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Alkaline Phosphatase (Liquid) Reagent Set

Intended Use
For the quantitative determination of alkaline phosphatase in human serum using the Mindray BS-200 analyzer. For *in vitro* diagnostic use only.

Clinical Significance
Serum alkaline phosphatase estimations are of interest in the diagnosis of two groups of conditions; hepatobiliary disease and bone disease associated with increased osteoblastic activity.[1]

Test Summary
Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified conditions. ρ-Nitrophenyl Phosphate is one such phosphate ester and was introduced as a substrate by Fujita in 1939.[2]
Bessey, Lowry, and Brock published an endpoint procedure in 1946[3] while Bowers and McComb reported a kinetic procedure in 1966.[4] The kinetic procedure has undergone several modifications and been recommended for routine analysis.[5,6] This liquid reagent is based on the recommended method of the AACC.[7]

Principle
$$\rho\text{-NPP} + H_2O \xrightarrow{\text{Alk. Phos.}} \rho\text{-Nitrophenol} + H_3PO_4$$

ρ-Nitrophenyl phosphate is hydrolyzed to ρ-nitrophenol and inorganic phosphate. The rate at which the p-NPP is hydrolyzed, measured at 405 nm, is directly proportional to the alkaline phosphatase activity.

Reagent Composition
After combining R1 and R2 as directed the reagent contains: AMP Buffer (pH 10.45), ρ-NPP ≤16mM, Magnesium ions ≥1.0mM, activators and preservatives.

Reagent Preparation
The reagents are ready to use.

Reagent Storage and Stability
Store reagent set at 2-8°C. The reagents are stable until the expiration date if stored as directed. Protect from direct light and avoid microbial contamination. **NOTE:** The R2 reagent is temperature sensitive and can be affected by prolonged exposure to room temperature. Return reagent to 2-8°C as soon as possible after use.

Precautions
1. This reagent set is for *in vitro* diagnostic use only.
2. Do not ingest any material, toxicity not determined.
3. Do not use if the initial absorbance of the working reagent is greater than 1.0 at 405 nm or if the reagent fails to meet the stated parameters of performance.
4. Reagent should not be used if it fails to recover stated values in control sera or shows evidence of microbial contamination.
5. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Ed., 1988, HHS Publication No. (CDC) 88-8395.

Specimen Collection and Storage
1. Use non-hemolyzed serum (plasma should not be used since anticoagulant agents inhibit alkaline phosphatase activity).[8,9]
2. Serum samples should be stored at 2-8°C and run within two days.[10]
3. Specimen collection should be carried out in accordance with NCCLS M29-T2.[11] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Interferences
1. Young, et al[8] provide a list of drugs and other substances that interfere with the determination of ALP activity.
2. The method is not influenced (< 10%) by hemoglobin values up to 500mg/dl, bilirubin levels up to 20mg/dl and lipemia / Triglycerides (Intralipid used to simulate) to 1000mg/dl. The studies were performed on the Hitachi 717™ analyzer following a modification of the guidelines contained in NCCLS document EP7-P.[12]

Materials Provided
Alkaline Phosphatase R1 Reagent
Alkaline Phosphatase R2 Reagent

Materials Required but not Provided
1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry control, catalog number C7592-100

BS-200 Test Parameters

Test :	ALP	R1:	180
No.:	002	R2:	45
Full Name:	Alkaline Phosphatase	Sample Volume:	4.5
Standard No.:		R1 Blank:	
Reaction Type:	Kinetic	Mixed Rgt. Blank:	
Pri. Wave:	405nm	Linearity Range:	0 - 1000
Sec. Wavel:	670nm	Linearity Limit:	0.2
Direction:	Increase	Substrate Limit:	
Reac. Time:	3 / 11	Factor:	2276
		Compensate: Slope 1.0	Intercept: 0
Incuba. Time:	3	□ Prozone check	
Unit:	U/L	q1: q2: q3: q4:	
Precision:	Integer	PC: Abs:	

Calibration Parameters

Rule:		Calibrator 1:	
Sensitivity:		Calibrator 2:	
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Coefficient:	0		

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Limitations

1. This methodology measures total alkaline phosphatase irrespective of tissue or organ of origin. Further tests may be necessary to assist in differential diagnosis.
2. Samples with values exceeding 1000 IU/L should be diluted with an equal volume of saline and re-assayed multiplying the results by two.

Calibration

The procedure is standardized by means of the millimolar absorptivity of ρ-nitrophenol (18.75 at 405nm) under the specified conditions. Results are based on the change in absorbance per unit of time; all parameters must be known and controlled.

Calculations (Example)

One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$(IU/L) = \frac{\Delta Abs./Min. \times 1000 \times 1.025}{18.75 \times 1 \times .025} = \Delta Abs./min. \times 2187$$

Where ΔAbs./Min. = Average absorbance change per minute
1000 = Conversion of IU/ml to IU/L
1.025 = Total reaction volume (ml)
18.75 = Millimolar absorptivity of ρ-nitrophenol
. 025 = Sample Volume (ml)
1 = Light path in cm

Example: If your ΔAbs/min. = 0.06

Then 0.06 x 2187 = 131 IU/L

NOTE: If test parameters are altered the factor has to be recalculated using the above formula.

SI Units: To convert to SI Units (nkat/L) multiply IU/L by 16.67.

Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal ALP activities and should be run with every working shift in which ALP assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values

Adults 35-123 IU/L at 37°C. This reference range is based on a study performed by the manufacturer using samples from 783 apparently healthy adults. Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.

Specific Performance Characteristics Data Generated on BS200

Assay Range: 0-1000 IU/L

Accuracy: Studies comparing the Liquid Alkaline Phosphatase Reagent method used on the Mindray BS200 and a similar analyzer yielded the following results:

Method	Alkaline Phosphatase
N	59
Mean Alkaline Phosphatase IU/L	198
Range IU/L	0-927
Standard Deviation IU/L	251
Regression Analysis	Y=1.077x – 8.9
Correlation Coefficient	0.993

Precision:
Within-Day precision for the Liquid Alkaline Phosphatase Reagent was determined following a modification of NCCLS document EP5-T2[17] using the Mindray BS200. Within-Day precision studies produced the following results:

Sample	LOW	HIGH
N	20	20
Mean Alkaline Phosphatase IU/L	61	171
Standard Deviation IU/L	2.5	2.6
Coefficient of Variation (%)	4.1	1.5

Day-to-Day precision was also determined following a modification of NCCLS document EP5-T2.[17] Day-to-Day precision studies run on the Mindray BS200 produced the following results:

Sample	LOW	HIGH
N	80	80
Mean Alkaline Phosphatase IU/L	81	204
Standard Deviation IU/L	2.6	4.1
Coefficient of Variation (%)	3.3	2.0

Sensitivity: 2SD Limit of Detection (95% Conf) = 0.828 IU/L

References

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