Laboratory Procedure Manual

Analyte: **N Telopeptide in Urine**

Matrix: **Urine**

Method: **Enzyme Immunoassay**

Method No.:

Revised:

as performed by: *Department of Laboratory Medicine*
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Important Information for Users

The University of Washington Medical Center 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.

Two different methods were 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.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
l11_b	URDNT	N-telopeptides (NTx) (nmol BCE)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Osteomark is a competitive inhibition enzyme-linked solid-phase immunosorbent assay for the quantitative measurement of the cross-linked N-telopeptides of type I bone collagen (NTx) in human urine. The solid phase consists of microwells onto which cross-linked telopeptides (antigen) are adsorbed. Urine controls, test samples, and calibrators are added to the antigen-coated 96-well plate. Antibody to the N-telopeptide cross-links that are conjugated to horseradish peroxidase is then added to each well. During an initial incubation period, antigen in the sample competes with the solid-phase antigen for binding to the antibody. The wells are then washed to remove unbound material.

Buffered substrate/chromogen reagent is then added to each well. During the final incubation, a blue color will develop when bound antibody-horseradish peroxidase conjugate is present in the well. The color intensity is a measure of the amount of conjugated antibody bound to the solid-phase antigen and is inversely proportional to the amount of antigen in the test sample. The reaction is stopped by the addition of stopping reagent (1N sulfuric acid), which results in a color change from blue to yellow. The absorbance values for the control, calibrators, and test samples are determined spectrophotometrically at 450 nm with a 650 nm reference filter by using a microtiter plate reader.

A standard curve is constructed for each assay by plotting absorbance versus concentration for each calibrator. The antigen concentrations of the samples and control are then read from the curve. Assay values are standardized to an equivalent amount of bone collagen and are expressed in nanomoles bone collagen equivalents (nM BCE/L) per liter. Often assay results are corrected for urinary dilution by urinary creatinine analysis and expressed in nanomoles bone collagen equivalents per liter (nM BCE/L) per millimole creatinine per liter (mM creatinine/L). This ratio is reported as nM BCE/mM creatinine.

Mammalian bone is continuously remodeled through a coupled process of osteoclast- mediated bone resorption, followed by osteoblast-mediated bone formation. This process is necessary for normal development and maintenance of the skeleton. Abnormalities in this tightly coupled process often result in changes in skeletal mass and shape. The measurement of specific degradation products of bone matrix provides analytical data of the rate of bone metabolism.

Approximately 90% of the organic matrix of bone tissue is type I collagen, a helical protein that is cross-linked at the N-terminal and C-terminal ends of the molecule. This protein forms the basic fabric and tensile strength of bone tissue.

The discovery of urinary cross-linked N-telopeptides of type I collagen (NTx) has provided a specific biochemical marker of human bone resorption. The NTx molecule is specific to bone due to the unique amino acid sequences and orientation of the cross-linked α-2 N-telopeptide. Generation of the NTx molecule is mediated by osteoclasts on bone and is found in the urine as a stable end-product of degradation.

Elevated levels of urinary NTx indicate elevated bone resorption. Clinical research has demonstrated that elevated bone resorption is the primary cause of age-related bone loss and that low bone mass often results in osteopenia and is the major cause of osteoporosis. Loss of bone mass occurs when bone resorption levels are elevated above bone formation levels. Osteoporotic fractures are reported to be the major source of increased morbidity and mortality in older women.

Clinical studies have supported the use of urinary NTx levels to monitor the response to treatment of patients with diagnosed osteoporosis. A study was conducted to determine the ability of the assay to monitor the effect of antiresorptive therapy in patients with Paget's disease. Paget's patients treated with antiresorptive therapy should have a > 30% reduction in urinary NTx levels from baseline after 3 months of treatment.

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 the prevalence rate in certain populations at 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.

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, Missouri). 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 for all reagents used in the performance of this assay, including but not limited to Staphene, sodium hydroxide, sodium hypochlorite, and sodium azide, are kept in the Immunology Division, University of Washington Medical Center (UWMC).

