For In Vitro Diagnostic Use
Rx Only

ANNUAL REVIEW

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

INTENDED USE

ASO- reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems CAL 5 Plus, is intended for the quantitative determination of antibodies to streptolysin-O concentrations in human serum or plasma.

CLINICAL SIGNIFICANCE

Streptolysin-O is one of several toxic immunogenic exoenzymes produced by group A β-hemolytic streptococci. An elevated ASO titer usually is an indication of a recent infection with a group A β-hemolytic streptococcus and as such has become routine for the diagnosis and management of acute rheumatic fever and acute glomerulonephritis. Approximately 80-85% of individuals, with a current streptococcal infection or their sequelae, will demonstrate an elevated ASO titer.

The ASO titer may decrease by the time a patient presents with acute rheumatic fever; therefore, a previous streptococcal infection cannot be ruled out due to a negative ASO result. Additionally, it is uncommon for a skin infection to produce a rise in ASO titer. Examining for antibodies to another streptococcal antigen such as DNAase B may be of value.

Laboratory results should always be interpreted in conjunction with other clinical findings.

METHODOLOGY

ASO- reagent is used to measure human antibodies to streptolysin-O concentration by a turbidimetric rate method. In the reaction streptolysin-O antibodies in the sample combine with latex particles coated with highly purified recombinant streptolysin-O resulting in agglutination and a change in absorbance.

The SYNCHRON System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio for antistreptolysin-o is one part sample to 50 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is directly proportional to the concentration of human ASO- antibodies in the sample and is used by the SYNCHRON System(s) to calculate and express the human ASO- antibody concentration.
based upon a single-point, linear calibration using a calibrator standardized in accordance with the World Health Organization.

CHEMICAL REACTION SCHEME

\[
\text{ASO- + Latex-streptolysin-O (Antigen)} \rightarrow \text{Latex-Antigen-Antibody Complex}
\]

SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.\(^5\) Freshly drawn serum or plasma are the preferred specimens. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood or urine are not recommended for use as a sample.

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.\(^6\)

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.\(^6\)

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 ASO- Reagent Cartridges (2 x 100 tests)

VOLUMES PER TEST

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Sample Volume</td>
<td>6 µL</td>
</tr>
<tr>
<td>ORDAC Sample Volume</td>
<td>3 µL</td>
</tr>
<tr>
<td>Total Reagent Volume</td>
<td>300 µL</td>
</tr>
<tr>
<td>Cartridge Volumes</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>250 µL</td>
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</table>
| B                      | 50 µL 
| C                      |  _ _  |

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Streptolysin-O Antigen Particle Reagent  7.4 mL
Streptolysin-O Reaction Buffer          37.4 mL

Also non-reactive chemicals necessary for optimal system performance.

NOTICE

The recombinant-based Streptolysin-O has the antigenic properties of the native protein with less than 0.1% of the hemolytic activity. The recombinant Streptolysin-O antigen is therefore safe to work with and is NOT classified as a Biohazard.
Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76). To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

Safety Data Sheet is available at techdocs.beckmancoulter.com.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems CAL 5 Plus
At least two levels of control material
Saline

REAGENT PREPARATION

1. Gently invert the cartridge several times prior to loading onto the system.
2. Check for bubbles or foam in compartments; break any bubbles.

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

ASO- 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 unless the expiration date is exceeded. Storage above +45°C will adversely affect performance of the reagent. DO NOT FREEZE.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems CAL 5 Plus

CALIBRATOR PREPARATION

No preparation is required.
CALIBRATOR STORAGE AND STABILITY

SYNCHRON® Systems CAL 5 Plus is stable until the expiration date printed on the calibrator bottle if capped and stored in the original container at +2°C to +8°C.

⚠️ 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.7

Calibrator storage location:

CALIBRATION INFORMATION

1. The system must have a valid calibration factor in memory before controls or patient samples can be run.

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

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

4. The system will 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 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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</table>

**TESTING PROCEDURE(S)**

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration may be 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 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 following reference intervals were taken from literature and a study performed on SYNCHRON Systems.\(^8\)

**Table 2.0 Reference intervals**

<table>
<thead>
<tr>
<th>INTERVALS</th>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Literature</td>
<td>Serum</td>
<td>less than 200 IU/mL (school age children)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>less than 100 IU/mL (adult and pediatric)</td>
</tr>
<tr>
<td>SYNCHRON</td>
<td>Serum</td>
<td>less than 145 IU/mL (adult)</td>
</tr>
</tbody>
</table>

\(^a\) With the SYNCHRON CAL 5 Plus calibrator standardized in accordance with the World Health Organization, 1 IU/mL is equivalent to 1 Todd unit. Therefore, the SYNCHRON adult reference intervals of less than 200 IU/mL is equivalent to less than 200 Todd units or less than a 1:199 dilution.
Refer to References (9, 10, 11) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

### PROCEDURAL NOTES

#### ANTICOAGULANT TEST RESULTS

If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

<table>
<thead>
<tr>
<th>ANTICOAGULANT</th>
<th>LEVEL TESTED FOR IN VITRO INTERFERENCE</th>
<th>AVERAGE PLASMA-SERUM BIAS (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Heparin</td>
<td>14 Units/mL</td>
<td>NSI(^a)</td>
</tr>
<tr>
<td>Sodium Heparin</td>
<td>14 Units/mL</td>
<td>NSI</td>
</tr>
</tbody>
</table>

\(^a\) NSI = No Significant Interference (within ± 20 IU/mL or 8%).

