

## Intended Use

For the quantitative determination of Inorganic Phosphorus in serum using the Mindray BS-480 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<sup>1</sup>, phenylhydrazine<sup>2</sup>, aminonaphtholsulfonic acid<sup>3</sup>, ascorbic acid<sup>4</sup>, p-methylaminophenolsulfate<sup>5</sup>, N-phenyl-p-phenylenediamine<sup>6</sup> and ferrous sulfate.<sup>7</sup> These methods suffered from color instability, deproteinization steps and complexity of performance<sup>8</sup>. 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.<sup>9</sup> Daly and Erlingshausen<sup>10</sup> adapted that technique for the determination of inorganic phosphorus in 1972. Amador and Urban<sup>11</sup> 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<sub>2</sub>SO<sub>4</sub> + 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 and Hazards

This reagent is for *in vitro* diagnostic use only.

### Hazards:

**Hazard Classifications:** Skin Corrosion/Irritation (Category 1), Serious eye damage/eye irritation (Category 1), Specific target organ toxicity, single exposure; Respiratory System (Category 1), Specific target organ toxicity, repeat exposure; Respiratory System (Category 1)

**Hazard Statements:** H314: Causes severe skin burns and eye damage, H319: Causes serious eye damage, H370: Causes damage to organs, H372: Causes damage to organs through prolonged or repeated exposure

### Precautionary Statements

**Prevention :** P260: Do not breathe dust/fume/gas/mist/vapors/spray, P264: Wash hands thoroughly after handling, P270: Do not eat, drink or smoke when using this product, P280: Wear protective gloves/protective clothing/eye protection/face protection

**Response :** P314: Get medical advice/attention if you feel unwell, P363: Wash contaminated clothing before reuse, P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting, P303 + P361 + P353: IF ON SKIN (or hair): remove/Take off immediately all contaminated clothing. Rinse SKIN with water/shower, P304 + P340: IF INHALED: Remove victim to fresh air and Keep at rest in a position comfortable for breathing, P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing, P307 + P311: IF exposed: call a POISON CENTER or doctor/physician.

**Storage :** P404 : Store in a closed container.

**Disposal :** P501 : Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations. Refer to the Safety Data Sheet for this product (SDS-PHO600) available at [www.medtestdx.com](http://www.medtestdx.com).



Signal Word: Danger

## Reagent Preparation

Reagent comes in a ready to use form.

## Reagent Storage and Stability

Store reagent at refrigerator temperature (2-8°C). The reagent is stable until the expiration date appearing on the label when stored as directed. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

## 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.<sup>12</sup>
3. Hemolyzed sample may give falsely high values.
4. Serum should be removed from the red cell clot as soon as possible.<sup>13</sup>
5. Serum inorganic phosphorus is stable for one week refrigerated and for three weeks frozen.<sup>13,14</sup>

## Interferences

For a comprehensive list of substances that interfere with the measurement of Inorganic Phosphorus see Young, et al.<sup>15</sup>

## Materials Provided

Inorganic Phosphorus Reagent

# Inorganic Phosphorus Reagent Set (UV)

## Materials Required but not Provided

1. Mindray BS-480 Analyzer
2. BS-480 operation manual
3. Chemistry Calibrator, catalog number CHEC480
4. Chemistry control, catalog number CHEQ480

## Calibration

Use MedTest DX Chemistry Calibrator (Catalog Number CHEC480). The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

## 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.

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

## Expected Values

Adults: 2.5-4.8mg/dl<sup>16</sup>

Children: 4.0-7.0mg/dl<sup>17</sup>

Values are decreased during menstrual period and after meals.<sup>17</sup> It is strongly recommended that each laboratory establish its own normal values.

