For In Vitro Diagnostic Use

ANNUAL REVIEW

<table>
<thead>
<tr>
<th>Reviewed by:</th>
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PRINCIPLE

INTENDED USE

C3 reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and SYNCHRON® Systems CAL 1, is intended for quantitative determination of complement c3 concentration in human serum or plasma by rate turbidimetry.

CLINICAL SIGNIFICANCE

Complement is a group of serum proteins which destroy infectious agents. Measurements of these proteins aid in the diagnosis of immunologic disorders, especially those associated with deficiencies of complement components.¹,²

METHODOLOGY

C3 reagent is used to measure the analyte concentration by a turbidimetric method.³ In the reaction, complement c3 combines with specific antibody to form insoluble antigen-antibody complexes.

The SYNCHRON® System(s) automatically dilutes sample and dispenses the appropriate sample and reagent volumes into a cuvette. The ratio used is one part diluted sample to 5.75 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is proportional to the concentration of complement c3 in the sample and is used by the SYNCHRON® System(s) to calculate and express the complement c3 concentration based upon a single-point calibration curve.

CHEMICAL REACTION SCHEME

\[
\text{Complement C3(sample) + Antibody } \rightarrow [\text{Complement C3(sample)-Antibody (aggregates)}]
\]

SPECIMEN

TYPE OF SPECIMEN

Serum samples are recommended. Plasma samples (EDTA, Lithium Heparin, and Sodium Heparin) can be used.
Serum or plasma samples should be collected in the manner routinely used for any clinical laboratory test. Freshly drawn serum or plasma from a fasting individual is preferred. Anticoagulants tested are listed in the PROCEDURAL NOTES section of this chemistry information sheet.

**SPECIMEN STORAGE AND STABILITY**

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 6 hours, and no longer than 24 hours at +2°C to +8°C. Specimens can be stored frozen at -15°C to -20°C for one week. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Additional specimen storage and stability conditions as designated by this laboratory:

**SAMPLE VOLUME**

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

**CRITERIA FOR UNACCEPTABLE SPECIMENS**

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

**PATIENT PREPARATION**

Special instructions for patient preparation as designated by this laboratory:
SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

Two Complement C3 Reagent Cartridges (2 x 100 tests)
One lot-specific Parameter Card

VOLUMES PER TEST

Sample Dilution Volumes

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume</td>
<td>15 µL</td>
</tr>
<tr>
<td>Diluent Volume</td>
<td>285 µL</td>
</tr>
</tbody>
</table>

Diluted Sample Volume (1:20 dilution)

<table>
<thead>
<tr>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 µL</td>
</tr>
</tbody>
</table>

Total Reagent Volume

<table>
<thead>
<tr>
<th>Total Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>230 µL</td>
</tr>
</tbody>
</table>

Cartridge Volumes

<table>
<thead>
<tr>
<th>Cartridge</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>200 µL</td>
</tr>
<tr>
<td>B</td>
<td>30 µL</td>
</tr>
<tr>
<td>C</td>
<td>– –</td>
</tr>
</tbody>
</table>

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Compartment A

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Buffer</td>
<td>44.0 mL</td>
</tr>
</tbody>
</table>

Compartment B

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody (polyclonal) Monospecific for complement c3</td>
<td>6.0 mL</td>
</tr>
</tbody>
</table>

Sodium Azide (used as a preservative) < 0.1% (w/w)

Also non-reactive chemicals necessary for optimal system performance.

⚠️ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).
EUROPEAN HAZARD CLASSIFICATION

C3 Reagent (Compartment A)  R52/53  Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S61  Avoid release to the environment. Refer to special instructions/Safety data sheets.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems CAL 1
DIL 1 Cartridge
At least two levels of control material
Saline

REAGENT PREPARATION

No preparation is required.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility’s acceptance criteria.

REAGENT STORAGE AND STABILITY

C3 reagent, when stored unopened at +2°C to +8°C, will obtain the shelf-life indicated on the cartridge label. Once opened, the reagent is stable for 60 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE.

