Principle

Intended Use

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 dialysate solutions.

Clinical Significance

Measurements of phosphorus (inorganic) are used 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. (1,2) 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.

Chemical Reaction Scheme

Specimen

Acceptable Sample Containers

13 x 75 PST, SST and Red Top BD tubes
PST, SST and Red Top BD microtainers
Dialysate solutions should be received in a 13 x 75 Clear Cap BD tube

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.(3) Freshly drawn serum or plasma or are the preferred specimens. Dialysate fluids should be received in a 13 x 75 Clear Cap BD tube Whole blood is not recommended for use as a sample.

Specimen Storage and Stability

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. (4)

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. (4)

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the *Primary Tube Sample Template*.

Unacceptable Specimens

Urine is not acceptable. Refer to the *Procedural Notes* section of this chemistry information sheet for further information on unacceptable specimens.

Reagents

Contents

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

Kit Reorder #467868

Volumes per Test

Sample Volume	8 µL
Total Reagent Volume	570 µL

Reactive Ingredients

Reagent Constituents

Ammonium Molybdate	3.2 mmol/L
pH	< 1.0

Also non-reactive chemicals necessary for optimal system performance.

CAUTION
Handle reagent with care. Reagent contains dilute SULFURIC ACID. Avoid contact with skin and clothing. In case of spill, flush with large amounts of water. In case of contact with eyes, wash at an eye bath for 10 to 15 minutes and seek medical attention immediately.

Materials Needed But Not Supplied With Reagent Kit

SYNCHRON® Systems AQUA CAL 1 and 2
At least two levels of control material
Saline

Reagent Preparation

1. Carefully pour 200 mL of molybdate reagent into the 1800 mL of diluent. Replace cap and mix at least ten times by gentle inversion. Record preparation date on the end label.
2. Date and initial reagent container and document in reagent log before loading each new bottle.

NOTICE
Do not reuse old reagent or mix fresh reagent with old reagent.

Acceptable Reagent Performance

The acceptability of this reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria, as defined in the Clinical Chemistry Quality Control Procedure #3000.T.

Reagent Storage and Stability

Phosphorus Reagent when stored unopened at 8°C to 30°C will remain stable until the expiration date indicated on each bottle. The combined Phosphorus Reagent is stable at room temperature for 30 days from the date of preparation, or by expiration date of either component, if sooner. **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.

NOTICE
Upon aging, the combined reagent may gradually turn light blue. This will not affect performance of the reagent.

Equipment

This test is performed on the Beckman UniCel DxC 800 systems; Beckman-Coulter, Brea, California.

Refer to the UniCel DxC 800 systems *Reference Manual* for detailed instructions.

Calibration

Calibrator Required

SYNCHRON® Systems AQUA CAL 1 and 2
Kit Reorder #s 471288 and 471291

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

If unopened calibrators should be stored at 2°C to 8°C until the expiration date printed on the calibrator bottle. Once opened, the calibrators are stable at room temperature for 30 days.

Repetitive refrigeration of the aqueous calibrators may facilitate crystal formation. Once removed from refrigerated storage, these calibrators should remain at room temperature.

Calibration Information

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

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 800 Systems *Instructions for Use* (IFU) manual.

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

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 print out with error codes and the system will alert the operator of the failure. The explanation of these error codes can be found in the UniCel DxC 800 System *Instructions For Use* (IFU) manual.

Traceability

Phosphorus (measurand) in these calibrators are traceable to the NIST* SRM 3139a.

**NIST - National Institute of Standards and Technology*

The set point values were established based upon the gravimetric addition of specific quantities of the measurand to achieve the appropriate concentration. The set point values for Phosphorus were established by human sample correlation to isotope dilution mass spectroscopy.

The values were verified using representative samples from this lot of calibrator and are specific to the assay methodologies of the SYNCHRON systems. Values determined by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

NOTICE

Benzoic acid crystal formation may result at lower temperatures in Cal 1 and Cal 2. Crystals may be redissolved by warming or maintaining at 18°C to 26°C for 24 to 48 hours.

Quality Control

At least two levels of control material should be analyzed each shift. In addition, these controls should be run with each new calibration, with each new bottle of reagent, and after specific maintenance or troubleshooting procedures as detailed in the UniCel DxC 800 System *Instructions For Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow.

The following controls should be used in accordance with the package instructions for use inserts. Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Quality Control Material

Control	Storage
MAS ChemTrak 1	2°C to 8°C*
MAS ChemTrak 3	2°C to 8°C*

*Controls are received frozen and stored at –10°C to –20°C. Bottles of controls in use are thawed and stored at +2°C to +8°C and are good for 14 days.

