Spectrum For Diagnostic Industries

Phosphorus UV –
Phosphomolybdate method

IVD

Cat. No.	Pack size
215 01 050	(1 x 50 ml) 50 tests
215 02 030	(2 x 30 ml) 60 tests
215 05 030	(5 x 30 ml) 150 tests

Intended Use

Phosphorus reagent is intended for the in-vitro quantitative and diagnostic determination of phosphorus in human serum, plasma or urine on both automated and manual systems.

Introduction

The body of human adult contains approximately 620 g of phosphorus entirely in the form of phosphates distributed equally between extracellular and intracellular compartments. About 85% of extracellular phosphate occurs in inorganic forms as hydroxyapatite. In plasma or serum, most phosphate exist in the inorganic form (Pi); this fraction is present as the mono-and dihydrogen forms, the relative proportions varying with the pH. Intracellularly, phosphate occurs mainly as phospholipids and phosphoproteins; this fraction is termed organic phosphate. Increased serum phosphate levels may occur in renal failure, hypervitaminosis D and hypoparathyroidism. Reduced serum phosphate levels are seen in vtamin D deficiency, rickets, hyperparathyroidism and fanconi's syndrome.

Method

UV – phosphomolybdate method.

Principle

Inorganic phosphate reacts with ammonium molybdate in presence of sulfuric acid to form non-reduced phosphomolybdate.

Phosphate + Ammonium molybdate $\xrightarrow{H_2SO_4}$ Nonreduced phosphomolybdate

The concentration of phoshomolybdate formed is directly proportional to the inorganic phosphate concentration. It is determined by measuring the increase in absorbance at 340 nm.

Reagents

Reagent

Ammonium molybdate	3.5 mmol/L
Sulphuric acid	750 mmol/L
Surfactants	1 %

(C)-Corrosive contains caustic materials.

R35 Causes severe burns.
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.

Standard
5 mg/dL 1.61 mmol/L

Reagents preparation, storage and stability

Reagent is supplied ready-to-use and stable till the expiration date stated on label when stored refrigerated at 2 - 8 °C.
Once opened, the reagent and the standard are stable for 3 months at specified temperature.

Deterioration

If absorbance of reagent measured at 340 nm against distilled water as reference is greater than 0.5, it should be discarded.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Specimen collection and preservation

Serum and plasma
Serum specimens collected from fasting patients are the preferable specimens since meals lower inorganic phosphate level. Heparin is the only acceptable anticoagulant. Serum and plasma should be separated from erythrocytes as soon as possible .

Stability: 1 day at 15 – 25 °C ; 4 days at 4 – 8 °C;
1 year at -20 °C

Urine
Urine specimens should be collected in an acid-washed, detergent-free container. Acidify with hydrochloric acid after collection (pH<3).
Stability : 2 days at 15 – 25 °C ; 6 months at 2 – 8 °C;
Urine samples should be diluted 1 : 10 (1 + 9) with distilled water before assay ; multiply the result by 10.

Procedure

Wavelength	340 nm
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample : Reagent Ratio	1 : 100
e.g.: Reagent volume	1 ml
Sample volume	10 µl
Temperature	15 – 25 °C or 37 °C
Zero adjustment	Reagent blank
Incubation time	10 minutes at 15 – 25 °C or 5 minutes at 37 °C
Reagent Blank Limits	Low 0.00 AU High 0.5 AU

	Reagent blank	Standard	Specimen
Reagent (R)	1.0 ml	1.0 ml	1.0 ml
Standard	____	10 µl	____
Specimen	____	____	10 µl

Mix, wait for 10 minutes at 15 – 25 °C or 5 minutes at 37 °C, then measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank within 30 minutes.

Calculation

Serum phosphorus conc. (mg/dL) = $\frac{A_{specimen}}{A_{standard}}$ x 5

Urine phosphorus conc. (mg/dL) = $\frac{A_{specimen}}{A_{standard}}$ x 5 x 10

Note: For turbid highly icteric sera , prepare a serum blank by adding 10 ml serum to 1 ml saline into a labeled test tube. Read absorbance of serum blank at 340 nm vs water and subtract from test absorbance before calculating results.

Quality control

Normal and abnormal control serum of known concentration should be analyzed with each run

Interference

Haemolysis
Avoid haemolysis since RBCs contain very high levels of inorganic phosphate .

Anticoagulants
EDTA, citrate and fluoride interfere with the test .

Lipemia
No significant interference.

Anticoagulants
EDTA, citrate and fluoride interfere with the test .

Expected Values

Serum (fasting)

1- Adults	: 2.7 – 4.5 mg/dL	(0.87 –1.45 mmol/L)
2- Children		
a) < 12 years	: 4.5 – 5.5 mg/dL	(1.45 – 1.78 mmol/L)
b) < 1year	: 4.5 – 6.7 mg/dL	(1.45 – 2.16 mmol/L)
3- Neonates	: 5.0 – 9.6 mg/dL	(1.60 – 3.10 mmol/L)

Urine (24 hrs) : 0.3 – 1.0 g/24 hrs (11 – 32 mmol/day)

Performance characteristics

A comparison between Phosphorus reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.947 was obtained.

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	5.49	8.92
SD	0.21	0.128
CV%	3.83	1.43

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	5.61	9.1
SD	0.29	0.133
CV%	3.97	1.5

Sensitivity

When run as recommended, the minimum detection limit of this assay is 1 mg/dL.

Linearity

The reaction is linear up to phosphorus concentration of 20 mg/dL. Specimens showing higher concentration should be diluted 1+4 using physiological saline and repeat the assay (result × 5).

Analytical Range

1 – 20 mg/dL.

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Frankel S:Electrolytes. In: Gradwhol's clinical laboratory methods and diagnosis, 6 th ed. S Frankel, S Reitman, Editors, Mosby, St. louis (MO).
2. Hanok A, Kao J: The stability of a reconstituted serum for the assay of fifteen chemical constituents.

SYMBOLS IN PRODUCT LABELLING

IVD For in-vitro diagnostic use
LOT Batch Code/Lot number
REF Catalogue Number
Consult instructions for use
Temperature Limitation
Use by/Expiration Date
CAUTION. Consult instructions for use
Manufactured by

Spectrum For Diagnostics Industries - Free Zone
Ismailia Free Zone , Block 5 .
Cairo- Port said Avenue.
Ismailia,Egypt
Tel: +2 064 3488 013 - +2 064 3488 014 Fax: +2 064 3488 015
www.sdi-fz.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

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