VITROS Products Chemistry

INSTRUCTIONS FOR USE

RF

VITROS Chemistry Products RF Reagent

Rheumatoid Factor

REF **680 1729**

Intended Use

For *in vitro* diagnostic use only.

VITROS Chemistry Products RF Reagent is used on the VITROS 5,1 FS Chemistry System, the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System to quantitatively measure rheumatoid factor (RF) concentration in human serum and plasma.

Summary and Explanation of the Test

Rheumatoid factor (RF) consists of autoantibodies of immunoglobulin isotypes IgM, IgA, IgG, and IgE.[1] The function of RF remains unclear, but it may play a role in the regulation of humoral and cellular immunity and protection against invading microorganisms.[2] Most patients with rheumatoid arthritis and Sjögren's syndrome have elevated levels of RF. RF may also be elevated in scleroderma, dermatomyositis, Waldenström's disease, sarcoidosis, and systemic lupus erythematosus.[3] There are also instances of elevated RF without apparent disease or definable clinical disorders.[2]

Principles of the Procedure

The quantitative measurement of rheumatoid factor is performed using the VITROS Chemistry Products RF Reagent in conjunction with the VITROS Chemistry Products Calibrator Kit 16 and VITROS Chemistry Products FS Calibrator 1 on the VITROS 5,1 FS/4600 Chemistry Systems and the VITROS 5600 Integrated System.

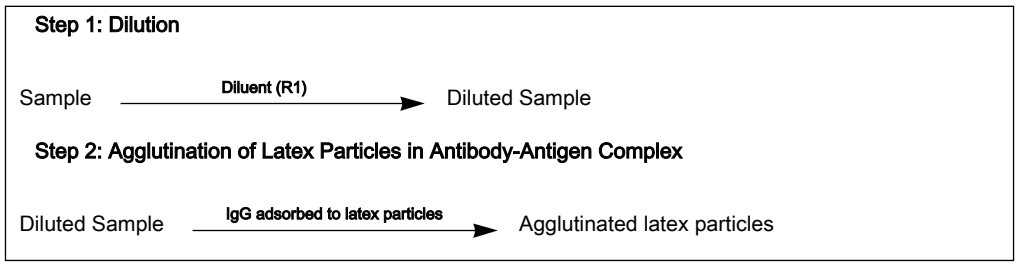
The VITROS RF Reagent is a dual chambered package containing stable, ready-to-use liquid reagents that are used in a two-step reaction to quantitatively measure rheumatoid factor. In the first step, sample containing rheumatoid factors is diluted with buffer contained in Reagent 1. In the second step, an antigen-antibody reaction occurs between rheumatoid factor from the sample and denatured human IgG adsorbed to latex particles in reagent 2 and agglutination results. The agglutination is detected as an absorbance change at 575 nm with the magnitude of the change being proportional to the quantity of RF in the sample. Once a calibration has been performed for each reagent lot, the RF concentration in each unknown sample can be determined using the stored calibration curve and the measured absorbance obtained in the assay of the sample.

Test Type and Conditions

Test Type	VITROS System	Approximate Incubation Time	Temperature	Wavelength	Reaction Sample Volume
Two-point Rate	5600, 4600, 5,1 FS	Incubation 1: 5.1 minutes Incubation 2: 1.5 minutes	37 °C (98.6 °F)	575 nm	5.0 µL

Not all products and systems are available in all countries.

Reaction Scheme



Warnings and Precautions

For *in vitro* diagnostic use only.

WARNING: ***HANDLE AS IF CAPABLE OF TRANSMITTING DISEASE.***
This product is prepared from human components. Testing at the individual donor level was nonreactive for hepatitis B surface antigen (HbsAg), antibody to HCV, and antibody to HIV using FDA approved methods. However, since no test can offer complete assurance that infectious agents are absent, this product should be handled following the recommendations made in CLSI Guideline M29[4], or other published biohazard safety guidelines.

