

Phosphorus Reagent

Catalog #: 43717

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of Inorganic Phosphorus in serum.

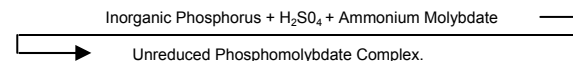
SUMMARY AND EXPLANATION¹

Calcium and phosphorus in serum usually exhibit a reciprocal relationship. An increase in one of these components is usually accompanied by a decrease in the other. Increased serum phosphorus levels may be found in hypervitaminosis (vitamin D), hypoparathyroidism, and renal failure. Decreased serum phosphorus levels may be found in rickets (vitamin D deficiency), hyperparathyroidism, and in the Fanconi syndrome, which is, among others, associated with a defect in reabsorption of phosphorus from the glomerular filtrate.

METHODOLOGY

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,² phenylhydrazine,³ aminonaphtholsulfonic acid,⁴ ascorbic acid,⁵ p-methylaminophenolsulfate,⁶ N-phenyl-p-phenylenediamine⁷ and ferrous sulfate.⁸ These methods suffered from color instability, deproteinization steps and complexity of performance.⁹ 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 1946,¹⁰ Daly and Ertingshausen¹¹ adapted that technique for the determination of Inorganic phosphorus in 1972. Amador and Urban¹² 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 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.

REAGENT COMPOSITION

Active Ingredients	Concentration
Ammonium Molybdate	0.81 mM
Sulfuric Acid	255 mM
Surfactant.	
pH 0.4 ± 0.2	

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.
3. DO NOT PIPET BY MOUTH.

REAGENT PREPARATION

Reagent is supplied ready to use.

REAGENT STORAGE

Store the reagent at 2-25°C.

REAGENT DETERIORATION

Do not use the reagent if:

1. Reagent read against water has an absorbance greater than 0.500 at 340 nm.
2. The reagent fails to meet stated parameters of performance.

SPECIMEN COLLECTION AND STORAGE

1. Unhemolyzed serum is specimen of choice. Serum should be removed from the red cell clot as soon as possible.¹⁴

2. Plasma should not be used since anticoagulants may produce falsely low values.¹³
3. Serum inorganic phosphorus is stable for one week refrigerated and for three weeks frozen.^{14,15}

INTERFERENCES

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference from hemoglobin up to 100 mg/dL.

Bilirubin:

No significant interference from bilirubin up to 7.5 mg/dL.

Lipemia:

No significant interference from lipemia up to 446 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of phosphorus. See Young, et al.¹⁶

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. SDI CA480 Clinical Chemistry System.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

Phosphorus

TEMPERATURE:	37°C
WAVELENGTH:	340 nm
ASSAY TYPE:	Endpoint
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 50
e.g. Sample Vol.	0.02 mL (20mL)
Reagent Vol.	1.0 mL
INCUBATION TIME:	5 Min

Procedure Note:

The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.

Calculations:
(A = Absorbance)

$$\frac{A(\text{patient})}{A(\text{standard})} \times \text{Concentration of standard} = \text{Phosphorus (mg/dL)}$$

Example:

A patient = 0.20
A standard = 0.29
Concentration of standard = 5 mg/dL

$$\frac{0.20}{0.29} \times 5 = 3.4 \text{ mg/dL Phosphorus}$$

Limitations:

1. Samples with values exceeding 15 mg/dL should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.
2. Detergents containing phosphate should not be used for cleaning glassware used in this procedure.

CALIBRATION

Use an aqueous Phosphorus standard or an appropriate serum calibrator. Refer to appropriate instrument operator manual for recommend calibrator interval.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Phosphorus values

EXPECTED VALUES

Adults : 2.5 - 4.8 mg/dL¹⁷
Children: 4.0 - 7.0 mg/dL¹⁸

Values are decreased during menstrual period and after meals.¹⁸

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 15 mg/dL

Method Comparison:

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	43
Range of samples:	0.7–11.9 (mg/dL)
Correlation Coefficient:	0.998
Slope:	1.03
Intercept:	-0.03 (mg/dL)

Precision:

Within Run	Level 1	Level 2
n=40		
Mean (mg/dL)	4.34	8.10
S.D. (mg/dL)	0.10	0.18
C.V. (%)	2.2	2.3

Total

n=40 (10 days / 2 runs per day / 2 replicates per run)		
Mean (mg/dL)	4.34	8.10
S.D. (mg/dL)	0.17	0.22
C.V. (%)	4.0	2.7

Sensitivity:

A calibration factor of approximately 15.2 was obtained, which is equivalent to a sensitivity of 0.066 D Abs per mg/dL.

* NOTE: Performance established on the Chiron 550 Express.

REFERENCES

1. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p. 638, (1970).
2. Osmond, M.F., Bull. Soc. Chim. 47:745 (1887)
3. Taylor, A.E., Miller, C.W., J. Biol. Chem. 18:215 (1914).
4. Fiske, C.H., Subbarow, Y., J. Biol. Chem. 66:275 (1925).
5. Lowry, O.H., Lopez, J.A., J. Biol. Chem. 162:421 (1945).
6. Power, M.H., Standard Methods of Clinical Chemistry New York, Academic Press, (1953).
7. Dryer, R.L., et al, J. Biol. Chem. 225:177 (1957).
8. Taussky, H.H., Shorr, E., J. Biol. Chem. 202:675 (1953).
9. Martinek, R.G., J. Am. Med. Tech. 32:337 (1970).
10. Simonsen, D.G. et al, J. Biol. Chem. 166:747 (1946)
11. Daly, J.A., Ertingshausen, G., Clin. Chem. 18:263 (1972).
12. Amador, E., Urban, J., Clin. Chem. 18:601 (1972).
13. Goldenberg, H. Fernandez, A. Clin. Chem. 12:871 (1966).
14. Henry, R.J., et al, Clinical Chemistry: Principles and Technics, New York, Harper & Row, pp. 122:143 (1964).
15. Hansk, A., Kao, J., Clin. Chem. 14:58 (1968).
16. Young, D.S., et al, Clin. Chem. 21:1D (1975).
17. Henry, R.J., et al, Clinical Chemistry: Principles and Technics, 2nd Ed., Hagarstown (MD), Harper & Row, p. 728, (1974).
18. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p. 917, (1976).

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