VITROS Products Chemistry

INSTRUCTIONS FOR USE C3

VITROS Chemistry Products C3 Reagent

Complement C3

REF 680 1735

Intended Use

For *in vitro* diagnostic use only.

VITROS Chemistry Products C3 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 complement C3 (C3) concentration in human serum and plasma. Measurements of these proteins aids in the diagnosis of immunologic disorders, especially those associated with deficiencies of complement components.

Summary and Explanation of the Test

C3 is the most abundant protein of the complement system. As one of the major mediators of inflammation, it serves as the functional link between the classical and alternative pathway of complement system activation. The activation of complement produces a number of biological effects such as the destruction of foreign agents (bacteria, yeast, viruses), release of histamine, and the release of leukocytes from the bone marrow. C3 levels may be used as an aid in the diagnosis of inherited or collagen vascular diseases such as active lupus nephritis, severe infections, and inflammation. Increased levels may be found after trauma or surgery, biliary obstruction, or focal glomerulosclerosis. [1]

Principles of the Procedure

The quantitative measurement of C3 is performed using the VITROS Chemistry Products C3 Reagent in conjunction with the VITROS Chemistry Products Calibrator Kit 20 and VITROS Chemistry Products FS Diluent Pack 2 on the VITROS 5,1 FS/4600 Chemistry System and the VITROS 5600 Integrated System. The VITROS Chemistry Products C3 Reagent is a dual chambered package containing ready-to-use liquid reagents. Samples, calibrators and controls are automatically diluted in saline and mixed with Reagent 1 containing a polymer. Addition of antisera specific for human C3 (Reagent 2) produces an immunochemical reaction yielding antigen/antibody complexes. The light scattering properties of the antigen/antibody complexes increase solution turbidity proportional to C3 concentration in the sample. The turbidity is measured spectrophotometrically at 340 nm. Once a calibration has been performed for each reagent lot, the C3 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
Blanked Endpoint	5600, 4600, 5,1 FS	Incubation 1: 5 minutes Incubation 2: 5 minutes	37 °C (98.6 °F)	340 nm	16.7 µL

Not all products and systems are available in all countries.

Reaction Scheme

Step 1: Dilution of C3 Sample

Sample —(Diluent/R1 Polymer)→ Diluted Sample

Step 2: Formation of C3/Antibody Complexes

Diluted Sample —(R2 Polyclonal antibodies)→ Antigen/Antibody Complexes

Warnings and Precautions

For *in vitro* diagnostic use only.

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

WARNING: ***Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and CLSI Guideline M29[2] or other published biohazard safety guidelines.***

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): Goat antisera to human C3 1 mL/mL

Other Ingredients

Reagent 1 (R1): Preservative, polymer, buffers, inorganic salt
Reagent 2 (R2): Preservative, buffer, inorganic salt

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 C3 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

IMPORTANT: *Certain collection devices have been reported to affect other analytes and tests.[3] 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

Plasma: EDTA

Serum and Plasma

Specimen Collection and Preparation

Collect specimens using standard laboratory procedures.[4, 5]

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

Centrifuge specimens and remove the serum or plasma from the cellular material within two hours of collection.[6]

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.

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	≤-20 °C (≤-4 °F)	≤ 4 weeks
Frozen[6]	≤-70 °C (≤-94 °F)	≤ 1 year

IMPORTANT: *Avoid repeated freeze-thaw cycles.*

Testing Procedure

Materials Provided

VITROS Chemistry Products C3 Reagent

Materials Required but Not Provided

- VITROS Chemistry Products Calibrator Kit 20
- Quality control materials, such as VITROS Chemistry Products Protein Performance Verifiers I, II, and III
- 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

On-Analyzer Sample Dilution

Refer to the operating instructions for your system for more information on the On-Analyzer Dilution Procedure. Use VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline) for the dilution.

Manual Sample Dilution

If C3 concentrations exceed the system's measuring (reportable) range or for samples that generate a T-Index Flag:

1. Dilute 1 part sample with 1 part saline using saline from VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline).
2. Reanalyze.
3. Multiply the results by 2 to obtain an estimate of the original sample's C3 concentration.

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

Calibration

Required Calibrators

VITROS Chemistry Products Calibrator Kit 20

Calibrator Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Calibrator 20.

