Phosphorus in Plasma/Serum or Urine

Purpose

This procedure provides instructions for performing PHOSPHORUS IN PLASMA/SERUM OR URINE. The PHOS method used on the Dimension® clinical chemistry systems is an *in vitro* diagnostic test intended for the quantitative determination of phosphorus in **serum**, **plasma** and **urine**.

Policy Statements

This procedure applies to all personnel running the Siemens Dimension Vista or RxL MAX

Principle

PRIMARY METHOD: Siemens Dimension Vista® 500 System

The Vista phosphorus (PHOS) method is a modification of the classical phosphomolybdate method introduced by Fiske and Subbarow and uses a mixture of p-methylaminophenol sulfate and bisulfite to reduce the phosphomolybdate as reported by Gomori and by Drewes. The PHOS method measures the absorption of the reduced phosphomolybdate complex at UV wavelengths to improve sensitivity. Interference caused by protein precipitation is eliminated using the solubilizing agent, lithium dodecyl sulfate. Because of the pre-blanking of the sample, bilirubin interference is considerably minimized.

Inorganic phosphate combines with molybdate (MoO_4) in an acid solution to form a complex that is reduced by p-methylaminophenol sulfate (PMAPS) and bisulfite. The 340 nm absorbance of the reduced phosphomolybdate solution is proportional to the inorganic phosphorus concentration and is measured using a bichromatic (340, 383 nm) endpoint technique.

SECONDARY METHOD:

The phosphorus (PHOS) method is a modification of the classical phosphomolybdate method introduced by Fiske and Subbarow. The PHOS method measures the absorption of the unreduced phosphomolybdate complex as reported by Daly and Ertingshausen. Inorganic phosphate reacts with ammonium molybdate in the presence of sulfuric acid to form a phosphomolybdate complex which is measured at 340 nm and blanked at 700 nm.

Clinical Significance

Measurements of phosphorus (inorganic) are used in the diagnosis and treatment of bone, parathyroid and renal disease. Most of the phosphorus found in the human body is found in the calcium phosphate salts that make up the inorganic substance of bone. The remainder is distributed throughout all of the other cells of the body. Practically all body phosphorus is present as the phosphate radical. The calcium phosphate salts of bone give them rigidity but also serve as a large storage depot for phosphate.

Serum phosphate is increased during periods of growth so the normal range for children is higher than the adult normal range. The highest levels are seen in newborn infants. Spuriously high values may be seen if the blood sample is not handled properly. Red blood cells contain high levels of organic phosphates so the serum should be quickly separated from the cells with care to avoid hemolysis. Abnormal serum phosphate levels are most commonly seen in kidney, bone, and parathyroid diseases. Phosphate is usually measured along with serum calcium since each measurement is useful in the interpretation of the other.

Clinical Significance (cont)

Increased Serum Phosphate
Kidney Disease: The ordinary diet contains far more phosphate than is required to maintain body structure and function. Normally the excess is excreted into the urine. Kidney disease from almost any cause impairs its ability to remove phosphate from plasma, so there is a gradual rise in serum phosphate levels and a secondary fall in serum calcium.
Hypoparathyroidism: In the absence of sufficient parathyroid hormone the kidney tends to retain phosphate ion, so the serum level is high. The serum calcium is low.
Hyperthyroidism: Serum phosphate will occasionally rise in this disease. The cause is not well understood, but it may be due to excessive mobilization of calcium phosphate from bone.
Other Causes: Elevated serum phosphate may be seen in diabetic acidosis, acromegaly, excess vitamin D intake, and acute osteoporosis.

Decreased Serum Phosphate
Hyperparathyroidism, Malabsorption Syndromes, Recovery from diabetic acidosis where serum phosphate may fall below normal due to the movement of glucose and phosphate from the blood into cells. Hypopituitarism (insufficient production of growth hormone) in children results in lower than normal phosphate levels for a child of the same age.

Analyzer

PRIMARY METHOD: Siemens Dimension Vista® 500 System
SECONDARY (BACKUP) METHOD: Siemens Dimension® clinical chemistry system

Sunquest Test Codes

PO4	Phosphorus in plasma or serum in mg/dL
UPO4	Urine Phosphorus in mg/dL
UPQ	Quantitative urine phosphorus in mg/ 24 hr, timed collection

Specimen

Plasma (lithium heparin) preferred, or Serum
Serum and plasma can be collected using recommended procedures for collection of diagnostic blood specimens by venipuncture.

Patient should preferably be fasting. Phosphate levels fluctuate following meals.

