Phosphorus

Phosphomolybdate. UV

Quantitative determination of phosphorus

CE IVD

PACKAGING

Ref.: 101-0458	Cont.: 2 x 100 mL

Store at 2 - 8º C

CLINICAL SIGNIFICANCE

Phosphorus is an essential mineral for tissue bone formation and is required by every cell in the body for normal function. Approximately 85 % of the body phosphorus is found in bone and in teeth. Low levels of phosphorus, can be caused by hypervitaminosis D, primary hyperparathyroidism, renal tubular disorders, antacids or malabsortion. High levels of phosphorus can be caused by diet, bone metastases, liver disease, alcohol ingestion, diarrhea and vomiting[1,5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Direct method for determining inorganic phosphate.

Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow color.

The intensity of the color formed is proportional to the inorganic phosphorus concentration in the sample[1,2].

REAGENTS

R Molybdic	Ammonium molybdate	0.40 mM
	Sulphuric acid (SO_4H_2)	210 mM
	Detergents	
PHOSPHORUS CAL	Phosphorus aqueous primary standard 5 mg/dL	

PRECAUTIONS

R: H314-Causes severe skin burns and eye damage.

Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Reagent and Standard are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm ≥ 0.54.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment [Note 1].

SAMPLES

- Serum or plasma[1,5]:
  Free of hemolysis. Serum or plasma should be removed from the clot as quickly as possible to avoid elevation of serum phosphorus from hydrolysis or leakage of phosphate present in erythrocytes.
  Stability: 7 days at 2 - 8º C.
- Urine[1,2] (24 h):
  Collect the specimen into a bottle containing 10 mL of 10 % v/v hydrochloric acid (HCl) to avoid phosphate precipitations. Adjust to pH 2. Dilute the sample 1/10 with distilled water. Mix. Multiply the result by 10 (dilution factor). Stability: 10 days at 2 - 8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

PHOSPHORUS CAL: Proceed carefully with this product because due its nature it can get contamined easily.

Most of the detergents and water softening products used in the laboratories contain chelating agents and phosphates. It is recommended to rinse glassware in diluted nitric acid and water before using.

Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

1. Assay conditions:
   Wavelength: . . . . . .340 nm
   Cuvette: . . . . 1 cm. light path
   Temperature . . . . . .37 / 30 / 25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard[Note 2,3] (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 5 minutes.
5. Read the absorbance (A) of the samples and Standard, against the Blank.

CALCULATIONS

Serum: $\frac{(A)\text{Sample}}{(A)\text{Standard}}$ x 5 (Standard conc.) = mg/dL of phosphorus

Urine 24 h: $\frac{(A)\text{Sample}}{(A)\text{Standard}}$ x 5 x vol. (dL) urine 24 h =mg/24 h of phosphorus

Conversion factor: mg/dL x 0.323 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems: Contro-N (Ref. 101-0083, 101-0252) and Contro-P (Ref. 101-0084, 101-0253).

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma:

Children	4,0 – 7,0 mg/dL	≅	1,29 – 2.26 mmol/L
Adults	2,5 – 5,0 mg/dL	≅	0.80 – 1.61 mmol/L

Urine:

Adults	0.4 – 1.3 g /24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.000 mg/dL to linearity limit of 35 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	4.09	7.12	4.11	7.09
SD	0.03	0.046	0.09	0.06
CV (%)	0.62	0.80	2.15	0.80

Sensitivity: 1 mg/dL = 0.0798 A.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.8577.
Regression equation: y= 0.724x + 0.837.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolyzed specimens are unacceptable because erythrocytes contain high concentrations of organic phosphate esters, which can be hydrolyzed to inorganic phosphate during storage. Inorganic phosphate increases by 4 to 5 mg/dL per day[5]. A list of drugs and other interfering substances with phosphorus determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY

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