**Intended Use**
For IN VITRO quantitative determination of inorganic phosphorus in serum or plasma using manual or automated applications.

**Clinical Significance**
Measurements of IP are primarily used for diagnosing hypervitaminosis D, hypoparathyroidism, renal failure, rickets and Fanconi syndrome, as well as for monitoring the causes and treatments. (1)

**Method History**
Most of the Inorganic Phosphorus methods presented in the literature are based upon the reduction of a phosphomolybdate complex to molybdenum blue. In 1972 Daly and Ertingshausen (2) found that an unreduced phosphomolybdate complex absorbs ultraviolet light. Acidified ammonium molybdate with a non-ionic surfactant was used to measure serum Inorganic Phosphorus. Daly’s procedure was modified and adapted for continuous flow instruments by Amador and Urban. (3) The Catachem Inorganic Phosphorus procedure is based upon the work of Amador and Urban.

**Method Principle**
Phosphorus from the serum sample mixes with the molybdate to form the phosphomolybdate complex. The increase in absorbance is monitored at 340nm. The reaction scheme illustrates the reaction that occurs in this method.

\[
\text{H}^+ + \text{Phosphorus} + \text{Ammonium Molybdate} \rightarrow \text{Phosphomolybdate Complex}
\]

**Reagent Content**
The concentrations of the active ingredients in the reagents will be approximately as follows:
- Inorganic Phosphorus Reagent:
  - Water: 0.30 mmol/L
  - Ammonium Molybdate: 0.14 mmol/L
- Nonreactive ingredients

**Precautions**
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

**Preparation Of Working Reagent**
Catachem Inorganic Phosphorus Reagent is packaged in ready-to-use form. No preparation is required.

**Reagent Storage And Stability**
Store the Inorganic Phosphorus Reagent at room temperature or refrigerated. When stored as directed, the reagent is stable until the expiration date stated on the label.

**Specimen Collection And Preparation**
Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay.

**Precautions**
Avoid contact of specimens with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

**Quality Control**
To monitor the performance of the working reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

**Interfering Substances**
Various substances have been reported to interfere with the inorganic phosphorus method. (4)

**Expected Values**
The range of expected values determined for this method in humans is 2.5 - 4.5 mg/dL. Values are species specific. It is recommended that each laboratory establish the normal range for the species under test and for the area in which the laboratory is located.

**Procedure**
Important: Read entire procedure instructions before proceeding with assay.

**Materials Required (Not Provided)**
- Spectrophotometer
- Match Cuvettes 1 cm light path
- Timer to time incubation time
- Pipette 2.0 ml for reagent
- Pipette 0.04 ml for sample

**Materials Provided**
- Inorganic Phosphorus Reagent
Analytical Parameters
Wavelength 340 nm
Pathlength 1 cm
Reaction Mode endpoint
Reaction Time 5 minutes
Reagent Volume 1.0 ml
Sample Volume 0.015 ml
Total Volume 1.015 ml
Sample-to-reagent ratio 1:68

Note: To eliminate interferences for lipemic and icteric samples and to maximize accuracy in the assay procedure, all samples should be blank corrected.

Assay Procedures
1. Pipette 1.0 ml of IP reagent into each of three cuvettes marked “Calibrator”, “Sample”, and “Blank”.
2. Pipette 0.015 ml of calibrator or sample into their respective cuvettes. Use 0.015 ml of distilled water for the blank. Mix all cuvettes well.
3. Incubate all cuvettes for 5 minutes at room temperature.
4. Set spectrophotometer wavelength at 340 nm and zero the instrument with blank.
5. Read the “Calibrator” and “Sample” absorbencies.
6. Calculate the IP concentration (mg/dL) in the sample(s), as shown in calculations and results.

Blank Procedure
Follow the Assay Procedure and replace the IP Reagent with 0.9% Sodium Chloride.

Calculations And Results

\[
\text{Sample Absorbance} = \frac{\text{Assay OD} - \text{Blank OD}}{\text{Calibrator Absorbance}}
\]

\[
\text{IP (mg/dL)} = \left( \frac{0.320 - 0.020}{0.250 - 0.015} \right) \times 5 \text{ mg/dL}
\]

\[
= 6.4 \text{ mg/dL}
\]

Method Performance Characteristics
Sensitivity: 0.040-0.051 absorbance units per mg/dL.
Linear Range: 0-10 mg/dL.
Precision: Within-run and day-to-day precision is summarized below.

<table>
<thead>
<tr>
<th>I. P.</th>
<th>Within-Run Precision</th>
<th>Total Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>1.7</td>
<td>1.6</td>
<td>0.11</td>
</tr>
<tr>
<td>5.2</td>
<td>0.16</td>
<td>3.0</td>
</tr>
<tr>
<td>9.0</td>
<td>0.16</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Correlation
A comparison of this method using an automated analyzer and reference method based upon the molybdate reaction resulted in the following regression statistics:

\[
\text{Range} = 1.6-7.3 \text{ mg/dL} \\
N = 196 \\
Y = 0.981x + 0.08 \\
r = 0.990 \\
Sy.x = 0.15
\]

References