Urine: Timed (24 hour) or random collection, clarified by centrifugation. Refer to 24 hour urine collection procedures. Urine testing is performed by the **PRIMARY METHOD** only

Minimum volume: 200 µL, minimum 100 µL: Actual test volume1.3 µL (Vista) 3 µL (MAX)

Stability:
Plasma/serum: RT / 8 hours, 2-8 °C / 7 days, < -20°C / 3 months, < 3 freeze thaw cycles

Urine: RT / 48 RT, 2 – 8 °C / 6 months when 24 hour specimens are acidified

Rejection criteria: Unlabeled tube, other than heparinized plasma, serum, or urine.

Specimen (cont)

Preparation:

1. Complete clot formation should take place before centrifugation.
2. Whole blood and body fluid specimens should be centrifuged according to Specimen Processing procedures prior to analysis.
3. Serum or plasma should be separated from red cells promptly because erythrocytes contain phosphate concentrations several times greater than those found in the serum.
4. Separate serum or plasma from cells with a maximum limit of 2 hours from the time of collection. Specimens should be free of particulate matter.
5. Transfer serum, plasma, or body fluid to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
6. Collections of 24-hour urine specimens may be made in containers with 10 mL of 6M HCI or:
    a. **Preferred:** Acidify a well-mixed aliquot to pH < 3. Prior to centrifugation, add 50 uL of 6M HCl to a 3 ml aliquot of urine. Verify pH is < 3. A maximum of 150 uL may be added to correct pH (max dilution factor = <5%). Allow to stand 1 hour before analysis for warming and dissolution.
    b. Measure timed urine collections for total volume.
    c. Record the collection date and time for the start and end of the collection.
    d. Enter this information into Sunquest by ordering the test PV on the same accession number.

Reagents

PRIMARY METHOD:

Product Description	*Product Code*	*Stability*
Dimension Vista ® PHOS Flex® reagent cartridge Liquid and ready to use.	K1060	**Store at:** 2 - 8 °C. **Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges. **On-board:** Sealed wells on the instrument are stable for 30 days. **Open well stability:** 7 days for wells 1 – 12
CHEM 2 CAL Traceable to NIST SRM 21861	KC120	**Store at:** 2 - 8 °C. **Unopened:** Refer to carton for expiration date. **On-board:** Once the vial stopper is punctured, assigned values are stable for 24 hours when stored on board the Dimension Vista® System **Opened:** Once the cap is removed, the assigned values are stable for 30 days when recapped and stored at 2 - 8 °C. Do not use this vial on board.

Risk and Safety

Precautions:
Irritant. Contains mixture of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

- Irritating to eyes and skin.
- Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
- Wear suitable gloves and eye/face protection.
- Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

Reagents (cont)

SECONDARY METHOD:

Product Description	*Product Code*	*Stability*
Dimension PHOS Flex® reagent cartridge Liquid and ready to use.	DF16A	**Store at:** 2 - 8 °C. **Unopened:** Refer to carton for expiration date of individual unopened reagent cartridges. **On-board:** Sealed wells on the instrument are stable for 30 days. **Open well stability:** 3 days for wells 1 – 6, 30 days for wells 7-8
CHEM II Calibrator	DC20	**Store at:** 2 - 8 °C **Unopened:** Refer to carton for expiration date. **Opened:** Use immediately
Contains: Sulphuric acid • May be corrosive to metals. Causes severe skin burns and eye damage. • Wear protective gloves/protective clothing/eye protection/face protection. • IF SWALLOWED immediately call a POISON CENTER or doctor/physician. • Do NOT induce vomiting. IF ON SKIN (or hair): • Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. • IF IN EYES immediately call a POISON CENTER or doctor/physician. • Rinse cautiously with water for several minutes. • Remove contact lenses, if present • Continue rinsing. Absorb spillage to prevent material damage. • Dispose of contents and container in accordance with all local, regional, and national regulations.		

Calibration

PRIMARY METHOD:

Assay Range:	0.1 – 9.0 mg/dL
Calibration Material:	CHEM 2 Calibrator (KC120)
Calibration Scheme:	2 levels, n=5 Level 1 (Calibrator A): 0 mg/dL Level 2 (Calibrator B): 10.0 mg/dL
Calibration Frequency:	• Every 90 days for any one lot. • For each lot of Flex® reagent cartridges • After major maintenance or service, if indicated by quality control results • As indicated in laboratory quality control procedures • When required by government regulations
Analytical Measuring Range (AMR)	• Cal Verification and AMR verification are performed at least once every six (6) months. • Touch Advanced → Calibrations → Calibrations by Lot, select method PHOS and “Order a Linearity Study” • See iGuide “Calibration by Lot” for more information.

