Laboratory Procedure Manual

Analyte: **Bone Alkaline Phosphatase**

Matrix: **Serum**

Method: Beckman Coulter Access Ostase assay

Method No.:

Revised:

as performed by: *University of Washington Medical Center*
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Important Information for Users

The University of Washington periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001–2002 data. There were two methods used to measure Bone Alkaline Phosphatase in NHANES 2001-2002. For NHANES 2001, the HybritechTandem-MP Ostase ImmunoEnzymetric assay was used for quantitative measurement of Bone Alkaline Phosphatase (BAP). For NHANES 2002, the Beckman Access Ostase assay was used to measure serum Bone Alkaline Phosphatase.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
l11_b	LBXBAP	Bone Alkaline Phosphatase (BAP) (µg/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access Ostase assay is an *in vitro* assay for the quantitative measurement of bone alkaline phosphatase (also called skeletal alkaline phosphatase, sALP), an indicator of osteoblastic activity in human serum and plasma. It is intended to be used as an aid in the management of postmenopausal osteoporosis and Paget's disease.

The Access Ostase assay is a one-step immunoenzymatic assay. A mouse monoclonal antibody specific to BAP is added to a reaction vessel with paramagnetic particles coated with goat anti-mouse polyclonal antibody. Calibrators, controls, and samples containing BAP are added to the coated particles; they bind to the anti-BAP monoclonal antibody. After the formation of a solid-phase/capture antibody/BAP complex, separation in a magnetic field and washing remove materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos*530, is added to the reaction vessel, and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of BAP in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Serum levels of sALP are believed to reflect the metabolic status of osteoblasts. An accurate assessment of bone metabolism is critical for determining the severity of metabolic bone disease and responses to therapy. Measurement of serum levels of sALP has been shown to be useful in evaluating patients with Paget's disease, osteomalacia, primary hyperparathyroidism, renal osteodystrophy, osteoporosis, and metastases to bone. Total alkaline phosphatase determinations have been the accepted method for the diagnosis and monitoring of patients with Paget's disease.

Paget's disease of bone is a common skeletal disorder in which there is a focal proliferation of the normal cellular components of bone. Paget's disease is more prevalent than once thought, with a prevalence rate in certain populations equal to 3–4% in middle-aged patients and 10–15% in the elderly. This disease does not affect young individuals. The majority of patients with Paget's disease have no symptoms and often go undiagnosed unless an abnormal X-ray or serum alkaline phosphatase level is found in the course of a medical evaluation for unrelated reasons. The most common complaints in symptomatic patients are pain and deformity.

The risk of osteoporosis, another bone remodeling disorder, depends in part upon skeletal development, the attainment of peak bone mass, and in later life, the amount of bone lost. In healthy children, bone formation is favored over bone resorption, which results in bone development and normal skeletal growth. In healthy young adults, bone formation and bone resorption are balanced, resulting in no net increase or decrease in skeletal mass. With advancing age, men and women experience an imbalance in bone remodeling in which resorption is slightly greater than formation, resulting in a continuous net loss of bone mass with time. If this imbalance persists, bone mass may decline until the skeleton is insufficient to withstand normal mechanical stresses, and it becomes abnormally susceptible to fractures. The excessive loss of bone mass with an increased susceptibility to fractures is a disorder known as osteoporosis.

The most common form of osteoporosis occurs in postmenopausal women and is the result of estrogen deficiency. Rapid bone loss accompanies the decline of estrogen levels at the onset of menopause or as a result of surgical removal of the ovaries. Rapid bone loss occurs as a result of the combined effects of imbalance in bone remodeling and an increase in bone turnover. In the United States, osteoporosis affects some 25 million postmenopausal women and is the cause of approximately 1.5 million fractures annually, including approximately 500,000 vertebral crush fractures, 250,000 hip fractures, and 200,000 distal radius fractures.