Testing Procedure

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 is required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operation.

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

Calculations

UniCel DxC Systems 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.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

Reporting Results

Equivalency between the SYNCHRON LX and UniCel DxC 800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

Reference Intervals

The following reference intervals were taken from literature and a study performed on SYNCHRON Systems.(7)

Reference Intervals

Interval	Sample Type	Conventional Units		
Literature	Serum or Plasma	2.7 - 4.5 mg/dL		
SYNCHRON	Serum or Plasma	2.4 - 4.7 mg/dL		
UCDMC	Serum or Plasma	Premature	At birth	5.6 - 8.0 mg/dL
	Serum or Plasma		6 - 10 d	6.1 - 11.7 mg/dL
	Serum or Plasma		20 - 25 d	6.6 - 9.4 mg/dL
	Serum or Plasma	Mature	< 3 d	5.0 - 7.8 mg/dL
	Serum or Plasma		3 - 6 d	5.8 - 9.0 mg/dL
	Serum or Plasma		6 days - 4 m	5.0 - 9.0 mg/dL
	Serum or Plasma		4 m - 1 yr	4.8 - 8.1 mg/dL
	Serum or Plasma		1 - 5 yrs	3.6 - 6.8 mg/dL
	Serum or Plasma		5 - 10 yrs	3.4 - 5.9 mg/dL
	Serum or Plasma		> 10 yrs	2.4 - 5.0 mg/dL

Refer to References (7,8,9) for guidelines on establishing laboratory reference intervals.

UCDMC Pediatric Reference Intervals are cited from literature. No in-house studies were performed. This information should serve as a general guideline.

There are no published reference intervals for dialysate solutions. These are custom solutions prepared for each patient and results from analysis are used to verify accuracy of dialysate solution preparations.

Critical Value

Phosphorus results **≤1.0** mg/dL is considered a critical value and should be called immediately to the attending physician or charge nurse.

Procedural Notes

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	Average Plasma-Serum Bias (mg/dL)
Ammonium Heparin	14 Units/mL	NSI[a]
Lithium Heparin	14 Units/mL	NSI
Sodium Heparin	14 Units/mL	NSI

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

Incompatible Anticoagulants

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

[a] Bias is based on worst case instead of the average.
Plus (+) or minus (-) signs signify positive or negative interference.

Limitations

None Identified.

Interferences

The following substances were tested for interference with this methodology:

Interferences

Substance	Source	Level Tested	Observed Effect[a]
Bilirubin (unconjugated)	Bovine	30 mg/dL	NSI[b]
Ditauro Bilirubin	Synthetic	20 mg/dL	+ 0.99 @ 2.1 mg/dL + 1.84 @ 8.9 mg/dL
Hemoglobin	RBC Hemolysate	250 mg/dL	+ 0.24 mg/dL
Lipemia	Intralipid[c]	500 mg/dL	NSI
	Human	Serum Index 8	NSI
Cefotaxime	Cefotaxime sodium salt	500 µg/dL	NSI
Ascorbic Acid	L-Ascorbic Acid	20 mg/dL	NSI
Fluorescein	Fluorescein Disodium Salt	300 mg/dL	NSI
Naficillin	NA[d]	50 mg/L	+ 0.3 mg/dL
Methylbenzethonium Chloride	NA	2.0 mg/dL	NSI
Rifampin	NA	2.5 mg/dL	- 0.3 mg/dL

[a] Plus (+) or minus (-) signs in this column signify positive or negative interference.
[b] Intralipid is a registered trademark of KabiVitrum, Inc.
[c] NSI = No Significant Interference (within ±0.3 mg/dl or 4%).
[d] NA = Not applicable

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, Waldenström's macroglobulinemia, and heavy chain disease. Some of these samples may precipitate when mixed with reagent. Results for these samples may be suppressed due to "rxn noise" or may result in "UL" or "DL" errors. Remisol will order a total protein on these samples to check total protein for abnormally elevated values.

An accurate phosphorus result may be obtained as follows.

1. Make 2 offline dilutions (X3 and X4) with a normal control (ChemTrak 1).
   Do not program the dilutions in Remisol.

   3(PHOS RESULT FROM MIX) + 2(PHOS MAS 1) = **PATIENT RESULT** FROM X3 DILUTION

   4(PHOS RESULT FROM MIX) + 3(PHOS MAS 1) = **PATIENT RESULT** FROM X4 DILUTION

2. Analyze the solutions. Manually calculate the results from the 2 dilutions. The 2 dilution results should match within +/- 0.2 mg/dL. If they do not match and the protein levels are elevated, the phosphorus result should not be released and the comment "Unable to determine due to elevated abnormal paraproteins." should be entered as the result comment. Do not over-dilute to below the analytical range.
3. Phosphorous determinations made in plasma are frequently subject to nonspecific interferences.(8) If a phosphorus result is unable to be determined by dilution and is not due to protein interference, the comment "Unable to determine due to nonspecific interferences" should be used.
4. Grossly lipemic samples should be ultracentrifuged and the analysis performed on the infranate.
5. Patients being treated with high dosages of drugs that use a phospholipid bilayer in a liposomal envelope as a delivery system may exhibit elevated serum/plasma results (e.g., AmBisome®)(10*)
6. Refer to References (11,12,13) for other interferences caused by drugs, disease and preanalytical variables.

