Phosphorus 5 mg/dL (1.61 mmol/L)

hyperparathyroidism…

An adult human body contains approximately 600 g of phosphates expressed as phosphorus, of which about 85% is bound to calcium in bones and the rest principally in other tissue cells. Most phosphate present in cells is organic and incorporated into phospholipids, nucleic acids, and high energy compounds. Serum/plasma contains approximately 1% of total phosphate as inorganic phosphate, the fraction measured in routine biochemical analysis.

An elevation of phosphorus in serum/plasma is often associated with bone diseases, renal failures, hypoparathyroidism, hypervitaminosis D…

Decreased serum/plasma phosphorus concentrations are found in case of osteomalacia, vitamin D deficiency, primary hyperparathyroidism…

Reagents are ready for use.

CLINICAL SIGNIFICANCE (1) (2)

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PRINCIPLE (4) (5)

Method without deproteinisation described by Daly and al. modified by Gamst O.K. and Try K.

In an acid medium, phosphate ions form a phosphomolybdic complex with the ammonium molybdate. The absorbance measured at 340 nm is proportional to the concentration of phosphate ions in the specimen.

REAGENTS COMPOSITION

Vial R1  MOLYBDATE REAGENT

Ammonium Molybdate 0.63 mmol/L
Sulfuric acid 210 mmol/L
Surfactant

XL, R36/38: Irritating to eyes and skin
S36/37/39: Wear suitable protective clothing, gloves and eye-face protection
S26/07/2011

STABILITY AND STORAGE

Store at 18-25°C in well recapped original vial and away from light.

• Standard (vial R2): transfer requested quantity, well recap the vial and store at 18-25°C.

• Stored and used as described, reagents (vial R1 and R2) are stable, when free from contamination, until expiry date stated on the label of the kit.

Discard any reagent if cloudy or if absorbance at 340 nm > 0.500.

Don’t use reagents after expiry date stated on the label of the kit.

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum or plasma:
Separate from erythrocytes within 1 hour after collection.

• Phosphorus is stable in serum for:
  ✓ several days at 2-8°C.
  ✓ several months at –15°C.

24h Urines:
Collect in acid washed, detergent free container. Acidified urines (pH < 3 with concentrated hydrochloric acid) should be diluted (1+9) in demineralised water free from phosphorus before performing the assay.

• Phosphorus is stable in acidified urines for:
  ✓ 6 months.

INTERFERENCES (3)

1. To avoid contamination with environmental phosphorus, it is recommended to use disposal glass or plastic ware, carefully cleaned material with hydrochloric acid 0.1 N and well rinsed with distilled water.

2. Gross hemolysis, lipemia or icterus may cause falsely elevated results unless a specimen blank is used.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

CALIBRATION (6)

• Standard (vial R2) provided in the kit or BIOLABO Multicalibrator REF 95015 traceable to SRM 3186a.

• Or any calibrator traceable to reference method or material.

The calibration frequency depends on proper instrument functions and on preservation of the reagent.

It is recommended to calibrate in the following cases:
1. When changing batch of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.
QUALITY CONTROL
- BIOLABO EXATROL-N Level I  REF 95010.
- BIOLABO EXATROL-P Level II  REF 95011.
- Other assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:
1. At least once a run.
2. At least once within 24 hours.
3. When changing vial of reagent.
4. After maintenance operations on the instrument.
5. If control is out of range, apply following actions:
   1. Repeat the test with the same control.
   2. If control is still out of range, prepare a fresh control serum and repeat the test.
   3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
   4. If control is still out of range, calibrate with a new vial of reagent.
   5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES

<table>
<thead>
<tr>
<th>In serum or plasma</th>
<th>Phosphorus (mg/dL)</th>
<th>(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In cord</td>
<td>3.7-8.1</td>
<td>[1.20-2.62]</td>
</tr>
<tr>
<td>Premature</td>
<td>5.4-10.9</td>
<td>[1.74-3.52]</td>
</tr>
<tr>
<td>0-10 days</td>
<td>4.5-9.0</td>
<td>[1.45-2.91]</td>
</tr>
<tr>
<td>10 days-24 months</td>
<td>4.5-6.7</td>
<td>[1.45-2.16]</td>
</tr>
<tr>
<td>24 months-12 years</td>
<td>4.5-5.5</td>
<td>[1.45-1.78]</td>
</tr>
<tr>
<td>12-60 years</td>
<td>2.7-4.5</td>
<td>[0.87-1.45]</td>
</tr>
<tr>
<td>&gt; 60 years, male</td>
<td>2.3-3.7</td>
<td>[0.74-1.20]</td>
</tr>
<tr>
<td>&gt; 60 years, female</td>
<td>2.8-4.1</td>
<td>[0.90-1.32]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In 24 h Urines</th>
<th>Phosphorus (g/24 h)</th>
<th>(mmol/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant daily diet (*)</td>
<td>&lt; 1</td>
<td>[&lt; 32.3]</td>
</tr>
<tr>
<td>Non-restricted diet</td>
<td>0.4-1.3</td>
<td>[12.9-42.0]</td>
</tr>
</tbody>
</table>

Each laboratory should establish its own normal range for the population that it serves.
(*)Constant daily diet: 0.9 at 1.5 g P (29-48 mmol P) and 10 mg Ca/Kg (0.25 mmol Ca/Kg).

PERFORMANCES CHARACTERISTICS

<table>
<thead>
<tr>
<th>Between run</th>
<th>Medium level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean mg/dL</td>
<td>3.62</td>
<td>7.53</td>
</tr>
<tr>
<td>S.D. mg/dL</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>C.V. %</td>
<td>3.3</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Detection limit: approximately 0.7 mg/dL.

Sensitivity for 5 mg/dL: approximately 0.500 Abs at 340 nm.

Comparison study with commercially available method:
y = 0.990 x + 0.0504  r = 0.9948

LINEARITY
The assay is linear up to 10 mg/dL (3.22 mmol/L).
Above, dilute specimen with demineralised water free from phosphorus and reassay taking into account dilution factor to calculate the result.

LINEARITY LIMIT DEPENDS ON SPECIMEN/REAGENT RATIO.

MANUAL PROCEDURE

<table>
<thead>
<tr>
<th>Pipette into well identified test tubes:</th>
<th>Blank</th>
<th>Specimen blank</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
<td></td>
</tr>
<tr>
<td>Saline Solution</td>
<td></td>
<td>1 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demineralised water</td>
<td>20 µL</td>
<td>20 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td>20 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen</td>
<td>20 µL</td>
<td>20 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix well. Incubate for 2 minutes at room temperature. Use a 1 cm path length cuvette and read Standard and assays absorbance at 340 nm (334-366) against Reagent blank.

Read Specimen blank against saline solution.

Notes:
- Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.
- Specimen blanking: for cloudy or icteric serum and for bovine control sera and calibrator which are usually very coloured.
- Target values of control sera and calibrator may have been obtained with or without specimen blanking.

CALCULATION

Calculate the result as follows:

Serum or plasma:
Result = \( \frac{\text{Abs (assay)} - \text{Abs (Specimen blank)}}{\text{Abs (Standard)}} \times \text{Standard concentration} \)

Urine diluted 1+9: Multiply the result by 10 (dilution factor).

REFERENCES
(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-456 to 3-462
(6) SRM: Standard Reference Material®