Phosphorus

Phosphomolybdate. Colorimetric
Quantitative determination of phosphorus

IVD

PACKAGING

Ref.: 101-0384	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

Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum colour.
The intensity of the color formed is proportional to the inorganic phosphorus concentration in the sample[1,2].

REAGENTS

R 1 Molybdic	Molybdate-Borate Sulphuric acid (H_2SO_4)	1.21 mmol/L 100 mmol/L
R 2 Catalyzer	1,2 Phenylenediamine	2.59 mmol/L
PHOSPHORUS CAL	Phosphorus aqueous primary standard 5 mg/dL	

PRECAUTIONS

R1: H314-Causes severe skin burns and eye damage.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Working reagent (WR):
Mix equal volumes of R 1 (Molybdic) and R 2 (Catalyzer)
Stability: 10 h at 2 - 8º C, protected from light.

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 710 nm ≥ 0.40.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 710 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment[(Note 1)].

SAMPLES

- Serum[1]:
  Free of hemolysis. Serum 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: . . . . . 710 nm (620 - 750)
   Cuvette: . . . . 1 cm. light path
   Temperature . . . . . 37º C / 15 - 25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.5	1.5	1.5
Standard[(Note 2-3)] (µL)	--	50	--
Sample (µL)	--	--	50

4. Mix and incubate for 10 min at 37º C or 30 min at room temperature (15 - 30º C).
5. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

Serum

$$\frac{(A)Sample-(A)Blank}{(A)Standard-(A)Blank} \times 5 \text{ (Calibrator conc.)} = \text{mg/dL of phosphorus in the sample}$$

Urine 24 h

$$\frac{(A)Sample-(A)Blank}{(A)Standard-(A)Blank} \times 5 \times \text{vol. (dL) urine 24 h} = \text{mg/24 h of phosphorus}$$

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: Contro-N (Ref. 101-0083, 101-0252) and Contro-P (Ref. 101-0084, 101-0253).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum:

Children	: 4.0 - 7.0 mg/dL	(1.3 – 2.2 mmol/L)
Adults	: 2.5 - 5.0 mg/dL	(0.8 – 1.8 mmol/L)

Urine: 300 - 1000 mg/24 horas (10 - 33 mmol/24h)

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

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,00 mg/dL to *linearity limit* of 13 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)	4.08	6.96	4.24	6.98
SD	0.02	0.02	0.09	0.26
CV (%)	0.54	0.31	2.03	3.66

Sensitivity: 1 mg/dL = 0,0972 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 $(r)^2$: 0.9927
Regression equation: y=0.9626x – 0.01366
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed with bilirubin up to 20 mg/dL, hemoglobin up to 150 mg/dL and ascorbic acid up to 30 mg/dL[1].
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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