SYNCHRON® System(s)
Chemistry Information Sheet

PHS
Phosphorus
REF A09426

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

PHS reagent, in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) and Synchron® Systems Multi Calibrator, is intended for the quantitative determination of inorganic phosphorus concentration in human serum, plasma or urine.

CLINICAL SIGNIFICANCE

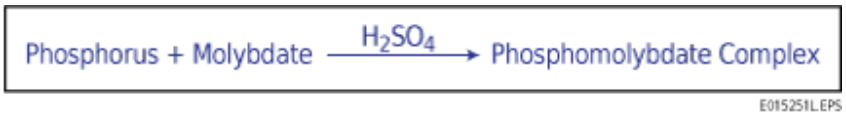
Measurements of phosphorus (inorganic) are used in the diagnosis and treatment of various disorders, including parathyroid gland and kidney diseases, and vitamin D imbalance.

METHODOLOGY

PHS reagent is used to measure the phosphorus concentration by a timed endpoint method.[1,2] In the reaction, inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a colored phosphomolybdate complex.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 67 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is directly proportional to the concentration of phosphorus in the sample and is used by the system to calculate and express the phosphorus concentration.

CHEMICAL REACTION SCHEME



SPECIMEN

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 are the preferred specimens. Freshly collected urine may also be used for testing. 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.[4]
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.[4]
3. It is recommended that urine specimens be collected in an acid-washed, detergent-free container. After collection, specimens should be acidified to pH<3 with hydrochloric acid (HCl).[5] Assays should be performed within 2 hours of collection. For timed specimens, the collection container should be kept in the refrigerator or on ice during the time period.[6]

ADDITIONAL SPECIMEN STORAGE AND STABILITY CONDITIONS AS DESIGNATED BY THIS LABORATORY:

SAMPLE PREPARATION

Sample preparation is not required prior to analysis on SYNCHRON® System(s). Urine samples are diluted (1:10) automatically by the system using the DIL1 cartridge.

SAMPLE VOLUME

A filled 0.5 mL sample cup is the optimum volume. For optimum volume in primary tube samples, or if urine specimens are sampled from test tubes, refer to Primary Sample Tube Chart Template.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

CRITERIA FOR SAMPLE REJECTION AS DESIGNATED BY THIS LABORATORY:

PATIENT PREPARATION

SPECIAL INSTRUCTIONS FOR PATIENT PREPARATION AS DESIGNATED BY THIS LABORATORY:

SPECIMEN HANDLING

SPECIAL INSTRUCTIONS FOR SPECIMEN HANDLING AS DESIGNATED BY THIS LABORATORY:

REAGENTS

CONTENTS

Each kit contains the following items:

Two PHS Reagent Cartridges (2 x 300 tests)

VOLUMES PER TEST

Sample Volume	4 µL
Total Reagent Volume	267 µL
Cartridge Volumes	
A	243 µL
B	24 µL
C	– –

Urine

Sample Dilution Volumes	
Sample Volume	20 µL
Diluent Volume	180 µL

Urine	
Total Reagent Volume	267 µL
Cartridge Volumes	
A	243 µL
B	24 µL
C	—

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Ammonium Molybdate	2.5 mmol/L
pH	< 1.0

Also non-reactive chemicals necessary for optimal system performance.

EUROPEAN HAZARD CLASSIFICATION

Molybdate Solution (Compartment B)	C;R35	Causes severe burns.
	S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
	S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
Phosphorus Diluent (Compartment A)	C;R35	Causes severe burns.
	S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
	S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Synchron® Systems Multi Calibrator
At least two levels of control material
Saline
DIL 1 for urine samples

REAGENT PREPARATION

No preparation is required.

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

PHS reagent, when stored unopened at room temperature, will remain stable until the expiration date indicated on the cartridge label. Once opened, the reagent is stable for 30 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE.

DIL 1 stored unopened at room temperature is stable until the expiration date indicated on each cartridge. Once opened, DIL 1 is stable for 60 days on instrument or until the expiration date, if sooner.

REAGENT STORAGE LOCATION:

CALIBRATION

CALIBRATOR REQUIRED

Synchron® Systems Multi Calibrator

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

If unopened, the Synchron® Systems Multi Calibrator may be stored at -15°C to -20°C until the expiration date printed on the calibrator bottle. Opened calibrators that are resealed and stored at +2°C to +8°C are stable for 20 days unless the expiration date is exceeded.

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[7]

CALIBRATOR STORAGE LOCATION:

CALIBRATION INFORMATION

1. The system must have a valid calibration curve in memory before control or patient samples can be run.
2. Under typical operating conditions the PHS reagent cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the SYNCHRON LX *Maintenance Manual and Instrument Log*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual. This assay has within-lot calibration available. Refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual for information on this feature.
3. For detailed calibration instructions, refer to the SYNCHRON LX *Operations Manual*, or 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 SYNCHRON LX *Diagnostics and Troubleshooting Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

TABLE 1 QUALITY CONTROL MATERIAL

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. If necessary, 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 operations.

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

CALCULATIONS

The system performs 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.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals listed below were taken from a study performed on SYNCHRON Systems.

TABLE 2 REFERENCE INTERVALS

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
SYNCHRON® Systems	Serum or Plasma	2.5 – 4.6 mg/dL	0.81 – 1.49 mmol/L
Literature[5]	Urine (non-restricted diet)	0.4 – 1.3 g/24 hrs	12.9 – 42 mmol/24 hrs

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Laboratory			

Refer to References (8,9,10) for guidelines on establishing laboratory-specific reference intervals.

