MEDTEST DX
A MEDTEST COMPANY

Inorganic Phosphorus Reagent Set (UV)

Intended Use
For the quantitative determination of Inorganic Phosphorus in serum using the Mindray BS-200 analyzer.

Method History
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 the choice of reducing agents: stannous chloride[1], phenylhydrazine[2], aminonaphtholsulfonic acid[3], ascorbic acid[4], ρ-methylaminophenolsulfate[5], N-phenyl-ρ-phenylenediamine[6] and ferrous sulfate.[7] These methods suffered from color instability, deproteinization steps and complexity of performance[8]. The addition of a surfactant eliminated the need to prepare a protein-free filtrate, accelerated color production, stabilized the color and simplified the procedure. Many of the components in these reagents were unstable and had to be stored separately. The quantitative measurement of unreduced phosphomolybdate complexes was first reported by Simonsen in 1946.[9] Daly and Ertingshausen[10] adapted that technique for the determination of inorganic phosphorus in 1972. Amador and Urban[11] modified this procedure further the same year. The present method is a modification of the above procedure using a single, stable reagent performing in the UV range.

Principle
Inorganic Phosphorus + H_2SO_4 + Ammonium Molybdate ----------➢ Unreduced Phosphomolybdate Complex

Inorganic phosphorus reacts with ammonium molybdate in an acid medium to form a phosphomolybdate complex that absorbs light at 340nm. The absorbance at this wavelength is directly proportional to the amount of inorganic phosphorus present in the sample.

Reagents
Ammonium Molybdate 0.48 mM, Sulfuric Acid 220 mM with surfactant

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. This reagent is an acid and is caustic. Avoid contact with skin. Flush with plenty of water if contact occurs. DO NOT PIPETTE BY MOUTH.

Reagent Preparation
Reagent comes in a ready to use form.

Reagent Storage
Store reagent at refrigerator temperature(2-8°C). The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration
Do not use reagent if:
1. Reagent read against water has an absorbance greater than 0.500 at 340 nm.
2. The reagent fails to recover stated control values.

Specimen Collection and Storage
1. Unhemolyzed serum is specimen of choice.
2. Plasma should not be used since anticoagulants may produce falsely low values.[12]
3. Hemolyzed sample may give falsely high values.
4. Serum should be removed from the red cell clot as soon as possible.[13]
5. Serum inorganic phosphorus is stable for one week refrigerated and for three weeks frozen.[13,14]

Interferences
For a comprehensive list of substances that interfere with the measurement of Inorganic Phosphorus see Young, et al.[15]

Materials Provided
Inorganic Phosphorus Reagent

Materials Required but not Provided
1. Mindray BS-200 Analyzer
2. BS-200 operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

Mindray BS-200 Test Parameters

Test:	PHOS	R1:	300
No.:	029	R2:	0
Full Name:	Phosphorus	Sample Volume:	3
Standard No.:		R1 Blank:	
Reac. Type:	Endpoint	Mixed Rgt. Blank:	
Pri. Wave:	340nm	Linearity Range:	0.0 – 12.0
Sec. Wave:		Linearity Limit:	
Direction:	Increase	Substrate Limit:	
Reac. Time:	0 / 8	Factor:	
		Compensate: Slope 1.0	Intercept: 0
Incuba. Time:	0	□ Prozone check	
Unit:	mg/dl	q1: q2: q3: q4:	
Precision:	0.1	PC:	Abs:

Calibration Parameters

Rule:	Two-point linear	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	Chem Cal
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Coefficient:	0		

Calibration
Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Quality Control
The integrity of the reaction should be monitored by use of normal and abnormal control sera with known concentrations of inorganic phosphorus. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

5449 Research Dr. • Canton MI 48188 • USA • Phone: 734-487-8300 • Toll Free: 800-445-9853 • Fax: 734-483-1592 • www.medtestdx.com

Calculation (Example)

Abs. = Absorbance

Abs. of Unknown x Concentration of = Inorganic Phosphorus (mg/dl)
Abs. of Standard Standard

Example: Abs. of Unknown = 0.20; Abs. of Standard = 0.29; Conc. of Standard = 5 mg/dl

Then: 0.20 x 5 = 3.4 mg/dl
0.29

SI Units

To obtain results in SI Units (mmol/L), multiply the results in mg/dl by the factor 0.323.

Example: 3.4 mg/dl x 0.323 = 1.09 mmol/L.

Limitations

Detergents containing phosphate should not be used for cleaning glassware used in this procedure.

Expected Values

Adults:	2.5-4.8mg/dl[16]
Children:	4.0-7.0mg/dl[17]

Values are decreased during menstrual period and after meals.[17]
It is strongly recommended that each laboratory establish its own normal values.

Performance

1. Linearity: 12 mg/dl
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer and method, resulting in a correlation coefficient of 0.994 with a regression equation of
   y = 0.902x + 0.07 (N=37).
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[18]

Within Run			Run to Run		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
3.21	0.12	3.8	3.54	0.07	1.98
7.17	0.21	3.0	7.99	0.20	2.50

References

1. Osmond, M.F., Bull. Soc. Chim. 47:745 (1887).
2. Taylor, A.E., Miller, C.W., J. Biol., Chem 18:215 (1914).
3. Fiske, C.H., Subbarow, Y., J. Biol. Chem. 66:275 (1925).
4. Lowry, O.H., Lopez, J.A., J. Biol. Chem. 162:421 (1946).
5. Power, M.H., Standard Methods of Clinical Chemistry New York, Academic Press, (1953).
6. Dryer, R.L., et al, J. Biol. Chem. 225:177 (1957).
7. Taussky, H.H., Shorr, E., J. Biol. Chem. 202:675 (1953).
8. Martinek, R.G., J. Am. Med. Tech. 32:337 (1970).
9. Simonsen, D.G., et al, J. Biol. Chem. 166:747 (1946).
10. Daly, J.A., Ertingshausen, G., Clin. Chem. 18:263 (1972).
11. Amador, E., Urban, J., Clin. Chem. 18:601 (1972).
12. Goldenberg, H. Ferrnandez, A. Clin. Chem. 12:871 (1966).
13. Henry, R.J., et al, Clinical Chemisty: Principles and Technics, New York, Harper & Row, pp.122:143 (1964).
14. Hansk, A., Kao, J., Clin. Chem. 14:58 (1968).
15. Young, D.S., et al, Clin. Chem., 21:1D, (1975).
16. Henry, R.J., et al, Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper & Row, p.728 (1974).
17. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p.917 (1976).
18. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Rev. 12/13 M803-PH0600-01