Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12550 R 1 x 50 ml
For 50 tests

Cat. No. 12551 R 2 x 50 ml
For 100 tests

Phosphorus UV

Ammonium molybdate UV method

Liquid Reagent

Test principle

Ammonium molybdate + Sulfuric acid $\xrightarrow{Phosphorus}$ Phosphomolybdic 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.

Concentrations in the test

Reagent R		
Sulfuric acid	210	mmol/L
Ammonium molybdate	0.3	mmol/L
Detergent		
Standard : The concentration as indicated on vial.		

Stability of reagent

Reagent R : *liquid, ready to use.*

The reagent is stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Note: Don't use if the reagent reading against water has an absorbance greater than 0.500 at 340 nm.

Specimen collection and handling

1. Serum, heparinized or EDTA plasma, free from hemolysis.
2. Serum, plasma should be removed from the red cell clot as soon as possible.
3. Serum inorganic phosphorus is stable for 1 week at 2 - 8 °C, and for 3 weeks at - 20 °C.
4. 24/ hr. Urine: Add 10 ml of 6N HCl to the urine container during the 24 hr collection. Dilute urine (1+9) with double distilled water before testing and multiply result by 10.
5. Urine inorganic phosphorus is stable for 6 months at 2 - 8 °C when acidified.

Calibrator / Standard

MediCal U Cat. No. 15011
Phosphorus STD. Cat. No. 16171

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 340 nm, Hg 334 nm
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator / Standard	Sample
Double dist. water	10 µl	--	--
Calibrator / Standard	--	10 µl	--
Sample	--	--	10 µl
Reagent R	1000 µl	1000 µl	1000 µl
Mix, incubate for 2 min. at 37°C or 5 min. at 20 - 25 °C. Read the absorbance (A). The absorbance is stable for 1 hour.			

Procedure notes

Lipemic and icteric samples require a serum blank. For maximum accuracy a serum blank should be run with each sample.

- Add 10 µl sample to 1000 µl saline solution.
- Adjust the spectrophotometer to zero at 340 nm with saline solution.
- Read and record absorbance of serum blank.
- Subtract the absorbance from test absorbance.

Calculation

$$Conc._{Phosphorus}\ (mg/L) = \frac{A_{Sample}}{A_{Cal./STD.}} \times Conc._{Cal./STD.}\ (mg/L)$$

$$mmol/L \xrightarrow[32.3 \times 10^{-3} \times]{X\ 31} mg/L$$

Linearity

Up to 150 mg/L (4.84 mmol/L).
If the result exceeds 150 mg/L, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

1. A number of drugs and substances affect phosphorus results, see Young ,et al.
2. Citrated plasma produce low false values.
3. Hemolyzed sample may give false high values.

Precautions

1. This reagent contains sulfuric acid, which is irritating to the eyes and skin. If solution comes into contact with skin, eyes or mucous membranes, flush immediately with large quantities of water.
2. To avoid contamination, either use glassware thoroughly rinsed with distilled water, or preferably use disposable material.

Reference range

Serum

Newborn	50 – 96	mg/L
2 – 12 mth.	50 – 108	mg/L
≥ 1 yr.	34 – 62	mg/L
Adults	27 – 45	mg/L

Urine

Urine /24 hr.	325 – 900	mg /24hr.

References

1. Daly, J. A., Erthingshausen G., Clin. Chem., 18, (1972), 263.
2. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
3. Fiske CH, SubbaRowY. The colorimetric determination of inorganic phosphorus. J Biol Chem 1925, 66:375 - 400.
4. Soldin SI, Hicks JM, eds. Paediatric reference ranges.Washington: AACC - Press, 1995: 110.