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 C3 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 340 nm after each incubation step (blank, endpoint) and the response calculated from the difference in absorbance values. Once a calibration has been performed for each reagent lot, C3 concentration in the unknown samples can be determined using the stored calibration curve and the calculated response 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 (mg/dL)	SI Units (mg/L)
40–380	400–3800

For out-of-range samples, refer to "Sample Dilution."

Traceability of Calibration

Values assigned to the VITROS Chemistry Products Calibrator Kit 20 for C3 are traceable to BAM-IRMM-LGC (Bundesanstalt für Materialforschung und -prüfung/Institute for Reference Methods and Materials/Laboratory of the Government Chemist) ERM-DA470 Reference Material. [7]

Quality Control

Quality Control Material Selection

IMPORTANT: *VITROS Chemistry Products Protein Performance Verifiers are recommended for use with the VITROS 5,1 FS/4600 Chemistry and VITROS 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 Protein Performance Verifiers may show a difference when compared with other C3 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*[8] 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 Protein Performance Verifier I, II, and III or to other manufacturer's product literature.

Results

Reporting Units and Unit Conversion

The VITROS Integrated and VITROS 5,1 FS/4600 Chemistry Systems may be programmed to report C3 results in conventional or SI units.

Conventional Units	SI Units
mg/dL	mg/L (mg/dL × 10)

Limitations of the Procedure

Known Interferences

None identified.

Other Limitations

- For samples that generate a Sample Integrity T-index flag, refer to the Sample Dilution section.
- No antigen excess effect was observed for samples with C3 concentrations up to 2000 mg/dL (20,000 mg/L).
- Certain drugs and clinical conditions are known to alter C3 concentration *in vivo*. For additional information, refer to one of the published summaries.[9, 10]

Expected Values

Reference Interval

The reference interval values are the central 95th percentile of results from an internal study of 122 apparently healthy adults.

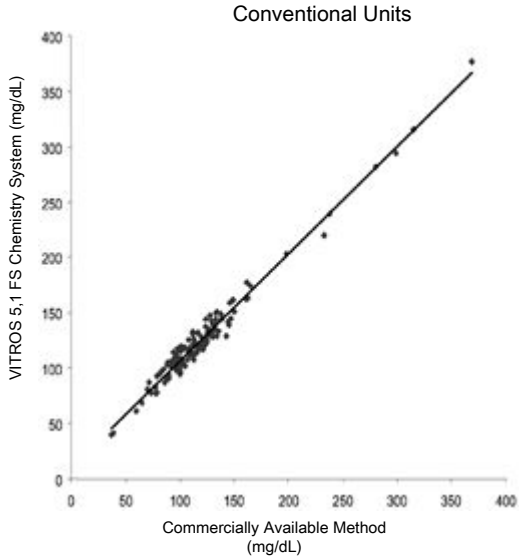
Conventional Units (mg/dL)	SI Units (mg/L)
88–165	880–1650

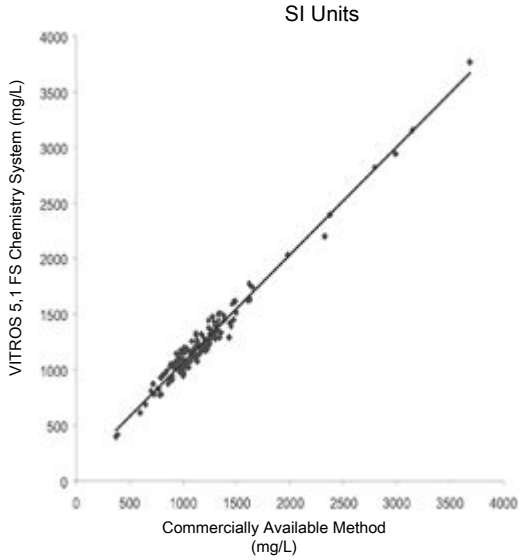
Each laboratory should confirm the validity of these intervals for the population it serves.