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

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

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

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

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	
NTX Date of Receipt	Mmddyyyy	Smalldatetime	LBXNTDR
NTX Run num	{test code}mmddyy.x(letter)	Char(10)	LBXNTBT
NTX Date of Analysis	Mmddyyyy	Smalldatetime	LBXNTDA
NTX Result	XXXXX	int	LBXNT
NTX Comment	XX	Smallint	LBXNTLC
NTX Analyst id	XXX	Char(3)	LBXNTTK
NTX 2.5% repeat	XXXXX	int	LBCNT

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

Data entry is checked for errors.

- After the NTx testing has been resulted and checked, the result file is stored as a comma delineated file and is transmitted electronically to NHANES WESTAT. Electronic and hard copies of the files and all primary data are kept in the laboratory.

- 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

No special preparation of the patient is necessary.

Either a single urine collection other than a first morning void or a 24 hour urine collection may be used.

The requested sample volume for the assay is 2.0 mL, and the minimum sample volume is 1.0 mL

DO NOT ADD PRESERVATIVE TO THE URINE SPECIMEN.

Specimens with visible whole blood contamination or visible hemolysis may interfere with the assay and should be discarded. Collection of a new specimen is recommended.

Specimen can be stored at room temperature for up to 24 hours or at 2–8°C for up to 72 hours. For longer storage < –20°C is required, specimens maybe frozen and thawed up to 3 times.

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

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

Not applicable for this procedure.

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

A. Reagents and standard materials.

(1) Osteomark NTx Kit, Cat. #9006, (Ostex International, Inc., Seattle, WA). Contents are as follows:

(2) Osteomark antigen-coated microtiter plate (#5048-A): 12 1x8 microwell strips with adsorbed antigen from human bone tissue. Ready to use, return unused strips to pouch and reseal. Store at 2–8°C.

(3) Osteomark Antibody conjugate diluent (#5043-A): A buffer containing protein stabilizers and preservative for use as diluent for antibody conjugate concentrate. Ready to use. Store at 2–8°C.

(4) Osteomark Antibody conjugate concentrate (#5042-A): Mouse monoclonal antibody horseradish peroxidase conjugated solution with protein stabilizers and preservative. Dilute into antibody conjugate diluent at a 1:101 dilution for each assay run. Store at 2–8°C . Protect from light.

(5) Calibrators : 1, 30, 100, 300, 1000, and 3000 nM BCE (#5036A-5041A): Antigen from human bone tissue in protein stabilizers with buffers and preservative. Ready to use. Store at 2–8°C .

(6) Osteomark Buffered Substrate (5044-A): Buffered hydrogen peroxide solution with dimethylsulfoxide (DMSO). Use as diluent for Chromogen reagent. Protect from light. Store at 2–8°C.

(7) Osteomark Chromogen Reagent (#5005-A): 3,3',5,5'-tetramethylbenzidine (TMB) with DMSO. Dilute in buffered substrate at 1:101 dilution for each assay run. Protect from light. Store at 2–8°C.

(8) Osteomark 30X wash concentrate (#5045-A): A detergent solution to be diluted X30 in deionized water. Store concentrate at 2–8°C. Make by adding 50 ml of wash concentrate to 1450 ml of deionized H_2O. Diluted wash solution is stable for one month at room temperature.

(9) Osteomark Stopping Reagent (#5023-A): 1N H_2SO_4 . Supplied ready to use.

(10) Deionized water (University of Washington Medical Center, Seattle, WA)

B. Reagent Preparation

(1) Allow all reagents to equilibrate to room temperature (18–28°C) for at least 1 hour before performing the assay.

(2) Prepare the working strength wash solution. Dilute the 30× wash concentrate with deionized water (1 part 30× wash concentrate to 29 parts of deionized water). Mix for a minimum of 5 minutes. Stable for 1 month at room temperature.

C. Instrumentation

(1) Bio Tek EL 808 Automated Microtiter Plate Reader is an 8-channel, automated, benchtop, general purpose, enzyme immunoassay analyzer which measures the optical density of solutions in a 96-well microtiter plate at 405 nm, 414 nm, 450 nm, 550 nm, or 650 nm. Data handling and reduction is performed using Bio Tek KC4 KinetiCalc for Windows. This software is a general data reduction package used to analyze data generated from colormetric microtiter plate assays as read on the Bio Tek EL808. (Bio Tek Instruments, Winooski, Vermont) This instrument includes a Hewlett Packard Laser Jet 6MP printer (Hewlett Packard, Boise ID).

