VITROS Products Chemistry

INSTRUCTIONS FOR USE C4

VITROS Chemistry Products C4 Reagent

Complement C4

REF 680 1736

Intended Use

For *in vitro* diagnostic use only.

VITROS Chemistry Products C4 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 C4 (C4) 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

C4 is one of the activating enzymes of the complement system. Activation of C4 leads to formation of the C3 convertase in the classical pathway. C4 plays a contributing role in tissue cell damage and inflammation in immunologic disorders. Depressed levels of C4 due to increased consumption may be found in systemic lupus erythematosus (SLE), hereditary angioedema, autoimmune hemolytic anemia, and autoimmune nephritides. A genetic deficiency of C4 is associated with a high prevalence of autoimmune or collagen vascular disease, especially SLE. As an acute-phase reactant, modestly increased levels may occur in inflammation, trauma, and tissue necrosis. [1]

Principles of the Procedure

The quantitative measurement of C4 is performed using the VITROS Chemistry Products C4 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 and VITROS 5600 Integrated Systems. The VITROS Chemistry Products C4 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 C4 (Reagent 2) produces an immunochemical reaction yielding antigen/antibody complexes. The light scattering properties of the antigen/antibody complexes increase solution turbidity proportional to C4 concentration in the sample. The turbidity is measured spectrophotometrically at 340 nm. Once a calibration has been performed for each reagent lot, the C4 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 C4 Sample

Sample —(Diluent/R1 Polymer)→ Diluted Sample

Step 2: Formation of C4/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 C4 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 C4 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 assays.*[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 assay.*

Specimens Not Recommended

None

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)	≤ 7 days
Frozen	≤-20 °C (≤-4 °F)	≤ 4 weeks
Frozen[6]	≤-70 °C (≤-94 °F)	Indefinitely

IMPORTANT: *Avoid repeated freeze-thaw cycles.*

Testing Procedure

Materials Provided

VITROS Chemistry Products C4 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 C4 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 C4 concentration.

IMPORTANT: *If using the 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.*

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 C4 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, C4 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)
8.0–60.0	80.0–600.0

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

Traceability of Calibration

Values assigned to the VITROS Chemistry Products Calibrator Kit 20 for C4 are traceable to the 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 C4 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 C4 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 C4 concentrations up to 600 mg/dL (6,000 mg/L).
- Certain drugs and clinical conditions are known to alter C4 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)
14–44	140–440

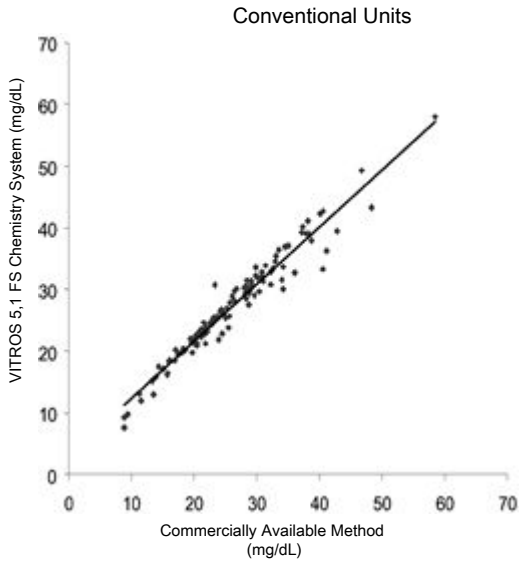
Each laboratory should confirm the validity of these intervals for the population it serves.

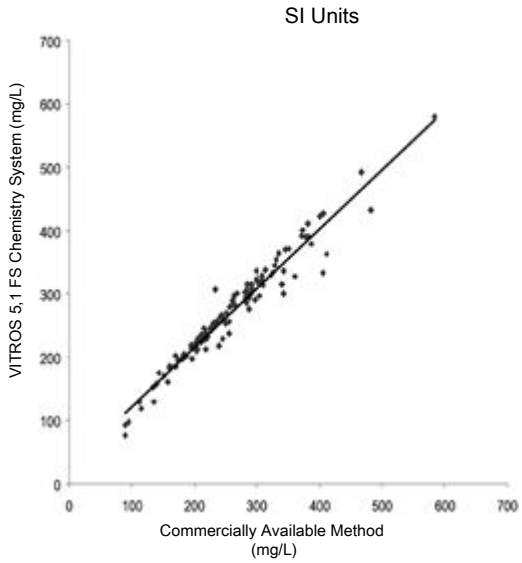
Performance Characteristics

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*	112	0.93	0.976	9.2–58.0	3.0	1.80	92.2–579.5	30.0	18.0
5600 vs. 5,1 FS†	108	0.99	0.997	9.3–58.9	0.9	1.03	93.0–589.0	9.0	10.3

* Dade Behring N Human C4 Complement 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 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.

