INTENDED USE
For the quantitative in vitro determination of Inorganic Phosphorous in serum and urine. This product is suitable for manual use.

Cat. No.  
PH 1016  
300 ml  
R1a. Blank Reagent 210 ml  
R1b. Molybdate 90 ml  
CAL. Standard 1 x 5.5 ml

CLINICAL SIGNIFICANCE
The human body contains approximately one kilogram of phosphorous. The calcium phosphate salts which comprise the inorganic substance of bone account for approximately 80% of the total phosphorous content. The remainder is distributed throughout other cells of the body primarily as organic phosphorous in phospholipids and phosphoproteins. In serum most inorganic phosphorous exists in a free form with approximately 15% bound to protein. Abnormal serum phosphates are most commonly seen in kidney, bone and parathyroid diseases. Phosphate is usually measured in conjunction with serum calcium since each measurement is useful in the interpretation of the other.

ASSAY PRINCIPLE
Inorganic phosphorous reacts with ammonium molybdate in the presence of sulphuric acid to form a phosphomolybdate complex which is measured at 340 nm.

SAMPLE COLLECTION AND PREPARATION
Serum is the recommended sample. Serum stable for 5 days when stored at +2 to 8°C. Stable for 3 months if stored frozen at -20°C. Avoid haemolytic samples as haemolysis interferes with the assay. Urine: 24 hour urine should be collected in an acid washed, detergent free bottle. Acidify after collection to pH<3.0. Dilute urine 1+19 with 0.9% NaCl solution. Multiply the result by 20.

REAGENT COMPOSITION

<table>
<thead>
<tr>
<th>Contents</th>
<th>Initial Concentration of Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1a. Blank Reagent</td>
<td>Sulphuric acid 0.36 mol/l</td>
</tr>
<tr>
<td></td>
<td>Sodium chloride 154 mmol/l</td>
</tr>
<tr>
<td>R1b. Molybdate Reagent</td>
<td>Ammonium molybdate 3.5 mmol/l</td>
</tr>
<tr>
<td></td>
<td>Sodium chloride 154 mmol/l</td>
</tr>
<tr>
<td>CAL. Standard</td>
<td>Potassium Phosphate See lot specific insert</td>
</tr>
</tbody>
</table>

SAFETY PRECAUTIONS AND WARNINGS
For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solutions R1a and R1b contain sulphuric acid. Avoid ingestion or contact with skin or mucous membranes.

The CAL contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety data sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS
All reagents are ready for use. Stable until expiry date when stored at +15 to +25°C.

STABILITY AND PREPARATION OF WORKING REAGENT
Mix one bottle of Blank Reagent R1a with one bottle of Molybdate Reagent R1b (300 ml of working reagent). For smaller volumes prepare working reagent according to the following table:

<table>
<thead>
<tr>
<th>Blank Reagent (R1a)</th>
<th>Molybdate Reagent (R1b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0 ml</td>
<td>3.0 ml</td>
</tr>
<tr>
<td>14.0 ml</td>
<td>6.0 ml</td>
</tr>
<tr>
<td>28.0 ml</td>
<td>12.0 ml</td>
</tr>
</tbody>
</table>

Stable for 8 weeks at +15 to +25°C.

MATERIALS PROVIDED
Blank Reagent  
Molybdate  
Standard

MATERIALS REQUIRED BUT NOT PROVIDED
Pipetting devices capable of delivering 10 µl and 1 ml. Timing device and water bath or heating block to maintain temperature at 20 - 25°C or 37°C. Spectrophotometer with wavelength capacity of 340 nm. Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532).

PROCEDURE
Wavelength: 340 nm  
Cuvette: 1 cm light path  
Temperature: 20-25°C/37°C  
Measurement: against reagent blank

Mix, incubate for 10 min at 20-25°C or 5 min at 37°C. Measure absorbance of the sample (A_sample) and standard (A_standard) against reagent blank.

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

Measure absorbance of the sample (A_sample) and standard (A_standard) against reagent blank.
NOTE
Icteric and slightly lipaemic samples require a sample blank to be carried out. Using the same pipetting scheme, mix 10 µl sample with 1000 µl of Blank Reagent. The absorbance of the Sample Blank (A_{sample blank}) is then subtracted from the absorbance of the Sample (A_{sample}).

CALCULATION
Inorganic phosphorus conc.

\[ \text{conc. of standard} \times \frac{A_{sample}}{A_{standard}} \]

QUALITY CONTROL
Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:
1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Support, Northern Ireland (028) 94422413.

INTERFERENCE
The following analytes were tested up to the following levels and were found not to interfere:
- Bilirubin 500 µmol/l
- Intralipid® 0.4%
- Triglycerides 4.05 mmol/l
- Haemoglobin 7 g/l

NORMAL VALUES\(^{(2,3)}\)

<table>
<thead>
<tr>
<th></th>
<th>Serum:</th>
<th>24 hour Urine:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.87 - 1.45 mmol/l (2.68 - 4.5 mg/dl)</td>
<td>12.9 - 42.0 mmol/d (0.4 - 1.3 g/d) Non restricted diet</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS
The following performance data were obtained using a Cobas Mira analyser at 37°C. Performance data and application sheets for other automated analysers are available on request from Randox Laboratories.

SENSITIVITY
The minimum detectable level has been determined as 0.325 mmol/l.

LINEARITY
The method is linear to 6.5 mmol/l (20 mg/dl). Samples with higher concentration should be diluted 1 + 4 with 0.9% (w/v) NaCl solution and reassayed, multiplying the result by 5.

METHOD COMPARISON (Serum)
The Randox method (Y) was compared to another commercially available test kit (X). Forty patient samples spanning the range 0.87 to 6.94 mmol/l were tested. Linear regression analysis of the data resulted in the following equation:

\[ Y = 0.996 \times X + 0.04 \] with a correlation coefficient \( r = 0.999 \)

REFERENCES