Brown Clinic Northridge
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Watertown, SD

Brown Clinic 506
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Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Phosphorus

PURPOSE:
The PHOS method used on the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative measurement of inorganic phosphorus in serum and plasma. Measurements of phosphorus (inorganic) are used in the diagnosis and treatment of bone, parathyroid and renal disease.

PRINCIPLE:
The phosphorus (PHOS) method is a modification of the classical phosphomolybdate method introduced by Fiske and Subbarow[1]. The PHOS method measures the absorption of the unreduced phosphomolybdate complex as reported by Daly and Ertingshausen. [2] 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

$$(NH_4)_2MoO_4 + PO4 \xrightarrow[\text{pH 1.1}]{} (NH_4)_3[PO_4(MoO_3)_{12}]$$

INSTRUMENT, REAGENTS & SUPPLIES:

Reagent Wells[a]	Form	Ingredient	Concentration
1–6	Liquid	Ammonium Molybdate Sulfuric Acid	3.8 mmol/L
7-8	Liquid	Sulfuric Acid Detergent	0.22mol/L

a. Wells are numbered consecutively from the wide end of the cartridge.

Risk and Safety:

H290, H314
P280, P301 + P310 + P331, P303 + P361 + P353 + P310, P305 + P351 + P338, P390, P501
Danger!
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. Immediately call a POISON CENTER or doctor/physician. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Absorb spillage to prevent material damage. Dispose of contents and container in accordance with all local, regional, and national regulations.
Contains: Sulphuric acid
Safety data sheets (MSDS/SDS) available on www.siemens.com/healthcare

Precautions: Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.
For *in vitro* diagnostic use

Reagent Preparation: All reagents are liquid and ready-to-use.

STORAGE & STABILITY:
Store at 2 – 8° C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed wells on the instrument are stable for 30 days.

Once open Well Stability : 3 days for wells 1–6
30 days for wells 7- 8

SPECIMEN REQUIREMENTS:

Specimen Collection and Handling:
Serum and plasma should be collected using recommended procedures for collection of diagnostic blood specimens by venipuncture.[3]
Follow the instructions provided with your specimen collection device for use and processing.[4]
For serum complete clot formation should take place before centrifugation. Serum or plasma should be separated from red cells within 1 hour because erythrocytes contain phosphate concentrations several times greater than those found in the serum.[5,6]

Thawed frozen specimens which are turbid must be clarified by centrifugation prior to testing.

The purpose of specimen handling and storage information is to provide guidance to users; however, users may validate their own procedures for handling and storing patient samples
Specimen:

- patient preparation: no patient preparation required
- specimen type: serum or heparinized plasma
- Stability: $\leq$ 8 hours at room temp, $\leq$ 7 days at 2-8oC. For longer storage, specimens may be frozen for $\leq$ 3 months at -20oC or colder.

- handling: specimens are stable for ≤ three freeze-thaw cycles

PROCEDURE:

Material Needed:

PHOS Flex® reagent cartridge, Cat. No. DF61A

Materials Required But Not Provided:

CHEM II Calibrator, Cat. No. DC20

Quality Control Materials

Test Steps

Sampling,[b] reagent delivery, mixing, processing and printing of results are automatically performed by the Dimension® clinical system. For details of this processing, refer to your Dimension® Operator's Guide.

The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus the dead volume; precise container filling is not required.

Test Conditions

Sample Size:	3 µL
Reagent 1 Volume:	50 µL
Reagent 2 Volume:	20 µL
Reagent 3 Volume:	20 µL
Diluent Volume:	350 µL
Temperature:	37°C
Wavelength:	340 - 700 nm
Type of Measurement:	Bichromatic endpoint

Calibration

The general calibration procedure is described in your Dimension® system manual (also see Appendix B).

The following information should be considered when calibrating the phosphorus method:

Assay Range for serum and plasma:	0.5 – 9.0 mg/dL [0.16–2.91 mmol/L]
Calibration Material	Dimension® CHEM II Calibrator, Cat. No. DC20
Calibration Scheme	3 levels, n=3
Units	mg/dL [mmol/L] (mg/dL x 0.323)= [mmol/L]
Typical Calibration Levels	2.0, 5.0, 8.0 mg/dL [0.65, 1.62, 2.58 mmol/L]
Calibration Frequency	Every 3 months for any one lot
A new calibration is required	-For each new lot of Flex® reagent -After major maintenance or service, if indicated by quality control results -As indicated in laboratory quality control procedures -When required by government regulations
Assigned Coefficients	C_0 -1.100 C_1 0.077

c. Système International d'Unités [SI Units] are in brackets.

Backup Process:
Refer to Brown Clinic Back-up Policy

CONTROLS:
At least once daily run solutions at two levels of a quality control material with known concentrations. For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

INTERPRETATION:
The instrument automatically calculates and prints the concentration of PHOS in mg/dL [mmol/L] using the calculation scheme illustrated in your Dimension® Operator's Guide.

REFERENCE RANGE:
Serum or Plasma: 2.5-4.9 mg/dL

Each reference interval was calculated non-parametrically and represents the central 95% of the population.

Each laboratory should establish its own reference intervals for PHOS as performed on the Dimension® system.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings

REPORTING:
report format: mg/dl

LIMITATIONS:

Results:	> 9.0 mg/dL [2.91 mmol/L]
Manual dilution:	Serum/Plasma: make appropriate dilution with Reagent grade water to obtain result within the assay range. Enter dilution factor. Reassay. Resulting readout is corrected for dilution.

Autodilution (AD) (for serum, plasma): Refer to your Dimension® system literature.

Serum and plasma samples with results less than 0.5 mg/dL [0.16 mmol/L] will produce an instrument flag "below assay range" and should be reported as "less than 0.5 mg/dL [0.16 mmol/L]".

Serum and plasma samples with results in excess of 9.0 mg/dL [2.91 mmol/L] are reported as "Above Assay Range" and should be repeated on dilution.

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® system manual.

Analytical Specificity
For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

Reference: PHOS Flex® reagent cartridge insert sheet PN 717061.001 Issue Date 2016-1-17

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Written By:_____Lori Murray MT(ASCP)_____Date: _8-8-12

Approved By: __Aaron Shives, MD_____ Date: __10/23/2017___
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	Changed instrumentation to EXL200
3-30-2016	Sam Legg	Changed to method per package insert dated 02-04-2015
10/18/17	Heather Hall	Updated to package insert dated 1/17/16, modified backup method