Alinity c ASO-05

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity c ASO assay is an automated latex enhanced immunoassay for the quantitative determination of antistreptolysin-O in human serum on the Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE TEST

Group A Streptococci produce various exotoxins, such as streptolysin-O, that can act as antigens. Streptolysin-O antibodies can be measured to determine whether a previous Group A Streptococcus infection has caused a poststreptococcal disease, such as scarlet fever, rheumatic fever, or glomerulonephritis.[1]

Refer to *Effects of Disease on Clinical Laboratory Tests* for a summary of causes of increased ASO concentration.[2]

PRINCIPLES OF THE PROCEDURE

The ASO latex reagent is a suspension of polystyrene latex particles of uniform size coated with streptolysin-O. When a sample containing antistreptolysin-O is mixed with the latex reagent and the reaction buffer included in the kit, agglutination occurs. The degree of agglutination is directly proportional to the concentration of ASO in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.[3], [4]

Methodology: Turbidimetric/Immunoturbidimetric

For additional information on system and assay technology, refer to the **Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity c ASO Reagent Kit 01R06

REF	01R0621	01R0631
Tests per cartridge	150	195
Number of cartridges per kit	2	4
Tests per kit	300	780
R1	41.0 mL	52.2 mL
R2	9.7 mL	11.6 mL
R1 Active Ingredients: Sodium dihydrogen phosphate dihydrate (0.6%). Inactive Ingredients: bovine serum albumin, buffer. Preservative: sodium azide (< 0.1%).		
R2 Active Ingredients: Suspension of polystyrene latex particles coated with streptolysin-O (0.03%), sodium dihydrogen phosphate dihydrate (0.6%). Inactive Ingredients: Bronidox (0.01%), bovine serum albumin. Preservative: sodium azide (< 0.1%).		

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[5], [6], [7], [8]

The following warnings and precautions apply to: R1 and R2	
Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS Alinity folder.

For a detailed discussion of safety precautions during system operation, refer to the **Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

- Reagents are shipped on wet ice.
- Upon receipt, place reagent cartridges in an upright position for 8 hours before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Prior to running, gently invert cartridge 5 times.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

Do not freeze.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Onboard	System	35 days	

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
	Temperature		
Opened	2 to 8°C	Until expiration date	Store in upright position. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity c ASO assay file must be installed on the Alinity c analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity ci-series Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity ci-series Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen type listed below was verified for use with this assay.

Other specimen types, collection tube types, and anticoagulants have not been verified with this assay.

Specimen Type	Collection Vessel
Serum	Serum tubes

Specimen Conditions

- Analyze fresh specimens if possible.
- For accurate results, serum specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross-contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Specimen Storage

Analyze fresh specimens if possible.

Specimen Type	Temperature	Maximum Storage Time
Serum	20 to 25°C	2 days[9]
	2 to 8°C	2 days[9, 10]
	-20°C	6 months[9]

Avoid multiple freeze/thaw cycles.

Guder et al. suggest storage of frozen specimens at -20°C for no longer than the time intervals cited above.[9]

Stored specimens must be inspected for particulates. If present, mix with a low speed vortex or by inversion and centrifuge the specimen to remove particulates prior to testing.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

PROCEDURE

Materials Provided

01R06 Alinity c ASO Reagent Kit

Materials Required but not Provided

- Alinity c ASO assay file
- 01R0602 Alinity c ASO Calibrator
- 04S0912 Alinity c ASO Control I Kit and 04S0913 Alinity c ASO Control II Kit or other commercially available controls
- Saline (0.85% to 0.90% NaCl) for specimen dilution

For information on materials required for operation of the instrument, refer to the **Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Minimum sample volume requirements:
  - Sample volume for single test: 3.0 µL.

    NOTE: This amount does not include the dead volume plus the additional over-aspiration volume. **For total sample volume requirements, refer to the Alinity ci-series Operations Manual, Section 4.**

- Refer to the Alinity c ASO Calibrator package insert and/or Alinity c ASO Control I Kit and Alinity c ASO Control II Kit, or commercially available controls for preparation and usage.
- For general operating procedures, **refer to the Alinity ci-series Operations Manual, Section 5.**
- For optimal performance, it is important to perform routine maintenance as described in the **Alinity ci-series Operations Manual, Section 9**. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

All samples should be initially tested using the STANDARD Dilution Protocol. If the result obtained is within the measuring interval 50.0 - 832.8 IU/mL, the sample should not be diluted.