WARNING: ***This product contains sodium azide. Disposal of product into sinks with copper or lead plumbing should be followed with plenty of water to prevent formation of potentially explosive metallic azides.***

WARNING: ***This product contains bovine serum albumin. This product should be handled using the same precautions as with any other blood or blood-derived product.***

For specific warnings and precautions for calibrators, quality control materials, and other components, refer to the Instructions for Use for the appropriate VITROS product, or to other manufacturer's product literature.

Reagents

Reactive Ingredients

Reagent 1 (R1): None
Reagent 2 (R2): Latex particles coated with human IgG, 0.17% (w/v)

Other Ingredients

Reagent 1 (R1): Buffers, inorganic salt, protein, preservative
Reagent 2 (R2): Buffer, inorganic salt, preservative

Reagent Handling

Caution: **Do not use reagent packs with damaged or incompletely sealed packaging.**

- Inspect the packaging for signs of damage.
- Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.
- The reagents are supplied ready for use.
- Avoid agitation, which may cause foaming or the formation of bubbles.

Reagent Preparation

1. Remove from refrigerated storage.
2. Immediately load into Supply 3.

IMPORTANT: *Do not loosen or remove caps prior to loading.*

Reagent Storage and Stability

VITROS Chemistry Products RF Reagent is stable until the expiration date on the carton when it is stored and handled as specified. Do not use beyond the expiration date.

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	Until expiration date
Opened	On-analyzer	System turned on	≤ 4 weeks
	On-analyzer	System turned off	≤ 30 minutes

Verify performance with quality control materials after reloading reagents that have been removed from Supply 3 and stored for later use.

Specimen Collection, Preparation and Storage

Specimens Recommended

- Serum
- Plasma:
  - Heparin
  - EDTA

IMPORTANT: *Certain collection devices have been reported to affect other analytes and tests.*[5] *Owing to the variety of specimen collection devices available, Ortho-Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this test.*

Specimens Not Recommended

None identified.

Serum and Plasma

Specimen Collection and Preparation

Collect specimens using standard laboratory procedures.[6, 7]

Note: For details on minimum fill volume requirements, refer to the operating instructions for your system.

Patient Preparation

No special patient preparation is necessary.

Special Precautions

- Refer to “Sample Dilution” for dilution instructions.
- Centrifuge specimens and remove the serum or plasma from the cellular material within two hours of collection.[8]

Specimen Handling and Storage

- Handle and store specimens in stoppered containers to avoid contamination and evaporation.
- Mix samples by gentle inversion and bring to room temperature, 18–28 °C (64–82 °F), prior to analysis.
- Frozen samples should be centrifuged before analysis to remove any particulate matter formed during storage.

Specimen Storage and Stability

Storage	Temperature	Stability
Room Temperature	18–28 °C (64–82 °F)	≤ 1 day
Refrigerated	2–8 °C (36–46 °F)	≤ 3 days
Frozen[3]	≤-20 °C (≤-4 °F)	≤ 6 months

IMPORTANT: *Avoid repeated freeze-thaw cycles.*

Testing Procedure

Materials Provided

VITROS Chemistry Products RF Reagent

Materials Required but Not Provided

- VITROS Chemistry Products Calibrator Kit 16
- VITROS Chemistry Products FS Calibrator 1
- Quality control materials, such as VITROS Chemistry Products ASO/RF Performance Verifiers I and II
- VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline)

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned workload.
- For additional information, refer to the operating instructions for your system.

IMPORTANT: *Bring all fluids and samples to room temperature, 18–28 °C (64–82 °F), prior to analysis.*

Sample Dilution

IMPORTANT: *If using system in On-Analyzer Dilution Mode, do not manually dilute samples for analysis. Refer to the operating instructions for more information on the On-Analyzer Dilution Procedure.*

For samples with RF concentration that exceed the system's measuring (reportable) range, or if the sample generates a DP code (antigen excess condition), refer to the operating instructions for more information on the On-Analyzer Dilution Procedure. Use VITROS Chemistry Products FS Diluent Pack 2 for the dilution.

