Phosphorus, Inorganic

Intended Use
Spectrum Diagnostics phosphorus reagent is intended for the in-vitro quantitative, diagnostic determination of phosphorus in human serum and urine on both automated and manual systems.

Background
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 hypophosphatemia. Reduced serum phosphate levels are seen in vitamin D deficiency, rickets, hyperparathyroidism, and Fanconi’s syndrome.

Method
UV – phosphomolybdate method.

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

Phosphate + Ammonium molybdate $\xrightarrow{H_2SO_4} \text{H}_2\text{PO}_4^{-} + \text{phosphomolybdate}$

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

Reagents

| Standard phosphorus (ST) | 5 mg/dl | 1. 61 mmol/L |
| Reagent (R) amm. molybdate | 3.5 mmol/L |
| Sulphuric acid | 750 mmol/L |
| Surfactants | 1% |
(C)-Corrosive contains caustic materials.

R35 Causes severe burns.
R41 Risk of serious damage to eyes.
S28 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

For further information, refer to the Phosphorus, Inorganic reagent material safety data sheet.

Precautions and Warnings
Do not ingest or inhale. 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.

Reagent Storage and Stability
Reagents are supplied ready-to-use and stable until expiration date stated on label when stored refrigerated at 2 - 8 °C.

Deterioration
If absorbance of reagent measured at 340 nm against distilled water as reference is greater than 0.5, it should be discarded.

Specimen Collection and Preservation

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 °C; 4 days at 4 – 8 °C;
1 year at -20 °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 °C; 6 months at 2 – 8 °C;
Urine samples should be diluted 1 : 10 (1 + 9 ) with distilled water before assay; multiply the result by 10.

System Parameters

Wavelength: 340 nm
Optical path: 1 cm
Assay type: End-point
Direction: Increase
Sample : Reagent Ratio: 1 : 100
Temperature: 15 – 25 °C or 37 °C
Zero adjustment: Reagent blank
Incubation time: 10 minutes at 15 – 25 °C or 5 minutes at 37 °C
Reagent Blank Limits: Low 0.00 AU
High 0.5 AU
Sensitivity: 1 mg/dL
Linearity: 20 mg/dL (6.4 mmol/L)

Procedure

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10 μl</td>
<td>-----</td>
</tr>
<tr>
<td>Standard</td>
<td>10 μl</td>
<td>-----</td>
</tr>
<tr>
<td>Sample</td>
<td>10 μl</td>
<td>-----</td>
</tr>
</tbody>
</table>

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

Calculation

Serum phosphorus conc. (mg/dl) = $A_{\text{specimen}}$ / $A_{\text{standard}}$ x 5

Urine phosphorus conc. (mg/dl) = $A_{\text{specimen}}$ / $A_{\text{standard}}$ x 5 x 10

Note: For turbid highly icteric sera, prepare a ream blank by adding 10 ml 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.

Quality Control
Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision: Within run (Repeatability)
<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>5.49</td>
<td>8.92</td>
</tr>
<tr>
<td>SD</td>
<td>0.21</td>
<td>0.128</td>
</tr>
<tr>
<td>CV%</td>
<td>3.83</td>
<td>1.43</td>
</tr>
</tbody>
</table>

**Run to run (Reproducibility)**

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>5.61</td>
<td>9.1</td>
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<tr>
<td>SD</td>
<td>0.29</td>
<td>0.133</td>
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<tr>
<td>CV%</td>
<td>3.97</td>
<td>1.5</td>
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</table>

**Methods Comparison**

A comparison between Spectrum Diagnostics Phosphorus reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.947 was obtained.

**Sensitivity**

When run as recommended, the minimum detection limit of this assay is 1 mg/dL.

**Linearity**

The reaction is linear up to phosphorus concentration of 20 mg/dL. Specimens showing higher concentration should be diluted 1+4 using physiological saline and repeat the assay (result \( \times 5 \)).

**Interfering Substances**

(Serum, plasma)

- **Haemolysis**
  Avoid haemolysis since RBCs contain very high levels of inorganic phosphate.

- **Icterus**
  No significant interference up to a bilirubin level of 30 mg/dL.

- **Lipemia**
  No significant interference.

- **Anticoagulants**
  EDTA, citrate and fluoride interfere with the test.

**Expected Values**

- **Serum (fasting)**
  - Adults: 2.7 – 4.5 mg/dL (0.87 – 1.45 mmol/L)
  - Children < 12 years: 4.5 – 5.5 mg/dL (1.45 – 1.78 mmol/L)
  - Children < 1 year: 4.5 – 6.7 mg/dL (1.45 – 2.16 mmol/L)

- **Neonates**
  5.0 – 9.6 mg/dL (1.65 – 3.10 mmol/L)

- **Urine (24 hrs)**
  0.3 – 1.0 g/24 hrs (11 – 32 mmol / day)

**Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.**

**Analytical Range**

1 – 20 mg/dL.

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**Waste Disposal**

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

**SS6**: dispose of this material and its container at hazardous or special waste collection point.

**SS7**: use appropriate container to avoid environmental contamination.

**SS61**: avoid release in environment. Refer to special instructions/safety data sheets.

**References**


**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
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<tbody>
<tr>
<td>294 001</td>
<td>4 x 25 ml</td>
</tr>
<tr>
<td>294 002</td>
<td>2 x 100 ml</td>
</tr>
<tr>
<td>294 003</td>
<td>4 x 100 ml</td>
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</table>

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IFUFCC34 Rev.(3), 1/1/2007