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INORGANIC PHOSPHORUS REAGENT (COLOR METHOD)

INTENDED USE
Inorganic phosphorus reagent is used for the quantitative determination of inorganic phosphorus in human serum.

INTRODUCTION
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, and renal failure. Reduced serum phosphorus levels are seen in rickets (vitamin D deficiency) hyperparathyroidism, and Fanconi's syndrome.

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] P-methylaminophenolsulfate,[5] N-Phenyl-p-phenylenediamine[6] and Ferrous Sulfate.[7] These methods suffered from color instability, deproteinization steps and complexity of performance.[5] The addition of a surfactant eliminated the need to prepare a protein-free filtrate, accelerated color production, stabilized the color and simplified the procedure. Our method is based on a modification of the above method using Ferrous Ammonium Sulfate as the reducing agent.

PRINCIPLE
Inorganic phosphorus reacts with ammonium molybdate in an acid medium to form a phosphomolybdate complex. This complex is reduced by ferrous ammonium sulfate to produce a molybdenum blue complex. The color produced is measured at 675 nm and its intensity is directly proportional to the concentration of inorganic phosphorus present.

MATERIALS PROVIDED
1. Inorganic Phosphorus Reagent: Containing Ammonium Molybdate 2.4 mM, Sulfuric Acid 750mM, Ferrous Ammonium Sulfate 10.2 mM, Surfactant.
2. Inorganic Phosphorus Standard (Aqueous Solution): Containing Potassium Phosphate in distilled water (5 mg/dl).

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate pipetting devices.
2. Test tubes/rack.
3. Timing device.
4. Spectrophotometer with ability to read at 675 nm (650 - 700).

PRECAUTIONS
1. The reagents are 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 and standard at refrigerator temperature (2 - 8°C).

REAGENT DETERIORATION
The reagent should be clear to blue. Any precipitate or change of color to green would indicate contamination and the reagent should not be used.

SPECIMEN COLLECTION AND STORAGE
1. Unhemolyzed serum is specimen of choice.
2. Plasma should not be used since anticoagulants may produce falsely low values. [10]
3. Hemolyzed samples may give falsely high values.
4. Serum should be removed from the red cell clot as soon as possible.[11]
5. Serum inorganic phosphorus is stable for one (1) week refrigerated and for three (3) weeks frozen.[11, 12]

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

PROCEDURE (AUTOMATED)
See appropriate instrument application instructions.

PROCEDURE (MANUAL)
1. Label test tubes Blank, Standard, Control, Patient, etc.
2. Transfer 1.0 ml of reagent into each tube. Allow to come to room temperature (25°C).
3. Transfer 0.020 ml (20 µl) of sample to respective tubes and mix by inversion. (Alternate volumes: 100 ul/3 ml reagent)
4. Allow tubes to stand for at least ten (10) minutes.
5. Zero spectrophotometer at 675 nm with the reagent blank. Read and record absorbencies of all tubes.(Wavelength range: 600-675nm).

* TC - MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

PROCEDURE NOTES
1. Final color is stable for at least thirty (30) minutes.
2. Specimens with values above 14 mg/dl should be diluted 1:1 with saline, re-assayed and the result multiplied by two (2).
3. Severely lipemic and icteric samples require a serum blank. To 1.0 ml of saline add 0.02 ml (20 æl) of sample mix and read at 675 nm blanked against saline. Subtract the absorbance reading from the test absorbance. Calculate as usual.

CALIBRATION
Use the stable aqueous standard provided in the kit for the calibration. The concentration of inorganic phosphorus should be validated by comparison with commercially available standard. The linearity of this procedure extends to 14 mg/dl.

QUALITY CONTROL
1. It is recommended that high and low values of inorganic phosphorus are included in each set of assays.
2. Commercially available control materials with established inorganic phosphorus values may be used for quality control.

CALCULATIONS

Abs. = Absorbance

$\frac{\text{Abs. Of Unknown}}{\text{Abs. Of Standard}} \times \text{Conc. Of Std.} = \text{Inorganic Phosphorus (mg/dL)}$

Example: Abs. of Unknown = 0.20
Abs. of Standard = 0.290
Conc. of Standard = 5 mg/dl

Then: $\frac{0.20}{0.290} \times 5 = 3.4$ mg/dl

To obtain results in SI units (mmol/L), multiply the results in mg/dl by ten (10) to convert dl to liter and divide the value by thirty (30), the molecular weight of inorganic phosphorus.

Example: $\text{mg/dl} \times \frac{10}{3} = \text{mg/dl} \times 0.3333 = \text{mM/L}$

$3.4 \times 0.3333 = 1.13$ mM/L

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

EXPECTED VALUES

Adults: [14] 2.5 - 4.8 mg/dl
Children:[15] 4.0 - 7.0 mg/dl

Values are decreased during menstrual period and after meals.[15]
It is strongly recommended that each laboratory establish its own normal values.

PERFORMANCE

1. Linearity: 14 mg/dl
2. Sensitivity: Based on an instrument resolution of A - 0.001, the present procedure has a sensitivity of 0.02 mg/dl.
3. Comparison: A comparison study was performed between the present method and one based on the UV method. The correlation coefficient was 0.95 with a regression equation of Y = 0.89X - 0.13 (N=40).
4. Precision:

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

Mean	S.D.	C.V.(%)
3.1	0.1	3.2
6.8	0.2	2.9

   Day-to-Day Precision: Two commercial control sera were assayed for a period of thirty (30) days the following day to day precision was obtained.

Mean	S.D.	C.V.(%)
3.2	0.2	6.2
7.3	0.5	6.8

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