C3

OSR6159	**4 x 10 mL**	**R1**
	4 x 8 mL	**R2**

Intended Use
System reagent for the quantitative determination of Complement 3 (C3) in human serum on Beckman Coulter AU analyzers.

Summary
The Complement system is composed of a series of circulating blood proteins that serve as mediators of the inflammatory response. C3 comprises about 70% of the total protein in the complement system and is central to activation of both the classical and alternate pathways.[1] Measurements of this protein aids in the diagnosis of immunologic disorders, especially those associated with deficiencies of complement components.

Methodology
Immune complexes formed in solution scatter light in proportion to their size, shape and concentration. Turbidimeters measure the reduction of incident light due to reflection, absorption, or scatter.

In the procedure, the measurement of the decrease in light transmitted (increase in absorbance) through particles suspended in solution as a result of complexes formed during the antigen-antibody reaction, is the basis of this assay.

System Information
For AU400/400^{e}/480, AU600/640/640^{e}/680 and AU2700/5400/AU5800 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

Tris buffer (pH 7.2)	62 mmol/L
Polyethylene glycol 6000	1.6 % w/v
Goat anti-C3 antiserum	
Also contains preservatives.	

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation of reagents
The C3 reagent is ready for use. No preparation is required.

Storage and stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened bottles of reagent are stable for 90 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Visible signs of microbial growth, turbidity, precipitate, or change in color in the C3 reagents may indicate degradation and warrant discontinuation of use.

Specimen Collection And Preparation
Serum is the recommended specimen. Prior to centrifugation, allow the sample to clot 15 - 30 minutes at room temperature, then refrigerate the sample 2 - 8°C and allow clotting to continue for 30 - 60 minutes.[1] To separate serum: rim the clot and centrifuge in refrigerated centrifuge. Serum should then be separated into multiple aliquots and frozen (≤ -20°C) to avoid thawing and refreezing.

If cryoprecipitating antibodies are suspected, clot formation and centrifugation should proceed at 37°C as a complement fixation may occur if the specimen is chilled. In certain sera, chilling will significantly lower complement titer.[2]

Sample Storage and Stability
Serum samples can be stored at 2 - 8°C or at -20°C for up to 8 days without any loss of activity. Samples should be refrigerated or frozen if the assay cannot be run within 4 hours.[7]

Interfering Substances
Results of studies[3] show that the following substances may interfere with this C3 procedure:

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin:	No significant interference up to 40 mg/dL Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 500 mg/dL Intralipid*

No interference was observed from RF positive specimens (15 - 1838 IU/mL) with this procedure.
* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young for a compilation of reported interferences with this test.[4]

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Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer**.**

Materials Provided
C3 Reagent

Materials required but not provided
Serum Protein Multi-Calibrator (Cat # ODR3021)
MC Cal A (Cat # ODR30037) for Mastercurve enabled systems

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzers automatically compute every determination at the same time interval.

Calibration
The frequency of calibration for the C3 turbidimetric procedure is every 90 days. Calibration of this C3 procedure is accomplished by use of the Serum Protein Multi-Calibrator (Cat # ODR3021), which is traceable to IFCC International Reference Preparation CRM470 (RPPHS).
Use MC Cal A Cat. No. ODR30037 for Mastercurve enabled systems only. Please refer to User Guide for further instructions.

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate Immunology control should be tested a minimum of once a day. In addition, these controls should be performed after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in mg/dL at 37°C.

Dynamic Range

The C3 turbidimetric procedure is linear from 15 - 500 mg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Expected Values

Serum:[6] 87 - 200 mg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the C3 Reagent on Beckman Coulter AU analyzers according to established procedures.

Precision[8]
Estimates of precision, based on CLSI recommendations,[5] are consistent with typical performance. The within run precision is less than 3% CV and the total precision is less than 5% CV. Assays of serum pools and control sera were performed and the data reduced following CLSI guidelines above.

N= 100	Within run		Total	
Mean, mg/dL	SD	CV%	SD	CV%
62.6	0.4	0.7	0.9	1.5
120.2	1.0	0.8	1.7	1.4

Method Comparison[8]
Patient samples were used to compare this C3 Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	AU600
Slope	0.983
Intercept	- 0.7
Correlation Coeff. (r)	0.9991
No. of Samples (n)	177
Range (mg/dL)	40 – 324

References

1. Tilzer, L., et. al., Laboratory Test Handbook, Third Edition, Lexi-comp., Cleveland OH, 1994.
2. Henry, J.B., et. al., Clinical Diagnosis and Management by Laboratory Methods, Nineteenth Edition, WB Saunders, 1996.
3. CLSI/NCCLS, Interference Test in Clinical Chemistry, EP7-P, 1986.
4. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
5. CLSI/NCCLS Evaluation Protocol EP5-T2, 1992.
6. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
7. World Health Organization, Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO/DIL/LAB/99.1 Rev.2.
8. Data is on file for specific AU analyzers.

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