PHOSPHORUS(Molybdate)

INTENDED USE

Bioline Inorganic phosphorus reagent is for the quantitative determination of inorganic phosphorus in human serum.

CLINICAL SIGNIFICANCE

The majority of the body's phosphorus is found in the bone as hydroxyapatite. The remaining phosphate is present as inorganic phosphate and phosphate esters. Phosphorus is involved in the intermediary metabolism of carbohydrates and is a component of other physiologically important substances. Thus, increased serum phosphorus may occur in hypervitaminosis, hypoparathyroidism, andrenalfailure.Reduced serum phosphorus levels are seen in rickets (vitamin Ddeficiency) hyperparathyroidism, and Fanconi's syndrome.

METHOD AND PRINCIPLE

The determination of inorganic phosphorus has been based on the reaction of molybdate with phosphate to produce the phosphomolybdate complex ,which is measured photometrically. in UV range 340 nm.

Inorganic Phosphorus+H_2SO_4+AmmoniumMolybdate----------→ PhosphomolydateComplex.

Inorganic phosphorus reacts with ammonium molybdate in an acid medium to form a phosphomolybdate complex, which absorbs light at340 nm. The absorbance at this wavelength is directly proportional to the amount of inorganic phosphorus present in the sample.

REAGENT COMPOSITION

Inorganic Phosphorus Reagent: Ammonium Molybdate 0.4mM, Sulfuric Acid 210 mM with surfactant.
Inorganic Phosphorus Standard: (5.0 mg/dl)

WARNINGS AND PRECAUTIONS

1. The reagents are for "In Vitro" diagnostic use only.
2. Do not pipette by mouth. Avoid contact of reagents with skin, eyes and clothing.

REAGENT PREPARATION

Reagent comes in a ready to use format.

REAGENT STORAGE AND STABILITY

Store reagent and standard at Room temperature.

REAGENT DETERIORATION

Do not use if:

1. Reagent without serum added has an absorbance greater than 0.500 at 340 nm.
2. The reagent fails to recover stated control values.

SPECIMEN COLLECTION AND STABILITY

1. Use only clear, unhemolyzed serum, separated from the erythrocytes as soon as possible. Erythrocytes contain organic phosphates that can hydrolyze on standing or can be enzymatically cleaved by phosphatases. Inorganic phosphates can then leak through the cell walls, increasing the concentration.
2. Once the serum has been separated, the phosphate content will not change for at least a week when stored in the refrigerator (2-8°C)

INTERFERENCES

For a comprehensive list of substances that interfere with the Measurements of Inorganic Phosphorus see Young , et al.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength : 340 nm
Temperature : 37 °C

	Blank	Standard	Test
Reagent	1000 µL	1000 µL	1000 µL
Distilled water	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

Mix and read the absorbance of standard and Test against reagent blank after 5 minutes of incubation at 37⁰C. The final colour is stable for at least 1 hour.

PROCEDURE NOTES

1. Lipemic and icteric samples require a serum blank. For maximum accuracy a serum blank should be run with each sample.
   a. Add sample to Saline solution.
   b. Zero spectrophotometer at 340 nm with Saline solution.
   c. Read and record absorbance of serum blank.
   d. Subtract absorbance from test absorbance.
2. Samples with values exceeding 12.0mg/dl must be diluted 1:1with saline, re-run, and result multiplied by two (2).

CALCULATIONS

Abs. =Absorbance

$$\frac{\text{Abs. of Unknown - Abs. of reagent Blank}}{\text{Abs. of Standard- Abs. of reagent Blank}} \times \text{Std Conc.} = \text{Phos(mg/dl)}$$

Example:

Abs. of reagent Blank = 0.536
Abs. of Unknown = 0.918
Abs. of Standard = 1.012
Conc. of Standard = 5 mg/dl

$$\frac{0.918 - 0.536}{1.012 - 0.536} \times 5 = \frac{0.382}{0.476} \times 5 = 4.0 \text{mg/dl}$$

CALIBRATION

The procedures are calibrated with the standard solution, which is included with each series of tests. Its absorbance is used to calculate the results. It is recommended to establish a linearity curve up to 12 mg/dl with other available commercial standard solutions to verify the performance of instruments and reagents

LIMITATIONS

Most commonly employed detergent and disposable wipes used in the laboratory contain phosphates ,and the use of improperly rinsed glassware may result in elevated inorganic phosphorus values.

QUALITY CONTROL

It is recommended that controls be included in each set of assays.
Commercially available control material with established inorganic phosphorus values may be routinely used for quality control.The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction or procedural errors.

EXPECTED VALUES
Adults :2.5-4.8 mg/dl
Childrens: 4.0-7.0 mg/dl

This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values since differences exist between instruments, laboratories and local populations.

PERFORMANCE CHARACTERSTICS

1. Linearity:12 mg/dl. For samples above the linearity range dilute and ru-run the sample with distilled water. Multiply the obtained result with the dilution factor to get correct result.
2. Sensitivity: Based on an instrument resolution of A - 0.001, the present procedure has a sensitivity of 0.01 mg/dl.
3. Comparison: A comparison study performed between this method and one based on the same methodology yielded correlation coefficient of 0.99 with a regression equation of y =1.01x - 0.06.
4. Precision:
   Day -to-Day Precision: Two commercial control sera were assayed for a period of thirty (30) days and the following day today precision was obtained.

N=20	Low control	High control
Mean (mg/dl)	3.2	7.2
SD	0.2	0.3
CV%	6.6	4.1

Within Run Precision Two commercial control sera were assayed twenty(20) times and the following within run precision was obtained.

N=20	Low control	High control
Mean (mg/dl)	3.0	7.4
SD	0.5	0.4
CV%	6.7	4

General Technical Parameters

Mode	**End Point**
Wavelength (Filter)	**340 nm**
Reaction Direction	**Increasing**
Reagent Blank	**Yes**
Sample Vol.	**10 µL**
Reagent Vol.	**1000 µL**
Measuring Time	**5 min**
Reagent Blank Abs.(Max)	**NMT 0.500 Abs**
Calibration Method	**1- Point**
Standard (Conc.)	**5.0 mg/dL**
Linearity	**12 mg/dL**
Decimal Places	**2**
Temp.	**37 °C**
Unit	**mg/dL**
Ref. Low adult	**2.5 mg/dL**
Ref. High adult	**4.8 mg/dL**

REFERENCES

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3. Amador,E., Urbon, J.: *Clin. Chem*. 18, 601 (1972).
4. Henry,R.J., etal., *Clinical Chemistry*:Principles and Techniques409, New York, Harper and Row 122 - 143 (1964).