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ALKALINE PHOSPHATASE
(KINETIC PROCEDURE)

ALKALINE PHOSPHATASE REAGENT SET
For the quantitative determination of alkaline phosphatase in human serum.

INTRODUCTION:
Distributed in almost every tissue of the body, serum alkaline phosphatase (ALP) levels are of interest in the diagnosis of hepatobiliary disorder and bone disease.[1] Most of the ALP in the normal adult serum is from the liver or biliary tract.[2] Normal alkaline phosphatase levels are age-dependent, and are elevated during periods of active bone growth. Moderate elevations of ALP (not involving the liver or bone) may be attributed to Hodgkin's disease, congestive heart failure, and abdominal bacterial infections.[3] Elevations also occur in the third trimester of pregnancy.

Alkaline phosphatase is determined by measuring the rate of hydrolysis of various phosphate esters. p-Nitrophenyl Phosphate is one such ester that was used as a substrate by Fujita in 1939.[4] Bowers and McComb further modified the procedure to a kinetic assay.[5] In 1974, the Committee on enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology adopted a modification of the above procedure as the recommended procedure.[6] The present method is a modification of the reference methods of the above committee and the American Association for Clinical Chemistry.[7]

PRINCIPLE
The enzymatic sequence employed in the assay of Alkaline Phosphatase is as follows:

$$p\text{-Npp} + H_2O \xrightarrow{\text{Alk. Phos.}} p\text{-Nitrophenol} + H_3PO_4$$

p-Npp is colorless but p-Nitrophenol has strong absorbance at 405 nm. The rate of increased absorbance at 405 nm is proportional to the enzyme activity.

REAGENT COMPOSITION
When reconstituted as directed, the reagent for Alkaline Phosphatase contains the following:
(Alkaline Phosphatase Reagent): p-Nitrophenyl Phosphate 17mM, Magnesium Ions 4mM, Buffer (pH 10.2 ± 0.2), activator and binder.

WARNINGS AND PRECAUTIONS:
1. For in vitro diagnostic use.
   CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
2. Specimens should be considered infectious and handled appropriately.
3. Use distilled or deionized water indicated.

STORAGE AND STABILITY
Store reagent set at 2-8°C (refrigerated). Reconstituted reagent is stable for thirty (30) days when stored at 2 - 8°C and twenty-four (24) hours at room temperature.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. Moisture has penetrated the vial and caking has occurred.
3. The reconstituted reagent has an absorbance against water greater than 0.8 at 405nm.

SPECIMEN COLLECTION
Unhemolyzed serum is the preferred sample. Heparinized plasma may also be used. Oxalate, fluoride and EDTA inhibit alkaline phosphatase, so are unsuitable as anticoagulants.[8] Samples should be kept cold and assayed as soon as possible after collection. A timed routine for sample collection and analysis should be established in each laboratory because ALP levels in serum or plasma, or in reconstituted control serum, rise significantly when stored at 2° - 8°C or at room temperature.

INTERFERING SUBSTANCES
EDTA, citrate, fluoride and oxalate inhibit alkaline phosphatase. Young et al. give a list of drugs and other substances which may interfere with the determination of ALP activity.[9]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipetting devices.
2. Test tubes/rack.
3. Timer.
4. Spectrophotometer with a temperature controlled cuvette.
5. Heating bath/block.

GENERAL INSTRUCTIONS
The reagent for Alkaline Phosphatase is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
Consult the appropriate instrument application guide available from us.

PROCEDURE (MANUAL)
1. Reconstitute reagent according to instructions.
2. Pipette 1.0 ml of reagent into appropriate tubes and allow to equilibrate to 37°C.
3. Zero spectrophotometer with water at 405 nm.
4. Transfer 0. 025 ml (25 ul) of sample to reagent. Mix well.
5. After one (1) minute, measure the absorbance. Return tube to 37°C. Repeat readings every minute for the next two (2) min.*
6. Calculate the average absorbance difference per minute (Δ abs/.min)
7. The Δabs./min. multiplied by the factor 2187 (see Calculations) will yield results in IU/L.
8. Samples with values above 800 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two (2).

* Note: If the spectrophotometer being used is equipped with a temperature controlled cuvette. The reaction mixture may be left in the cuvette while the absorbance readings are taken.

VOLUMES
If the spectrophotometer, being used requires a final volume greater than 0.50ml for accurate readings, follow the "ALTERNATE PROCEDURE."
Unit: One unit is the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under the specified conditions.

$$\text{IU/L} = \frac{\Delta\ \text{Abs./ min.} \times 1000 \times \text{TV}}{\in \times \text{LP} \times \text{SV}} = \frac{\Delta\ \text{Abs./min.} \times 1000 \times 1.025}{18.75 \times 1 \times 0.025}$$

= Δ Abs./min. x 2187

Where:

Δ Abs./min	=	Absorbance change
1000	=	Conversion of IU/ml to IU/L
TV	=	Total reaction volume (0.1.025 ml)
$\in$	=	Millimolar absorptivity of p-Nitrophenol 18.75
LP	=	Light path in centimeters (cm) (1.0)
SV	=	Sample volume (0.01 ml)

Example: If the Δ Abs./min. = 0.007 then 0.007 x 2187 = 19.04 U/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.

ALTERNATE PROCEDURE

1. Reconstitute reagent according to instructions.
2. Pipette 0.50ml (500 µL) of reagent into appropriate tubes and allow to equilibrate to 37°C.
3. Zero spectrophotometer with water at 405nm.
4. Add 0.010 ml (10µL) of sample to reagent, mix well.
5. After one (1) minute, measure the absorbance. Return tube to 37°C. Repeat readings every minute for the next two (2) min.*
6. Calculate the average absorbance difference per minute (Δ abs/.min.).
7. The ΔAbs./min. multiplied by the factor 2720 (see Calculations) will yield results in IU/L.
8. Samples with values above 800 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two (2).

PROCEDURE CALCULATION

Unit: One unit is the mount of enzyme that catalyzes the transformation of one micromole of substrate per minute under the specified conditions.

$$\frac{(A_1\text{-}A_2) \times 1000 \times 0.510}{1 \times 18.75 \times 1 \times 0.010} = (A_1\text{-}A_2) \times 2720$$

Where:

$(A_1\text{-}A_2)$	=	Absorbance Change
1000	=	Conversion of IU/ml to IU/L
TV	=	Total reaction volume (mL) (0.510)
1 min.	=	Time interval between readings
1 cm	=	Light path in centimeters
18.75	=	Millimolar absorptivity of p-Nitrophenol
SV	=	Sample volume (mL) (0.025)

Example: A_1 = 0.40, A_2 = 0.60
then: (0.60 - 0.40) = 0.2 x 2720 = 146 IU/L

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

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

PROCEDURAL LIMITATIONS

This methodology measures total Alkaline Phosphatase irrespective of tissue or organ of origin. Further tests may be necessary to assist in differential diagnosis.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established Alkaline Phosphatase values may be used for quality control. The assigned value of the control material must be con/L, by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

Adults 25 - 90 IU/L at 37°C. Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.[10]

PERFORMANCE CHARACTERISTICS

1. Linearity: Within Run
   900 IU/L
2. Comparison: A study performed between the present procedure and one commercial product resulted in a coefficient of correlation of 0.99 with a regression of y = 0.95x + 3.50.
3. Precision studies:

Mean U/L	S.D.	C.V.
76.1	2.1	2.7%
331.3	15.4	4.6%

Run to Run

Mean IU/L	S.D.	C.V.
75.3	5.1	6.7%
328.6	11.26	3.4%

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