Calibration

Required Calibrators

- VITROS Chemistry Products Calibrator Kit 16
- VITROS Chemistry Products FS Calibrator 1

Calibrator Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Calibrator 16 and VITROS Chemistry Products FS Calibrator 1.

Calibration Procedure

Refer to the operating instructions for your system.

When to Calibrate

Calibrate:

- When the reagent lot number changes.
- When critical system parts are replaced due to service or maintenance.
- When government regulations require.
  For example, in the USA, CLIA regulations require calibration or calibration verification at least once every six months.

The VITROS RF assay may also need to be calibrated:

- If quality control results are consistently outside acceptable range.
- After certain service procedures have been performed.

For additional information, refer to the operating instructions for your system.

Calculations

Absorbance is measured at 575 nm after a fixed incubation time. Once a calibration has been performed for each reagent lot, RF concentration in the unknown samples can be determined using the stored calibration curve and the measured absorbance obtained in the assay of each sample.

Validity of a Calibration

Calibration parameters are automatically assessed by the system against a set of quality parameters detailed in the Review Assay Data screen (found via Options → Review/Edit Calibrations → Review Assay Data). Failure to meet any of the pre-defined quality parameters results in a failed calibration. The calibration report should be used in conjunction with quality control results to determine the validity of a calibration.

Measuring (Reportable) Range

Conventional Units (IU/mL)	SI Units (kIU/L)
8.6–120.0	8.6–120.0

Traceability of Calibration

Values assigned to the VITROS Chemistry Products Calibrator Kit 16 and VITROS Chemistry Products FS Calibrator Kit 1 for rheumatoid factor are traceable to the *International Reference Preparation of Rheumatoid Arthritis Serum*, WHO 1st British Standard, NIBSC 64/2. [9]

Quality Control

Quality Control Material Selection

IMPORTANT: *VITROS Chemistry Products ASO/RF Performance Verifiers are recommended for use with the VITROS 5,1 FS/4600 Chemistry and Integrated Systems. Evaluate the performance of other commercial control fluids for compatibility with this assay before using for quality control.*

Control materials other than VITROS Chemistry Products ASO/RF Performance Verifiers may show a difference when compared with other RF methods if they:

- Depart from a true human matrix.
- Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives.

Quality Control Procedure Recommendations

- Choose control levels that check the clinically relevant range.
- Analyze quality control materials in the same manner as patient samples, before or during patient sample processing.
- To verify system performance, analyze control materials:
  - After calibration.
  - According to local regulations or at least once each day that the assay is being performed.
  - After specified service procedures are performed. Refer to the operating instructions for your system.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- For general quality control recommendations, refer to CLSI *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Third Edition*[10] or other published guidelines.
- For additional information, refer to the operating instructions for your system.

Quality Control Material Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products ASO/RF Performance Verifiers I and II or to other manufacturer's product literature.

Results

Reporting Units and Unit Conversion

The VITROS 5,1 FS/4600 Chemistry and Integrated Systems may be programmed to report RF in conventional or SI units.

Conventional Units	SI Units
IU/mL	kIU/L (IU/mL x 1)

Limitations of the Procedure

Known Interferences

Pathologically high levels of IgG (>2500 mg/dL) may interfere with quantitation of RF.[11]

Other Limitations

- For samples that generate an Antigen Excess flag or for samples that generate both a Sample Integrity T-index flag and an Antigen Excess flag, refer to “Sample Dilution”.
- No antigen excess effect was observed for samples with rheumatoid factor concentrations up to 7982 IU/mL.
- Certain drugs and clinical conditions are known to alter Rheumatoid Factor concentration *in vivo*. For additional information, refer to one of the published summaries.[12, 13]

Expected Values

Reference Interval

The reference interval is defined as the 97.5 percentile value of results from a study of 507 apparently healthy adults.