Calibration (cont)

SECONDARY METHOD:

Assay Range:	0.5 – 9.0 mg/dL
Reference Material:	Dimension® CHEM II Calibrator, Cat. No. DC20
Calibration Scheme:	3 levels, N = 3 2.0, 5.0, 8.0 mg/dL
Calibration Frequency:	• 3 months for any one lot. • For each lot of Flex® reagent cartridges • After major maintenance or service, if indicated by quality control results • As indicated in laboratory quality control procedures • When required by government regulations
Analytical Measuring Range (AMR)	• Once every 6 months confirm the reportable range by analyzing Maine Standards Validate GC1 according to the manufacturer's instructions. • Enter data using Maine Standards MSDRx free software, and send reports to technical specialist for review. • Investigate unacceptable results.

Quality Control (Plasma/Serum)

PRIMARY METHOD: Biorad Multiqual (Human) Levels 1 & 3

Frequency: Two levels each day of use

Stability: Stable until the date on vial when stored at -20 to -50 °C protect from light. Thawed and unopened, 7 days at 2 -8 °C. 7 days on board the Vista, and 5 days opened and stored at 2 – 8 °C

Preparation: Allow the control to stand at 18 – 25 °C for 30 minutes until completely thawed. Gently mix the vials until homogeneous to dissolve any precipitate.

Sunquest Control names: Level 1 = C-MQ1, Level 2 = C-MQ3

SECONDARY METHOD: Biorad Liquichek™ Unassayed Chemistry Control (Human) Levels 1 & 2

Frequency: Two levels each day of use

Stability: Refer to the current lot product insert

Sunquest Control names: C-X1, C-X2

Quality Control (Urine)

PRIMARY METHOD: Biorad Liquichek® **Urine Chemistry** Control Levels 1 & 2 in Vista ® vials

Frequency: Day of use or daily

Stability: Unopened at 2-8 C/ date on vial, opened on board Vista/ 30 days.

Preparation: Gently swirl contents prior to loading on Vista

Sunquest Control names: Urine Level 1 = C-UR1, Level 2 = C-UR2

Quality Control Acceptable ranges

- Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.
- If a control value is outside the confidence interval, the determination must be repeated. If the repeated determination confirms the deviation, a new reference curve should be established.
- Do not release patient results until the cause of deviation has been identified and corrected
- When a new lot of control is received, validate the manufacturer's insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range

Calculations

UPQ = PO4 in mg /24 Hrs = $\frac{\text{PO4 in mg/dL} \times \text{Total Volume in mL}}{100}$

Interferences

PRIMARY METHOD:

Hemolysis, Icterus & Lipemia (HIL) Index Values:

H	I	L
7	7	5

HIL:

- Hemoglobin at 1000 mg/dL increases PHOS results by 15.6% at 4.2 mg/dL of phosphorus.
- Bilirubin (unconjugated) at 60 mg/dL decreases PHOS results by 11.2% at 4.2 mg/dL of phosphorus
- Lipemia above 600 mg/dL decreases phosphorus by up to 10-20% at normal PHOS concentrations
- Mannitol at concentrations of 500 mg/dL or greater causes a decrease of 10% or more.
- Creatine phosphate and phosphoenol pyruvate liberate phosphorus under the reaction conditions of this method and can thereby increase results.

SECONDARY METHOD:

- Hemoglobin at 1000 mg/dL increases PHOS results by 17% at a phosphorus concentration of 2.5 mg/dL
- Lipemia (Intralipid®) at 3000 mg/dL increases PHOS results by 71% and 30% at a phosphorus concentration of 2.5 mg/dL and 6.5 mg/dL respectively.
- Albumin at 6.0 g/dL increases PHOS results by 19% at a concentration of 2.5 mg/dL

Refer to the product insert for a list of substances that have been shown to have no measurable effect on the PO4 result at typical concentrations

Reference Range

Serum/Plasma:

Age	Phosphorus
0 – 4 days	4.6 – 8.0 mg/dL
5 days – 3 years	3.7 – 6.5 mg/dL
4 – 10 years	3.6 – 5.6 mg/dL
11 – 15 years	2.9 – 5.4 mg/dL
> 15 years	2.5 – 4.5 mg/dL

Reference ranges adapted from Clinical Biochem Reference Range Handbook[10], and Children's Hospital patients.

Urine:
500 - 1,500 mg/24 hr

The reference values are for a 24-hour collection. Random specimens and timed specimens collected for other than a 24-hour time period are reported in units of mg/dL, for which reference values are not established.

Critical Values

Plasma/Serum: < 2.0 or > 10.0 mg/dL. Critical values must be called according to the Critical Limit Test Value Policy.