Hormone replacement therapy is currently the most widely prescribed therapy for the prevention of osteoporotic fractures in postmenopausal women. However many women cannot, or will not, avail themselves of hormone replacement therapy because of the potential for the increased risk of cancer and the resumption of menstrual bleeding. For this reason, other compounds such as bisphosphonates, a standard treatment for Paget's disease of bone, have been developed to treat osteoporosis. The anti-resorptive properties of bisphosphonates decrease bone remodeling and, consequently, decrease the overall loss of bone.

Biochemical markers are useful in monitoring metabolic bone disease. Urinary hydroxyproline and total serum alkaline phosphatase have been used for monitoring the treatment of Paget's disease.

Osteoporosis, however, represents a more subtle modification of the bone resorption process; therefore, more specific and sensitive markers are needed.

Osteomalacia is the term used to describe a pathological condition in bone in which the osteoid matrix (the proteinaceous scaffolding in bone) remains uncalcified. The most common condition causing osteomalacia is vitamin D deficiency, resulting in rickets in children or osteopenia with bone fractures in adults. Elevated serum alkaline phosphatase is a hallmark of this condition. Recent data suggests that mild degrees of vitamin D deficiency may be very common in the population, and could contribute to osteopenia in patients diagnosed with osteoporosis.

The Ostase assay is an *in vitro* device for the quantitative measurement of sALP in human serum. Changes in sALP have been shown to be useful in patients undergoing therapy for metabolic bone disorders. It may also be an indicator of vitamin D deficiency in some patients.

2. SAFETY PRECAUTIONS

Consider all samples received for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions. Wear gloves, lab coat, and safety glasses when handling all human blood products and infectious viruses. Place disposable plastic, glass, paper, and gloves that contact blood in a biohazard bag or discard pan to be autoclaved. Disinfect all work surfaces with a 1:200 dilution of Staphene (Calgon Vestal Laboratories, St. Louis, MS) Dispose diluted specimens and any other potentially contaminated materials in a biohazard bag at the end of the analysis to be autoclaved prior to final disposal. Autoclaved or disinfect other non- disposable material at the end of the working day.

Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wash hands thoroughly after removal of personal protective devices used in handling specimens and kit reagents.

Material safety data sheets (MSDSs) for all reagents used in the performance of this assay are kept in the Immunology Division, University of Washington Medical Center (UWMC).

3. COMPUTERIZATION: DATA SYSTEM MANAGEMENT

A. Each shipment of specimens received from the NHANES IV mobile unit arrives with a corresponding transmittal sheet and a Send File [a comma delineated (CSV) text file] transmitted electronically (labeled boxnum.shp). This file contains the following information:

Send File

Field	Type
Sample ID	XXXXXXXXX
Slot Number	XXX
Sample Collection Date	mm/dd/yyyy hh:mm:ss
MEC Comment Code	XX

B. The information from the shipping file is imported into a result file with the following format:

Results File: Bone Alkaline Phosphatase-Vessel ID 19

Field	Format	Type	Item ID
Sample ID	XXXXXXXXX	Int	
Slot Number	XXX	smallint	
Sample Collection Date	mm/dd/yyyy hh:mm:ss	Smalldatetime	
MEC Comment Code	XX	Smallint	
Bone Alk. Date of Receipt	mmddyyyy	Smalldatetime	LBXBAPDR
Bone Alk. Run num	{test code}mmddyy. x(letter)	Char(10)	LBXBAPBT
Bone Alk. Date of Analysis	mmddyyyy	Smalldatetime	LBXBAPDA
Bone Alk. Result	XXXXX.X	Numeric(6,1)	LBXBAP
Bone Alk. Comment	XX	Smallint	LBXBAPLC
Bone Alk. Analyst id	XXX	Char(3)	LBXBAPTK
Bone Alk. 2.5% repeat	XXXXX.X	Numeric(6,1)	LBCBAP

C. After the testing is completed, the run number, date of analysis, sALP result, sALP comment, sALP analyst, and the sALP 2.5% repeat results are entered into the result file.

D. Data entry is checked for errors.

E. After the sALP testing has also been completed, resulted, and checked, the result file is stored as a CSV file and is transmitted electronically to NHANES WESTAT. Electronic and hard copies of the files and all primary data are kept in the laboratory.

