POINTE SCIENTIFIC, INC.
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# Inorganic Phosphorus Reagent Set (UV)

## Intended Use
For the quantitative determination of Inorganic Phosphorus in serum. For *in vitro* diagnostic use only.

## Method History
The measurement of inorganic phosphorus in serum has been 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] p-methylaminophenolsulfate,[5] N-phenyl-p-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 two-part, 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
After combining R1 and R2 the reagent contains: ammonium molybdate >0.48mM, sulfuric acid <220mM 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
The reagents (R1 and R2) are ready to use.

## Reagent Storage
Store reagents at room temperature (15-30°C).

## Reagent Deterioration
Do not use reagent if:
1. Reagents show evidence of microbial contamination..
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 (R1 and R2).

## Materials Required but not Provided
1. Beckman Coulter AU™ analyzer
2. Instrument application and Operation manuals.
3. Calibrators and controls

## Procedure (Beckman AU™400 application)

**SPECIFIC TEST PARAMETERS**

TEST NUMBER: **#** TEST NAME: **Phosphorus** ∇ TYPE:**Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **3** DIL. VOL.: **0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **150** DIL. VOL.: **0** MIN. OD MAX. OD
R2 VOLUME: **60** DIL. VOL.: **0** L H
REAGENT OD LIMIT:
WAVELENGTH: PRI. **340** ∇ SEC. **380** ∇ FIRST L: **-0.100** FIRST H: **0.900**
METHOD: **END** ∇ LAST L: **-0.100** LAST H: **0.900**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **0** LAST: **27** L: **#** H: **#**
MEASURING POINT 2: FIRST: **0** LAST: **10** CORRELATION FACTOR:
LINEARITY: % A: **1.000** B: **0.000**
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: **#**

**SPECIFIC TEST PARAMETERS**

VALUE FLAG: **#** ∇ LEVEL L: **#** LEVEL H: **#**

| NORMAL RANGES: | SEX | AGE L YEAR | AGE L MONTH | AGE H YEAR | AGE H MONTH | L | H |
|---|---|---|---|---|---|---|---|
| ○ 1. | # ∇ | # | # | # | # | # | # |
| ○ 2. | # ∇ | # | # | # | # | # | # |
| ○ 3. | # ∇ | # | # | # | # | # | # |
| ○ 4. | # ∇ | # | # | # | # | # | # |
| ○ 5. | # ∇ | # | # | # | # | # | # |
| ○ 6. | # ∇ | # | # | # | # | # | # |
| 7. | NONE SELECTED | | | | | # | # |
| 8. | OUT OF RANGE | L | H | | | # | # |

PANIC VALUE: **#** **#** UNIT: **mg/dl** DECIMAL PLACES: **1**

**CALIBRATION SPECIFIC PARAMETERS**

CAL TYPE: **AB** ∇ FORMULA: **Y=AX+B** ∇ COUNTS: **2** PROCESS: **CONC.** ∇

| CAL. NO. | OD | CONC. | FAC/OD-L | FAC/OD-H |
|---|---|---|---|---|
| POINT 1. | # | # | **-9999999** | **9999999** |
| POINT 2. | | | | |
| POINT 3. | | | | |
| POINT 4. | | | | |
| POINT 5. | | | | |
| POINT 6. | | | | |
| POINT 7. | | | | |

1-POINT CAL. POINT: ○ WITH CONC-0
MB TYPE FACTOR: CALIBRATION STABILITY PERIOD: **#**

**#: User-Defined**

# Inorganic Phosphorus Reagent Set (UV)

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

**NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853.**

## Calibration
Use an appropriate serum-based standard or calibrator.

## Quality Control
The integrity of the reaction should be monitored by the use of normal and abnormal control sera with known concentrations of inorganic phosphorus.

## Calculation
Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Concentration of Standard} = \text{Inorganic Phosphorus (mg/dl)}$$

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

Then: $\frac{0.20}{0.29} \times 5 = 3.4$ mg/dl

## 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 = 1.10 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 comparison study performed between the Beckman Coulter AU400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.993 with a regression equation of y = 1.018x – 0.04 . (n=38, range 2.7 – 11.8 mg/dl).
3. Precision:
   Within - day precision study was performed using three levels of material.
   Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

| Within Run (N=20) | | | Day to Day | | |
|---|---|---|---|---|---|
| Mean | S.D. | C.V.% | Mean | S.D. | C.V.% |
| 0.9 | 0.04 | 4.4 | 3.4 | 0.05 | 1.5 |
| 3.3 | 0.08 | 2.4 | 7.0 | 0.13 | 1.9 |
| 6.1 | 0.17 | 2.8 | | | |

Precision and Linearity studies were performed following modifications of CLSI Protocols EP-5 and EP6[18] using a Beckman AU™400 analyzer.

## 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. CLSI protocols EP5-A2, Vol. 24 No. 25, 2nd Ed, (2004) and CLSI EP6-A, Vol. 23 No. 16 (2003)



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