Reactivos GPL

Barcelona, España

CE

- Phosphorus-UV -

PHOSPHORUS
Phosphomolybdate. U.V.

Store at: +2+8ºC.

Presentation:
Cod. SU027 CONT: *R 2 x 125 mL.+ CAL 1 x 5 mL.*
Cod. SU027-SP CONT: *R 2 x 50 mL.+ CAL 1 x 5 mL.*

Procedure

Quantitative determination of phosphorus.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
Direct method for determining inorganic phosphate.
Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow colour.
The intensity of the colour formed is proportional to the inorganic phosphorus concentration in the sample[1,2].

REAGENTS COMPOSITION

R (Molybdic)	Ammonium molybdate Sulphuric acid (SO_4H_2) Detergents	0.40 mM. 210 mM.
Phosphorus Cal	Phosphorus aqueous primary calibrator	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.

REAGENT PREPARATION AND STABILITY
Reagent (R) and calibrator (Phosphorus Cal) are ready to use.
Phosphorus Cal: Proceed carefully with this product because due its nature it can get contaminated easily.
Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm. ≥ 0.54

All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use. Do not use reagents over the expiration date.

SPECIMEN
- Serum or plasma[1,5]:

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 2-8ºC.
- 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 2-8ºC.

MATERIAL REQUIRED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 340 nm.
- Matched cuvettes 1.0 cm. light path.

General laboratory equipment *(note 1)*

TEST PROCEDURE
1. Assay Conditions
   - Wavelength: ..... 340 nm.
   - Cuvette: ..... 1 cm light path.
   - Temperature .....37 / 30 / 25ºC.
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Calibrator	Sample
R.1 (mL.)	1.0	1.0	1.0
Calibrator (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. The colour is stable for at least 30 minutes.

CALCULATIONS
Serum:

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

Urine 24 h:

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

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

QUALITY CONTROL
Control sera are recommended to monitor the performance of the procedure, Control H Normal Ref. QC003 and Control H Pathological Ref. QC004. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions

REFERENCE VALUES1
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).

It is suggested that each laboratory establish its own reference range.

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.

REAGENT PERFORMANCE
- *Measuring Range*:
  From detection limit of 0 mg/dL. to linearity limit of 35 mg/dL., under the described assay conditions.
  If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply 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	0.04	0.09	0.06
CV	0.62	0.80	2.15	0.08

- *Sensitivity:* 1 mg/dL. = 0.0798A
- *Accuracy:*
  Results obtained GPL 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): 0.8577
  Regression Equation: y= 0.724x + 0.837
  The results of the performance characteristics depend on the analyzer used.

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

NOTES
1. 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.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

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