DXC 800 (PHOSM) PHOSPHORUS

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☐ St. Clare Hospital Lakewood, WA	☐ Highline Medical Center Burien, WA	☐ PSC

PURPOSE

To provide instructions for the quantitative determination of phosphorus on the DXC 800.

PRINCIPLE

PHOSm reagent, in conjunction with UniCel® DxC 800 System and the SYNCHRON® Systems AQUA CAL 1 and 2, is intended for the quantitative determination of inorganic Phosphorus concentration in human serum, plasma or urine.

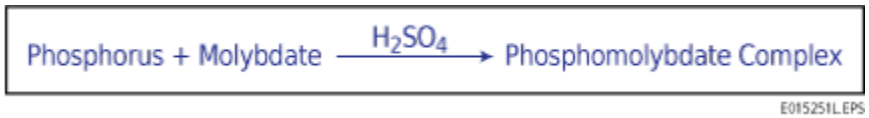
BACKGROUND

Clinical Significance

Measurements of phosphorus (inorganic) are used to in the diagnosis and treatment of various disorders, including parathyroid gland and kidney diseases, and vitamin D imbalance.

Methodology

PHOSm reagent is used to measure the phosphorus concentration by a timed rate method. In the reaction, inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a colored phosphomolybdate complex.
A precise volume of sample (8 microliters) is injected in a reaction cup containing a molybdate solution. The ratio used is one part sample to 72 parts reagent. The phosphomolybdate method consists of measuring the rate change in absorbance of an acidic ammonium molybdate reagent following the addition of sample. The system monitors the change in absorbance of yellow phosphomolybdate at 365 nanometers. The rate measurement between 19 and 25 seconds after sample introduction has been shown to be directly proportional to the concentration of the inorganic phosphorus in the sample and is used by the SYNCHRON System to calculate and express the phosphorus concentration.



RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CH-0820	DXC 800 Controls
J-F-CH-0826	DXC 800 Calibrators
J-F-CH-1940	DXC Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum, plasma or properly collected urine (random/timed) are the preferred specimens. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood is not recommended for use as a sample.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

3. It is recommended that urine assays be performed within 2 hours of collection. For timed specimens, the collection container is to be kept in the refrigerator or on ice during the time period. Urine should be acidified with 25 mL of 6 N HCl added to the container before collection begins.

Sample Type	Volume	Sample Stability
Plasma/Serum/Urine	0.5mL	• Separate serum from cells within 2 hours. • Room Temp 8 hours • Refrigerated 48 hours • Frozen 3 months. • URINE RECOMMENDED TO BE TESTED WITHIN 2 HOURS OR KEPT REFRIGERATED OR ON ICE. For timed collections, 25 mL of 6N HCL should be added to the container before collection begins.

Criteria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

SAMPLE PREPARATION

Urine Sample Preparation

All urine samples, including urine controls, must be diluted one part sample with nine parts normal saline prior to analysis on UniCel DxC 800 Systems. These dilutions should be made according to the following table:

URINE SAMPLE DILUENT

Sample	Dilution	Volume of Sample	Volume of Diluent
Controls	1:10	100 µL	900 µL
Samples	1:10	100 µL	900 µL

All urine results reported by the UniCel DxC 800 System must be multiplied by a correction factor of 10 (see CALCULATIONS Section of this chemistry information sheet).

SAMPLE VOLUME

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

Contents

Each kit contains the following items:
Two Molybdate Reagent Bottles (2 x 200 mL)
Two Phosphorus Diluent Bottles (2 x 1800 mL)

Volume per Test	
Sample Volume	8 µL
Total Reagent Volume	570uL

Reactive Ingredients	
Ammonium Molybdate	3.2 mmol/L
pH	< 1.0

Also non-reactive chemicals necessary for optimal system performance.

Reagent Preparation

Carefully pour 200 mL of molybdate reagent into the 1800 mL of diluent. Replace cap and mix at least ten times by gentle inversion. Do not reuse old reagent or mix fresh reagent with old reagent.

Acceptable Reagent Performance

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility`s acceptance criteria.

Reagent Storage and Stability

PHOSm reagent when stored unopened at 8 C to 30 C will remain stable until the expiration date indicated on each bottle. The combined reagent is stable on instrument for 30 days from the date of preparation. Do not freeze or refrigerate.
If reagent is frozen in transit, thaw completely, warm to room temperature and mix thoroughly by gently inverting bottle a least 10 times.

CALIBRATION

Calibrator Required

SYNCHRON® Systems AQUA CAL 1 and 2

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

If unopened, the SYNCHRON® Systems AQUA CAL 1 and 2, may be stored at +2°C to +8°C until the expiration date printed on the calibrator bottle. Opened calibrators are stable at room temperature for 30 days unless the expiration date is exceeded.

Calibration Information

1. The system must have a valid calibration in memory before controls or patient samples can be run.

2. Under typical operating conditions the PHOSm assay must be calibrated every 72 hours or with each new bottle of reagent and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 600/800 Systems *Instructions for Use* (IFU) manual.

3. For detailed calibration instructions, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

Traceability

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls

STEPS

1. If necessary prepare reagent as defined in the Reagent Preparation section of this chemistry information sheet and load the reagent onto the system.

