Laboratory Procedure Manual

Analyte: **N Telopeptide in Urine**

Matrix: **Urine**

Method: **Vitros Immunoassay**

Method No.:

Revised:

as performed by: *Department of Laboratory Medicine*
University of Washington Medical Center

Contact: *Dr. Mark Wener, M.D.. Director*
University of Washington Medical Center
Seattle, Washington

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

The Vitros NTx assay is a competitive immunoassay technique using a synthetic NTx peptide which has been coated on the reaction wells provided in the reagent pack. This assay depends on competition between this synthetic peptide and the NTx present in the specimen being tested. These two sources of peptide compete for binding with a horse radish peroxidase (HRP) -labeled antibody conjugate (mouse monoclonal anti-NTx). The conjugate is captured by the peptide coated on the wells. Any unbound materials are removed through a washing step.

The bound HRP conjugate is then measured through the addition of a luminescent substrate. This signal reagent contains the luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative. This produces a light signal. The electron transfer agent (a substituted acetanilide) increases the level of the light and prolongs its emission. This light signal is read by the Vitros. The level of HRP conjugate bound is indirectly proportional to the concentration of NTx present.

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.

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 or personal protective devices used in handling specimens and kit reagents.

Material safety data sheets for all reagents used in the perfomance 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

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 text file) transmitted electronically (labeled *boxnum.*shp). This file contains the following information:

Send File

Field	Type
Sample ID	XXXXXXXXX

Field	Type
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: NTX-Vessel ID 47

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

C. 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.

D. Data entry is checked for errors.

E. 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.

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

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

D. DO NOT ADD PRESERVATIVE TO THE URINE SPECIMEN.

E. 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.

F. 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 $\leq$ -20°C is required, specimens maybe frozen and thawed up to 3 times.

G. 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. Vitros Immunodiagnostic Products NTx Reagent Pack: Store at 2-8 degrees C. Ortho-Clinical Diagnostics, 100 Indigo Creek Drive, Rochester, NY 14626-5101 Product # 680 0030
   a. 100 coated wells (streptavidin, bonds > 3 ng biotin/well and biotin – synthetic NTx peptide, coated at > 18 ng/well.
   b. 23 ml, conjugate reagent (HRP-mouse monoclonal anti-NTx binds > 740 moles BCE NTx) in buffer with antimicrobial agent.
   c. NOTE: contains bovine serum albumin.
2. Vitros Immunodiagnostics Products NTx Calibrators – matched to Reagent Pack lot Ortho-Clinical Diagnostics, 100 Indigo Creek Drive, Rochester, NY 14626-5101 Product # 680 0031. Store at 2-8 degrees C unopened. Ready for use. Do not use beyond expiration date. After opening, store for up to 13 weeks at 2-8 degrees C or 13 weeks at –20 degrees C (With no more than 1 freeze thaw cycle).
3. Vitros High Sample Diluent A Reagent Pack: Ortho-Clinical Diagnostics, 100 Indigo Creek Drive, Rochester, NY 14626-5101 Product # 843 0373. Store at 2-8 degrees C
4. Vitros Signal Reagent Pack: Ortho-Clinical Diagnostics, 100 Indigo Creek Drive, Rochester, NY 14626-5101 Product # 107 2693. Store at 2-8 degrees C. Expires 7 days after initial use.
5. Vitros Wash Solution: Ortho-Clinical Diagnostics, 100 Indigo Creek Drive, Rochester, NY 14626-5101 Product # 838-9793. Store at room temperature, protect from light.
6. Vitros Maintenance Packs: Ortho-Clinical Diagnostics, 100 Indigo Creek Drive, Rochester, NY 14626-5101 Product # 183-1312. Store at room temperature.

B. Reagent Preparation

1. Allow all calibrators to equilibrate to room temperature (18 – 28 º C) for at least 15 minutes before performing a calibration.

2. Signal reagent should 35-40 minutes RT before use.

3. All reagents are to be considered to be homogenous and should not be mixed prior to loading them onto the instrument (may cause bubbles that would interfere with the sampling).

4. Do not allow reagent packs to warm prior to loading them onto the instrument.

C. Instrumentation

1. Vitros Eci instrument:
   Ortho-Clinical Diagnostics 100 Indigo Creek Drive, Rochester, NY 14626-5101

2. Vitros specimen trays:
   Ortho-Clinical Diagnostics 100 Indigo Creek Drive, Rochester, NY 14626-5101

3. Specimen holders (if needed due to aliquot tube size) created in laboratory using 16x100 disposable culture tubes and caps for aliquot tubes

4. Transfer pipettes: any vendor

5. Specimen cups and caps: Vitros microsample cup:
   Ortho-Clinical Diagnostics 100 Indigo Creek Drive, Rochester, NY 14626-5101. Cat# 121 3115

6. Centrifuge (Jouan, Winchester, VA)

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

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 ^{o}C -28 ^{o}C).