F. Technical support for this system is provided by Westat, Rockville, MD (1-301-294-2036)

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

A. No special preparation of the patient is necessary.

B. A whole blood specimen should be obtained by acceptable medical technique. For serum samples, allow the blood to clot and separate the serum by centrifugation.

C. The requested sample volume for the assay is 1.0 mL, and the minimum sample volume is 0.5 mL.

D. Serum and plasma (lithium heparin and sodium heparin) are the recommended sample types.

E. If the serum sample is to be assayed within 48 hours after collection, the specimen can be stored in a refrigerator at 2–8°C.

F. Specimens held for longer times (up to 2 months) should be frozen at –20°C or colder.

G. Specimens should be collected in such a way as to avoid hemolysis.

H. Turbid serum samples or samples containing particulate matter should be centrifuged prior to use. Contamination or introduced particulate matter can lead to erroneous results.

I. Specimens may be stored in glass or plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample.

J. Avoid repeat freeze/thaw cycles.

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

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRTORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

A. Reagents and standard materials.

(1) Access Ostase Reagent Packs, cat no # 37300, 2 packs 50 tests/pack, (Beckman Coulter Inc., Fullerton, CA). When unopened, packs are stable until manufacturer expiration date. Once opened, stable for 28 days. Store upright at 2–8°C. Must be refrigerated for at least 2 hours before use on the instrument.

R1a: Paramagnetic particles coated with goat anti-mouse polyclonal antibody suspended in Tris-buffered saline, with surfactant, bovine serum albumin (BSA), protein (goat), <0.1% sodium azide, and 0.1% ProClin300.

R1b: Anti-BAP mouse monoclonal antibody diluted in Tris-buffered saline, with surfactant, BSA, <0.1% sodium azide, and 0.1% ProClin300.

(2) Access Ostase Calibrators, cat. # 37305 (Beckman Coulter Inc., Fullerton, CA). Provided at zero, and approx. 7, 15, 30, 60, and 120 µg/L. Stable until manufacturer expiration date. Store at 2–8°C.

(3) Access Sample Diluent A, cat. # 81908. (Beckman Coulter Inc., Fullerton, CA). Stable until manufacturer expiration date. Store at 2–8°C.

(4) Access Substrate, cat. # 81906. (Beckman Coulter Inc., Fullerton, CA). Stable until manufacturer expiration date when unopened and stored at 2–8°C. Can store at room temperature for up to 14 days prior to use. Once opened and placed into use on the instrument, stable for an additional 14 days.

(5) Access Wash Buffer, cat. # 81907. (Beckman Coulter Inc., Fullerton, CA). Stable until manufacturer expiration date. Store at room temperature.

(6) Stock human BAP, cat. # 1003 (Cliniqa, Fallbrook, CA). Store frozen –70 ºC. Used to prepare control material.

(7) Gibco normal human serum (NHS) (Gibco, Gaithersburg, MD). Used to prepare control material.

B. Reagent Preparation

Kit reagents are ready to use as provided. Thoroughly mix reagents by gentle mixing before loading them onto the instrument. Substrate should be at room temperature at least 18 hours (but less than 14 days) prior to loading it onto the instrument.

C. Instrumentation

(1) Access or Access II Immunoanalyzer (Beckman Coulter, Inc., Fullerton, CA)

(2) Centrifuge (Jouan, Winchester, VA)

(3) Computer (Dell Computer Systems, Round Rock, TX).

(4) Transfer pipettes, cat. no. #7524 (Becton-Dickinson, Franklin Lakes, NJ).

(5) Disposable tip precision pipettors: fixed volume or adjustable for 20 and 180 µL (± 5%) (Any vendor).

(6) Test tubes for sample dilutions (Any vendor)

(7) Conical sample cups, 2 ml, cat. no. #02-544 (Fisher Scientific, Pittsburgh, PA)

D. Standards/Calibrator Preparation

Calibrators are received in a liquid ready to use format. No further preparation is required prior to use.