OR

(1) Rosys Plato 3301 is fully automated, benchtop, general purpose, enzyme immunoassay analyzer which performs all sample, dilution, plate rotation,, and reagent handling steps including movement of plates, mixing of samples, incubation, washing and measuring the optical density of solutions in a 96-well microtiter plate at 405 nm, 414 nm, 450 nm, 550 nm, or 650 nm. Data handling and reduction is performed using resident Rosys Plato 3301 software (Rosys Anthos Instruments, New Castle, DE) This instrument includes a Hewlett Packard Laser Jet 6MP printer (Hewlett Packard, Boise ID).

(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 and tips: fixed volume or adjustable for 25, 100, and 200 µL Optionally, a multichannel pipette can be used together with disposable V-shaped troughs for addition of NTx assay conjugate, substrate and quench reagents. (Any vendor)

(6) Skatron Macrowell Tube Strips (1.0 ml minitubes), cat #5776 (Genetic Systems Sanofi, Woodinville, WA)

(7) Microplate washer and Aspiration device (Nunc, Cambridge, MA)

(8) Timer, minimum range of 5–90 minutes in 1 minute intervals (any vendor)

(9) Container for storage of Wash Solution, 2 liter (any vendor)

(10) Containers for preparing Conjugate and Substrate Reagents, approximately 25 ml (any vendor)

(11) Plate Sealers, cat# 3095 (Corning Inc., Corning, NY)

D. Standards/Calibrator Preparation

Assay calibrators are received in a liquid ready-to-use format. No further preparation is required prior to use other than bringing to room temperature (18°C–28°C).

E. Preparation of Quality Control Materials

(1) The Immunology Division prepares two levels of control from normal and/or pooled patient urine. Both pools are analyzed with each assay.

(2) Prepare in sufficient quantity to provide control material for at least 2 years. Prior to aliquoting and defining, test the stock once for approximate value and adjust if necessary.

(3) 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.

(4) Divide the stock control material into 10-mL tubes containing a volume for a 3–4 month supply and label with 'l #' and freeze at < –70°C. As needed, thaw a stock control tube and divide into approximately 100 uL aliquots to be stored for a maximum of 3–4 months at –70°C. Thaw and use one aliquot of control material for each run.

(5) As new stock control is prepared, define a new control range by assigning the first value observed as the mean and assigning a large standard deviation. Append TEMP to the control lot number name. Prepare new blank Levey-Jennings table using these temporary limits.

(6) After 20 parallel runs, use the data from the Levey-Jennings chart to assign a permanent mean and standard deviation. Normal acceptance ranges are determined as mean + 2 standard deviations.

(7) Stock control material is aliquoted into individual use bullets. The aliquot bullet 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.

(8) The lot name should include an identifying name, the date the control was prepared (month and year), and information about the control range (temp or date of calculation or recalculation).

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibration Curve

Calibration materials are run with each patient run. Each run is evaluated using run specific observations. See following for example of observed absorbances and curve construction.

	Description	Absorbance. 450 nm (650 nm reference)	Mean Absorbance
1 2	1 nM BCE Calibrator	1.792 1.852	1.822
3 4	30 nM BCE Calibrator	1.602 1.634	1.618
5 6	100 nM BCE Calibrator	1.336 1.314	1.325
7 8	300 nM BCE Calibrator	0.903 0.953	0.928
9 10	1000 nM BCE Calibrator	0.548 0.528	0.538
11 12	3000 nM BCE	0.229 0.230	0.230

The standard curve is constructed using a 4-parameter curve-fitting program. The assay results are valid if the following criteria are met:

- The mean absorbance of the 1 nM BCE standard is > 1.500
- The span of the calibrator curve (difference between the absorbance values of the 1 nM BCE and 3000 nM BCE standards) must be > 1.300
- The correlation coefficient is > 0.99

B. Verification

The instruments used to read assay results are equipped to analyze the two different level controls for each test series. If, within a testing series, these 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) The procedure for the Osteomark NTx assay is performed at room temperature. Bring all kit components to room temperature (18–28°C) and gently mix before use. Larger bottles should be removed from the box to facilitate warming.

(2) Bring urine specimens to room temperature. Frozen urine specimens may be thawed at 37°C. Gently mix specimens, then centrifuge specimens at approximately 500 g for 15 minutes. Specimens should be transferred to minitubes prior to testing to reduce pipetting time.

