POINTE SCIENTIFIC, INC.
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Inorganic Phosphorus Reagent Set (UV)

Intended Use
For the quantitative determination of Inorganic Phosphorus in serum.

Method History
The measurement of inorganic phosphorus in serum is usually accomplished by forming a phosphomolybdate complex and in turn reducing it to a molybdenum blue color complex. Methods differ as to the choice of reducing agents: stannous chloride[1], phenylhydrazine[2], aminonaphtholsulfonic acid[3], ascorbic acid[4], ρ-methylaminophenolsulfate[5], N-phenyl-ρ-phenylenediamine[6] and ferrous sulfate.[7] These methods suffered from color instability, deproteinization steps and complexity of performance[8]. The addition of a surfactant eliminated the need to prepare a protein-free filtrate, accelerated color production, stabilized the color and simplified the procedure. Many of the components in these reagents were unstable and had to be stored separately. The quantitative measurement of unreduced phosphomolybdate complexes was first reported by Simonsen in 1946.[9] Daly and Ertingshausen[10] adapted that technique for the determination of inorganic phosphorus in 1972. Amador and Urban[11] modified this procedure further the same year. The present method is a modification of the above procedure using a single, stable reagent performing in the UV range.

Principle
Inorganic Phosphorus + H_2SO_4 + Ammonium Molybdate ----------➢ Unreduced Phosphomolybdate Complex

Inorganic phosphorus reacts with ammonium molybdate in an acid medium to form a phosphomolybdate complex that absorbs light at 340nm. The absorbance at this wavelength is directly proportional to the amount of inorganic phosphorus present in the sample.

Reagents
Ammonium Molybdate 0.48 mM, Sulfuric Acid 220 mM with surfactant.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. This reagent is an acid and is caustic. Avoid contact with skin. Flush with plenty of water if contact occurs. DO NOT PIPETTE BY MOUTH.

Reagent Preparation
Reagent comes in a ready to use form.

Reagent Storage
Store reagent at refrigerator temperature (2-8°C).

Reagent Deterioration
Do not use reagent if:
1. Reagent read against water has an absorbance greater than 0.500 at 340 nm.
2. The reagent fails to recover stated control values.

Specimen Collection and Storage
1. Unhemolyzed serum is specimen of choice.
2. Plasma should not be used since anticoagulants may produce falsely low values.[12]
3. Hemolyzed sample may give falsely high values.
4. Serum should be removed from the red cell clot as soon as possible.[13]
5. Serum inorganic phosphorus is stable for one week refrigerated and for three weeks frozen.[13,14]

Interferences
For a comprehensive list of substances that interfere with the measurement of Inorganic Phosphorus see Young, et al.[15]

Materials Provided
Inorganic Phosphorus Reagent.

Materials Required but not Provided
1. Accurate pipetting devices
2. Test tubes/rack
3. Timer
4. Spectrophotometer able to read at 340 nm.

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Label test tubes “blank”, “control”, “patient”, etc.
2. Transfer 1.0 ml of reagent into each tube. Allow to come to room temperature (25°C).
3. Add 0.02 ml (20ul) sample to respective tubes, mix and allow to stand for 5.0 minutes at room temperature.
4. Zero spectrophotometer with the reagent blank at 340nm.
5. Read and record absorbances of all tubes.
6. See “Calculations” to obtain results.

Procedure Notes
1. For spectrophotometers requiring a larger total volume for accurate reading, a 3.0ml reagent to 0.100ml (100 ul) sample ratio may be used.
2. Lipemic and icteric samples require a serum blank. For maximum accuracy a serum blank should be run with each sample.
    a) Add 0.02ml (20ul) sample to 1.0ml saline solution.
    b) Zero spectrophotometer at 340nm with saline solution.
    c) Read and record absorbances of serum blanks.
    d) Subtract absrobances form test absorbances.
3. Samples with values exceeding 12.0 mg/dl must be diluted 1:1 with saline, re-run, and results multiplied by two.

Calibration
Use an appropriate protein-based standard (5mg/dl) or calibrator.

Quality Control
The integrity of the reaction should be monitored by use of normal and abnormal control sera with known concentrations of inorganic phosphorus.

Calculation
Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Concentration of Standard} = \text{Inorganic Phosphorus (mg/dl)}$$

Example: Abs. of Unknown = 0.20; Abs. of Standard = 0.29; Conc. of Standard = 5 mg/dl

Then: $\frac{0.20}{0.29} \times 5 = 3.4$ mg/dl

SI Units

To obtain results in SI Units (mmol/L), multiply the results in mg/dl by the factor 0.323.

Example: 3.4 mg/dl x 0.323 = 1.09 mmol/L.

Limitations

Detergents containing phosphate should not be used for cleaning glassware used in this procedure.

Expected Values

Adults: 2.5-4.8mg/dl[16]
Children: 4.0-7.0mg/dl[17]
Values are decreased during menstrual period and after meals.[17]
It is strongly recommended that each laboratory establish its own normal values.

Performance

1. Linearity: 12 mg/dl
2. Comparison: A study performed between the present method and one based on the same methodology yielded a correlation coefficient of 0.999 with a regression equation of y = 1.02x – 0.06 (N=61).
3. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
3.5	0.05	1.4	3.5	0.08	2.3
7.8	0.07	0.9	7.7	0.21	2.7

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