Conventional Units (IU/mL)	SI Units (kIU/L)
< 12	< 12

Each laboratory should confirm the validity of this interval for the population it serves.

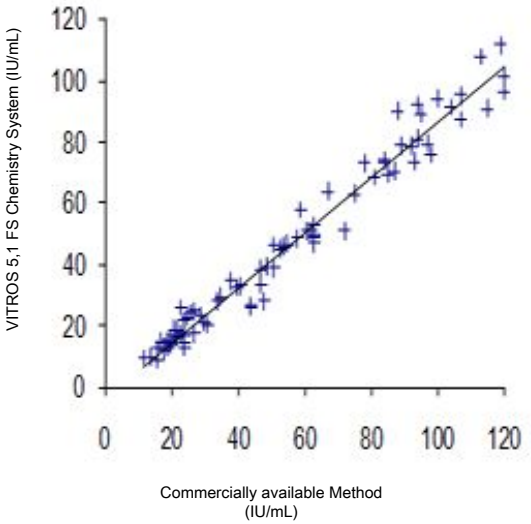
Performance Characteristics

Method Comparison

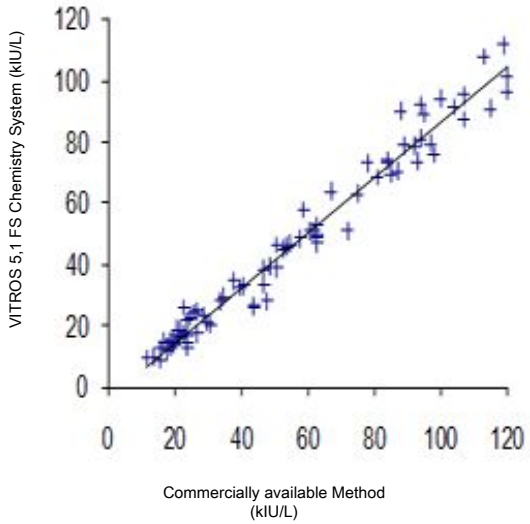
The plots and table show the results of a comparison of serum samples analyzed on the VITROS 5,1 FS Chemistry System with those analyzed using a commercially available method. Testing followed NCCLS Protocol EP9. [14]

The table also shows the results of comparisons with serum and plasma samples on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System. The testing followed NCCLS Protocol EP9. [14]

Conventional Units



SI Units



	n	Slope	Correlation Coefficient	Conventional (IU/mL) and SI (kIU/L) Units: Range of Sample Conc.	Intercept	Sy.x
5,1 FS† vs. commercially available method*	88	0.90	0.986	8.8–111.8	-3.66	4.91
5600 vs. 5,1 FS †	98	1.00	0.999	8.7–112.3	-0.42	1.19

* Immunoturbidimetric method

† Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

Precision

Precision was evaluated with quality-control materials on the VITROS 5,1 FS System following NCCLS Protocol EP5. [15]

Precision was also evaluated with quality-control materials on the VITROS 5600 Integrated System following NCCLS Protocol EP5. [16]

These results are guidelines. Variables such as instrument maintenance, environment, reagent storage/handling, control material reconstitution, and sample handling can affect the reproducibility of test results.

System	Conventional (IU/mL) and SI (kIU/L) Units			Within-Lab CV%**	No. Observ.	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD**			
5,1 FS†	15.3	0.72	1.22	8.0	100	26
	28.1	0.78	1.03	3.7	100	26
	81.1	1.50	2.13	2.6	100	26
5600	16.1	0.52	0.68	4.2	88	22
	28.3	0.41	0.66	2.3	88	22

* Within Day precision was determined using two-runs/ day with two replications per run.

** Within Lab precision was determined using a single lot of reagents and at least four calibrations.

† Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

Specificity

Substances That Do Not Interfere

The substances listed in this table were tested on the VITROS Chemistry Products RF Reagent at RF nominal concentrations of approximately 25 IU/mL (25 kIU/L) using protocols based on NCCLS Protocol EP7 [17], and found not to interfere, bias <3.4 IU/mL (<3.4 kIU/L), at the concentrations shown.

