Phosphorus

MODIFIED METOL METHOD

Code : 10015 (15 Tests)

(For the Analyser / Colorimetric Estimation of Inorganic Phosphorus in Serum & Urine)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

The metabolism of phosphorus is closely related with the metabolism of calcium. Almost 80% of total body phosphorus is present in bones and the remaining 20% is present as organic phosphate esters, with only a small but significant amount of phosphorus present in plasma as inorganic phosphate. The daily urinary excretion of phosphorus is almost entirely inorganic and varies greatly with dietary intake and with clinical variation. Determination of inorganic phosphorus is useful in the diagnosis of hyper/hypoparathyroidism.

Various methods for the determination of phosphorus are based on the reaction of phosphate with molybdic acid to form phosphomolybdic acid, which is subsequently reduced by using various reducing agents to give a colored complex. Older methods involve a protein precipitation step also.

PHOSPHORUS Kit has a simple, rapid one step procedure, based on a modified metol method. The proteins are maintained in solution under acidic conditions of assay by the use of a surfactant. The amount of blue colored complex formed is directly proportional to the concentration of inorganic phosphorus in Serum / Urine Sample.

PRINCIPLE:

Ammonium molybdate under acidic conditions reacts with phosphorus to form phosphomolybdate complex which is reduced to blue colored complex by metol. The absorbance of color developed is proportional to the inorganic phosphorus concentration.

REAGENTS:

1.	Catalyst Reagent	50 ml.
2.	Molybdate Reagent	50 ml.
3.	Metol Reagent	50 ml.
4.	Standard (5 mg%)	3 ml.

The reagents are ready to use and usable to the expiration date at room temperature till the expiry date mentioned on the label.

SAMPLE :

1. Serum : Hemolysed/grossly lipemic sera should not be used. Inorganic phosphorus is stable for 4 days at R.T. and for one week at 2 - 8^{o}C.
2. Urine : 24 hours urine collection; dilute the urine sample 1 : 10 with deionized water before use.

EXPECTED RANGE:

1. Serum Phosphorus : 2.5 - 4.5 mg%
2. Urine Phosphorus : 0.4 - 1.3 gms/24 hours Urine

LINEARITY:

This method is linear Upto 15 mg%

INSTRUCTIONS:

1. Glassware must be thoroughly washed and cleaned. Use of detergents containing phosphorus may interfere in assay, unless removed properly.
2. Serum should be seperated from the clot as early as possible.

DIRECTIONS FOR USE ON ANALYSERS:

Reaction Type	:	End point with std.
Reaction Slope	:	Increasing
Wave Length	:	680 nm (red filter)
Incubation Temp	:	Room Temperature
Incubation Time	:	5 min.
Standard	:	5 mg%
Linearity	:	15 mg%
Unit	:	mg%

PROCEDURE:

Pipette into clean dry test tubes labeled Blank (B), Standard (S), and Test (T).

	B	S	T
Catalyst Reagent (1)	1.0 ml	1.0 ml	1.0 ml
Molybdate Reagent (2)	1.0 ml	1.0 ml	1.0 ml
Deionised Water	0.1 ml	-	-
Standard (4)	-	0.1 ml	-
Serum/Dilute urine	-	-	0.1 ml
Metol Reagent (3)	1.0 ml	1.0 ml	1.0 ml

Mix well and incubate at room temperature for five minutes. Measure the absorbance of Standard(s) and Test(T) against Blank (B) either on a photocolorimeter with red filter or on Spectrophotometer at 680 nm, within 30 minutes.

CALCULATIONS :

a) Serum phosphorus in mg% = $\frac{\text{A of (T)}}{\text{A of (S)}}$ x 5 (Std. Conc)

b) Urine phosphorus in mg% = $\frac{\text{A of (T)}}{\text{A of (S)}}$ x 0.5

c) Urine phosphorus in gm/24 hrs. : (b)x24 hrs. Urine volume in Litres.

SI conversion factor mMol/L = ~~mg%~~ x 0.323

NOTES :

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY :

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2. Daly J.A. (1972) Clin. Chem. 18, 263.
3. Gomorri G. (1942) J. Lab. Clin. Med. 27, 995.

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