E. Preparation of Quality Control Materials

The Immunology Division prepares two levels of control from normal and/or pooled patient serum. Both pools are analyzed with each assay. Prepare in sufficient quantity to provide serum samples for 2 years. Prior to aliquoting and defining, test the stock once for approximate value and adjust if necessary. For the normal control, use normal human serum. (Gibco, Gaithersburg, MD) Mix well prior to aliquoting. For the high control, dilute normal human serum with an elevated patient serum or purchased human BAP.

Analyze newly prepared control material for at least 20 runs in parallel with the current control to determine acceptance ranges. Acceptance ranges must be determined prior to using control material for any patient run evaluations.

Divide the stock control material into 10–15 mL tubes containing a volume for a 3–4 month supply and label with 'I #' and freeze at –85°C. As needed, thaw a stock control tube and divide into approximately 900 µL aliquots to be stored for a maximum of 3–4 months at –70°C. Thaw and use aliquot within 1 week, recap and store at 2–8°C between uses. The aliquot vial label should include the date of preparation and a letter indicating sequential aliquot. (Examples: 9/90-A for the first time this control is aliquoted, 9/90-B for the second time). Record the label on the quality control material record sheet.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration Curve

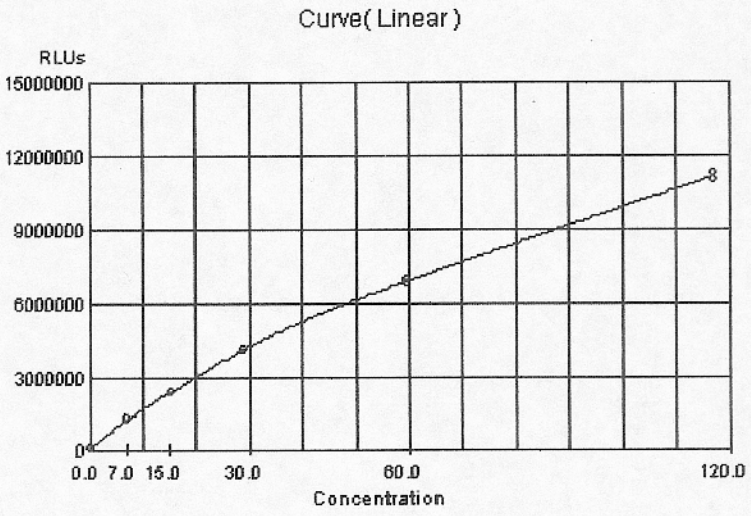
Calibration is required every 42 days to maintain an active calibration curve. Refer to the Operator's Guide and Reference Manual for complete instructions on calibration procedures.

Example of Access Bone Alkaline Phosphatase calibration curve and data:

Calibration Data Report
Access 2 Immunoassay System
S/N 500476

Test Name:	Ostase	Curve:	Last Run; Active
Reagent Lot:	106444	Status:	**Passed**
Calibrated:	07/08/02 08:41	Reason:	
Expiration:	08/19/02 08:41	Cal Lot:	105791
Comment:			

Stated Conc. ug/L	RLU	1 SD	%CV	Calculated Conc. ug/L	Flags
S0 0.00	8177			No Value	
S0 0.00	8238			No Value	
Mean	**8207.67**	**43.39**	**0.53**	**No Value**	
S1 7.10	1204700			7.22	
S1 7.10	1229130			7.38	
Mean	**1216915.00**	**17274.62**	**1.42**	**7.30**	
S2 15.50	2331360			15.36	
S2 15.50	2296350			15.09	
Mean	**2313855.00**	**24755.81**	**1.07**	**15.23**	
S3 29.00	4069330			29.19	
S3 29.00	4065800			29.16	
Mean	**4067565.00**	**2496.09**	**0.06**	**29.17**	
S4 59.70	6859340			60.22	
S4 59.70	6759070			58.92	
Mean	**6809205.00**	**70901.60**	**1.04**	**59.57**	
S5 117.00	11206600			No Value	
S5 117.00	10984300			115.48	
Mean	**11095450.00**	**157189.84**	**1.42**	**115.48**	



B. Verification

The instruments used to read assay results are equipped to analyze the positive and negative controls for each test series. If, within a testing series, positive or negative controls do not conform to specifications as defined in the quality control manual, the entire series is invalidated.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

(1) Check reagent and system supplies, load any needed supplies onto the instrument. Mix reagent pack contents by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs — mix reagents by swirling gently. Check instrument zone temperature.

