Fosfatemia UV

AA

UV method for the determination of inorganic phosphorus (iP) in serum, plasma or urine

SUMMARY

Phosphorus is found in the body forming part of organic compounds (proteins, lipids, carbohydrates, nucleic acids, etc.) or as inorganic phosphates, performing several functions (energy transport, tissue structure, pH control in body fluids). Bone and muscular tissues contain it as the main constituent and its participation in the nervous tissue formation is remarkable.

Its circulating concentration is regulated, among other factors, by vitamin D levels and endocrine glands. Physiologic variations are observed according to age, dietary habits, physical activity, pregnancy, etc. This balance may be altered by some pathological conditions, producing abnormalities in the circulating phosphorus concentration.

High levels of serum phosphorus are found on hypoparathyroidism, while hyperparathyroidism leads to the opposite situation. Hypervitaminosis D and several renal disorders may also cause hyperphosphatemia, while hypophosphatemia is related to vitamin D deficiencies and defects on phosphorus absorption at renal level.

PRINCIPLE

Inorganic phosphorus (IP) reacts in acid medium with molybdate to form a phosphomolybdic complex spectrophotometrically measured at 340 nm.

PROVIDED REAGENTS

A. Reagent A: 2 mmol/l ammonium molybdate solution in 1% sulfuric acid.

S. Standard*: stabilized phosphate solution equivalent to 4 mg/dl inorganic phosphorus.

NON-PROVIDED REAGENTS

Wiener lab.'s **Calibrador A plus**.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.

The Reagent A is corrosive. H315+H320: Causes skin and eye irritation. H314: Causes severe skin burns and eye damage. P262: Do not get in eyes, on skin, or on clothing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P280 :Wear protective gloves/ protective clothing/eye protection/face protection.

Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable at room temperature until the expiration date shown on the box.

INSTABILITY OR DETERIORATION OF REAGENTS

When spectrophotometer has been set to zero O.D. with distilled water, absorbance reading of Reagent A should not be over 0.500 O.D. Discard otherwise.

SAMPLE

Serum, plasma or urine

a) Collection: obtain serum in the usual way or plasma with EDTA or citrate.

Urine may also be tested. In this case, collect 24 hs urine in a recipient containing 2 ml concentrated hydrochloric acid. Homogenize and measure diuresis. Centrifuge or filter an aliquot and perform dilution 1:10 in distilled water (1 ml urine + 9 ml distilled water). Follow the instructions indicated under PROCEDURE.

b) Additives: if plasma is used as sample, the use of Wiener lab.'s **Anticoagulant W** or **TP** is recommended.

c) Known interfering substances:

- No interferences are observed from bilirubin up to 58 mg/l.

- The lipemic or hemolyzed samples may produce erroneous results. To avoid interference it is recommended to process a Sample Blank.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: separate serum or plasma from red blood cells within 2 hours from collection, since erythrocytes contain labile organic phosphates which may generate falsely increased results.

Inorganic phosphorus is stable in serum for 8 hours at room temperature. If sample is not assayed within this period, it can be refrigerated (2-10ºC) up to 7 days.

The 24 hours urine is stable for 7 days refrigerated (2-10ºC).

REQUIRED MATERIAL (non-provided)

- Spectrophotometer
- Micropipettes and pipettes for measuring stated volumes
- Spectrophotometric square cuvettes
- Watch or timer

** Non-provided with all kit sizes*

ASSAY CONDITIONS
- Wavelength: 340 nm (Hg 334 or 366 nm)
- Reaction temperature: room temperature
- Reaction time: 10 minutes
- Sample volume: 10 ul
- Final reaction volume: 1.01 ml

PROCEDURE
In three cuvettes labeled B (Blank), S (Standard) and U (Unknown), add:

	B	S	U
Standard	-	10 ul	-
Sample	-	-	10 ul
Reagent A	1 ml	1 ml	1 ml

Incubate for 10 minutes at room temperature. Then read in spectrophotometer at 340 nm (Hg 334 or 366 nm), setting instrument to zero O.D. with blank.