(3) Remove the appropriate number of microwell strips from the sealed pouch. Promptly returned unused strips to pouch with desiccant.

(4) Prepared adequate wash solution and working conjugate for the run. The conjugate is diluted 1:101 in conjugate diluent within 1 hour prior to use.

Total number of strips	Conjugate Reagent (L)	Conjugate Diluent (ml)
3	70	7.0
4	90	9.0
5 – 6	120	12.0
7 – 8	150	15.0
9 – 10	180	18.0
11 – 12	220	22.0

(5) All calibrators, controls, and specimens should be tested at the same time and run in duplicate. Because the termination of each incubation stops a reaction that is in progress (i.e., antibody binding or substrate turnover), reliable calibration of the assay depends on ensuring that the incubation times are essentially the same for all wells.

B. Assay procedure:

(1) Pipette 25 L of calibrators, controls and all specimens into duplicate antigen-coated microwells, using a multichannel pipette. The two blank wells should remain empty. Swirl the plate gently on a flat surface for 5–10 seconds to ensure even coating.

Note: specimen dilutions are not routinely required.

(2) Pipette 200 L of working conjugate solution into each microwell using a multichannel pipette. A clean disposable reagent boat is used each time reagents are dispensed onto the plate. Apply a plate sealer and swirl the plate gently on a flat surface for 5–10 seconds to ensure mixing.

(3) Incubate at room temperature for 90 ± 5 minutes.

(4) Chromogen buffered substrate should be prepared during the last 10 minutes of the 90-minute incubation period, before beginning of the wash procedure. The chromogen reagent contains dimethylsulfoxide and has a high freezing temperature which may solidify upon refrigerated storage, but will be liquid at room temperature. Mix the bottle of chromogen reagent immediately prior to pipetting. Make a 1:101 dilution of chromogen reagent into buffered substrate using the volumes in the table below. First pipette buffered substrate into a clean plastic disposable tube and then add the chromogen reagent. Invert gently to mix. Do not vortex or shake vigorously. This solution should be colorless. Protect from light until used. Use within 30 minutes of preparation.

Total number of strips	Chromogen Reagent (L)	Buffered Substrate (ml)
3	70	7.0
4	90	9.0
5 – 6	120	12.0
7 – 8	150	15.0
9 – 10	180	18.0
11 – 12	220	22.0

(5) At the end of the incubation period, carefully remove and discard the plate sealer. Using a microtiter plate washing device, aspirate the conjugate solution then dispense wash solution into the first strip, move to the next strip and do the same. Complete all strips in the plate in this fashion. Return to the first strip and repeat this procedure on the entire plate 4 more times. After completing the fifth wash cycle, aspirate all fluid out of each strip, then pound out any excess liquid onto a stack of paper towels. Immediately proceed to the next step, do not allow the plate to dry.

(6) Pipette 200 L of working chromogen-buffered substrate into each well using a multichannel pipette. Use a clean disposable reagent boat to hold the chromogen-buffered substrate. Cover the plate with a plate sealer.

(7) Incubate at room temperature for 15 ± 1 minutes. A blue color will develop in wells containing bound antibody-horseradish peroxidase conjugate.

(8) At the end of the incubation period, add 100 L of stopping reagent to each well using a multichannel pipettor. The wells that have developed will turn yellow.

(9) Swirl the plate gently on a flat surface to ensure mixing. Allow the plate to set at room temperature for 5 minutes before reading absorbance levels.

(10) Within 30 minutes of adding stopping reagent, read absorbance on a microplate reader at 450 nm with a reference filter of 650 nm. Refer to separate operating instructions for the proper use of the microplate reader.

C. Calculations

(1) Results are calculated using a computer capable of 4-parameter curve fitting. NTx concentrations are inversely proportional to the absorbance. KC4 software (or Rosys Plato 3301) automatically calculate and print analytical results as derived from run specific calibration data.

(2) Samples with results above the top standard (3000 nM BCE) should be diluted and re-assayed. A urine sample with NTx results that are known to be in the range of 200–500 nM BCE is used as the diluent. The diluent value is confirmed by retesting it as a specimen on the same plate as used the diluted unknown specimen. The observed concentration of the diluted patient specimen must be corrected for the dilution factor using the following formula. (Note: When performing a 1:5 dilution the diluent represents a 4/5th contribution, the specimen provides 1/5th of the sample volume).

