Alinity c Aspartate Aminotransferase-06

Prepared by: Yusra Othman /Director/Supervisor-Chem **Date:** May/20/2024
signature/title

Reviewed by: Jordan Dillard /Instructor **Date:** July 10 2024
signature/title

Approved by: Stanford V. Cauley, M.D /Chairman **Date:** July 12 2024
signature/title

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity c Aspartate Aminotransferase assay is used for the quantitation of aspartate aminotransferase in human serum or plasma on the Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE TEST

Aspartate aminotransferase (AST), also referred to as glutamate oxaloacetate transaminase (GOT), is one of a group of enzymes which catalyzes the interconversion of amino acids and α-keto acids by transfer of amino groups. Both AST and alanine aminotransferase (ALT) are normally found in most body fluids, but not in urine except in instances of kidney lesions. The greatest concentrations of AST are found in heart, liver, muscle, and kidney tissues. Damage to these tissues can greatly elevate serum AST levels. Following myocardial

infarction, AST in serum begins to increase within 6 to 8 hours of onset of pain, reaching a peak within 18 to 24 hours and falling to normal by the fourth or fifth day. Serum values may increase to 10 to 15 times normal levels and the increase is roughly proportional to the degree of tissue damage.[1], [2]

PRINCIPLES OF THE PROCEDURE

AST present in the sample catalyzes the transfer of the amino group from *L*-aspartate to α-ketoglutarate, forming oxaloacetate and *L*-glutamate. Oxaloacetate in the presence of NADH and malate dehydrogenase (MDH) is reduced to *L*-malate. In this reaction, NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD.

Methodology: Enzymatic (NADH (without P-5'-P))

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

REAGENTS

Kit Contents

Alinity c Aspartate Aminotransferase Reagent Kit 08P17

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	**08P1720**
Tests per cartridge	360
Number of cartridges per kit	10
Tests per kit	3600
R1	68.1 mL
R2	21.0 mL
R1 Active ingredients: β-NADH (0.16 mg/mL), Malate Dehydrogenase (0.64 U/mL), Lactate Dehydrogenase (0.64 U/mL), *L*-Aspartate (232 mmol/L).	
R2 Active ingredients: α-Ketoglutarate (51.3 mmol/L), *L*-Aspartate (100 mmol/L).	

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.[3], [4], [5], [6]

The following warnings and precautions apply to: R2*	
H316	Causes mild skin irritation.
P332+P313	If skin irritation occurs: Get medical advice / attention.

* Not applicable where regulation EU 1272/2008 (CLP) or OSHA Hazard Communication 29CFR 1910.1200 (HCS) 2012 have been implemented.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

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 refrigerated or 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 8 hours before use to allow bubbles that may have formed to dissipate.
- 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

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

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Onboard	System Temperature	30 days	
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 Aspartate Aminotransferase 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 types listed below were 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	Special Conditions
Serum	Serum tubes (with or without gel barrier)	
Plasma	Collection tubes Acceptable anticoagulants are: Lithium heparin (with or without gel barrier) Sodium heparin	Do not use ammonium heparin.[7]

Specimen Conditions

- For accurate results, serum and plasma 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.
- For accurate results, plasma specimens should be free of platelets and other particulate matter. Ensure centrifugation is adequate to remove platelets.
- 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

Specimen Type	Temperature	Maximum Storage Time
Serum/Plasma	20 to 25°C	4 days[8]
	2 to 8°C	7 days[8], [9]
	-20°C	12 weeks[8]

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.[8]

Each laboratory may establish a range around -20°C from either the freezer manufacturer's specifications or your laboratory standard operating procedure(s) for specimen storage.

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

08P17 Alinity c Aspartate Aminotransferase Reagent Kit

Materials Required but not Provided

- Alinity c Aspartate Aminotransferase assay file
- Commercially available controls containing aspartate aminotransferase
- 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: 5.3 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 commercially available control material package insert 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

Samples with an aspartate aminotransferase value exceeding 4202 U/L are flagged with the code "> 4202 U/L" and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

If using an automated dilution protocol, the system performs a dilution of the sample and automatically calculates the enzyme activity value by multiplying the result by the dilution factor. For details on configuring automated dilutions, refer to the Alinity ci-series Operations Manual, Section 2.

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 enzyme activity value 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 flagged indicating it is less than the lower value of the measuring interval of 3 U/L, 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 **30 days (720 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 by 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.*10*

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 Aspartate Aminotransferase assay utilizes the Factor data reduction method to generate a calibration and results.

The calibration factor for the Alinity c Aspartate Aminotransferase assay is 8141.

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 U/L which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity c Aspartate Aminotransferase assay is 3 U/L to 4202 U/L.

LIMITATIONS OF THE PROCEDURE

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

EXPECTED VALUES

Manufacturer provided reference ranges adopted, effort made to verify locally.

Reference Range

Serum/Plasma[11]

	Range (U/L)
Adult	5 to 34

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. Testing was conducted using 1 lot of the Alinity c Aspartate Aminotransferase Reagent Kit, 1 lot of commercially available controls and 1 instrument. Three controls and one human serum panel were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.[12]

			Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	**n**	**Mean (U/L)**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	120	42	0.5	1.2	0.6	1.4
Control Level 2	120	122	0.7	0.6	1.3	1.1
Control Level 3	120	251	0.8	0.3	1.4	0.6
Panel	119	177	0.7	0.4	0.8	0.5

[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. Testing was conducted using 3 lots of the Alinity c Aspartate Aminotransferase 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.*13*

	U/L
LoB[a]	1
LoD[b]	3
LoQ[c]	3

[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 was met.

Linearity

A study was performed based on guidance from CLSI EP06-A.*14*

This assay is linear from **3 to 4202 U/L.**

Interference

This study was performed on the AEROSET System.

Potentially Interfering Endogenous Substances

Interference studies were conducted using NCCLS EP7-P. Interference effects were assessed by Dose Response and Paired Difference methods, at the medical decision level of the analyte.[15]

Potentially Interfering Substance	Interferent Level		Target Level (U/L)	Recovery (% of Target)
	Default Units	Alternate Units		
Bilirubin	30 mg/dL	513 µmol/L	72.2	95.8
	60 mg/dL	1026 µmol/L	72.2	91.9
Hemoglobin	62 mg/dL	0.62 g/L	64.5	105.7
	125 mg/dL	1.25 g/L	64.5	111.6
Intralipid	550 mg/dL	5.5 g/L	69.0	95.4
	625 mg/dL	6.25 g/L	69.0	103.9

The following drugs were tested on the ARCHITECT system for interference at the concentrations indicated using an acceptance criteria of ± 10% from the target value.

Potentially Interfering Substance	Interferent Level		Target Level (U/L)	Recovery (% of Target)
	Default Units	Alternate Units		
Sulfapyridine	300 mg/L	1204.8 µmol/L	14.4	101.2
Sulfasalazine	300 mg/L	753.8 µmol/L	14.4	98.4
Temozolomide	20 mg/L	103.1 µmol/L	35.9	106.0

Interferences from medication or endogenous substances may affect results.[16]

Method Comparison

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

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity c Aspartate Aminotransferase vs ARCHITECT Aspartate Aminotransferase	Serum	U/L	114	1.00	0.15	0.96	4 - 4025

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