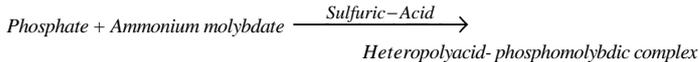


For the quantitative determination of serum and urine inorganic phosphorus. UV method.

Phosphorus in blood exists in two forms as inorganic phosphate and organophosphate esters mainly 2,3-diphosphoglyceric acid, ATP etc. The inorganic phosphate is the fraction of clinical interest. Serum inorganic phosphorus levels are closely tied to bone metabolism, renal function, vitamin D levels and parathyroid hormone status.

Principle

Inorganic phosphate reacts with molybdate to form a heteropolyacid complex. The sulfuric acid eliminates the need to prepare a protein free filtrate. The absorbance at 340 nm is directly proportional to the inorganic phosphorus level in the sample.



Reference values

Serum	0.87-1.45	mmol/l (2,7-4,5 mg/dl)
Urine	10.4-43.0	mmol/24 h (32,24-133,3 mg/dl/24h)

It is recommended that each laboratory should assign its own normal range.

Reagents

1.Reagent (R1)

Sulfuric acid	210 mmol/l
Ammonium molybdate	650 µmol/l

Do not pipette by mouth!

2. Phosphorus standard

Ready for use. For details please check the insert.

Available only in Cat. No.: 41341S

Samples

Serum free of haemolysis.

Urine, diluted with distilled water (1:10).

PROCEDURE

Preparation of working reagent

The reagent is ready for use.

If the absorbance of working reagent is higher than 0.4 at 334 nm the reagent can not be used.

Assay conditions

Wavelength:	334 nm or 340 nm
Temperature:	37°C
Cuvette:	1 cm light path
Read against:	reagent blank
Measure:	end point

Pipette into cuvette

	Blank	Standard	Sample
	10 µl		
Standard		10 µl	
Sample			10 µl
Reagent	1 ml	1 ml	1 ml

Mix and read the optical density after 2 minutes incubation.

Calibration: (37°C, UV method)

S1: Distilled water

S2: Inorganic phosphorus standard Cat. No.: 52101 or

Roche C.F.A.S. (Calibrator for automated system)

Randox Calibration Serum Level I

Calibration frequency

Two point calibration is recommended:

- after reagent lot change,

- as required following quality control procedures.

Calculation using calibration

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

A = Absorbance

C = Concentration

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer (37°C).

Linearity

The test is linear up to 6,49 mmol/l (20,1 mg/dl)

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.006 mmol/l (0,02mg/dl) phosphorus concentration at 334 nm.

Precision

	Reproducibility		
	Average concentration (mmol/l)	SD	CV%
Sample I.	1.93	0.015	0.79
Sample II.	1.23	0.008	0.62

	Repeatability		
	Average concentration (mmol/l)	SD	CV%
Sample I.	0.96	0.016	1.62
Sample II.	1.44	0.021	1.46

Correlation

Comparative studies were done to compare our reagent with another commercial Phosphorus reagent.

The results from these studies are detailed below:

Correlation coefficient: $r = 0.9982$

Linear regression: $y \text{ (mmol/l)} = 0.957x + 0.129$

(x= other commercial reagent , y=own reagent).

Specificity

Bilirubin 855 µmol/l (50 mg/dl), lipid 100 mg/dl, glucose 55.5 mmol/l (1000 mg/dl) and ascorbic acid 2.84 mmol/l (50 mg/dl) don't interfere with the assay up to the given levels.

Note

Reagent contains a strong acid which causes irritation to the eyes, skin and mucous membranes. If the serum is lipaemic make a serum blank. Sample blanks must be run to correct for non-specific absorbance in the serum. Drugs and other substances may affect inorganic phosphorus determinations.

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only.

The following symbols are used on labels

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

Bibliography

Daly J., Erthlingshausen G.: Clin. Chem. 18, 263 (1972)