Specimen NTx concentration =
Specimen NTx result-{[(dilution factor – 1)/dilution factor] × diluent NT × result} × dilution factor

Example calculation:
Unknown specimen diluted 1:5 in diluent gave an NTx result of 1040 nM BCE
The diluent has an NTx result of 300 nM BCE

Specimen NTx = [1040 nM BCE – (4/5 x 300 nM BCE)] × 5 = 4000 nM BCE

Calculating results for diluted specimens can be simplified by defining the calculation formula on a spreadsheet using Excel software (Microsoft Corporation, Redmond WA).

(3) Final results are reported as nmoL BCE/mmoL Creatinine.
Example calculation:

Test sample Osteomark assay value = 360nM BCE

Urinary creatinine = 60 mg/dL /11.3* = 5.3 mM creatinine

$$\frac{360 \text{ nM BCE}}{5.3 \text{ mM creatinine}} = 68 \text{ nmoL BCE/mM creatinine}$$

* (11.3 = conversion factor used to convert mg/dL to mM for creatinine).

D. 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 into the assay specific Levey-Jennings table and plot if they are found to be in compliance with Westgard rules The evaluated copy of the table is printed out and checked for accuracy of data entry.

9. REPORTABLE RANGE OF RESULTS

Report results to the nearest whole number. The reportable range is from 20–3000 nM BCE. The upper reportable value is determined by the calibration material supplied with the kit from the manufacturer. Specimens with results exceeding this upper limit are repeated on dilution on a following run until the uncorrected values fall between 20–3000 nM BCE. Specimens with results less than 20 nM BCE are repeated to confirm the result.

10. QUALITY CONTROL (QC) PROCEDURES

A. Good laboratory practices include the use of control specimens within an assay run to ensure that all reagents and protocols are performing properly.

B. Recovery of control concentration should fall within the stated range. If the controls are out of range:

- Verify that the microplate reader is correctly programmed.
- Verify that the controls have not exceeded the expiration date.
- Check the control ranges for accuracy.
- Verify that the order of addition has not been changed.

C. The coefficient of variation (% CV) of the duplicate readings for each calibrator, control, and patient samples should be less than 20%.

D. All pipettes used in testing clinical specimens should be checked for calibration every 3 months. Pipettes that do not conform to specifications should be autoclaved and sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records should be kept for each pipette by serial number.

E. Estimates of imprecision can be generated from long-term quality control pool results. Bench quality controls are used in this analytical method. Bench quality control specimens are inserted by the analyst at least once in 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.

F. The bench controls are prepared in sufficient quantity to provide urine samples for all the assays for 2 years. Ranges are established after 20 parallel runs with previously established controls. Ranges are established by using the formulas for statistical calculation data. The quality control pools comprise two levels of concentration spanning the low and high ranges for urine NTx.

G. Standards and 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 means of the duplicate determinations 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 mean from a single pool falls outside the 99% confidence limits.
- The means from two pools fall either both above or both below the 95% confidence limits.
- The means 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. Report values to the nearest whole number. Reportable range is from 20 µg nM BCE to approximately 3000 nM BCE prior to correction for urine creatinine levels. 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 values, prior to correction for dilution, fall between 20 nM/BCE to approximately 3000 nM/BCE.

B. The lower limit of detection of NTx of this assay is 20 mM/BCE. The upper limit of detection is limited by the level of the highest standard provided in the kit, approximately 3000 nM/BCE.

C. Various urine components and microorganisms were evaluated for interfering effect in the Osteomark assay. The organisms tested, *E. Coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *C. albicans* (ATCC 14053), did not interfere with the assay. Human albumin, bilirubin, glucose, and vitamin C did not interfere with assay performance.

D. Urine specimens obviously contaminated with whole blood or that have visible hemolysis may interfere with assay performance.

13. REFERENCE RANGES (NORMAL VALUES)

Reference ranges according to the Hansen article (Reference 1) are as follows:

Reference ranges	
Children, years:	
0 – 1	102–4769 nM BCE/mM Creatinine
2 – 5	34–1752 nM BCE/mM Creatinine
6 – 10	90–1356 nM BCE/mM Creatinine
11 – 15	34–2158 nM BCE/mM Creatinine
16 – 20	34–780 nM BCE/mM Creatinine
Adult Males:	14–87 nM BCE/mM Creatinine
Premenopausal females[§]:	5—65 nM BCE/mM Creatinine (N = 258)

[§] Female reference ranges taken from Ostex's package insert.