System	Conventional Units (mg/dL)			SI Units (mg/L)			Within Lab CV%**	No. Observ.	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**			
5,1 FS†	13.4	0.18	0.27	134.4	1.82	2.69	2.0	87	22
	25.6	0.16	0.43	255.9	1.57	4.28	1.7	87	22
	45.1	0.37	0.69	450.6	3.72	6.89	1.5	88	22
5600	13.2	0.57	0.68	131.7	5.70	6.79	5.2	92	23
	19.4	0.33	0.53	194.2	3.33	5.28	2.7	92	23
	48.6	0.50	1.02	485.7	5.04	10.23	2.1	92	23

* 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 reagent 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 C4 Reagent at C4 concentration of approximately 22 mg/dL (220 mg/L) using protocols based on NCCLS Protocol EP7, [14] and found not to interfere, bias <3.5 mg/dL (<35 mg/L), at the concentrations shown. Hemoglobin and bilirubin were tested on the VITROS Chemistry Products C4 Reagent at C4 concentration of approximately 26 mg/dL (260 mg/L) using protocols based on NCCLS Protocol EP7, and found not to interfere, bias <3.8 mg/dL (<38 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
Theophylline	250 µg/mL	1388 µmol/L
Triglyceride	600 mg/dL	6.8 mmol/L
Valproic acid	500 µg/mL	3465 µmol/L

References

1. Burtis C, Ashwood, ER (ed). *Tietz Textbook of Clinical Chemistry, 3rd ed.* Philadelphia, PA; WB Saunders Co; 506; 1999.
2. CLSI. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Third Edition.* CLSI document M29-A3 (ISBN 1-56238-567-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2005.
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.
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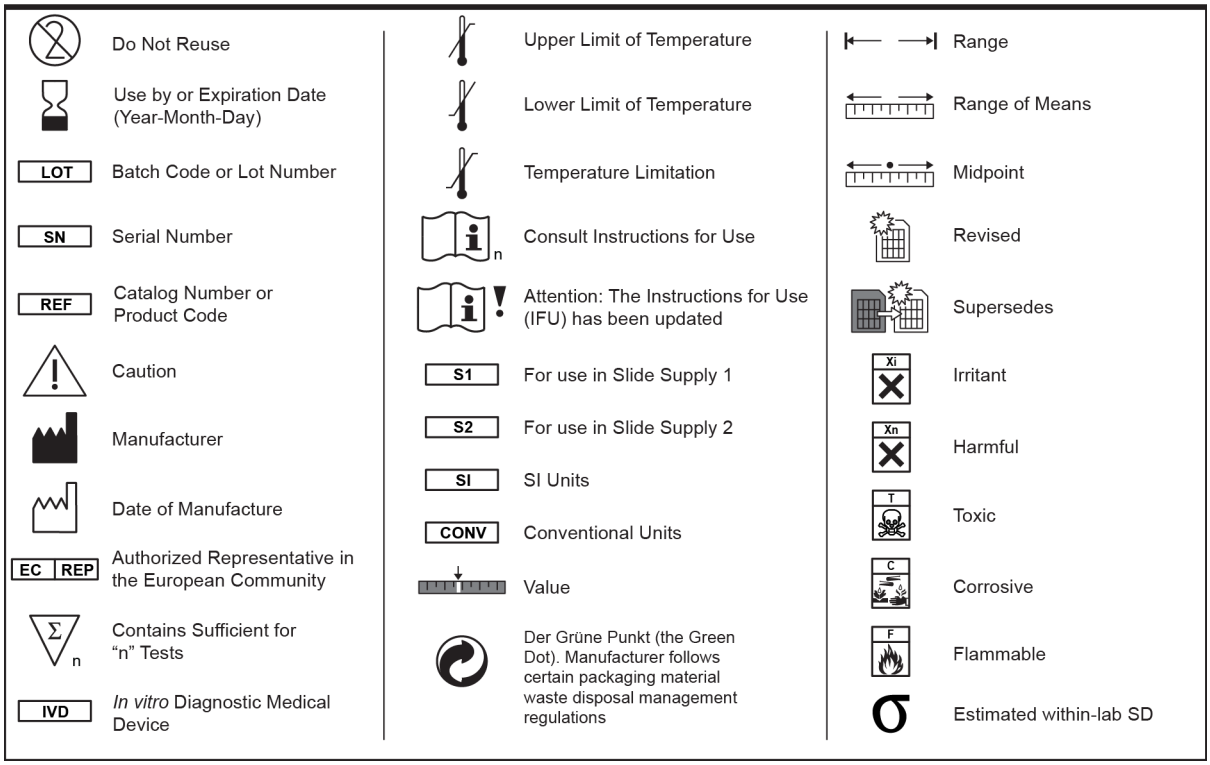
13. NCCLS. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. NCCLS document EP5-A2 [ISBN 1-56238-542-9]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2004.
14. NCCLS. *Interference Testing In Clinical Chemistry*; Proposed 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.



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*
2013-08-01	4.1	Materials Required but Not Provided: corrected product name — FS Diluent Pack 2
2012-02-28	4.0	Glossary of Symbols: updated
2010-11-01	3.0	Added information for the VITROS 4600 Chemistry System
2009-03-25	2.0	• Added information for the VITROS 5600 Integrated System • Test Type and Conditions – Added statement • Traceability of Calibration – Updated • 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

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