#### LIMITATIONS

None identified

#### INTERFERENCES

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

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>SOURCE</th>
<th>LEVEL TESTED</th>
<th>OBSERVED EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>RBC hemolysate</td>
<td>500 mg/dL</td>
<td>NSI(^a)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Bovine</td>
<td>30 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>Lipemia</td>
<td>Human</td>
<td>4+</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 ± 20 IU/mL or 8%).

2. Refer to References (12, 13, 14) 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 ranges:

Table 5.0 Analytical Range

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>CONVENTIONAL UNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum or Plasma</td>
<td>25 – 800 IU/mL</td>
</tr>
<tr>
<td>Serum or Plasma (ORDAC)a</td>
<td>640 – 1600 IU/mL</td>
</tr>
</tbody>
</table>

a Overrange Detection and Correction. Refer to the UniCel DxC 600/800 System Instructions For Use (IFU) manuual for more details on this function.

Samples with concentrations outside of the analytical range will be reported as "<25 IU/mL" or ">800 IU/mL" (ORDAC "<640 IU/mL" or .">1600 IU/mL").

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

Samples reported out as "SUPPRESSED" due to RXN ERROR should be reanalyzed.

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 6.0 Reportable Range

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

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for ASO- determination is 25 IU/mL.

EQUIVALENCY

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

Serum or plasma (in the range of <25 to 861 IU/mL):

\[ Y \text{ (SYNCHRON LX Systems)} = 0.997X + 6.67 \]

\[ N = 70 \]

\[ \text{MEAN (SYNCHRON LX Systems)} = 180 \]

\[ \text{MEAN (SYNCHRON CX7 DELTA)} = 173 \]

\[ \text{CORRELATION COEFFICIENT (r)} = 0.999 \]

Refer to References (15) for guidelines on performing equivalency testing.

PRECISION

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

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ASO- Page 8 of 11

Chemistry Information Sheet A18459 AN

DECEMBER 2015
Table 7.0 Precision Values

<table>
<thead>
<tr>
<th>TYPE OF PRECISION</th>
<th>SAMPLE TYPE</th>
<th>1 SD IU/mL</th>
<th>CHANGEOVER VALUE IU/mL</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>Serum/Plasma</td>
<td>10</td>
<td>250</td>
<td>4.0</td>
</tr>
<tr>
<td>Total</td>
<td>Serum/Plasma</td>
<td>15</td>
<td>250</td>
<td>6.0</td>
</tr>
</tbody>
</table>

a 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.

Refer to References (16) for guidelines on performing precision testing.

Comparative performance data for a SYNCHRON LX® System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below. Each laboratory should characterize their own instrument performance for comparison purposes.

Table 8.0 NCCLS EP5-T2 Precision Estimate Method

<table>
<thead>
<tr>
<th>TYPE OF IMPRECISION</th>
<th>SAMPLE TYPE</th>
<th>No. Systems</th>
<th>No. Data Pointsa</th>
<th>Test Mean Value (IU/L)</th>
<th>EP5-T2 Calculated Point Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>Serum Control 1</td>
<td>1</td>
<td>80</td>
<td>84.9</td>
<td>SD 4.8 %CV 5.6</td>
</tr>
<tr>
<td></td>
<td>Serum Control 2</td>
<td>1</td>
<td>80</td>
<td>195.4</td>
<td>SD 4.1 %CV 2.1</td>
</tr>
<tr>
<td></td>
<td>Serum Control 3</td>
<td>1</td>
<td>80</td>
<td>375.5</td>
<td>SD 4.2 %CV 1.1</td>
</tr>
<tr>
<td>Total</td>
<td>Serum Control 1</td>
<td>1</td>
<td>80</td>
<td>84.9</td>
<td>SD 5.2 %CV 6.1</td>
</tr>
<tr>
<td></td>
<td>Serum Control 2</td>
<td>1</td>
<td>80</td>
<td>195.4</td>
<td>SD 4.7 %CV 2.4</td>
</tr>
<tr>
<td></td>
<td>Serum Control 3</td>
<td>1</td>
<td>80</td>
<td>375.5</td>
<td>SD 5.5 %CV 1.5</td>
</tr>
</tbody>
</table>

a 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.

**NOTICE**

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

**ADDITIONAL INFORMATION**

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

Beckman Coulter, the Beckman Coulter Logo, Synchron, UniCel and DxC are trademarks of Beckman Coulter, Inc and are registered in the USPTO.

**SHIPPING DAMAGE**

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

**REVISION HISTORY**

Revision AG

Revised Reagent Preparation section.
Revision AH
Updated corporate address; removed EDTA as an Acceptable Anticoagulant claim.

Revision AJ
Added Revision History.

Revision AK
Added new language requirement: Czech, and Korean.

Revision AL
Removed references to CX and LX systems as they are discontinued effective 12/2013.
Added Beckman Coulter trademark statement and disclaimer.

Revision AM
Added GHS Classification information

Revision AN
Added new language requirement: Romanian
REFERENCES


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