Alinity c Alkaline Phosphatase (AlkP)-03

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity c Alkaline Phosphatase assay is used for the quantitation of alkaline phosphatase in human serum or plasma on the Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE TEST

Human alkaline phosphatase (AlkP, EC.3.1.3.1) consists of a group of at least five tissue-specific isoenzymes which catalyzes the hydrolysis of phosphate mono-esters at alkaline pH. A variety of disease processes can result in the release of increased quantities of alkaline phosphatase into the blood.

PRINCIPLES OF THE PROCEDURE

Several substrates have been used to measure alkaline phosphatase activity such as glycerophosphate,[1] phenyl phosphate,[1] and *p*-nitrophenyl phosphate.[2] Bowers and McComb[3] improved the method of Bessey et al. to include a kinetic measurement. Tietz et al.[4] optimized this method to include a chelated metal-ion buffer of zinc, magnesium, and HEDTA. This Alkaline Phosphatase procedure is a modification of this method. Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless *p*-nitrophenyl phosphate (*p*-NPP) to give *p*-nitrophenol and inorganic phosphate. At the pH of the assay (alkaline), the *p*-nitrophenol is in the yellow phenoxide form. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample.

Methodology: Para-nitrophenyl Phosphate

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

REAGENTS

Kit Contents

Alinity c Alkaline Phosphatase Reagent Kit 08P20

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

REF	08P2020	08P2030
Tests per cartridge	400	1075
Number of cartridges per kit	10	10
Tests per kit	4000	10 750
R1	27.7 Ml	68.2 mL
R2	14.3 mL	33.0 mL

R1 Active ingredients: 2-amino-2-methylpropanol (> 1.2 mol/L), Magnesium acetate (> 7.2 mmol/L), Zinc sulfate (> 3.6 mmol/L), HEDTA (> 7.2 mmol/L).

R2 Active ingredients: 4-nitrophenyl phosphate (> 171.6 mmol/L). Preservatives: ProClin 300 (0.05%), ProClin 950 (0.10%).

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	
	
WARNING	Contains 2-amino-2-methylpropanol and zinc sulfate heptahydrate.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
H402*	Harmful to aquatic life.
H412	Harmful to aquatic life with long lasting effects.
Prevention	
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
P273	Avoid release to the environment.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.
P302+P352	IF ON SKIN: Wash with plenty of water.
P332+P313	If skin irritation occurs: Get medical advice / attention.

P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

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

The following warnings and precautions apply to: R2	
	
WARNING	Contains 4-hydroxybenzoic acid* and methylisothiazolones.
H317	May cause an allergic skin reaction.
H316*	Causes mild skin irritation.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* 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/and SDS folder .

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

Reagent Handling

- Upon receipt, place reagent cartridges in an upright position for 1 hour 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.
- 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.
Onboard	System Temperature	8 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.

The R2 reagent is light sensitive. Always store reagents protected from light.

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 Alkaline Phosphatase 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.

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit*
U/L	1.0651	U/L

* The Default Result Unit for the Alkaline Phosphatase assay is U/L. The corresponding Alternate Result Unit is U/L when using International Federation of Clinical Chemistry (IFCC) units.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

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

Specimen Type	Collection Vessel
Serum	Serum tubes (with or without gel barrier)
Plasma	Collection tubes Acceptable anticoagulants are: Lithium heparin (with or without gel barrier) Sodium heparin

- The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

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	7 days[9]
	2 to 8°C	7 days[9], [10]
	-20°C	2 months[9]

Avoid multiple freeze/thaw cycles.

Allow specimens to reach room temperature prior to analysis.[11]

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

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

08P20 Alinity c Alkaline Phosphatase Reagent Kit

Materials Required but not Provided

- Alinity c Alkaline Phosphatase assay file
- Commercially available controls containing alkaline phosphatase
- 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: 7 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 alkaline phosphatase value exceeding 4555 U/L (4852 U/L using IFCC units) are flagged with the code "> 4555 U/L" (> 4852 U/L using IFCC units) 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 concentration 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 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 9 U/L (10 U/L using IFCC units), 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 **8 days (192 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.[12]

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 Alkaline Phosphatase assay utilizes the Factor data reduction method to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

The calibration factor for the Alinity c Alkaline Phosphatase assay is 2150 (2290 using IFCC units).

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 (U/L using IFCC units) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity c Alkaline Phosphatase assay is 9 to 4555 U/L (10 to 4852 U/L using IFCC units).

LIMITATIONS OF THE PROCEDURE

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

EXPECTED VALUES

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

Serum/Plasma[13]

		Range (U/L)
Male	1 to 12 years	< 500
	12 to 15 years	< 750
	> 20 years	40 to 150
Female	1 to 12 years	< 500
	> 15 years	40 to 150

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 Alkaline Phosphatase Reagent Kit, 1 lot of commercially available controls and 1 instrument. Three controls were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.[14]

			Repeatability		Within-Laboratory Precision[a]	
Sample	**n**	**Mean (U/L)**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	119	67	1.2	1.7	2.1	3.1
Control Level 2	120	187	1.4	0.8	3.0	1.6
Control Level 3	120	360	1.2	0.3	4.6	1.3

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

			Repeatability		Within-Laboratory Precision[a]	
Sample	**n**	**Mean (U/L using IFCC units)**	**SD**	**%CV**	**SD**	**%CV**
Control Level 1	119	71	1.3	1.8	2.2	3.1
Control Level 2	120	199	1.4	0.7	3.0	1.5
Control Level 3	120	383	1.3	0.3	5.0	1.3

[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 Alkaline Phosphatase 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.[15]

	U/L	U/L (using IFCC units)
LoB[a]	4	4
LoD[b]	6	6
LoQ[c]	9	10

[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.[16]

This assay is linear across the measuring interval of 9 to 4555 U/L (10 to 4852 U/L using IFCC units).

Interference

This study was performed on the AEROSET System.

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07-A2.[17]

Potentially Interfering Substance	Interferent Level		Alkaline Phosphatase	
	Default Units	Alternate Units	Target Level (U/L)	Recovery (% of Target)
Bilirubin	30 mg/dL	513 µmol/L	155.6	102.4
	60 mg/dL	1026 µmol/L	155.6	102.7

Potentially Interfering Substance	Interferent Level		Alkaline Phosphatase	
	Default Units	Alternate Units	Target Level (U/L)	Recovery (% of Target)
Hemoglobin	750 mg/dL	7.5 g/L	150.8	95.3
	1000 mg/dL	10.0 g/L	150.8	94.3
Intralipid	750 mg/dL	7.5 g/L	144.3	100.4
	1000 mg/dL	10.0 g/L	144.3	100.2

Interferences from medication or endogenous substances may affect results.[18]

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[19]

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity c Alkaline Phosphatase vs ARCHITECT Alkaline Phosphatase	Serum	U/L	123	1.00	0.15	1.03	13 - 3934
		U/L (using IFCC units)	123	1.00	0.22	1.03	14 - 4190

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