0. Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2002 data.

Two laboratories performed this testing during 2001-2002. In order to maintain confidentiality of the participants the quality control summary statistics and graphs were combined to mask the individual analysis dates from the two laboratories. Methods for both labs are included in this release. Most methods for Lab18 analytes are in one combined file. Methods Lab40 are described in a separate file for each analyte tested.

A list of the released analytes follows:

Lab	Analyte	SAS Label	Description
l40_b	LBDSPH	Phosphorus (mg/dL)	Phosphorus
l40_b	LBDSPHSI	Phosphorus (mmol/L)	

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The LX system uses a timed-rate method to determine the concentration of phosphorus in serum, plasma and urine. In the reaction, inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a colored phosphomolybdate complex. The system monitors the change in absorbance at 365 nm at a fixed-time interval. This change in absorbance is directly proportional to the concentration of phosphorus in the sample.

Phosphorus measurements are used in the diagnosis and treatment of kidney disease and disorders involving the parathyroid gland.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Sani-Cloth HB, Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Microsoft Excel software on a PC and our Laboratory Information Systems (L.I.S.) are used to manage the data. The test is analyzed on a Beckman Synchron LX20. When all ordered tests are completed for each sample, the results are printed out by Beckman Synchron LX20 instrument.
The LX20 is interfaced to the Laboratory Information Systems (L.I.S.) with a bi-directional interface. After results have printed at the LX20 printer, the results will go to the L.I.S. Host Interface Workstation to be collated and then certified by qualified analyst.

b. A statistical evaluation of the runs is accomplished with Microsoft Excel software on a PC. An Adhoc report of the completed runs data is saved to a floppy disk in a comma delimited format (CSV) text file. The file is opened and copied to an Excel spreadsheet for evaluation. The Excel spreadsheet results file data are copied to the shipment file and saved as a comma delimited file (CSV) and e-mailed to Westat within 21 days of sample receipt.

c. The Excel files containing all raw data and results are backed up once a week using a CD writer or Zip drive for storage. Files stored on the L.I.S. network are automatically backed up nightly to tape.

d. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. Interferences:
   1) No interference from ≤30 mg/dL bilirubin.
   2) No interference from <2+ hemolysis.

b. Separated serum or plasma should not remain at +15°C to +30°C 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.

c. Fasting is not required.

d. A minimum of 0.6 mL serum is needed for the Multi-Analyte Panel.

e. Sample volume for individual test is 8 µl added to 570 µl reagent.

f. Sample is run singly as part of Multi-analyte Biochemistry Panel.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Instrumentation: Beckman Synchron LX20

b. Materials
   1) Beckman Synchron CX Micro Sample Tube (Part #448774)
   2) S/P Plastic Transfer Pipet (Cat. #P5214-10)
   3) S/P Brand Accutube Flange Caps (Cat. #T1226-37)

c. Reagent Preparation: Synchron LX Phosphorus (PHOSm) Reagent (Part #467868)
   1) Pour 200 mL bottle of molybdate reagent into the 1800 mL bottle of diluent. Recap and mix at least 10 times. (Technical tip: Let prepared reagent stand at least 2 hours prior to loading on LX).
   2) Unopened reagent when stored at room temperature will remain stable until the expiration date.
   3) Combined reagent is stable for 30 days unless the expiration date is exceeded.
   4) Do not freeze or refrigerate.
   5) If reagent is frozen in transit, thaw completely, warm to room temperature and mix thoroughly by inverting at least 10 times.
   6) Irritating to skin and eyes. Avoid contact with reagent. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

d. Standards Preparation: No preparation required.
   Synchron LX Aqua Calibrators 1, 2 (Part #471288 and #471291).