(2) Inspect fluidics module. Clean exterior of the substrate, dispense and aspirate probes.

(3) Thaw and mix specimens. Centrifuge samples that may have particulate matter.

(4) Run controls to verify that the assay is performing as expected.

B. Instrument Operation: (see Access Operators Guide for details).

(1) Load specimens into specimen racks, with the barcode in the open slot. Make sure there are no bubbles. Alternately, use the barcode wand to identify the specimens, and then load samples into the appropriate sample cups. Load the racks onto the instrument.

(2) Select the Ostase test, initiate testing.

(3) The instrument automatically calculates all results. After testing is completed, results are printed and reviewed by the technologist. Samples with results above the top standard (approximately 120 µg/L are diluted 1:10 off-line using Access diluent A and repeated, and results are corrected for the dilution factor. Samples with results < 0.1 µg/L are repeated to confirm. Do not rerun samples that have sat on the Access for more than 60 minutes. Pour fresh aliquots before rerunning.

(4) Remove specimens and controls. Return controls to the refrigerator and refreeze specimens.

(5) Perform scheduled instrument maintenance (daily, weekly, and monthly) as outlined on the maintenance log. See the operator's manual for specific instructions.

C. Recording of Data

(1) Analytical Results Data:

Specimen results are entered into the assay specific results table created from the send file corresponding to the specific sample box using Excel software (Microsoft Corporation, Redmond WA). A copy of this table is printed out and checked for accuracy of data entry.

(2) Quality Control Data:

Control results are entered to the Assay Specific QC/Levey-Jennings Table using the Excel program. Compliance with the Westgard rules is evaluated. A copy of this table is printed out and checked for accuracy of data entry.

D. Replacement and Periodic Maintenance of Key Components

(1) Daily Maintenance:

Start-up:

- Inspect fluidics module.
- Check system supplies and replace as needed.
- Clean exterior of substrate, dispense, and aspirate probes.
- Prime substrate.
- Verify temperature.

- Shut-down:
- Check waste containers, empty if needed
- Perform special cleaning

(2) Weekly Maintenance:

- Change probes and clean them
- Clean exterior of the analyzer
- Clean upper portion of the main pipettor with alcohol wipe
- Inspect waste filter bottle for fluid
- Run system check

(3) Periodic Maintenance: to be performed by the manufacturer's service engineer.

E. Calculations

Patient test results are determined automatically by the system software. The amount of analyte in a sample is determined from the measured light production by means of a stored nonlinear calibration curve. Patient test results can be reviewed using the Sample Results screen. Refer to the Operator's Guide for complete instructions on reviewing results.

9. REPORTABLE RANGE OF RESULTS

Report values to the nearest 0.1 µg/L. Reportable range is from 0.1 µg sALP/L to approximately 120 µg sALP/L. The upper reportable value is determined by the calibration material supplied with the kit from the manufacturer. Values exceeding this upper limit are repeated on dilution on a following run until uncorrected values fall between 20 µg sALP/L to approximately 120 µg sALP/L. Report values <0.1 as <0.1 µg sALP/L.

10. QUALITY CONTROL (QC) PROCEDURES

A. Bench quality controls are used in this analytical method. Bench quality control specimens are tested with each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

B. The bench controls are prepared in sufficient quantity to provide serum samples for all the assays for approximately 1 year. Ranges are established after 20 parallel runs with previously established controls. The quality control pools comprise two levels of concentration spanning the low and high ranges for BAP.

C. Bench quality controls are placed at the beginning of each analytical run. After analysis, the long-term quality control charts (Levey-Jennings) for each control material are consulted to determine if the system is "in control." The Levey-Jennings chart plots the quality control material observations on the *y*-axis and the date of the observation on the *x*-axis. Quality control material observations are compared with the 95 and 99% confidence limits as well as with the center line (the overall mean of the characterization runs) prior to reporting any results. The system is out of control if any of the following events occur for any one of the quality control materials:

The observation from a single pool falls outside the 99% confidence limits.

