or HIV-1 Ag.

| The following warnings and precautions apply to: R1 and R2 | |
|---|---|
| | Contains sodium azide. |
| EUH032 | Contact with acids liberates very toxic gas. |
| **Disposal** | |
| P501 | Dispose of contents / container in accordance with local regulations. |

Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

For the most current hazard information, see the product Safety Data Sheet.

Safety Data Sheets are available at www.corelaboratory.abbott or/and SDS folder.

For a detailed discussion of safety precautions during system operation, refer to **the Alinity ci-series Operations Manual, Section 8.**

**Reagent Handling**

- Reagents are shipped on wet ice.
- Upon receipt, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Prior to running, gently invert cartridge 5 times.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent

aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

## Reagent Storage

| | Storage Temperature | Maximum Storage Time | Additional Storage Instructions |
|---|---|---|---|
| **Unopened** | 2 to 8°C | Until expiration date | Store in upright position. |
| **Onboard** | System Temperature | 30 days | |
| **Opened** | 2 to 8°C | Until expiration date | Store in upright position.<br>Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance. |

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, refer to the Alinity ci-series Operations Manual, Section 5.

## Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

**For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.**

# INSTRUMENT PROCEDURE

The Rheumatoid Factor assay file must be installed on the Alinity c system prior to performing the assay.

The Alinity c Rheumatoid Factor assay file is also known as RheumF assay file.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity ci-series Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity ci-series Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

### Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

| Default Result Unit | Conversion Factor | Alternate Result Unit |
|---|---|---|
| IU/mL | 1 | kU/L |

## SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

### Specimen Types

The specimen type listed below was verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

| Specimen Type | Collection Tube |
|---|---|
| **Serum** | Serum (with or without gel barrier) |

For specimen type limitations, refer to the LIMITATIONS OF THE PROCEDURE section of this package insert.

The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

### Specimen Conditions

- For accurate results, serum specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

### Preparation for Analysis

- Follow the tube manufacturer’s processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

### Specimen Storage

| Specimen Type | Temperature | Maximum Storage Time |
|---|---|---|
| Serum | Room temperature (20 to 25°C) | 1 day |
| | 2 to 8°C | 3 days |

For additional information on sample handling and processing, refer to CLSI GP44-A4.[6] The storage information provided here is based on references or data maintained by the manufacturer.

Stored specimens must be inspected for particulates. If present, mix with a low speed vortex or by inversion and centrifuge the specimen to remove particulates prior to testing.

### Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

Do not exceed the storage limitations listed above.

## PROCEDURE

### Materials Provided

04R99 Rheumatoid Factor Reagent Kit

### Materials Required but not Provided

- Rheumatoid Factor assay file
- 04S0002 Alinity c Rheumatoid Factor Calibrator Kit
- Commercially available controls containing rheumatoid factor
- Saline (0.85% to 0.90% NaCl) for specimen dilution

For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

### Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- Minimum sample cup volume is calculated by the system and printed on the Order List report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Minimum sample volume requirements:
  - Sample volume for single test: 3 µL.

    NOTE: This amount does not include the dead volume plus the additional over-aspiration volume. For total sample volume requirements, refer to the Alinity ci-series Operations Manual, Section 4.
- Refer to the Alinity c Rheumatoid Factor Calibrator Kit package insert REF 04S0002 and/or commercially available control material package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

## Sample Dilution Procedures

All samples should be initially tested using the STANDARD Dilution Protocol. If the result obtained is within the analytical measuring interval 13.0 to 200.0 IU/mL, the sample should not be diluted.

Samples with a rheumatoid factor value exceeding 200.0 IU/mL are flagged with the code "> 200.0 IU/mL" and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Samples that are flagged with the **message code 1041 may have excess antigen**. Dilute the sample and rerun following either the Automated Dilution Protocol or the Manual Dilution Procedure. Samples were tested for excess antigen up to 5557.6 IU/mL.

### Automated Dilution Protocol

The system performs a **1:5 or 1:10** dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

### Manual Dilution Procedure

Dilute the sample with saline (0.85% to 0.90% NaCl).

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than the lower value of the analytical measuring interval of 13.0 IU/mL, do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

## Calibration

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration is stable for approximately **60 days (1440 hours)**, but is required with each change in reagent lot. Verify calibration with at least 2 levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

## Quality Control Procedures

As appropriate, refer to your laboratory standard operating procedure(s) and/or quality assurance plan for additional quality control requirements and potential corrective actions.

- At least two levels of controls (normal and abnormal) are to be run every day testing performed.
- If quality control results do not meet the acceptance criteria defined by laboratory quality controls procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

### Quality Control Guidance

Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[7]

### Verification of Assay Claims

For protocols to verify package insert claims, **refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.**

# RESULTS

## Calculation

The Rheumatoid Factor assay utilizes the Spline data reduction method to generate a calibration and results.

## Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

## Reportable Interval

Based on representative data for the limit of quantitation (LoQ) and the limit of detection (LoD), the ranges over which results can be reported are provided below according to the definitions from CLSI EP34.[8]

| | **IU/mL (kU/L)** |
|---|---|
| Analytical Measuring Interval (AMI)[a] | **13.0 - 200.0** |
| Extended Measuring Interval (EMI)[b] | 200.0 - 2000.0 |
| Reportable Interval[c] | 13.0 - 2000.0 |

[a] AMI: The AMI extends from the LoQ to the upper limit of quantitation (ULoQ). This is determined by the range of values in IU/mL (kU/L) that demonstrated acceptable performance for linearity, imprecision, and bias.

[b] EMI: The EMI extends from the ULoQ to the ULoQ × dilution factor. The value reflects a 1:10 dilution factor.

[c] The reportable interval extends from the LoD to the upper limit of the EMI.

NOTE: The default Low Linearity value in the assay file corresponds to the lower limit of the reportable interval.

# LIMITATIONS OF THE PROCEDURE

Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

Plasma specimens are not acceptable for use with this assay.[9]

# EXPECTED VALUES

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

**Reference Range**

**Serum**[9]

| | Range (IU/mL) |
|---|---|
| Adult | < 30 |

Abbott has not evaluated reference ranges in the pediatric population.

# SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity c system, ARCHITECT c System, and the AEROSET System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity c system.

## Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.[10] Testing was conducted using 1 lot of the Rheumatoid Factor reagent kit, 1 lot of the Alinity c Rheumatoid Factor Calibrator Kit, and 1 lot of commercially available controls and 1 instrument. Two controls and 2 serum panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

| | | Mean IU/mL | Within-Run (Repeatability) | | Within-Laboratory (Total)[a] | |
|---|---|---|---|---|---|---|
| **Sample** | **n** | **(kU/L)** | **SD** | **%CV** | **SD** | **%CV** |
| Control Level 1 | 120 | 26.0 | 0.66 | 2.5 | 0.71 | 2.7 |
| Control Level 2 | 119 | 34.2 | 0.61 | 1.8 | 0.81 | 2.4 |
| Panel A | 118 | 14.4 | 0.98 | 6.8 | 1.06 | 7.3 |
| Panel B | 119 | 81.4 | 0.51 | 0.6 | 0.67 | 0.8 |

[a] Includes within-run, between-run, and between-day variability.

## Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.[11] Testing was conducted using 3 lots of the Rheumatoid Factor reagent kits on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.

| | IU/mL (kU/L) |
|---|---|
| LoB[a] | 9.6 |
| LoD[b] | 13.0 |
| LoQ[c] | 13.0 |

[a] The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b] The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met.

## Linearity

A study was performed based on guidance from CLSI EP06-A.*12*

This assay is linear across the analytical measuring interval of **13.0 to 200.0 IU/mL** (13.0 to 200.0 kU/L).

## Analytical Specificity

### Interference

This study was performed on the AEROSET System.

Potentially Interfering Endogenous Substances

A study was performed based on guidance from NCCLS EP7-P.*13*

Interference effects were assessed by Dose Response and Paired Difference methods at the medical decision level of the analyte.

| Potentially Interfering Substance | Interferent Level | | Rheumatoid Factor | |
|---|---|---|---|---|
| | Default Units | Alternate Units | Target Level IU/mL (kU/L) | Observed (% of Target) |
| Bilirubin | 30 mg/dL | 513 µmol/L | 27.1 | 97.2 |
| | 60 mg/dL | 1026 µmol/L | 27.1 | 96.4 |
| Hemoglobin | 750 mg/dL | 7.5 g/L | 24.0 | 97.0 |
| | 1000 mg/dL | 10.0 g/L | 24.0 | 95.7 |
| | 2000 mg/dL | 20.0 g/L | 24.0 | 93.0 |
| Intralipid | 750 mg/dL | 7.5 g/L | 24.2 | 100.4 |

| Potentially Interfering Substance | Interferent Level | | Rheumatoid Factor | |
|---|---|---|---|---|
| | Default Units | Alternate Units | Target Level IU/mL (kU/L) | Observed (% of Target) |
| | 1000 mg/dL | 10.0 g/L | 24.2 | 97.4 |
| | 2000 mg/dL | 20.0 g/L | 24.2 | 94.0 |

Pharmaceuticals listed below may affect rheumatoid factor concentration.[14]

1. Interferon Alfa-2a and methotrexate may decrease serum rheumatoid factor levels.
2. Penicillamine, pentopril, and timegadine have no significant effect on serum rheumatoid factor levels.
3. Methyldopa, oral contraceptives, and oxyphenisatin may increase serum rheumatoid factor levels.
4. Nonsteroidal anti-inflammatory drugs may decrease or have no significant effect on serum rheumatoid factor levels.

Interferences from medication or endogenous substances may affect results.[15]

## Method Comparison

A study was performed based on guidance from CLSI EP09-A3[16] using the Passing-Bablok regression method.

**Rheumatoid Factor on Alinity c system vs Rheumatoid Factor on ARCHITECT c System**

| | n | Units | Correlation Coefficient | Intercept | Slope | Concentration Range |
|---|---|---|---|---|---|---|
| Serum | 119 | IU/mL (kU/L) | 1.00 | 0.70 | 1.01 | 16.6 to 199.0 |

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