Samples that are flagged with the code ">" may have excess antigen. Dilute the sample and rerun following either the Automated Dilution Protocol or the Manual Dilution Procedure. Samples were tested for excess antigen up to 1590.0 IU/mL.

Automated Dilution Protocol

The system performs **a 1:5** dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

After the automatic 1:5 dilution is performed, if the sample concentration is > 4164.0 IU/mL, dilute the sample 1:20 and run using the Manual Dilution Procedure.

Manual Dilution Procedure

Dilute the sample with saline (0.85% to 0.90% NaCl).

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than the lower value of the measuring interval of 50.0 IU/mL, do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the **Alinity ci-series Operations Manual, Section 5.**

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Calibration is stable for approximately **35 days (840 hours)**, but is required with each change in reagent lot. Verify calibration with at least 2 levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

- At least two levels of controls (normal and abnormal) are to be run every day testing performed.
- If quality control results do not meet the acceptance criteria defined laboratory Quality control procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[11]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity c ASO assay utilizes the Spline data reduction method to generate a calibration and results.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the **Alinity ci-series Operations Manual, Section 5**.

Measuring Interval

Measuring interval is defined as the range of values in IU/mL which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity c ASO assay is **50.0 to 832.8 IU/mL**.

LIMITATIONS OF THE PROCEDURE

Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

There is no prozone interference for undiluted samples containing up to 1590.0 IU/mL of ASO. Sample concentrations higher than 1590.0 IU/mL have not been tested.

EXPECTED VALUES

Effort will be made to verify the provided manufacturer reference range.

Reference Range

Although normal values can vary with age, season of the year, and geographical area, the "upper limit of normal" antistreptolysin-O titers for preschool children is less than 100 IU/mL, and in school age children or young adults is usually between 166 and 250 IU/mL.

In any case, the average can be established at less than 200 IU/mL.*12*, *13*

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity c analyzer and the ARCHITECT c System and AEROSET System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity c analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.*14* Testing was conducted using 1 lot of the Alinity c ASO Reagent Kit, 1 lot of the Alinity c ASO Calibrator, and 1 lot of the Alinity c ASO Control I Kit and Alinity c ASO Control II Kit and 1 instrument. Two controls and 2 human serum panels were assayed in a minimum of 2 replicates at 2 separate times per day/4 replicates once per day on 20 different days.

			Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	**n**	**Mean (IU/mL)**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	126	175.9	2.44	1.4	4.03	2.3
Control Level 2	120	375.2	3.38	0.9	6.83	1.8

Sample	n	Mean (IU/mL)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Panel 1	126	55.7	2.70	4.8	2.82	5.1
Panel 2	120	775.9	6.70	0.9	21.14	2.7

[a]Includes within-run, between-run, and between-day variability.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2.*15* Testing was conducted using 3 lots of the Alinity c ASO Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.

	IU/mL
LoB[a]	6.0
LoD[b]	11.2
LoQ[c]	**18.6**

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum allowable precision of **20 %CV** and a maximum allowable bias of 20% were met.

Linearity

A study was performed based on guidance from CLSI EP06-A.*16*

This assay is linear across the measuring interval of **50.0 to 832.8 IU/mL**.

Interference

This study was performed on the AEROSET System.

Interference studies were conducted using an acceptance criteria **of ± 5% deviation** from the target value.

Interfering Substance	Interferent Concentration	Target (IU/mL)	Observed (% of Target)

Interfering Substance	Interferent Concentration	Target (IU/mL)	Observed (% of Target)
Bilirubin	20.8 mg/dL (355 µmol/L)	257.9	99.9
Hemoglobin	482 mg/dL (4.8 g/L)	255.4	100.1
Lipemia (chyle)	2.1 AU/cm at 660 nm	258.9	100.7
Rheumatoid factor	800 IU/mL (800 000 U/L)	108.1	104.2
Lipemia (triglyceride)	1327 mg/dL (14.99 mmol/L)	270.5	103.6

Method Comparison

A study was performed based on guidance from CLSI EP09-A3*17* using the Passing-Bablok regression method.

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity c ASO vs ARCHITECT ASO	Serum	IU/mL	119	1.00	-4.54	1.04	56.0 - 744.4

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