DXC (ALP) ALKALINE PHOSPHATASE

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PURPOSE

To provide instructions for the quantitative determination of alkaline phosphatase on the DXC 600/800.

PRINCIPLE

ALP reagent, when used in conjunction with UniCel® DxC 600/800 System(s), is intended for the quantitative determination of Alkaline Phosphatase activity in human serum or plasma.

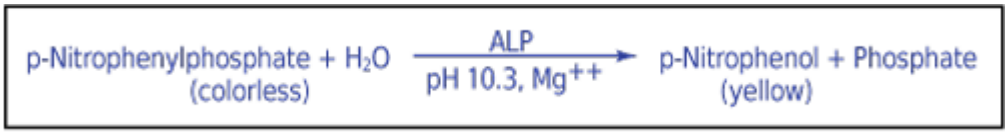
BACKGROUND

Clinical Significance

Alkaline phosphatase measurements are used in the diagnosis and treatment of liver, bone, parathyroid, and intestinal diseases.

Methodology

ALP reagent is used to measure alkaline phosphatase activity by a kinetic rate method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction, alkaline phosphatase catalyzes the hydrolysis of the colorless organic phosphate ester substrate, p-nitrophenylphosphate, to the yellow colored product, p-nitrophenol, and phosphate. This reaction occurs at an alkaline pH of 10.3.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 50 parts reagent. The system monitors the change in absorbance at 410 nanometers. This change in absorbance is directly proportional to the activity of ALP in the sample and is used by the System to calculate and express ALP activity.



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RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CH-0820	DXC 800 Controls
M-F-CH-0820	Chemistry Controls
J-F-CH-0826	DXC 800 Calibrators
M-F-CH-0826	Chemistry Calibrators
M-F-CH-1940	DXC 600 (AMR) Analytical Measurement Range
J-F-CH-1940	DXC 800 (AMR) Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma is the specimen of choice. Acceptable anticoagulants are listed in PROCEDURAL NOTES section of this chemistry information sheet. Whole blood is 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.

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, or 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.

Sample Type	Volume	Sample Stability
Plasma/Serum	0.5mL	• Separate serum from cells within 2 hours • Room Temp 8 hours • Refrigerated 48 hours • Frozen 3 months

Criteria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

Sample Volume

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

Contents

Each kit contains the following items:
Two ALP Reagent Cartridges (2 x 200 tests) or (2 x 400 tests)

Volume per Test	
Sample Volume	5 µL
Ordac Sample Volume	3 µL
Total Reagent Volume	250 µL
Cartridge Volumes	A 228 µL B 22 µL C – –

Reactive Ingredients	
p-Nitrophenylphosphate	15 mmol/L
2-Amino-2-methyl-1-propanol	350 mmol/L

Also non-reactive chemicals necessary for optimal system performance.

Reagent Preparation

No preparation is required.

Acceptable Reagent Performance

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

NOTE: New lots of reagent require lot to lot correlation studies. Refer to Related Documents section for related work instructions/forms.

Reagent Storage and Stability

ALP 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 10 days at +2°C to +8°C. Do not use beyond the manufacturer's expiration date. DO NOT FREEZE.

CALIBRATION

Calibrator Required

Calibration is not required.

TRACEABILITY

This measurand (analyte) is traceable to the manufacturer's selected Measurement Procedure as described in the Methodology section.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls & M-F-CH0820 Chemistry Controls

STEPS

1. If necessary, load the reagent onto the system.

2. Program controls for analysis.

3. After loading controls onto the system, follow the protocols for system operation. To load samples manually refer to the FHS DXC Series Manual Sample Programming procedure. For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

SYNCHRON® System(s) perform 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.

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:

Anticoagulant	Level Tested for In Vitro Interference
Ammonium Heparin	29 Units/mL
Lithium Heparin	29 Units/mL
Sodium Heparin	29 Units/mL

PERFORMANCE CHARACTERISTICS

Reference Range

Sample Type	Male		Female	
	Age (yrs)	Range	Age (yrs)	Range
Serum/ Plasma	0-6	72-307 IU/L	0-6	72-307 IU/L
Serum/ Plasma	6-9	133-340 IU/L	6-9	133-340 IU/L
Serum/Plasma	9-15	103-429 IU/L	9-13	99-453 IU/L
Serum/Plasma	15-18	49-210 IU/L	13-15	53-186 IU/L
Serum/Plasma	>18	35-115 IU/L	>15	35-115 IU/L

Analytic Range

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

Sample Type	Conventional Units
Serum or Plasma	5 – 1000 IU/L
Serum or Plasma (Ordac)	800-1650 IU/L

Samples with activities exceeding the high end of the analytical range should be rerun with ORDAC enabled or diluted with saline and reanalyzed. The appropriate dilution factor should be applied to the reported result.

Reporting results outside of analytical range

Lower limit of detection	5 IU/L	Results below 5; Report as <5 IU/L
Upper limit of range	1650 IU/L	Results >1650 should be diluted with 0.9% saline, reanalyzed and dilution factor applied. The maximum allowable dilution is X5. Results >8250 are reported as >8250 IU/L.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for ALP determination is 5 IU/L (0.08 µkat/L).

LIMITATIONS

None identified.

Interferences

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

Substance	Source	Level Tested	Observed Effect
Hemoglobin	RBC hemolysate	300 mg/dL INDEX of 8	-7 @ 34 IU/L
		400 mg/dL	-13 @ 170 IU/L
		500 mg/dL	No significant interference (within ± 6 IU/L or 7%)
Bilirubin (unconjugated)	Bovine	30 mg/dL INDEX of 20	No significant interference (within ± 6 IU/L or 7%)
Lipemia	Intralipid	500 mg/dL INDEX of 10 Airfuge recommended	No significant interference (within ± 6 IU/L or 7%)

2. Inhibitors of alkaline phosphatase activity include: oxalates, Hg^{++}, excess inorganic phosphate, bile acids, some amino acids (e.g., phenylalanine), and urea.

3. Refer to References (**Error! Reference source not found.**,**Error! Reference source not found.**,**Error! Reference source not found.**) for other interferences caused by drugs, disease and preanalytical variables.

ADDITIONAL INFORMATION

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

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
3/10/14 – New header/format. Changed from R to M document. Added Purpose and Related Doc sections. Changed formatting to match ACTM procedure			
Committee Approval Date	☒ Date: 8/26/14 ☐ NA – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 8/26/14