STABILITY OF FINAL REACTION
Final reaction is stable for 20 minutes, thus absorbance should be read within that period.

CALCULATIONS
Serum or plasma:

Inorganic phosphorus (Pi) (mg/dl) = U x f

where $f = \frac{4 \text{ mg/dl}}{S}$

Urine:

$$Pi \text{ (g/24 hours)} = \frac{U}{S} \times 0.040 \times 10 \times V = \frac{U}{S} \times 0.4 \times V$$

where:
0.040 g/l = 4 mg/dl = Standard concentration
10 = dilution factor
V = diuresis volume expressed in liters/24 hours

QUALITY CONTROL METHOD
Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known phosphorus concentration. If running urine samples, a urine-based control should be used.

SI SYSTEM UNITS CONVERSION
iP (mg/dl) x 0.323 = iP (mmol/l)

REFERENCE VALUES
Serum or plasma
Adults: 2.5 - 5.6 mg/dl

This range was obtained from samples of 120 individuals from Rosario (Argentina), from both sexes (between 20 and 45 years old), without presenting symptoms of parathyroid, renal or hepatic disease or vitamin D deficiency.

Children: 4.0 - 7.0 mg/dl

Urine
0.3 - 1.0 g/24 hours

However, each laboratory should establish its own references values.

PROCEDURE LIMITATIONS
- See Known interfering substances under SAMPLE.
- The glassware used (even for sample collection) should be free from phosphates. The use of Wiener lab.'s **Noion** is recommended for washing.
- The use of one Sample Blank is recommended with each test replacing the Reagent for saline solution, in order to achieve a better performance of results. This procedure is essential in case of icteric, turbid, lipemic or hemolyzed samples. Sample Blank absorbance must be subtracted from the Unknown absorbance to perform calculations.

PERFORMANCE
The assays were performed in an Express Plus analyzer(*).
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Level	S.D.	C.V.
3.6 mg/dl	± 0.09 mg/dl	2.64 %
7.5 mg/dl	± 0.16 mg/dl	2.18 %

Performing the same assay on different days, the following results were obtained:

Level	S.D.	C.V.
3.5 mg/dl	± 0.11 mg/dl	3.09 %
7.3 mg/dl	± 0.22 mg/dl	2.98 %

b) Recovery: when adding known amounts of inorganic phosphate to different samples, a recovery between 98.4 and 103.5% was obtained.
c) Linearity: reaction is linear up to 16 mg/dl. If higher values of inorganic phosphorus are obtained, sample must be diluted 1:2 with saline solution. Repeat testing multiplying the obtained result by 2.
d) Detection limit: the minimum detectable concentration change will be of 0.11 mg/dl.

PARAMETERS FOR AUTOANALYZERS
For programming instructions check the user manual of the autoanalyzer in use. For calibration use Wiener lab's **Calibrador A plus**, following the autoanalyzer requirements.

WIENER LAB. PROVIDES
- 100 ml (Cat. Nº 1382321).
- 4 x 20 ml (Cat. Nº 1009311).
- 6 x 20 ml (Cat. Nº 1009256).
- 6 x 20 ml (Cat. Nº 1009614).

REFERENCES

- Henry, R.J.; Cannon, D.C.; Winkelman, J.W. - "Clinical Chemistry, Principles and Techniques" - Harper and Row, Publishers, 1974.
- Daly, J. A. and Ertingshausen - Clin. Chem. 18:263, 1972.
- Amador, E. and Urban, J. - Clin. Chem. 18:601, 1972.

(*) TM Ciba Corning Diagnostics

- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

SYMBOLS

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.


This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices


Authorized representative in the European Community


"In vitro" diagnostic medical device


Contains sufficient for <n> tests


Use by


Temperature limitation (store at)


Do not freeze


Biological risks

Volume after reconstitution

Contents

Batch code


Manufactured by:


Harmful


Corrosive / Caustic


Irritant


Consult instructions for use

Calibrator

Control

Positive Control

Negative Control

Catalog number

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