Compound	Concentration	
Acetaminophen	200 µg/mL	1324 µmol/L
N-Acetyl-L-cysteine	100 mg/dL	6.13 mmol/L
Amoxicillin	20 µg/mL	1.15 mmol/L
Ascorbic acid	3 mg/dL	0.17 mmol/L
Bilirubin	60 mg/dL	1.03 mmol/L
Carbamazepine	120 µg/mL	507.6 µmol/L
Dipyrone	30 mg/dL	0.85 mmol/L
Ethamsylate	3 mg/dL	0.11 mmol/L
Gentamicin sulfate	120 µg/mL	251 µmol/L
Hemoglobin	1000 mg/dL	10 g/L
Ibuprofen	400 µg/mL	1940 µmol/L
Lidocaine	60 µg/mL	256 µmol/L
Methotrexate	10 mmol/L	10 mmol/L
Procainamide	100 µg/mL	423 µmol/L
Propanolol	5000 ng/mL	19300 nmol/L
Rantidine	200 µg/mL	637 µmol/L
Salicylic acid	500 µg/mL	3.6 mmol/L
Simvastatin	16 µg/mL	1.2 mmol/L
Theophylline	250 µg/mL	1388 µmol/L
Valproic acid	500 µg/mL	3465 µmol/L

References

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2. Ritchie, RF (ed). *Serum Proteins in Clinical Medicine, Volume 1 Laboratory Section*. Scarborough, ME: Foundation for Blood Research; 11.06.02-2; 1996.
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4. CLSI. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Third Edition*. CLSI Document M29 [ISBN 1-56238-567-4]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2005.
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14. NCCLS. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition*. NCCLS document EP9-A2 (ISBN 1-56238-472-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2002.
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16. NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition*.NCCLS document EP5-A2 [ISBN 1-56238-542-9]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2004.
17. NCCLS. *Interference Testing In Clinical Chemistry; Proposed Guideline*. NCCLS document EP7-A [ISBN 1-56238-480-5]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2002.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.

	Do Not Reuse		Upper Limit of Temperature		Range
	Use by or Expiration Date (Year-Month-Day)		Lower Limit of Temperature		Range of Means
LOT	Batch Code or Lot Number		Temperature Limitation		Midpoint
SN	Serial Number	n	Consult Instructions for Use		Revised
REF	Catalog Number or Product Code		Attention: The Instructions for Use (IFU) has been updated		Supersedes
	Caution	S1	For use in Slide Supply 1	Xi	Irritant
	Manufacturer	S2	For use in Slide Supply 2	Xn	Harmful
	Date of Manufacture	SI	SI Units	T	Toxic
EC REP	Authorized Representative in the European Community	CONV	Conventional Units	C	Corrosive
n	Contains Sufficient for "n" Tests		Value	F	Flammable
IVD	*In vitro* Diagnostic Medical Device		Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations	σ	Estimated within-lab SD

Revision History

Date of Revision	Version	Description of Technical Changes*
2014-09-05	8.0	Glossary of Symbols: added Date of Manufacture
2012-02-28	7.0	Glossary of Symbols: updated
2010-11-01	6.0	Added information for the VITROS 4600 Chemistry System
2009-10-20	5.0	• Measuring (Reportable) Range – Updated data • Method Comparison – Updated data
2009-04-03	4.0	• Added information for the VITROS 5600 Integrated System • Test Type and Conditions – Added statement • Method Comparison – Added information on sample types • References – Updated • Glossary of Symbols – Updated • Minor wording and formatting changes
2007-07-02	3.0	• Performance Characteristics – Corrected units • References – Updated M29, H3, H4, C24
2005-07-29	2.0	• Specimen Requirements, Special Precautions: Wording update • Reportable Range: Updated data
2004-09-15	1.0	First release of document

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature

Obsolete Date

CE

EC REP

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