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INORGANIC PHOSPHORUS REAGENT SET (UV METHOD)

INTENDED USE
Inorganic phosphorus (UV method) reagent is for the quantitative determination of inorganic phosphorus in human serum.

INTRODUCTION
The majority of the body's phosphorus is found in the bone as hydroxyapatite. The remaining phosphate is present as inorganic phosphate and phosphate esters. Phosphorus is involved in the intermediary metabolism of carbohydrates and is a component of other physiologically important substances. Thus, increased serum phosphorus may occur in hypervitaminosis, hypoparathyroidism, and renal failure. Reduced serum phosphorus levels are seen in rickets (vitamin D deficiency) hyperparathyroidism, and Fanconi's syndrome[1].

The determination of inorganic phosphorus has been based on the reaction of molybdate with phosphate to produce the phosphomolybdenum blue complex, which is measured photometrically. However, many of the components in these reagents are unstable and had to be stored separately[2]. Unreduced phosphomolybdate complex is measured directly in UV range 340 nm in the present method.

PRINCIPLE
Inorganic Phosphorus + H_2SO_4 + Ammonium Molybdate →
Unreduced Phosphomolydate Complex.

Inorganic phosphorus reacts with ammonium molybdate in an acid medium to form a phosphomolybdate complex, which absorbs light at 340 nm. The absorbance at this wavelength is directly proportional to the amount of inorganic phosphorus present in the sample.

REAGENTS
Inorganic Phosphorus Reagent: Ammonium Molybdate 0.4 mM, Sulfuric Acid 210 mM with surfactant.
Inorganic Phosphorus Standard: (5.0 mg/dl) Potassium Phosphate in dilute acid with an albumin base.

PRECAUTIONS
1. The reagents are for "In Vitro" diagnostic use only.
2. Do not pipette by mouth. Avoid contact of reagents with skin, eyes and clothing.

REAGENT PREPARATION
Reagent comes in a ready to use form.

REAGENT STORAGE
Store reagent and standard at refrigerator temperature (2 - 8°C).

REAGENT DETERIORATION
Do not use if:
1. Reagent without serum added has an absorbance greater than 0.500 at 340 nm.
2. The reagent fails to recover stated control values.

SPECIMEN COLLECTION AND STORAGE
1. Use only clear, unhemolyzed serum, separated from the erythrocytes as soon as possible. Erythrocytes contain organic phosphates that can hydrolyze on standing or can be enzymatically cleaved by phosphatases. Inorganic phosphates can then leak through the cell walls, increasing the concentration.
2. Once the serum has been separated, the phosphate content will not change for at least a week when stored in the refrigerator (2-8°C).[6]

INTERFERENCES
For a comprehensive list of substances that interfere with the Measurements of Inorganic Phosphorus see Young , et al.

MATERIALS PROVIDED
1. Inorganic Phosphorus reagent.
2. Inorganic Phosphorus standard.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate pipetting devices.
2. Test tubes/rack.
3. Timing device.
4. Spectrophotometer with ability to read at 340 nm.

PROCEDURE (AUTOMATED)
See appropriate instrument application instructions.

PROCEDURE (MANUAL)
1. Label test tubes Blank, Standard, Control, Patient, etc.
2. Pipette 1.0 ml of reagent into each tube. Allow to come to room temperature (25°C).
3. Add 0.02 ml (20 ul) sample to respective tubes, mix, and allow to stand for five (5) minutes at room temperature.
4. Zero spectrophotometer with distilled water at 340 nm.
5. Read and record absorbencies of all tubes.

* TC - MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

PROCEDURE NOTES
1. For spectrophotometers requiring a larger total volume for accurate reading, a 3.0 ml reagent to 0.1 ml (100 ul) sample ratio may be used.
2. Lipemic and icteric samples require a serum blank. For maximum accuracy a serum blank should be run with each sample.
   a. Add sample to Saline solution.
   b. Zero spectrophotometer at 340 nm with Saline solution.
   c. Read and record absorbencies of serum blanks.
   d. Subtract absorbencies from test absorbencies.
3. Samples with values exceeding 12.0 mg/dl must be diluted 1:1 with saline, re-run, and result multiplied by two (2).

CALCULATIONS
Abs. = Absorbance

Abs. of Unknown - Abs. of reagent Blank	× Conc.	= Inorg. Phos.
Abs. of Standard - Abs. of reagent Blank	of Std	(mg/dl)

Example:
Abs. of reagent Blank = 0.536
Abs. of Unknown = 0.918
Abs. of Standard = 1.012
Conc. of Standard = 5 mg/dl

$\frac{0.918 - 0.536}{1.012 - 0.536} \times 5 = \frac{0.382}{0.476} \times 5 = 4.0$

To obtain results in SI units (mmol/L), multiply the result in mg/dl by the factor 0.323

Example: 4.0 mg/dl × 0.323 = 1.296 mmol/L

LIMITATIONS
Most commonly employed detergent and disposable wipes used in the laboratory contain phosphates, and the use of improperly rinsed glassware may result in elevated inorganic phosphorus values.

CALIBRATION
It is not necessary to determine a standard curve since the reaction is linear in a range up to 12 mg/dl. However, a reagent blank and standard should be employed with each set of unknown assayed.

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with established inorganic phosphorus values may be routinely used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction or procedural errors.

EXPECTED VALUES[1, 8]
Adults: 2.5 - 4.8 mg/dl

This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values since differences exist between instruments, laboratories and local populations.

PERFORMANCE
1. Linearity: 12 mg/dl
2. Sensitivity: Based on an instrument resolution of A - 0.001, the present procedure has a sensitivity of 0.01 mg/dl.
3. Comparison: A comparison study performed between this method and one based on the same methodology yielded correlation coefficient of 0.99 with a regression equation of y = 1.01x - 0.06.
4. Precision:

   Day-to-Day Precision: Two commercial control sera were assayed for a period of thirty (30) days and the following day to day precision was obtained.

Day-to-Day (N = 21)

Mean (mg/dl)	S.D.	C.V.(%)
3.2	0.2	6.6
7.2	0.3	4.1

Within Run Precision: Two commercial control sera were assayed twenty (20) times and the following within run precision was obtained.

Within Run (N = 21)

Mean (mg/dl)	S.D.	C.V.(%)
3.0	92	7.7
7.4	0.5	6.7

REFERENCES

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