Limitations

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension Operator's Guide for troubleshooting.

Dilutions

PRIMARY METHOD:

Maximum Dilution:	1:2
Surplus Rack:	Samples with results >**9.0 mg/dL** automatically reflex to a 1:2 dilution.
Limited Rack:	Samples with results >**9.0 mg/dL** should be repeated as an Add-On Test with a Special Dilution of 1:2.

Phosphorus Urine

Automated Urine Dilution:	1:10
Manual Dilution	Do not manually prepare urine dilutions.

SECONDARY METHOD: serum / plasma

Maximum Dilution:	1:2
Automated Dilution:	Samples with results >**9.0 mg/dL** automatically reflex to a 1: 1.5 dilution which extends the serum/plasma reportable range to 13.5 mg/dL
Manual Dilution	Manually prepare a 1:2 dilution with Reagent grade water Enter dilution factor in RxL MAX and reassay

Result Reporting

PRIMARY METHOD:

- Serum/Plasma results between 0.1 – 9.0 mg/dL without error messages are released
- Serum/Plasma results below 0.1 mg/dL: report as < 0.1 mg/dL.
- Serum/Plasma results greater than 9.0 mg/dL without error messages are reported following a maximum dilution of 1:2
- Serum/Plasma results with "above assay range" appended following a maximum dilution of 1:2 are reported as >18.0 mg/dL
- Append the appropriate comment "HP" or "LIN" to samples with HIL flags. Refer to CH5.101 HIL on Dimension Vista 500

- Urine results between 1 – 90.0 mg/dL without error messages are released
- Urine results with "above assay range" appended are reported as >90.0 mg/dL
- Timed Urine collection results: The Laboratory Information System calculates urine phosphorus on timed and random collections when all information is present without messages. Report the numerical value in Sunquest.
- Timed Urine collection with results >90.0 mg/dL: answer the UPQ result with the comment NCAL

Result Reporting (cont)

SECONDARY METHOD:

- Serum/Plasma results between 0.5 – 9.0 mg/dL without error messages are released
- Serum/Plasma results below 0.5 mg/dL: report as < 0.5 mg/dL.
- Serum/Plasma results greater than 9.0 mg/dL without error messages are reported following a maximum dilution of 1:2
- Serum/Plasma results with "above assay range" appended following a maximum dilution of 1:2 are reported as >18.0 mg/dL

Specimen Storage

Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer.

References

1. PHOS Flex® reagent cartridge insert sheet Siemens Healthcare Diagnostics, Inc. 2008-03-27 717061.001 – US, Rev L
2. Siemens Dimension Vista system Chem 2 Cal product insert, Siemens Healthcare Diagnostics Inc. Newark DE 19714, PN 1751150.001-US, 03/2008
3. PHOS Flex® reagent cartridge insert sheet PN 781061.001 Issue Date 2015-04-27 Rev. F
4. Siemens Dimension clinical chemistry system Chem II Cal product insert, Siemens Healthcare Diagnostics Inc. Newark DE 19714, PN 1751150.001-US, 2010
5. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc, Hudson, OH, 5th Edition, 2001
6. Biorad Liquid Assayed Multiqual Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618
7. Biorad Liquichek Urine Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618
8. Should acidification of urine be performed before the analysis of calcium, phosphate and magnesium in the presence of crystals?, Clin Chim Acta. 2013 Nov 15;426:46-50. doi: 10.1016/j.cca.2013.08.025. Epub 2013 Sep 5,
9. Acidification and urine calcium: is it a preanalytical necessity? Ann Clin Biochem. 2009 Nov; 46(Pt 6):484-7. doi: 10.1258/acb.2009.009027. Epub 2009 Sep 3
10. Clinical Biochemistry Reference Range Handbook, East Sussex Healthcare, Paul Eaton, 7/2/2013

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.	Unknown	Unknown	Phosphorus on Dimension AR, Initial Version
2.	Deane L Riedel	August 2000	
3.	L. Lichty	August 2003	Phosphorus on Dimension RxL
4.	L. Lichty	July 31, 2006	Phosphorus in Plasma, Serum or Urine
5.	D. Helfinstine/ L. Lichty	April 1, 2011	New Format, Updated package insert information. Renumbered from CH 3.37
6.	L. Lichty	March 19, 2013	Clarify maximum dilution reporting, sample preparation
7.	L. Lichty	12/17/2013	Siemens CLSI PHOS procedure for Vista, issue date 8/20/2013
8.	L. Lichty	December 24, 2014	Revised for Vista
9.	L. Lichty	July 6, 2016	Phosphorus configured on RXL as backup. Revised reference ranges