The observations from two pools fall either both above or both below the 95% confidence limits.

The observations from eight successive runs for one pool fall either all above or all below the center-line.

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

If the run is declared "out of control", the system (instrument, calibration standards, reagents etc.) are investigated to determine the root of the problem before any results are released. Consult with the supervisor for appropriate actions.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Heterologous Antibody Interference: Some individuals have antibodies to mouse protein (human antimouse antibodies, HAMA), which can cause interference in immunoassays that employ antibodies derived from mice. This is most like to be seen in the samples from patients who have undergone therapy or diagnostic procedures that include infusion of mouse monoclonal antibody. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples. This assay has been formulated to minimize the effects of these antibodies on the assay. However, carefully evaluate results from patients suspected of having such antibodies.

B. Serum samples with significant elevations of liver ALP activity may yield elevated results in this assay. 100 U/L of BAP yields a result of 37.5 µg/L in this assay. In comparison: 100 U/L of liver ALP gives a result of 5.5 µg/L, 100 U/L of placental ALP gave a result of 0.03 µg/L, and 100 U/L of intestinal ALP gave a result of 1.8 µg/L.

C. Patients with metabolic bone disorders who have low levels of disease activity may have skeletal ALP levels that fall within the expected values.

D. These results should be used only in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures. Therefore, this assay is not recommended for use as a screening procedure to detect the presence of osteoporosis in the general population. Furthermore, this assay cannot be used to assess the rate of bone formation or bone remodeling.

E. Hemoglobin and bilirubin, which were tested up to 500 and 20 mg/dL, respectively, do not interfere with this assay. Triglycerides, which were tested at concentrations up to 2000 mg/dL, do not interfere. Total protein did not interfere tested at concentrations between 3.0 and 15.6 g/dL.

F. Various concentrations of drugs were added to three separate serum pools containing sALP and assayed in quadruplicate. The drugs and range of concentrations added were: acetaminophen (35 mg/dL), alendronate (8 mg/dL), aspirin (50 mg/dL), calcitonin-salmon (112 IU/dL), calcium (20 mg/dL), estrogen (10 mg/dL), etidronate (105 mg/dL), ibuprofen (40 mg/dL), pamidronate (18 mg/dL), progesterone (25 mg/dL), residronate (6 mg/dL), raloxifene (12 mg/dL), and vitamin D (80,500 IU/dL). These drugs did not interfere with the recovery of sALP from the serum pools.

13. REFERENCE RANGES (NORMAL VALUES)

Male:	3.7–20.9 µg/L
Premenopausal female:	2.9-14.5 µg/L
Postmenopausal female:	3.8-22.6 µg/L

This reference range is based on studies involving an apparently healthy adult population 20–89 years of age at six test sites

14. CRITICAL CALL RESULTS (PANIC VALUES)

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens should be maintained at 20–25°C during testing. After testing, the samples are stored at <–70°C.

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

There are no acceptable alternative methods of analysis. Specimens may be stored at 4–8°C for no longer than 2 days. Otherwise, specimens should be stored at <–70°C until the system is returned to functionality.

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

Not applicable to this procedure.

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

Standard record keeping should be used for tracking specimens. The primary results include daily test results as well as stored quality control results.

