PHOSPHORUS
UV Method

MONOTEST

DIATEK

CLINICAL SIGNIFICANCE :

The majority of the body's phosphorous is found in the bone as hydroxyapatite. The remaining phosphate is present as inorganic phosphate esters. Phosphorous is involved in the intermediary metabolism of carbohydrates and is a component of other physiologically important substances. Thus, increased serum phosphorous may occur in hypervitaminosis, hypoparathyroidism, and renal failure. Reduced serum phosphorous levels are seen in rickets (vitamin D deficiency) hyperparathyroidism, and Fanconi`s syndrome1.

TEST PRINCIPLE :

Ammonium molybdate + Sulphuric acid + Phosphate

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Phosphomolybdic complex

Inorganic phosphorous reacts with ammonium molybdate in the presence of an acid to form an ammonium phosphomolybdate complex, which absorbs light at 340nm. The increase in absorbance is measured spectrophotometrically and is proportional to the amount of inorganic phosphorous present.

REAGENTS COMPOSITION :

Reagent-1 (R1) : Sulphuric acid < 180 mmol/L
Ammonium molybdate 400 µmol/L, Dertergent > 1 %

Phosphorus Standard : 5 mg/dl

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 analyzer at approximately 10°C.

KIT CONTENTS :

CODE No.	PH01
Pack size :	(25x1 ml) MONOTEST
Reagent-1 (R1) Molybdate Reagent	25 x 1 ml
Phosphorus Standard (Conc. : 5.0 mg/dl)	1 x 1 ml

SAMPLES :

Serum free of hemolysis, Heparinised plasma.
Collected Urine
(Acidify before collection with 10 ml of 10% HCl to prevent precipitations. Dilute sample at 1 : 10 with distilled water. Multiply result by 10)

MATERIALS REQUIRED BUT NOT PROVIDED :

Phosphorus Control (Use of assayed QC sera is recommended to validate test result).

ASSAY CONDITIONS:

Wavelength :	340 nm
Cuvette:	1 cm light path
Constant temperature	37ºC
Reaction	End Point
Standard Conc.	5.0
Unit	mg/dl
Linearity	20 mg/dl
Unit	mg/dl
Slope of Reaction	Increasing
Blanking	Reagent

PROCEDURE :

For MONOTEST vial, no need to pipette out the reagent.
Take 3 vials of MONOTEST Reagent and labeled on the top as Blank (B), Standard (S) and Test (T).

Pipette into test tubes labeled Blank (B), Standard (S) and Test (T) as follows **for other pack sizes.**

	B	S	T
Molybdate Reagent (R1)	1.0 ml	1.0 ml	1.0 ml
Phosphorus Standard		10µl	
Specimen			10µl

Mix and incubate for 5 mint at 37°C. Read absorbance of Standard (S) and Test (T) against Blank (B) with 340 nm. The final transparent color is stable for 1 hour at R.T.

CALCULATIONS :

$$\text{Phosphorus in mg/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times 5$$

REFERENCE RANGE :

Serum, plasma (Adult) : 2.6 - 4.5 mg/dL
Higher values found in Children

Urine : 400 -1300 mg/24 h

The above reference range is guideline and all the laboratories must establish their age and sex specific own 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 fresh micropipette tips while pipetting Molybdate Reagent and Phosphorus Standard. Replug Standard vial after immediate use.**
3. Avoid contamination of the reagent during the assay process.
4. Before the assay begins, bring all the reagents to room temp.
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/standard.
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 Phosphorus concentration of 20 mg/dl. The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 2 with Normal Saline and multiply the result by 2.

BIBLIOGRAPHY :

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 241-7.
2. Endres DB, Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1395-1457.
3. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company;1999. p. 1829.

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