Performance Characteristics

Method Comparison

The plots and data below show the results of a method comparison study with serum samples analyzed on the VITROS 5,1 FS Chemistry System and a commercially available system, based on NCCLS Protocol EP9. [11]
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. [11]





				Conventional Units (mg/dL)			SI Units (mg/L)		
	n	Slope	Correlation Coefficient	Range of Sample Conc.	Intercept	Sy.x	Range of Sample Conc.	Intercept	Sy.x
5,1 FS† vs. Commercially Available method*	139	0.95	0.98	40–316	+11	7.2	400–3156	+114	71.5
5600 vs. 5,1 FS†	107	1.03	1.00	47–364	-2	5.2	470–3640	-20	52.0

* Beckman IMMAGE Immunochemistry Systems Complement C3 Assay

† 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. [12]
Precision was also evaluated with quality-control materials on the VITROS 5600 Integrated System following NCCLS Protocol EP5. [13]

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.

	Conventional Units (mg/dL)			SI Units (mg/L)					
	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**	Within Lab CV%**	No. Observ.	No. Days
5,1 FS†	74	0.7	1.6	737	6.9	16.1	2.2	88	22
	142	2.1	4.5	1415	21.4	45.1	3.2	88	22
	292	9.6	13.7	2917	96.4	136.9	4.7	87	22
5600	75	1.4	3.2	753	14.4	32.5	4.3	88	22
	128	1.5	3.2	1279	14.9	31.9	2.5	88	22
	253	4.8	6.6	2532	47.9	65.5	2.6	88	22

* Within Day precision was determined using two runs per 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

The substances listed in this table were tested with the VITROS Chemistry Products C3 Reagent at C3 concentration of approximately 97 mg/dL (970 mg/L) using protocols based on NCCLS Protocol EP7 [14] and found not to interfere, bias <9 mg/dL (<90 mg/L), at the concentrations shown. Hemoglobin and bilirubin were tested with the VITROS Chemistry Products C3 Reagent at C3 concentration of approximately 70 mg/dL (700 mg/L) using protocols based on NCCLS Protocol EP7 [14], and found not to interfere, bias <7 mg/dL (<70 mg/L), at the concentrations shown.

Compound	Concentration	
Acetominophen	200 µg/mL	1324 µmol/L
N-Acetyl-L-cysteine	100 mg/dL	6.13 mmol/L
Amoxicillin	20 µg/mL	55 µmol/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	91 mg/dL	2 mmol/L
Procainamide	100 µg/mL	368 µmol/L
Propranolol	5000 ng/mL	19.3 µmol/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
Triglyceride	600 mg/dL	6.8 mmol/L
Theophylline	250 µg/mL	1388 µmol/L
Valproic acid	500 µg/mL	3465 µmol/L

References

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3. Calam RR. Specimen Processing Separator Gels: An Update. *J Clin Immunoassay*. 11:86–90; 1988.

4. CLSI. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard – Sixth Edition.* CLSI document H3-A6 (ISBN 1-56238-650-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 2007.
5. NCCLS. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard – Fifth Edition.* NCCLS document H4-A5 [ISBN 1-56238-538-0]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.
6. *Clinical Laboratory Handbook for Patient Preparation and Specimen Handling*. Fascicle VI: Chemistry/Clinical Microscopy. Northfield, IL: College of American Pathologists; 1992.
7. European Commission. *The Certification of a Matrix Reference Material for Immunochemical Measurement of 15 Serum Proteins, ERM-DA470*. Report EUR 15243 EN and 16882 EN, European Communities, 2004.
8. CLSI.*Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline – Third Edition.* CLSI document C24-A3 (ISBN 1-56238-613-1). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2006.
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11. 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.
12. NCCLS. *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline*. NCCLS document EP5-A [ISBN 1-56238-368-X]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999.
13. 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.
14. 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	5.0	Glossary of Symbols: added Date of Manufacture

Date of Revision	Version	Description of Technical Changes*
2012-02-28	4.0	Glossary of Symbols: updated
2010-11-01	3.0	Added information for the VITROS 4600 Chemistry System
2009-03-17	2.0	• Added information for the VITROS 5600 Integrated System • Test Type and Conditions – Added statement • Traceability of Calibration – Updated data • Method Comparison – Added information on sample types • References – Updated • Glossary of Symbols – Updated • Minor wording and formatting changes
2004-10-05	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

Ortho-Clinical Diagnostics
50-100 Holmers Farm Way
High Wycombe
Buckinghamshire
HP12 4DP
United Kingdom

Ortho-Clinical Diagnostics, Inc.
100 Indigo Creek Drive
Rochester, NY 14626

Ortho Clinical Diagnostics