INORGANIC 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.

BACKGROUND
80% of the phosphorus contained in the body is localized in bone in the form of calcium phosphate as the apatite Ca₁₀(PO₄)₆(OH)₂. 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 phosphorus 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 phosphorus causes a decrease in the calcium level. The mechanism is influenced by interaction between parathormone and vitamin D. Hypophosphatemia, vitamin D intoxication and renal failure with decreased glomerular phosphate filtration give rise to hyperphosphatemia. Hypophosphatemia occurs in rickets, hyperparathyroidism and Fanconi's syndrome.

METHOD
One step colorimetric endpoint method without deproteinization.

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, ferrous sulfate, aminonaphtholsulfonic acid, ascorbic acid, N-phenyl-p-phenylenediamine and ferrous sulfate. These methods suffered from color instability, deproteinization steps and complexity of performance. 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.

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

<table>
<thead>
<tr>
<th>Specimen</th>
<th>IN</th>
<th>OUT</th>
<th>REPORTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (fasting)</td>
<td>4.5 – 6.7 mg/dl</td>
<td>1.45 – 2.16 mmol/l</td>
<td>2.5 – 5.0 mg/dl</td>
</tr>
<tr>
<td>Children (&lt; 2 years)</td>
<td>4.5 – 5.5 mg/dl</td>
<td>1.45 – 1.78 mmol/l</td>
<td>(0.87 – 1.45 mmol/l)</td>
</tr>
<tr>
<td>Children (2-12 years)</td>
<td>2.5 – 5.0 mg/dl</td>
<td>1.45 – 1.78 mmol/l</td>
<td>(0.87 – 1.45 mmol/l)</td>
</tr>
<tr>
<td>Adults (&gt;12 years)</td>
<td>0.4 – 1.3 g/day</td>
<td>(12.9 – 42 mmol/day)</td>
<td></td>
</tr>
</tbody>
</table>

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

**REAGENTS**

R₁: Phosphorus standard 1.0 ml
Sulfuric acid 1.0 ml
Ferrous ammonium sulfate 0.1 ml
Ferrous nitrate 0.1 ml
Ammonium molybdate 0.1 ml

R₂: Reagent preparation & Stability

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

**SPECIMEN**

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

**PROCEDURE**

- 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 acidic urine specimens is stable if stored at room temperature, refrigerated or frozen.
- Stored urine specimens must be mixed well and diluted 1:10 in distilled water prior analysis.

**CALCULATION**

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

\[
\text{Serum inorganic phosphorus Conc.} = \frac{A_{\text{specimen}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times \text{Standard value}
\]

Where:

- \(A_{\text{specimen}}\) = absorbance of specimen
- \(A_{\text{blank}}\) = absorbance of blank
- \(A_{\text{standard}}\) = absorbance of standard

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

\[
\text{Urine inorganic phosphorus Concentration mg/24 hrs} = \frac{A_{\text{specimen}}}{\text{Absorbance of Standard}} \times 5 \times 10^3 \times d \times V
\]

Where:

- \(A_{\text{specimen}}\) = absorbance of specimen
- \(A_{\text{standard}}\) = absorbance of standard
- \(d\) = dilution factor
- \(V\) = volume of specimen

**MAINTENANCE**

- The only acceptable anticoagulant is heparin.
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- The only acceptable anticoagulant is heparin.

**ASSAY PRINCIPLE**

This complex is reduced by ferrous ammonium sulfate to produce a molybdenum blue complex.

**SPECIMEN PREPARATION & STABILITY**

- Serum or plasma
  - Fresh serum collected in the fasting state is the preferred specimen as 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.
  - Avoid the leakage of inorganic phosphorus and phosphate esters into the plasma media.
  - Inorganic phosphorus in serum is stable 7 days refrigerated at 4°C and for three weeks when frozen.

- Urine
  - Specimen
  - Blank
  - Standard
  - Specimen

- specimen
- blank
- standard
- specimen

- R₂
- Blank
- 1.0 ml
- 1.0 ml
- 1.0 ml

- Standard
- 1.0 ml
- 1.0 ml

- Specimen
- 1.0 ml
- 1.0 ml

- Mix and incubate for 15 minutes at room temperature. Measure the absorbance of specimen (A_{\text{specimen}}) and standard (A_{\text{standard}}) against reagent blank.

- The color is stable for 60 minutes.

- Automated Procedure

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

- Date of Revision : 10/2012
- Article #: 133-EN
(10°) converts mg/dl to mg/l. (d) dilution factor (10)
(V) the 24 hours urine values in liter.

**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 et al. in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.

**WARNING & PRECAUTION**

- **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:

<table>
<thead>
<tr>
<th></th>
<th>Within Run</th>
<th>Between Day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>Level I</td>
<td>Level II</td>
</tr>
<tr>
<td>Number of samples</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Mean (mg/dl)</td>
<td>3.6</td>
<td>6.43</td>
</tr>
<tr>
<td>SD (mg/dl)</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.9</td>
<td>2.9</td>
</tr>
</tbody>
</table>

**Method Comparison**

Comparison studies were carried 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:

- 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.

**BIBLIOGRAPHY**


**SYMBOL DECLARATION**

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

**ORDERING INFORMATION**

<table>
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<tr>
<th>REF</th>
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<tbody>
<tr>
<td>1341</td>
<td>2 X 50 ml</td>
</tr>
<tr>
<td>1342</td>
<td>2 X 100 ml</td>
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</tbody>
</table>

**Technical Support:**

Technical Support:
+202 26439699
Order: +202 26439698
orders: order@vitroscient.com
info@vitroscient.com

**Vitro Scientific**

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**Published on:**

Technical Support:
+202 26439699
info@vitroscient.com

**Manufactured in Egypt by:**

Vitro Scientific

**Number of sample pairs:**

40

**Range of sample results:**

20.5 - 79.5 mg/dl

Mean of reference method results 3.8 mg/dl

**Date of Revision:**

10/2012

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