## Performance

1. Assay Range: 0.0-12.0 mg/dL.
2. Correlation: A study was performed between the Mindray BS-480 and a similar analyzer using this method, resulting in the following:

Method	Phosphorus
N	96
Mean Phosphorus (mg/dL)	4.30
Range (mg/dL)	0.5-9.9
Standard Deviation	1.83
Regression Analysis	$y = 0.936x + 0.25$
Correlation Coefficient	0.9724

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.<sup>18</sup>

Sample	Within Day			Total		
	LOW	MID	HIGH	LOW	MID	HIGH
N	20	20	20	40	40	40
Mean	3.78	7.31	10.89	3.81	7.38	11.06
Standard Deviation	0.04	0.04	0.04	0.13	0.31	0.31
Coefficient of Variation (%)	1.1%	0.6%	0.4%	3.5%	4.2%	2.8%

4. Sensitivity: 2SD limit of detection (95% Conf) = 0.0 mg/dL

## 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. Tausky, 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. Fernandez, A. Clin. Chem. 12:871 (1966).
13. Henry, R.J., et al, Clinical Chemistry: 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, 2<sup>nd</sup> 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", 2<sup>nd</sup> Ed. (1992).



**CHEMISTRY PARAMETERS**

Chem:	PHOS	No.:	227	Sample Type:	Serum
Chemistry:	Inorganic Phosphorus			Print Name:	PHOS
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	340			Sec Wave:	
Unit:	mg/dL			Decimal:	0.1
Blank Time:	10	12		Reaction Time:	27 29
	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	1.5 ul	--- ul	--- ul	R1:	150 ul --- ul
Decreased:	--- ul	--- ul	--- ul	R2:	--- ul -- ul
Increased:	--- ul	--- ul	--- ul	R3:	--- ul -- ul
	<input type="checkbox"/> Sample Blank	<input checked="" type="checkbox"/> Auto Rerun		R4:	--- ul --- ul
<b><u>Slope/Offset Adjustment</u></b>					
Slope: 1		Offset: 0			

Linearity Range (Standard)	0	12	Linearity Limit:
Linearity Range (Decreased)	---	---	Substrate Depletion:
Linearity Range (Increased)	---	---	Mixed Blank Abs:
R1 Blank Abs:	---	---	Uncapping Time
Blank Response:	---	---	Reagent Alarm Limit:
Twin Chemistry:			<input type="checkbox"/> Enzyme Linear Extension
<input type="checkbox"/> Prozone Check		<input type="radio"/> Rate Check	<input type="radio"/> Antigen Addition
Q1:	Q2:	Q3:	Q4:
PC:	ABS:		

# Inorganic Phosphorus Reagent Set (UV)

## CALIBRATION PARAMETERS

<b>Calibrator Definition</b>						
Calibrator:	*	Lot No.:			*	
Exp Date:	*					
<b>Carousel</b>	<b>Pos</b>					
Sample Carousel 1	*					
Sample Carousel 2						
Sample Carousel 3						
<b><u>Reagent/Calibration</u></b>						
<u>Calibrator</u>	<u>Pos</u>	<u>Lot No</u>	<u>Exp Date</u>	<u>Chem</u>	<u>Conc</u>	<u>Unit</u>
Water	W	*	*	PHOS	0	mg/dL
Chemistry Calibrator	*	*	*	PHOS	*	mg/dL
<b><u>Calibration Setup</u></b>						
Chem:	PHOS					
<b><u>Calibration Settings</u></b>						
Math Model:	Two-Point Linear					
Factor:	Replicates:		2			
<b><u>Acceptance Limits</u></b>						
Cal Time:	*	Hour				
Slope Diff:	---	SD:	---			
Sensitivity :	---	Repeatability:	---			
Deter Coeff:	---					
<b><u>Auto Calib.</u></b>						
<input type="checkbox"/> Bottle Changed	<input type="checkbox"/> Lot Changed	<input type="checkbox"/> Cal Time				

It is recommended that two levels of control material be assayed daily.  
\* Indicates user defined parameter.

**REF** PHO480



Manufactured for MedTest DX  
5449 Research Drive Canton, MI 48188



**IVD**

### Symbol Key

Use by (YYYY-MM-DD)	<b>LOT</b> Lot and batch code	<b>REF</b> Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	<b>IVD</b> <i>In vitro</i> diagnostic medical device	