   *AmBisome is a registered trademark of Gilead Sciences, Inc.

Performance Characteristics

Analytical Measurement Range

The SYNCHRON LX Systems and UniCel DxC Systems method for the determination of phosphorus provides the following analytical range:

Analytical Measurement Range

Sample Type	Conventional Units	S.I. Units
Serum/Plasma/Fluid	0.5 - 12.0 mg/dL	0.2 - 3.9 mmol/L

Clinical Reportable Range:

Clinical Reportable Range

Sample Type	Conventional Units	S.I. Units
Serum or Plasma/Fluid	0.5 - diluted result mg/dL	0.2 - diluted result mmol/L

Samples with concentrations below the AMR and CRR (0.5 mg/dL) will be reported as "**< 0.5 mg/dL**".
Samples with concentrations greater than the AMR should be diluted with saline and reanalyzed.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

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 or dialysate solutions.

Equivalency

Equivalency was assessed by correlation analysis of patient samples to accepted clinical methods as determined by Beckman.

Serum or Plasma (in the range of 0.9 to 12.3 mg/dL):

Y (SYNCHRON LX Systems)	= 0.970X + 0.29
N	= 96
MEAN (SYNCHRON LX Systems)	= 5.63
MEAN (SYNCHRON CX ® 7 DELTA)	= 5.51
CORRELATION COEFFICIENT (r)	= 0.9979

Urine (in the range of 6.5 to 116 mg/dL):

Y (SYNCHRON LX Systems)	= 0.935X + 0.75
N	= 75
MEAN (SYNCHRON LX Systems)	= 48.6
MEAN (SYNCHRON CX® 7 DELTA)	= 51.1
CORRELATION COEFFICIENT (r)	= 0.9983

Serum or Plasma (in the range of 0.5 to 11.6 mg/dL):

Y (UniCel DxC Systems)	= 1.004X + 0.02
N	= 198
MEAN (UniCel DxC Systems)	= 4.7
MEAN (SYNCHRON LX Systems)	= 4.7
CORRELATION COEFFICIENT (r)	= 0.999

Diluted Urine (in the range of 5.5 to 136.1 mg/dL):

Y (UniCel DxC Systems)	= 1.009X + 0.02
N	= 80
MEAN (UniCel DxC Systems)	= 45.6
MEAN (SYNCHRON LX Systems)	= 45.2
CORRELATION COEFFICIENT (r)	= 0.999

Refer to references (14) for guidelines on performing equivalency testing.

Equivalency assessed by correlation analysis of patient samples at UCDMC

Serum or Plasma (in the range of 2.0 to 11.5 mg/dL):

Y (UniCel DxC800-4118)	= 0.981X + 0.08
N	= 32
MEAN (UniCel DxC800-4118)	= 4.21
MEAN (UniCel DxC800-1805)	= 4.21
CORRELATION COEFFICIENT (r)	= 0.9986

Serum or Plasma (in the range of 2.0 to 11.5 mg/dL):

Y (UniCel DxC800-4427)	= 0.977X + 0.13
N	= 32
MEAN (UniCel DxC800-4427)	= 4.24
MEAN (UniCel DxC800-1805)	= 4.21
CORRELATION COEFFICIENT (r)	= 0.9987

Serum or Plasma (in the range of 2.0 to 11.5 mg/dL):

Y (UniCel DxC800-4449)	= 0.976X + 0.11
N	= 32
MEAN (UniCel DxC800-4449)	= 4.22
MEAN (UniCel DxC800-1805)	= 4.21
CORRELATION COEFFICIENT (r)	= 0.9989

Serum or Plasma (in the range of 2.0 to 11.3 mg/dL):

Y (UniCel DxC800-4427)	= 0.995X + 0.05
N	= 32
MEAN (UniCel DxC800-4427)	= 4.24
MEAN (UniCel DxC800-4118)	= 4.21
CORRELATION COEFFICIENT (r)	= 0.9993

Serum or Plasma (in the range of 2.0 to 11.5 mg/dL):

