**Intended Use**
For the quantitative determination of Inorganic Phosphorus in serum using the Pointe c2000 and Mindray BS-200 analyzers.

**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, phenylhydrazine, aminonaphtholsulfonic acid, ascorbic acid, p-methylnaphtholsulfate, N-phenyl-p-phenylenediamine, and ferrous sulfate. These methods suffered from color instability, deproteinization steps and complexity of performance. 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. Daly and Ertingshausen adapted that technique for the determination of inorganic phosphorus in 1972. Amador and Urban 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 + H₂SO₄ + 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). The reagent is stable until the expiration date appearing on the label when stored as directed.

**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.
3. Hemolyzed sample may give falsely high values.
4. Serum should be removed from the red cell clot as soon as possible.
5. Serum inorganic phosphorus is stable for one week refrigerated and for three weeks frozen.

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

**Materials Provided**
Inorganic Phosphorus Reagent

**Materials Required but not Provided**
1. Analyzer
2. Operation Manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

**Test Parameters**

<table>
<thead>
<tr>
<th>Test</th>
<th>PHOS</th>
<th>R1:</th>
<th>R2:</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.:</td>
<td>029</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Full Name:</td>
<td>Phosphorus</td>
<td>Sample Volume:</td>
<td>3</td>
</tr>
<tr>
<td>Standard No.:</td>
<td></td>
<td>R1 Blank:</td>
<td></td>
</tr>
<tr>
<td>Reac. Type:</td>
<td>Endpoint</td>
<td>Mixed Rgt. Blank:</td>
<td></td>
</tr>
<tr>
<td>Pht. Wave:</td>
<td>340nm</td>
<td>Linearity Range:</td>
<td>0.0 – 12.0</td>
</tr>
<tr>
<td>Sec. Wave:</td>
<td>Linearity Limit:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direction:</td>
<td>Increase</td>
<td>Substrate Limit:</td>
<td></td>
</tr>
<tr>
<td>Reac. Time:</td>
<td>0 / 8</td>
<td>Factor:</td>
<td></td>
</tr>
<tr>
<td>Incuba. Time:</td>
<td>0</td>
<td>Compensate: Slope 1.0</td>
<td>Intercept: 0</td>
</tr>
<tr>
<td>Unit:</td>
<td>mg/dl</td>
<td>q1:</td>
<td>q2:</td>
</tr>
<tr>
<td>PC:</td>
<td>Abs:</td>
<td>q3:</td>
<td>q4:</td>
</tr>
<tr>
<td>Precision:</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Calibration Parameters**

<table>
<thead>
<tr>
<th>Rule:</th>
<th>Two-point linear</th>
<th>Calibrator 1: Deionized Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity:</td>
<td>Calibrator 2: Chem Cal</td>
<td></td>
</tr>
<tr>
<td>Replicates:</td>
<td>2</td>
<td>Calibrator 3:</td>
</tr>
<tr>
<td>Interval (day):</td>
<td></td>
<td>Calibrator 4:</td>
</tr>
<tr>
<td>Difference Limit:</td>
<td></td>
<td>Calibrator 5:</td>
</tr>
<tr>
<td>SD:</td>
<td></td>
<td>Calibrator 6:</td>
</tr>
<tr>
<td>Blank Response:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error Limit:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient:</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Calibration**
Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer’s calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

**Quality Control**
The integrity of the reaction should be monitored by use of normal and abnormal control sera with known concentrations of inorganic phosphorus. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.
Calculation (Example)

Abs. = Absorbance

Abs. of Unknown x Concentration of = Inorganic Phosphorus (mg/dl)

Abs. of Standard

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

Then: 0.20 x 5 = 3.4 mg/dl

0.29

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: 2.5-4.8 mg/dl

Children: 4.0-7.0 mg/dl

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

Performance

1. Linearity: 12 mg/dl

2. Comparison: A study was performed between the Pointe c2000 / Mindray BS-200 and a similar analyzer and method, resulting in a correlation coefficient of 0.994 with a regression equation of

   \[ y = 0.902x + 0.07 \] (N=37).

3. Precision: Precision studies were performed using the Pointe c2000 / Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.

<table>
<thead>
<tr>
<th>Within Run</th>
<th>Run to Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>3.21</td>
<td>0.12</td>
</tr>
<tr>
<td>7.17</td>
<td>0.21</td>
</tr>
</tbody>
</table>

References