ADDITIONAL REPORTING INFORMATION AS DESIGNATED BY THIS LABORATORY:

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 at least 40 healthy volunteers:

TABLE 3 ACCEPTABLE ANTICOAGULANTS

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	DEMING REGRESSION ANALYSIS
Lithium Heparin	14 Units/mL	Y = 1.004X - 0.09; r = 0.998

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	DEMING REGRESSION ANALYSIS
Sodium Heparin	14 Units/mL	Y = 1.002X - 0.07; R = 0.999

LIMITATIONS

None identified.

INTERFERENCES

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

TABLE 4 INTERFERENCES

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT[a]
Bilirubin	Porcine	30 mg/dL	NSI[b]
Hemoglobin	RBC Hemolysate	250 mg/dL	NSI
		375 mg/dL	+0.5 mg/dL
Lipemia	Human	4+ (visual)	NSI
Cefotaxime	Cefotaxime sodium salt	50 mg/dL	NSI
Ascorbic Acid	L-Ascorbic Acid	20 mg/dL	NSI
Fluorescein	Fluorescein Disodium Salt	75 mg/dL	+0.7 mg/dL
Methotrexate	NA[c]	2 mmol/L	NSI
Nafcillin	NA	2.5 mg/dL	NSI
		3.75 mg/L	+ 0.7 mg/dL
Methylbenzethonium Chloride	NA	5 mg/dL	NSI
Rifampin	NA	10 mg/dL	NSI

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 may be suppressed. An accurate result may be obtained as follows.

   A. Prepare a 12% aqueous solution of trichloroacetic acid (TCA).

   B. Combine one part of the original patient sample with one part of the prepared TCA solution and mix well.

   C. Centrifuge for 10 minutes at 1200 x g at room temperature.

   D. Analyze the supernatant. Multiply the result by 2.

3. Phosphorus determinations made in plasma are frequently subject to nonspecific interferences.[9]

4. 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®) [11d]

5. Refer to References (10,12,13,14,15) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

Analytic Range

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

TABLE 5 ANALYTICAL RANGE

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Serum/Plasma	1.0 – 12.0 mg/dL	0.3 – 3.9 mmol/L
Urine	10 – 120 mg/dL	3.2 – 38.7 mmol/L

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

REPORTABLE RANGE (as determined on site):

TABLE 6 REPORTABLE RANGE

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the PHS determination is 1.0 mg/dL (0.3 mmol/L) for serum or plasma and 10 mg/dL (3.2 mmol/L) for urine.

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

Serum or Plasma (in the range of 1.0 to 12 mg/dL):

Y (SYNCHRON LX Systems)	= 0.984X + 0.12
N	= 112
MEAN (SYNCHRON LX Systems PHS Reagent)	= 5.6
MEAN (SYNCHRON CX Systems PO4 Reagent)	= 5.6
CORRELATION COEFFICIENT (r)	= 0.999

Urine (in the range of 10 to 120 mg/dL):

Y (SYNCHRON LX Systems)	= 1.041X - 0.62
N	= 78
MEAN (SYNCHRON LX Systems PHS Reagent)	= 49
MEAN (SYNCHRON CX Systems PO4 Reagent)	= 48
CORRELATION COEFFICIENT (r)	= 0.995

Refer to References (16) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:

TABLE 7 PRECISION VALUES

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[e]		% CV
		mg/dL	mmol/L	mg/dL	mmol/L	
Within-run	Serum/Plasma	0.2	0.06	10.0	3.23	2.0
	Urine	2.0	0.65	100.0	32.3	2.0
Total	Serum/Plasma	0.3	0.1	10.0	3.23	3.0
	Urine	3.0	0.97	100.0	32.3	3.0

Comparative performance data for the SYNCHRON LX® System evaluated using the NCCLS Approved Guideline EP5-A appears in the table below.[17] Each laboratory should characterize their own instrument performance for comparison purposes.

TABLE 8 NCCLS EP5-A PRECISION ESTIMATE METHOD

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points	Test Mean Value (mg/dL)	EP5-A Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	2.0	0.05	2.4
	Serum	Control 2	1	80	6.6	0.09	1.4
	Urine	Control 1	1	80	41.1	0.43	1.0
	Urine	Control 2	1	80	78.3	0.91	1.2
Total	Serum	Control 1	1	80	2.0	0.05	2.7
	Serum	Control 2	1	80	6.6	0.10	1.5
	Urine	Control 1	1	80	41.1	0.61	1.5
	Urine	Control 2	1	80	78.3	1.26	1.6

NOTICE

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

ADDITIONAL INFORMATION

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

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

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2. Dryer, R. L., Routh, J. I., "Determination of Serum Inorganic Phosphorus", *Clin. Chem.*, 4:191 (1963).

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4. National Committee for Clinical Laboratory Standards, *Procedures for the Handling and Processing of Blood Specimens*, Approved Guideline, NCCLS publication H18-A, Villanova, PA (1990).

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10. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).

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16. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Tentative Guideline, NCCLS publication EP9-T, Villanova, PA (1993).

17. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices*, 2nd Edition, Approved Guideline, Vol. 19, No. 2, NCCLS publication EP5-A, Villanova, PA (1999).

EC REP Beckman Coulter Ireland Inc., Mervue Business Park, Mervue, Galway, Ireland (353 91 774068)

Beckman Coulter, Inc., 250 South Kraemer Blvd., Brea, CA 92821

ENDNOTES

a Plus (+) or minus (-) signs in this column signify positive or negative interference.

b NSI = No Significant Interference (within ±0.4 mg/dL or 4%).

c NA = Not applicable.

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

e 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 guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.