ALKALINE PHOSPHATASE AMP (IFCC)

IFCC Method

DIATEK

CLINICAL SIGNIFICANCE :

Human ALP consists of a group of enzymes which hydrolyse phosphates at an alkaline pH. ALP is found in practically all tissues of the body but in high concentration in the osteoblasts of bone, liver, placenta, kidney, intestinal wall and lactating mammary glands. In adults the ALP normally found circulating in the serum is largely derived from the liver. In children or in adolescents going through pubertal growth spurts there is an additional contribution from bone and this accounts for the higher reference interval for these groups. Pregnancy also raises the normal values of ALP. Raised ALP levels are often observed in bone disease or liver disease involving the biliary tract. If the source of the isoenzyme is not apparent then estimation of GGT may help differentiate between the two. A raised GGT in the presence of a raised ALP would suggest the liver is the primary source. Increased ALP (usually normal GGT) is seen in Osteomalacia and Rickets, primary hyperparathyroidism with bone involvement, Pagets disease, secondary carcinoma in bone and some cases of osteogenic sarcoma. Increased levels of ALP (usually with a raised (GGT) is seen in cholestasis, hepatitis, cirrhosis, space occupying lesions and malignancy with bone or liver involvement or direct production. Low levels of ALP may be observed in conditions which cause arrested bone growth or in hypophosphatasia.

TEST PRINCIPLE :

This alkaline phosphatase (ALP) method is based on the recommendations of the International Federation of Clinical Chemistry (IFCC). This method utilises 4-nitrophenyl-phosphate as the substrate. Under the optimised conditions ALP present in the sample catalyses the following transphosphorylation reaction.

$$4\text{-NPP} + H_2O \xrightarrow[\text{Mg / Alkaline pH}]{\text{ALP}} \text{4-NPO} + \text{Phosphate}$$

REAGENTS COMPOSITION :

Reagent 1 (R1) : 2-Amino-2-Methyl-1-Propanol (pH=10.4) 0.33mol/l, Zinc sulphate 1.4mmol/l, Magnesium Acetate, 2.8mmol/l, Surfactant and Stabilisers, pH=10.4 (±0.2)

Reagent 2 (R2) : p-Nitrophenylphosphate 15mmol/l, AMP buffer pH=10.4 (±0.2) and Stabilisers

KIT CONTENTS :

	CODE No. AL01	CODE No. AL02	CODE No. Al03
Pack size :	(5x10 ml)	(5 x 25 ml)	(110ml)
Reagent 1	5 x 8 ml	5 x 20 ml	2 x 44 ml
Reagent 2	1 x 10 ml	1 x 25 ml	2 x11 ml

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, and contaminations prevented during their use. Do not use reagents over the expiration date. Once opened the reagent is stable for 1 month On-board the analyser at approximately 10°C.

MATERIALS REQUIRED BUT NOT PROVIDED :
ALP IFCC Control (Use of assayed QC sera is recommended to validate test result).

SAMPLES : Serum or heparinised Plasma, Fasting preferable
Serum should be separated from blood as soon as possible.
Sample must be free of hemolysis

INTERFERENCES

The following analyze were tested up to the levels indicated and found not to interfere with Hemoglobin: 1000 mg/dl Intralipid 2000 mg/dl, Bilirubin: 50 mg/dl, Ascorbic Acid: 50 mg/dl, Glucose: 1000mg/dl

ASSAY CONDITIONS:

Wavelength : ..... 405 nm
Cuvette: ..... 1 cm light path
Constant temperature 37ºC
Reaction (Mode)..... Kinetic
Kinetic Factor..... 2757
Delay ..... 60 sec
Read time..... 180 sec
Linearity..... 700 U/L
Unit..... U/L
Blanking..... D. Water
Slope of reaction..... Increasing

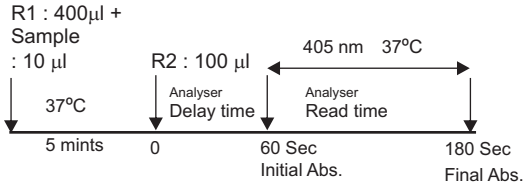
PROCEDURE :

Pipette into test tubes labeled Test (T) as follows:

	T
Reagent R1	400µl
Specimen	10µl
Mix and incubate for 5 mint at 37 ºC,	
Reagent R2	100µl

Mix and read the change of absorption (∆A) between 60 sec and 120 sec at 37ºC

Assay Procedure summary:



CALCULATIONS :

ALP Activity in U/L = ∆ Abs of Test x 2757

REFERENCE RANGE :

Adults : 45 - 135 U/L
Normally increased ALP values are found in growing Children

The above reference range is guideline and all the laboratories must establish their own Age sepcific normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.
2. Use clean glassware and microtips while pipetting 3. Avoid contamination of the reagent during the assay process.
4. Before the assay begins, bring all the reagents to room temperature.
5. If a larger volume of reagent is required for the absorbance reading, requisite volume can be taken in multiples, keeping the same ratio of reagent to specimen
6. Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.
7. Programmes for specific autoanalysers are available on request.
8. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.
9. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY AND DETECTION LIMIT :

The assay is linear up to ALP activity upto 700 IU/L. The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 5 with Normal Saline and multiply the result by 5.

BIBLIOGRAPHY

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Medical Device Insert / Kit Insert / Instruction for use (IFU)
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