SPECTRUM

The Creative Approach to Bioscience

INORGNIC PHOSPHORUS

REF 295 001 2x 50 ml 100 test
REF 295 002 2x100 ml 200 test

Colorimetric method without deproteinization

INTRODUCTION & PRINCIPLE

Spectrum Inorganic Phosphorus Reagent Used For The In-Vitro Quantitative Determination Of Inorganic Phosphorus In Human Serum, Plasma And Urine On Both Of Manual And Automation. The Measurement Of Inorganic Phosphorus In Serum Is Usually Accomplished By Forming A Phosphomolybdate Complex And In Turn Reducing It To A Molybdenum blue Color Complex
Methods Differ As To Choice Of Reducing Agent
Stannous chloride, Phenyl hydrazine, Aminonaphthoisulfonic Acid, Ascorbic Acid, Pmethylaminophenolsulfte,
N-Phenyl-P-Phenylenediamine And Ferrous Sulfate. These Methods Suffered From Color Instability.
Deproteinization Steps And Complexity Of Performance. Spectrum Regents Eliminated The Need To Prepare A Protein Free Filtrate, Accelerated Color Production, Stabilized The Color And Simplified The Procedure: Inorganic Phosphorus Reacts With Ammonium Molybdate In An Acidic Medium To Form A Phosphomolybdate Complex, Whic Reduced By Ferrous Ammonium Sulfte To Produce A Molybdenum Blue Complex.
The Intensity Of Color Measured Photometrically At 600 – 675 Nm, And Its 00000000000 Is Directly Proportional To Inorganic Phosphorus Concentration In The Specimen.

REAGENTS

S	PHOSPHORUS STANDARD	5.0mg/Dl
R1	**Reducing Reagent**	
	SULFURIC ACID	1.1 N
	FERROUS AMMONIUM SULFATE	100 G/L
	FERROUS NITRATE	2.0 G/L
R2	**Color Reagent**	
	AMMONIUM MOLYBDATE	4.5 G/L
	SULFURIC ACID	1.1 N

REAGENTS PREPARATION AND STABILITY

All reagents are supplied ready to use and stable up to the expiry date given on the label When stored at 2-8C

Specimen Collection and preservation

SPECTRUM Inorganic phosphorus kit can be used with
Plasma, serum and urine.

Serum and Plasma

Fresh serum collected in the fasting state is the preferred specimen, since serum inorganic phosphorus level are lower after meals. Heparin is the only anticoagulant which can be used and serum or plasma should be separated from blood cells as soon as possible to avoid the leakage of inorganic phosphorus and phosphate esters into the plasma media.
Inorganic phosphorus in serum is stable for 7 days at 4◦C and
For 3 weeks when frozen.

Urine

Urine specimens should be collected in acid-washed containers (5ml of 6.0mol/l HCL).
Stored Urine specimen must be mixed well and diluted 1:10 in distilled water prior analysis and Inorganic phosphorus stable if stored at room temp., refrigerated or frozen.

PROCEDURE

Wavelength	600-675 nm
Cuvette	1 cm light path
Temperature	20-25 ◦C
Zero adjustment	Reagent Blank
Specimen	Serum, Plasma or Diluted Urine

	Blank	Standard	Sample
R1	0.5 ml	0.5 ml	0.5 ml
R2	0.5 ml	0.5 ml	0.5 ml
Standard (µL)		50 µL	
Sample (µL)			50µL
Mix and Incubate for 15 min at 20-25°C. Then Read the absorbance of the sample (A_1) and Standard (A_2) against the Reagent Blank. (The color is stable for 60 mins.			

CALCULATION

$$\frac{\textbf{A1}\ \text{Sample}}{\textbf{A2}\ \text{Standard}} \times \text{Standard conc.} = \text{mg/dl of inorganic phosphorus}$$

Unit Conversion

mmol/l =mg/dl x 0.323

CALCULATION for 24 hr Urine

$$\frac{\textbf{A1}\ \text{Sample}}{\textbf{A2}\ \text{Standard}} \times 5 \times 10 \times V = \text{mg/day of inorganic phosphorus}$$

Where:
5.0 Standard concentration
10 Dilution Factor
V 24 hr.urine volume in litre

Expected Values

For Fasting Serum

Children	<2 years	4.5-6.7 mg/dl (1.45-2.16 mmol/l)
Children	2-12 years	4.5-5.5 mg/dl (1.45-1.78 mmol/l)
Adults	>12 years	2.5-5.0 mg/dl (0.81-1.62 mmol/l)

For Urine

On Non restricted diet	0.4-1.3 g/day (12.9-42 mmol/l)

Linearity

The assay is linear up to 14 mg/dl
Above this value should be diluted with 0.9% NaCl
Or Distilled water and re-assayed, then multiply the Result by the dilution factor.

Sensitivity

The sensitivity of the inorganic phosphorus Is 0.3 mg/dl

Quality Control

It is recommended that controls (normal and abnormal) be included in each set of assays, or at least once a start, or, when a new reagent is used, or, after preventive maintenance is performed or a clinical component is replaced.

Interfering Substances

Anticoagulants

The only accepted one is the heparin.
Complexing anticoagulants such as EDTA, CITRATE AND OXALATE Must be avoided.

BILIRUBIN

No significant interference from free or conjugated bilirubin up to a level 30mg/dl.

Haemolysis

Avoid haemolysed specimen since high amount of phosphate is liberated during rupture of erythrocytes.
Lypemia
No significant interference

Drugs

Young in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.

Warning & Precaution

SPECTRUM INORGANIC PHOSPHORUS reagent is for in-vitro diagnostics use only. Normal precautions with handling laboratory reagents should be followed.
Reagent is an acid and is caustic, so, avoid contact with skin. Flush with plenty of water if contact occurs.
Don´t pipette by mouth.
It is recommended that disposable the PVC tubes are used for this assay.

Imprecision

Reproducibility was determined using in an internal protocol.
The following results were obtained

Within Run		
Control	Level 1	Level 2
No. of Samples	50	50
Mean(mg/dl)	4.0	6.8
SD(mg/dl)	0.03	0.06
CV (%)	2.7	2.7

Between Day		
Control	Level 1	Level 2
No. of Samples	50	50
Mean(mg/dl)	4.2	6.0
SD(mg/dl)	0.07	0.09
CV (%)	4.9	2.6

Method Comparison

Comparison studies were carried out by using similar commercially available inorganic phosphorus reagents as a reference.
Ser m, plasma (Heparin) and urine samples were assayed in parallel and the results compared by least squares regression.
The following statistics were obtained:

SERUM/PLASMA

No. of sample pairs 50
Range of sample results 2.5- 7.4 mg/dl
Mean of reference method results 4.3 mg/dl
Mean of inorganic phosphorus results 4.1mg/dl
Slope 0.93
Intercept o.21mg/dl
Correlation Coefficient 1.00

BIBLIOGRAPHY

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2. Tietz, NW. ed.,(1990) Clinical Guide to Laboratory Tests,2nd Philadelphia Pa: WB Saunders company:444-447
3. Young, DS (1990): Effects of Drugs on. Clinical Laboratory Tests
Third Edition 1990:3:6-12.

ORDERING INFORMATION	
CATALOG NO.	QUANTITY
295 001	100 test
295 002	200 test

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