N.S. BIO-TEC

INORGANIC PHOSPHORUS

REF. PHO-MC – 0420 (4 X 20 ml)
PHO-MC-0620 (6X 20 ml)

INTENDED USE

NS Biotec 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

CLINICAL SIGNIFICANCE

The body of human adult contains approximately 620 g of phosphorus entirely in the form of phosphates distributed fairly equally between extracellular and intracellular compartments. About 85 % of extracellular phosphate occurs in inorganic forms as hydroxyapatite. In plasma or serum, most phosphate exist in the inorganic form (Pi); this fraction is present as the mono-and dihydrogen forms, the relative proportions varying with the pH. Intracellularly, phosphate occurs mainly as phospholipids and phosphoproteins; this fraction is termed organic phosphate. Increased serum phosphate levels may occur in renal failure, hypervitaminosis D,and hypoparathyroidism. Reduced serum phosphate levels are seen in vtamin D deficiency, rickets, hyperparathyroidism, and fanconi's syndrome.

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ASSAY PRINCIPLE

Inorganic phosphate reacts with ammonium molybdate in presence of sulfuric acid to form nonreduced phosphomolybdate.

Phosphate + Ammonium molybdate $\xrightarrow{H_2SO_4}$ Nonreduced phosphomolybdate

the concentration of phoshomolybdate formed is directly proportional to the inorganic phosphate concentration. It is determined by measuring the increase in absorbance at 340 nm.

EXPECTED VALUES

Serum (fasting)		
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)	2.7 – 4.5 mg/dL	(0.87 –1.45 mmol/l)
Urine		
On nonrestricted diet		
0.3 – 1.0 g/day		(11 – 32 mmol/day)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the phosphorus results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	phosphorus standard	5.0	mg/dl
R_2	ammonium molybdate	3.5	mmol/L
	Sulphuric acid	750	mmol/L
	Surfactants	1	%

Reagent Preparation & Stability

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

SPECIMEN

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

Specimen Preparation & Stability

Serum and plasma

Serum specimens collected from fasting patients are the preferable specimens since meals lower inorganic phosphate level. The heparin is the only acceptable anticoagulant. EDTA, fluoride and citrate lower the inorganic phosphate level.Serum and plasma should be separated
from erythrocytes as soon as possible to avoid the leakage of inorganic phosphate and phosphate esters into the plasma media. The biological half-life in serum is few minutes.
Stability: 1 day at 15 – 25 ^{o}C ; 4 days at 4 – 8 ^{o}C;
1 year at -20 ^{o}C

Urine

Urine specimens should be collected in an acid-washed, detergent-free container. Acidify with hydrochloric acid after collection (pH<3).
Stability : 2 days at 15 – 25 ^{o}C ; 6 months at 2 – 8 ^{o}C;
Urine samples should be diluted 1 : 10 (1 + 9) with distilled water before assay ; multiply the result by 10.

PROCEDURE

- **Manual Procedure**

Wavelength	340 nm
Cuvette	1 cm light path
Temperature	15-25 ^{0}C or 37 ^{0}C
Zero adjustment	Reagent blank
Specimen	Serum , Plasma or Urine

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	……	10 µ l	……
Specimen	……	……	10 µ l

Mix, wait for 10 minutes at 15 – 25 ^{o}C or 5 minutes at 37 ^{o}C, then measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank within 30 minutes.

Automated Procedure

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

CALCULATION

Serum and plasma :

Phosphorus conc. (mg/dl)=

$$\frac{\text{Absorbance of Specime}}{\text{Absorbance of Standard}} \times 5$$

Urine :

Phosphorus conc. (mg/dl)=

$$\frac{\text{Absorbance of Specime}}{\text{Absorbance of Standard}} \times 5 \times 10$$

Note: For turbid highly icteric sera , prepare a seum blank by adding 10 µl serum to 1 ml saline into a labeled test tube. Read absorbance of serum blank at 340 nm vs water and subtract from test absorbance before calculating results.

LINEARITY

When run as recommended, the assay is linear up to 20 mg/dl.

If result exceeds 20 mg/dl, specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

SENSITIVITY

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

When run as recommended the sensitivity of this assay is 1 mg/dL.

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 NS Biotec Technical Services.

INTERFERING SUBSTANCES

- **Haemoglobin:**
  Avoid haemolysis since RBCs contain very high levels of inorganic phosphate
- **Icterus:**No significant interference up to abilirubin level of 30mg/dL.
- **Lipemia:** No significant interference**.**
- **Anticoagulants:** EDTA, citrate and fluoride interfere with the test .

WARNING & PRECAUTIONS

- NS Biotec Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
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BIBLIOGRAPHY

1. **Daly JA, Ertingshausen G: Direct method for determination of inorganic phosphate in serum with the centerifichem. Clin Chem 18:263, 1972.**
2. **Frankel S:Electrolytes. In: Gradwhol's clinical laboratory methods and diagnosis, 6 th ed. S Frankel,S Reitman, Editors, Mosby, St. louis (MO), 1963, p 188,1963 .**

	Consult Instruction for Use
	Caution Consult Accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative In The European Community
REF	Catalogue Number
LOT	Batch Code
	Use By

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