e. Control Material
   1) Beckman Triad Custom Unassayed Chemistry Control Serum (Part #465405)
      - In use through August 23, 2002.
   2) Bio-Rad Liquid Unassayed Multiqual (Cat. #697, 699).
      - In use from August 24, 2002
      - Thaw new bottle weekly. Mix very well, using rocker prior to use.
      - Thawed control is stable 7 days. Mix well prior to each use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibrators: Synchron LX Aqua Calibrators 1 and 2 (Part #471288 and #471291). Refer to LX Operation Procedure for storage and stability information.
b. Calibration:
   1) Required with new bottle of reagent and after certain parts replacement or maintenance procedures.
   2) Calibration frequency: 72 hours.
   3) Refer to LX Operation Procedure for programming calibration.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries
   1) Enter test in L.I.S. as a part of a panel according to procedure listed in this document (See Attachment A).
b. Sample Preparation
   1) Procedure for labeling CX sample tubes and transferring serum (See Attachment B).
c. Operation
   1) Refer to Operation Procedures for programming controls/patients and loading sectors/racks in the Beckman LX20 Chemistry Information Manual, 2001 (See Attachment C for specific procedure for NHANES samples).
d. Recording of Data
   1) Operator will review results and collate and certify in the L.I.S.
   2) Operator will place printouts in box labeled for NHANES samples.
   3) Project supervisor will do an Adhoc Report onto a floppy disk in a comma delimited text file from the L.I.S.
   4) Comma delimited file is opened in Excel on a PC and copied into another Excel file to further evaluate the data.
   5) A printout of the Excel spreadsheet for each container ID results is made and comments noted.
   6) Project supervisor reviews the results. If problems noted with patient results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.
   7) Daily log sheets are completed and any problems or issues noted.
   8) Repeat values are used when match the original results within 3 CSV's.
e. Replacement and Periodic Maintenance of Key Components
   (See Attachment AB for LX20 Maintenance Schedule).
f. Calculations
   Synchron LX 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.

9. REPORTABLE RANGE OF RESULTS

a. Analytical Range:
   1) 0.5 - 12.0 mg/dL
      a) Samples out of analytical range high should be diluted with saline and reanalyzed. Enter dilution factor at sample information screen or multiply printout by dilution factor to obtain the final result.
      b) If phosphorus result is $\leq$1.6 mg/dL and patient does not have a known monoclonal gammopathy, result may be low due to non-fasting.
      c) Any out of range low result (provided there is no known monoclonal gammopathy) should be reported as "<0.5 mg/dL".

2) Limits of detection (LOD) are established by Beckman-Coulter and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.
3) 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.
4) 0 is not a reportable value.

10. QUALITY CONTROL (QC) PROCEDURES

a. Blind QC Specimens are included in the samples received from NHANES.
b. Beckman Triad Custom Unassayed Chemistry Controls Levels 2 and 3 are assayed in early A.M. and if a new reagent pack is loaded, controls are assayed again. One level is assayed in middle of the day and both control levels are assayed after running NHANES sample.
c. BioRad Liquid Unassayed Multiqual Controls Levels 1 and 3 are substituted for Beckman Triad controls as of August 24, 2002 for CDC-NHANES runs to allow long term control use. Multiqual controls are analyzed at beginning and end of runs with CDC-NHANES samples.
d. Acceptable Answer:
   1) Controls must be within ±2 S.D.
   2) Refer to Quality Control Flow Chart for action decisions guidelines (See Attachment I).

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. Hemolysis greater than 2+ demonstrates a positive interference Hemoglobin.
b. Bilirubin (unconjugated) has no significant interference.
c. Lipemia has no significant interference.
d. Interference, due to turbidity, may occur in patients with plasma cell dyscrasias and lymphoreticular malignancies. Phosphorus results may be “suppressed” (from “rxn noise”) due to presence of paraproteins which may precipitate when mixed with reagent.
e. Cefotaxime, Ascorbic Acid, Fluorescein and Methylbenzethonium Chloride have no significant interference.
f. Naficillin demonstrates a positive interference.
g. Monoclonal gammopathy can cause interference from serum paraprotein.
h. Refer to References for other interferences caused by drugs, disease and preanalytical variables.

13. REFERENCE RANGES (NORMAL VALUES)

Phosphorus

Serum/Plasma Age Group	Reference Range mg/dL
0-5 Y	3.4-5.9

5-10 Y	2.9-5.9
10-12 Y	3.3-6.2
>15 Y	2.6-4.4

Reference Range values were established from wellness participants with an age mix similar to our patients. These data were analyzed using non-parametric techniques described by Reed (Clin Chem 1971;17:275) and Herrara (J Lab Clin Med 1958;52:34-42) which are summarized in recent editions of Tietz' textbook. Descriptions appear in Clin Chem 1988;34:1447 and Clinics in Laboratory Medicine June 1993;13:481.
Pediatric Reference Range Guidelines for Synchron Systems- Multicenter study using data from Montreal, Quebec, Miami, FL and Denver, CO. Beckman 1995

14. CRITICAL CALL RESULTS ("PANIC VALUES")

There are no critical call back values for phosphorus.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive refrigerated. Specimens are kept refrigerated until ready to transfer to CX multi sample tubes. Capped CX sample tubes are kept refrigerated until ready to put on instrument.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of pour off tubes. Specimen vial container is placed in -70°C Freezer after testing is complete. CX sample tubes are refrigerated, then frozen after analysis.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Samples will remain in refrigerator until instrument is back in operation.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

The collaborating agency with access to patient identifiers or the responsible medical officer is notified by FAX by the Project Supervisor of any critical values. Copies of Faxes sent concerning abnormal results are kept in a folder by the supervisor for the duration of the study.

Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an comma delimited file, either through electronic mail or other electronic means.

All data are reported electronically to Westat within 21 days of receipt of specimens.

Internet FTP transfers of files or dial up modem transfer options are available.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

In general, when specimens are received, the specimen ID number, and a name identifying the container ID and slot number is entered into the Laboratory Information System (L.I.S.) database. New barcodes are printed and the specimens stored in a refrigerator. Samples are aliquoted to a CX-Micro Sample tube with the new barcodes. The specimen ID is read off of the tube by a barcode reader. Tracked in the database are the date and time of entry into the L.I.S., date and time analysis completed, and who certified the results.

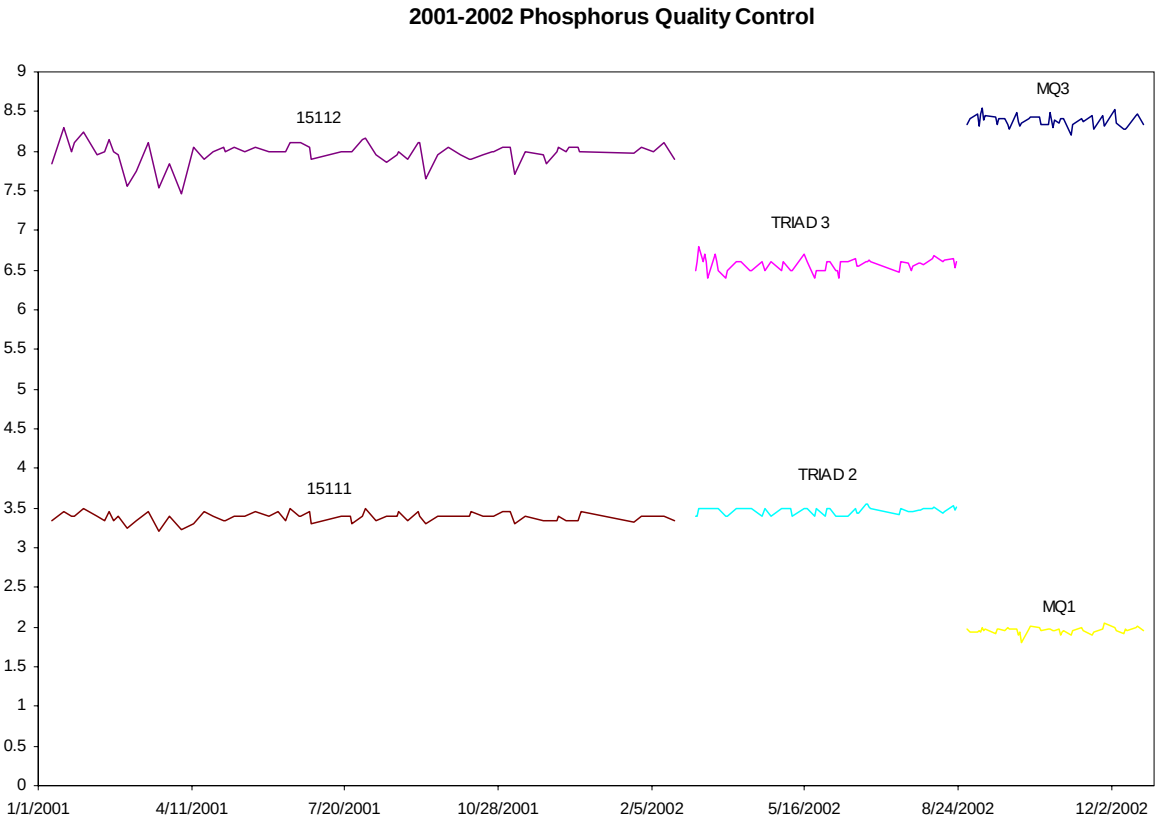
Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Phosphorus by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
15111	71	1/10/2001	2/20/2002	3.39	0.058	1.7
15112	71	1/10/2001	2/20/2002	7.98	0.144	1.8
TRIAD 2	58	3/6/2002	8/23/2002	3.47	0.05	1.4
TRIAD 3	58	3/6/2002	8/23/2002	6.57	0.08	1.2
MQ1	46	8/30/2002	12/23/2002	1.96	0.04	2.0
MQ3	46	8/30/2002	12/23/2002	8.38	0.07	0.9



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