DIL 1 stored unopened at room temperature is stable until the expiration date indicated on each cartridge. Once opened, DIL 1 is stable for 60 days on instrument or until the expiration date, if sooner.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems CAL 1

CALIBRATOR PREPARATION

No preparation is required.
CALIBRATOR STORAGE AND STABILITY

SYNCHRON® Systems CAL 1 is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C. DO NOT FREEZE.

Calibrator storage location:

⚠️ CAUTION
Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.5

CALIBRATION INFORMATION

1. The system must have a lot-specific parameter card and a valid calibration adjustment in memory before controls or patient samples can be run.

2. Under typical operating conditions the C3 reagent cartridge must be calibrated every 30 days and also with certain parts replacements or maintenance procedures, as defined in the SYNCHRON LX Maintenance Manual and Instrument Log, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual. This assay has within-lot calibration available. Refer to the SYNCHRON LX Operations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual for information on this feature.

3. For detailed calibration instructions, refer to the SYNCHRON LX Operations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

4. The system automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the SYNCHRON LX Diagnostics and Troubleshooting Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.
The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

### Table 1.0 Quality Control Material

<table>
<thead>
<tr>
<th>CONTROL NAME</th>
<th>SAMPLE TYPE</th>
<th>STORAGE</th>
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<tbody>
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### TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system. A lot-specific parameter card must be loaded one time for each lot.
2. After reagent load is completed, calibration is required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

For detailed testing procedures, refer to the SYNCHRON LX Operations Manual, or the UniCel DxC 600/800 System Instructions For Use (IFU) manual.

### CALCULATIONS

The SYNCHRON® System(s) performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

### REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

### REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The reference interval values for C3 were established using the IMMAGE® Immunochemistry System C3 assay for a population of 123 apparently healthy, non-smoking, ≥ 18 years of age, male and female adults from Southern California, and were verified with the SYNCHRON® System(s) C3 assay.

### Table 2.0 Reference intervals

<table>
<thead>
<tr>
<th>INTERVALS</th>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS</th>
<th>S.I. UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Coulter</td>
<td>Serum or Plasma (Adult)</td>
<td>79 – 152 mg/dL</td>
<td>790 – 1520 mg/L</td>
</tr>
</tbody>
</table>
Refer to References (7,8,9) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

### PROCEDURAL NOTES

#### ANTICOAGULANT TEST RESULTS

The following anticoagulants were assessed by Deming regression analysis with a minimum of 123 paired serum and plasma samples. Values of serum (X) ranging from 98.0 mg/dL to 216.7 mg/dL were compared with the values for plasma (Y) yielding the following results:

**Table 3.0 Anticoagulant Test Results**

<table>
<thead>
<tr>
<th>ANTICOAGULANT</th>
<th>LEVEL OF ANTICOAGULANT TESTED</th>
<th>DEMING REGRESSION ANALYSIS (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Heparin</td>
<td>14 Units/mL</td>
<td>$Y = 0.966X + 0.28; \ r = 0.985$</td>
</tr>
<tr>
<td>Sodium Heparin</td>
<td>14 Units/mL</td>
<td>$Y = 0.979X - 0.95; \ r = 0.979$</td>
</tr>
</tbody>
</table>

#### LIMITATIONS

Activation of C3 can occur both in vivo and in vitro and results in the production of four major conversion products; C3a, C3b, C3c and C3d. C3c is the final stable conversion product present in the fluid phase. The antibody used in the Beckman Coulter C3 assay reacts with native C3 and the two major conversion products C3c and C3d. When compared to an assay which uses an antibody which recognizes native C3, an assay using an antibody specific only to C3c would typically give lower results when the pathway has not been activated and higher results when the pathway has been activated.

#### INTERFERENCES

1. The following substances were tested in serum for interference with this methodology:

**Table 4.0 Interferences**

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>SOURCE</th>
<th>LEVEL TESTED</th>
<th>OBSERVED EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>Porcine</td>
<td>30 mg/dL</td>
<td>NSI</td>
</tr>
<tr>
<td>Lipemia</td>
<td>Human</td>
<td>3+</td>
<td>NSI</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Human</td>
<td>500 mg/dL</td>
<td>NSI</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>Human</td>
<td>300 IU/mL</td>
<td>NSI</td>
</tr>
<tr>
<td>Paraprotein (IgM)</td>
<td>Human</td>
<td>500 mg/dL</td>
<td>NSI</td>
</tr>
</tbody>
</table>

a  NSI = No Significant Interference (within ±7.0 mg/dL or 9.0%).
2. Refer to References (10,11,12) for other interferences caused by drugs, disease and preanalytical variables.