14. CRITICAL CALL RESULTS (PANIC VALUES)

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens should be maintained at 20–28°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 72 hours. 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.

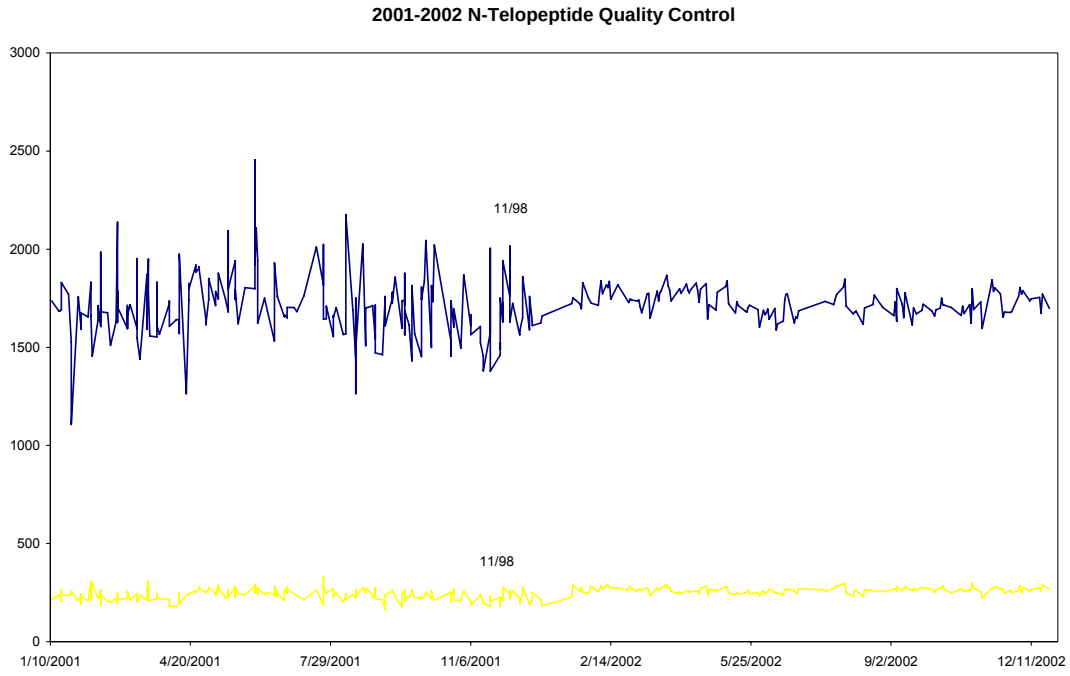
The original NHANES IV ship file is copied into a template Excel file and onto the hard drive of a PC computer. After the results are entered into the database and assay results transmitted electronically. Files are stored for 6 months on a server that is backed up on a daily basis. After 6 months, the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

The residual serum is stored at < –70°C for 6 months after analysis, then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. SUMMARY STATISTICS AND QC GRAPHS

A. N-Telopeptide in urine.

Summary Statistics for N Telopeptide in Urine by Lot						
Lot	**N**	**Start Date**	**End Date**	**Mean**	**Standard Deviation**	**Coefficient of Variation**
11/98	352	1/11/2001	12/24/2002	1713	135.7	7.9
11/98	353	1/11/2001	12/24/2002	247	27.0	10.9



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1. Hanson, Dennis A. et al. A Specific Immunoassay for Monitoring Human Bone Resorption: Quantitation of Type I Collagen Cross-linked N-Telopeptides in Urine. *J Bone Min Res*. 1992;7:1251–1258.

2. Wilson JD, Foster DW, Kronenberg HM, Larson PR. Williams Textbook of Endocrinology. 9th ed. Philadelphia: WB Saunders Co; 1998, p. 1220.

Other Sources:

1. Osteomark Elisa Immunoassay package insert. (Rev. 11/97)

2. Research performed by Roberta Ward, MT(ASCP) during the summer of 1994.