PHOSPHORUS

INTENDED USE

Vitro inorganic phosphorus reagent is intended for the in vitro quantitative determination of inorganic phosphorus in human serum, plasma and urine on both automated and manual systems.

METHOD

One step colorimetric endpoint method without deproteinization[1,2]

CLINICAL SIGNIFICANCE

88% of the phosphorus contained in the body is localized in bone in the form of calcium phosphate as the apatite $Ca^{2+}(Ca_3(PO))^{2-}{}_3$. The remainder is involved in intermediary carbohydrate metabolism and in physiologically important substances such as phospholipids, nucleic acids and ATP. Phosphorus occurs in blood in the form of inorganic phosphate and in organically bound phosphoric acid. The small amount of extracellular organic phosphorus is found almost exclusively in the form of phospholipids. The ratio of phosphate to calcium in the blood is approximately 6:10. An increase in the level of phosphorous causes a decrease in the calcium level. The mechanism is influenced by interaction between parathormome and vitamin D. Hypoparathyroidism, vitamin D intoxication and renal failure with decreased glomerular phosphate filtration give rise to hyperphosphatemia. Hypophosphatemia occurs in rickets, hyperparathyroidism and Fanconi's syndrome[3].

ASSAY PRINCIPLE

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[4], phenylhydrazine[5], aminonaphtholsulfonic acid[6], ascorbic acid[7], p-methylaminophenolsulfate[8], N-phenyl-p-phenylenediamine[9] and ferrous sulfate[1]. These methods suffered from color instability, deproteinization steps and complexity of performance[8]. Vitro reagent eliminated the need to prepare a protein free filtrate, accelerated color production, stabilized the color and simplified the procedure.

1. Inorganic phosphorus reacts with ammonium molybdate in an acidic medium to form a phosphomolybdate complex.
2. This complex is reduced by ferrous ammonium sulfate to produce a molybdenum blue complex.

Inorganic phosphorus+ ammonium molybdate —(Acidic medium)→ **Non-reduced phosphomolybdate**

Non-reduced phosphomolybdate + Ferrous ammonium sulfate → **Molybdenum blue complex**

The intensity of color measured photometrically at 600 - 675 nm, and its intensity is directly proportional to inorganic phosphorus concentration in the specimen.

EXPECTED VALUES

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

Serum (fasting)[10]	
Children (< 2 years)	4.5 – 6.7 mg/dl (1.45 - 2.16 mmol/l)
Children (2-12 years)	4.5 – 5.5 mg/dl (1.45 –1.78 mmol/l)
Adults (>12 years)	2.5 – 5.0 mg/dl (0.87 –1.45 mmol/l)
Urine	
On nonrestricted diet	0.4 - 1.3 g/day (12.9-42 mmol/day)

REAGENTS

R_1	Phosphorus standard	5.0 mg/dl
R_2	Sulfuric acid	1.1 N
	Ferrous ammonium sulfate	100 g/l
	Ferrous nitrate	2.0 g/l
	Ammonium molybdate	4.5 g/l

- **Reagent Preparation & Stability**

All reagents are stable up to the expiry date given on label when stored at 2 – 8^{0}C.

SPECIMEN

- Serum, plasma, and Urine.
- The only acceptable anticoagulant is heparin.

Specimen Preparation & Stability

Serum or plasma

- Fresh serum collected in the fasting state is the preferred specimen since serum inorganic phosphate levels are lower after meals.
- Use of anticoagulants other than heparin may interfere with the formation of the phosphomolybdate complex and should not be used.
- Serum or plasma should be separated from blood cells as soon as possible, to avoid the leakage of inorganic phosphorus and phosphate esters into the plasma media[11].
- Inorganic phosphorus in serum is stable 7 days refrigerated at 4^{0}C and for three weeks when frozen[9].

Urine

- Urine specimens should be collected in acid-washed bottles.
- 24 hours specimens should be collected in containers containing 5 ml of 6.0 mol/l HCl
- Inorganic phosphorus in acidified urine specimens is stable if stored at room temperature, refrigerated or frozen[10].
- Stored urine specimen must be mixed well and diluted 1:10 in distilled water prior analysis.

PROCEDURE

- **Manual Procedure**

Wavelength	600 - 675 nm
Cuvette	1 cm light path
Temperature	20-25 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum, plasma, or diluted urine

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	……	50 µl	……
Specimen	……	……	50 µl

Mix, and Incubate for 15 minutes at room temperature Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

The color is stable for 60 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Calculate the inorganic phosphorus concentration in serum by using the following formulae:

Serum inorganic phosphorus Conc.=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard value}$$

- **Unit conversion**

mg/dl x 0.323 = mmol/l

Calculate the inorganic phosphorus concentration in urine by using the following formulae:

Urine inorganic phosphorus Concentration mg/24 hrs=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times 5 \times 10^{*} \times d \times V$$

Where:
(5.0) inorganic phosphorus standard concentration.
(10^{*}) converts mg/dl to mg/l.
(d) dilution factor (10)
(V) the 24 hours urine values in liter.

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QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established inorganic phosphorus values may be routinely used for quality control.
Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  The only acceptable anticoagulant is heparin. Complexing anticoagulant such as EDTA, citrate, and oxalate must be avoided.
- **Bilirubin:**
  No significant interference from free or conjugated bilirubin up to a level of 30 mg/dl. Interference may increase or decrease sample results.
- **Haemolysis:**
  Avoid hemolyzed specimen since high amount of phosphate is liberated during rupture of erythrocytes.
- **Lipemia:**
  No significant interference.
- **Drugs :**
  Young[12] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.

WARNING & PRECAUTION

- Vitro phosphorous reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Reagent is an acid and is caustic. Avoid contact with skin. Flush with plenty of water if contact occurs. Don't pipette by mouth.
- It is recommended that disposable PVC tubes be used for this procedure.
- The reagent and specimen volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control

PERFORMANCE CHARACTERISTICS

Imprecision
Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level II	Level I	Level II
Number of samples	40	40	40	40
Mean (mg/dl)	3.6	6.43	3.9	5.95
SD (mg/dl)	0.04	0.06	0.08	0.07
CV (%)	2.9	2.9	5.9	3.6

Method Comparison
Comparison studies were carried out using a similar commercially available Inorganic Phosphorus reagent as a reference. Serum, plasma (Heparin) and urine samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Serum/Plasma:
Number of sample pairs 40
Range of sample results 2.05 - 7.95 mg/dl
Mean of reference method results 3.8 mg/dl
Mean of Inorganic Phosphorus results 3.7 mg/dl
Slope 0.91
Intercept 0.2 mg/dl
Correlation coefficient 1.00

Urine:
Number of sample pairs 40
Range of sample results 9.0-194.0 mg/dl
Slope 1.008
Intercept 1.0 mg/dl
Correlation coefficient 0.9995

Sensitivity

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.

When run as recommended the sensitivity of this assay is 0.3 mg/dl.

LINEARITY

When run as recommended, the assay is linear up to 14 mg/dl.

Specimens with values above 14 mg/dl should be diluted with 0.9% NaCl solution or distilled water and reassayed. Multiply the result by the dilution factor.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
13401	2 X 50 ml
13402	2 X 100 ml

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