2. After reagent load is completed, calibration may be required.

3. Program samples and controls for analysis.

4. After loading samples and controls onto the system, follow the protocols for system operation**.** To load samples manually refer to the FHS DXC Series Manual Sample Programming procedure. For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

SYNCHRON® System(s) perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

ANTICOAGULANT TEST RESULTS

If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Compatible Anticoagulants

Anticoagulant	Level Tested for In Vitro Interference
Ammonium Heparin	14 Units/mL
Lithium Heparin	14 Units/mL
Sodium Heparin	14 Units/mL

The following anticoagulant was found to be incompatible with this method:

Anticoagulant	Level Tested for In Vitro Interference	Plasma-Serum Bias (mg/dL)
EDTA	1.5 mg/dL	- 0.7
Potassium Oxalate/Sodium Fluoride	2.0 / 2.5 mg/mL	- 1.2

PERFORMANCE CHARACTERISTICS

Reference Range

Sample Type	Male		Female		Critical Low	Critical High
	Age	Range	Age	Range		
Serum/ Plasma	0-10 days	4.2-9.6 mg/dL	0-10 days	4.2-9.6 mg/dL	<3.0 mg/dL	>15.0 mg/dL
Serum/ Plasma	10 days-2 yrs	4.2-7.2 mg/dL	10 days-2 yrs	4.2-7.2 mg/dL	<3.0 mg/dL	>15.0 mg/dL
Serum/Plasma	2 yrs-12 yrs	4.2-5.9 mg/dL	2 yrs-12 yrs	4.2-5.9 mg/dL	<1.0 mg/dL	
Serum/Plasma	12-60 yrs	2.3-4.8 mg/dL	12-60 yrs	2.3-4.8 mg/dL	<1.0 mg/dL	
Serum/Plasma	>60 yrs	2.6-4.4 mg/dL	>60 yrs	2.1-3.9 mg/dL	<1.0 mg/dL	
Urine, timed	0->60yrs	400-1300mg/24 hr	0->60yrs	400-1300mg/24 hr	N/A	N/A
Urine, random	0->60yrs	N/A	0->60yrs	N/A	N/A	N/A

For Critical Value reporting protocol, refer to FHS Critical Policy

Analytic Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical range:

Sample Type	Conventional Units
Serum or Plasma	0.5 – 12.0 mg/dL
Urine	5 – 140 mg/dL

Samples with concentrations exceeding the high end of the analytical range should be diluted with saline and reanalyzed.

Reporting results outside of analytical range

Lower limit of range: serum / plasma	0.5 mg/dL	Result below 0.5, report as <0.5mg/dL
Upper limit of range: serum / plasma	12.0 mg/dL	Results >12.0 should be diluted with 0.9% saline, reanalyzed and dilution factor applied. The maximum allowable dilution is X2. Results >24.0 are reported as >24.0 mg/dL
Lower limit of range: urine	5 mg/dL	Result below 5, report as <5mg/dL
Upper limit of range: urine	140 mg/dL	Results >140 should be diluted with 0.9% saline, reanalyzed and dilution factor applied. The maximum allowable dilution is X10. Results >1400 are reported as >1400 mg/dL.

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the phosphorus determination is 0.5 mg/dL (0.2 mmol/L) for serum or plasma and 5 mg/dL (1.6 mmol/L) for urine.

LIMITATIONS

None identified.

Interferences

1. The following substances were tested for interference with this methodology:

Substance	Source	Level Tested	Observed Effect
Bilirubin (unconjugated)	Bovine	30 mg/dL INDEX of 20	No significant interference (within ± 0.3 mg/dL or 4%)
Ditauro Bilirubin	Synthetic	20 mg/dL	+ 0.99 @ 2.1 mg/dL
			+ 1.84 @ 8.9 mg/dL
Hemoglobin	RBC hemolysate	250 mg/dL INDEX of 7	+ 0.24 mg/dL
Lipemia	Intralipid	500 mg/dL	No significant interference (within ± 0.3 mg/dL or 4%)
	Human	Serum Index 8	No significant interference (within ± 0.3 mg/dL or 4%)
Cefotaxime	Cefotaxime sodium salt	500 µg/dL	No significant interference (within ± 0.3 mg/dL or 4%)
Ascorbic Acid	L-Ascorbic Acid	20 mg/dL	No significant interference (within ± 0.3 mg/dL or 4%)
Fluorescein	Fluorescein Disodium Salt	300 mg/dL	No significant interference (within ± 0.3 mg/dL or 4%)
Naficillin	NA	50 mg/L	+ 0.3 mg/dL
Methylbenzethonium Chloride	NA	2.0 mg/dL	No significant interference (within ± 0.3 mg/dL or 4%)
Rifampin	NA	2.5 mg/dL	- 0.3 mg/dL

2. Interference may occur with serum samples from patients diagnosed as having plasma cell dyscrasias and lymphoreticular malignancies associated with abnormal immunoglobulin synthesis, such as multiple myeloma, Waldenstöm`s macroglobulinemia, and heavy chain disease. Some of these samples may precipitate when mixed with reagent. Results for these samples will be suppressed due to "rxn noise". An accurate result may be obtained as follows.

- Prepare a 0.9% sodium chloride (NaCl) solution or use a commercial preparation.
- Combine one part of the original patient sample with one part of the prepared NaCl solution and mix well.
- Analyze the solution. Multiply the result by 2.

If the NaCl dilution still gives a suppressed result due to "rxn noise", SEND TO PAML.

3. Phosphorous determinations made in plasma are frequently subject to nonspecific interferences.
4. Lipemic samples with visual turbidity >3+, or with a Lipemia Serum Index >8, should be ultracentrifuged and the analysis performed on the infranate.
5. Refer to References for other interferences caused by drugs, disease and preanalytical variables.

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC Systems, refer to the appropriate system manual.

REFERENCES

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
Formatting, changed ref ranges to match LIS, added max dilutions, removed dilution protocol with TCA (no longer using), added send to PAML for interferring proteins.			
☐ *No significant change to process in above revision. Per CAP, this revision does not require further Medical Director approval.*			
Committee Approval Date	☐ Date: ☒ N/A – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 9/25/15