Y (UniCel DxC800-4449)	= 0.976X + 0.11
N	= 32
MEAN (UniCel DxC800-4449)	= 4.22
MEAN (UniCel DxC800-4118)	= 4.21
CORRELATION COEFFICIENT (r)	= 0.9989

Serum or Plasma (in the range of 2.0 to 11.4 mg/dL):

Y (UniCel DxC800-4449)	= 1.000X - 0.02
N	= 32
MEAN (UniCel DxC800-4449)	= 4.22
MEAN (UniCel DxC800-4427)	= 4.24
CORRELATION COEFFICIENT (r)	= 0.9995

Precision

A properly operating SYNCHRON System(s) and UniCel DxC system(s) should exhibit precision values less than or equal to the maximum performance limits in the table below. Maximum performance limits were derived by an examination of the imprecision of various methods, proficiency test summaries, and literature sources.

As determined by Beckman

Maximum Performance Limits

Type of Precision	Sample Type	1 SD	Changeover Value[a]	%CV
		mg/dL	mg/dL	
Within-run	Serum/Plasma	0.15	7.5	2.0
Total	Serum/Plasma	2.3	7.7	3.0
Within-run	Urine	2.0	100.0	2.0
Total	Urine	3.0	100.0	3.0

[a] When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test %CV to the %CV guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Within-run Precision established at UCDMC

Type of Precision	Sample Type	n	Mean (mg/dL)	1 SD	%CV
DxC800-4118 Within-run	SYNCHRON 1	20	1.80	0.00	0.0
	SYNCHRON 3	20	6.62	0.04	0.7
DxC800-4427 Within-run	SYNCHRON 1	20	1.82	0.04	2.3
	SYNCHRON 3	20	6.80	0.05	0.08
DxC800-4449 Within-run	SYNCHRON 1	20	1.81	0.04	2.2
	SYNCHRON 3	20	6.64	0.05	0.8

Type of Imprecision	Sample Type	n	Mean (mg/dL)	SD	%CV
DxC800-4118 Day to Day	MAS ChemTrak 1	1327	3.0	0.08	2.7
	MAS ChemTrak 3	1343	8.6	0.21	2.4
DxC800-4427 Day to Day	MAS ChemTrak 1	1321	2.9	0.09	3.1
	MAS ChemTrak 3	1334	8.5	0.22	2.6
DxC800-4449 Day to Day	MAS ChemTrak 1	1334	3.0	0.07	2.3
	MAS ChemTrak 3	1342	8.6	0.21	2.4

Comparative Performance

As determined by Beckman

Data for a SYNCHRON LX® System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.(15)

NCCLS EP5-T2 Precision Estimate Method.

Type of Imprecision	Sample Type		No. Systems	No. Data Points[h]	Test Mean Value (mg/dL)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	1.84	0.05	2.7
	Serum	Control 2	1	80	7.00	0.04	0.6
	Urine	Control 1	1	80	40.33	0.36	0.9
	Urine	Control 2	1	80	79.63	0.39	0.5
Total	Serum	Control 1	1	80	1.84	0.05	2.9
	Serum	Control 2	1	80	7.00	0.07	1.0
	Urine	Control 1	1	80	40.33	1.09	2.7
	Urine	Control 2	1	80	79.63	0.95	1.2

[a] The point estimate is based on the pooled data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.

NOTICE
These degrees of precision and equivalency were obtained in typical testing procedures on the SYNCHRON LX System and are not intended to represent the performance specifications for this reagent.

Additional Information

For more detailed information on UniCel DxC Systems, refer to the *Instructions for Use* and *Reference* manual.

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Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	October 2000	Reformatted

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
			11/27/2000	G. Kost
			12/28/2001	G. Kost
			10/16/2002	G. Kost
			10/10/2003	S. Devaraj
			10/25/2004	S. Devaraj
			11/28/2005	G. Kost
			09/26/2006	G. Kost
			11/05/2007	G. Kost
03/11/2008	Revised dilution procedure	M.Inn		
05/26/2008	Amphotericin B Therapy	M.Inn		
			06/16/2008	G. Kost
01/08/2009	General update	M.Inn		
			09/15/2009	G. Kost
			10/12/2010	G. Kost
12/2010	update	M. Inn	11/16/2011	G. Kost
06/22/2012	Added dialysate solutions as a sample type	M. Inn	06/22/2012	G. Kost
08/07/2013	No reference interval for spot/random urines	M. Inn	08/16/2013	G. Kost
			09/17/2013	G. Kost
			08/28/2015	J. Gregg
10/17/2015	Remove urine as an acceptable sample type	kdagang	10/26/2015	J. Gregg