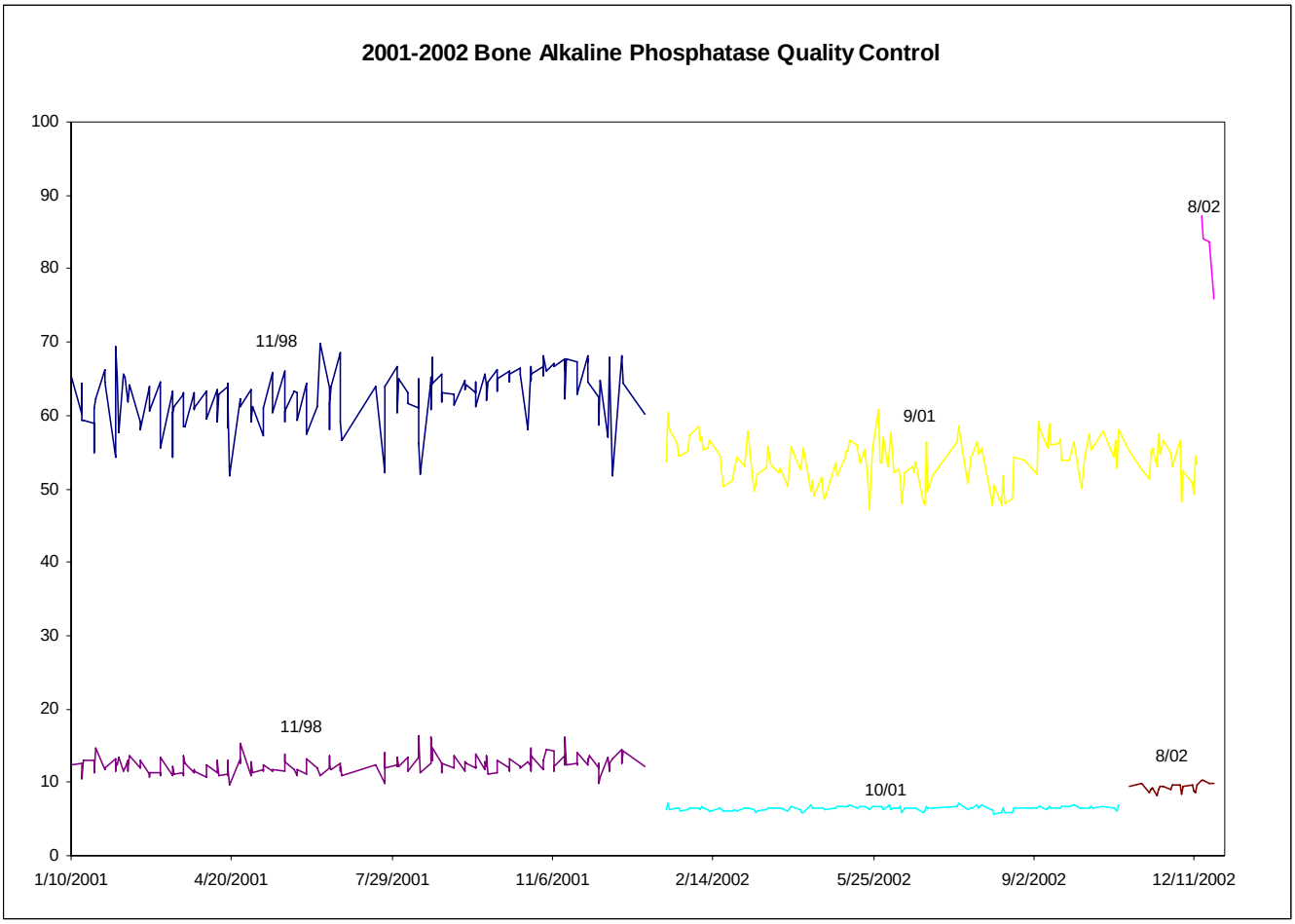
The original NHANES III ship file is copied into a template Excel file and onto the hard drive of the IBM computer. After the results are entered into the database and assay results transmitted electronically files are stored for 6 months on a server that is backed up on a daily basis. After 6 months, the result files are transferred onto a CD along with copies of the original ship files and QC information.

The residual serum is stored at <–70°C for 3 months after analysis; then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Bone Alkaline Phosphatase by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
11/98	172	1/10/2001	1/3/2002	62.7	3.57	5.7
11/98	178	1/10/2001	1/3/2002	12.3	1.13	9.1
9/01	133	1/16/2002	12/13/2002	53.8	2.96	5.5
10/01	114	1/16/2002	10/25/2002	6.5	0.30	4.7
8/02	23	11/1/2002	12/23/2002	9.3	0.56	5.9



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