**PERFORMANCE CHARACTERISTICS**

**ANALYTIC RANGE**

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical range:

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS</th>
<th>S.I. UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum or Plasma</td>
<td>10.0 – 350.0 mg/dL</td>
<td>100 – 3500 mg/L</td>
</tr>
</tbody>
</table>

Samples with concentrations outside of the analytical range will be reported as "<10 mg/dL" ("<100 mg/L") or ">350 mg/dL" (">3500 mg/L").

Samples reported out as greater than the analytical range should be confirmed by diluting with saline and reanalyzing. If a manual dilution is used the appropriate dilution factor should be applied to the reported result.

**REPORTABLE RANGE (AS DETERMINED ON SITE):**

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS</th>
<th>S.I. UNITS</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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</tbody>
</table>

**SENSITIVITY**

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for C3 determination is 10 mg/dL (100 mg/L).

**EQUIVALENCY**

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

**Serum (in the range of 13.4 to 220.5 mg/dL):**

\[
Y \text{ (SYNCHRON LX Systems)} = 1.025X + 2.31 \\
N = 134 \\
\text{MEAN (SYNCHRON LX Systems)} = 118.1 \\
\text{MEAN (Nephelometric Immunoassay)}^a = 113.0 \\
\text{CORRELATION COEFFICIENT (r)} = 0.992
\]

^a A product of Beckman Coulter, Inc.

Refer to References (8,13) for guidelines on performing equivalency testing.

**PRECISION**

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:
Table 7.0 Precision Values

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE TYPE</th>
<th>1 SD</th>
<th>CHANGEOVER VALUE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/dL</td>
<td>mg/L</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Within-run</td>
<td>Serum or Plasma</td>
<td>3.50</td>
<td>35.0</td>
<td>77.8</td>
</tr>
<tr>
<td>Total</td>
<td>Serum or Plasma</td>
<td>5.25</td>
<td>52.5</td>
<td>77.8</td>
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</tbody>
</table>

<sup>a</sup> When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance data for the SYNCHRON LX<sup>®</sup> System evaluated using the NCCLS Approved Guideline EP5-A appears in the table below.<sup>14</sup> Each laboratory should characterize their own instrument performance for comparison purposes.

Table 8.0 NCCLS EP5-A Precision Estimate Method

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE TYPE</th>
<th>No. Systems</th>
<th>No. Data Points</th>
<th>Test Mean Value (mg/dL)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EP5-A Calculated Point Estimates</th>
<th>SD</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum Control 1</td>
<td>1</td>
<td>80</td>
<td>55.6</td>
<td>0.97</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum Control 2</td>
<td>1</td>
<td>80</td>
<td>170.8</td>
<td>2.20</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum Control 3</td>
<td>1</td>
<td>80</td>
<td>237.4</td>
<td>2.27</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum Pool</td>
<td>1</td>
<td>80</td>
<td>28.9</td>
<td>0.49</td>
<td>1.71</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Serum Control 1</td>
<td>1</td>
<td>80</td>
<td>55.6</td>
<td>1.21</td>
<td>2.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum Control 2</td>
<td>1</td>
<td>80</td>
<td>170.8</td>
<td>2.44</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum Control 3</td>
<td>1</td>
<td>80</td>
<td>237.4</td>
<td>2.92</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum Pool</td>
<td>1</td>
<td>80</td>
<td>28.9</td>
<td>0.57</td>
<td>1.98</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The point estimate is based on the data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer’s instructions.

Refer to References (8,15) for guidelines on performing precision testing.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX<sup>®</sup> System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

For more detailed information on SYNCHRON LX Systems or UniCel DxC Systems, refer to the appropriate system manual.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.
REFERENCES