E. Preparation of Quality Control Materials

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

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.

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-mL tubes containing a volume for a 3-4 month supply and label with 'I #' and freeze at $\leq$ -70 ^{o}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 ^{o}C. Thaw and use one aliquot of control material for each run.

1. 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.

2. After 20 parallel runs, use the data from the Levey-Jenning chart to assign a permanent mean and standard deviation. Normal acceptance ranges are determined as mean $\pm$ 2 standard deviations.

3. 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.

4. 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 is required every 7 days. In addition, calibration is required whenever assay reagent and calibrator lots change and following specified service procedures (see VITROS ECi System Operator's Guide).

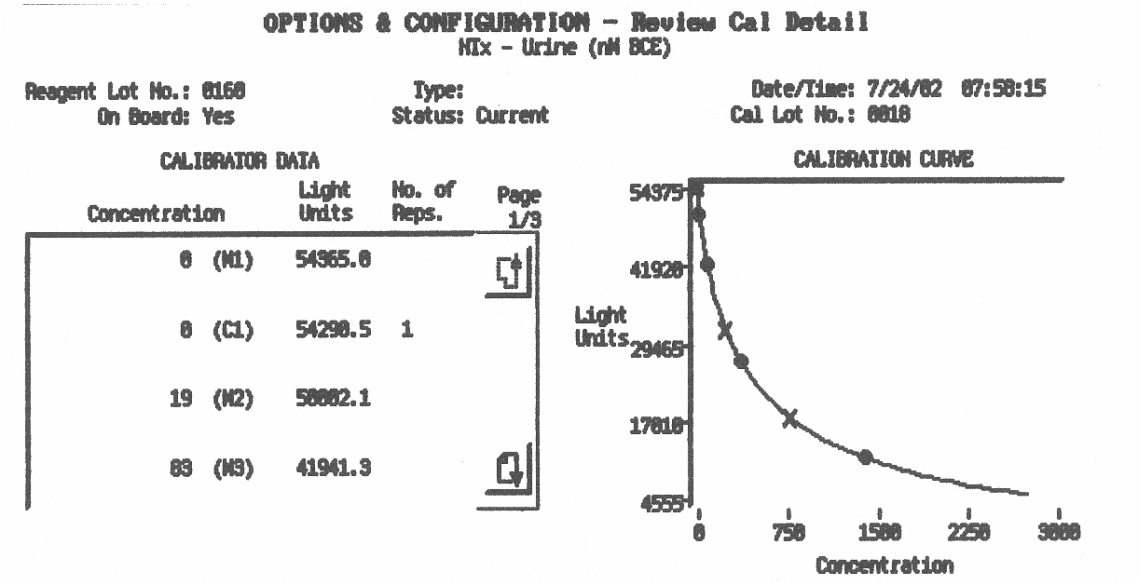
A master calibration is established for each reagent lot by the manufacturer. The master calibration process establishes a curve based on the generated light signals versus the concentration of six master calibrators. Multiple measurements of each master calibrator are made for each reagent lot. The mean light signals for each master calibrator concentration are calculated and encoded onto a magnetic stripe (mag) card, which constitutes the master calibration. This Reagent Lot Calibration card is provided for each assay reagent lot. The assay calibration is based on a modified four-parameter logistic curve fitting model. The calibrators are traceable to the Ostex Osteomark® NTx assay.

The laboratory uses three process calibrators, which are assigned concentration values from the master calibration. These concentrations are encoded onto the Reagent Lot Calibration card. The calibrator and regent lot numbers are dedicated (i.e. only one lot of calibrator can be used for each lot number of reagent).

After the weekly calibration the system compares the light signal expected from the master curve with the light scatter generated from the process calibrators and calculates the percentage difference between the two sets of signals. The system then adjusts or rescales the master curve and performs a quality check to validate the calibration curve. If the generated calibration curve falls within the quality guidelines, the calibration is successful. If not, the calibration fails.

If the calibration is successful, the system calculates the NTx concentrations for patient and quality control samples using the measured light units.

Sample NTx Calibration Curve



B. Calibration instructions:

Calibration of NTx test assay **without** the bar code reader on:

1. If this is a new lot of Reagent, download the information from the Lot Calibration Card provided with the Reagent pack using the magnestic scanning slot on the front of the keyboard consol. Ascertain that the light is green, insert the card and drawing it to the other end of the slot, turn the card 180 degrees, reinsert the card in the slot and slide it back in the opposite direction. The turining of the card and its reintroduction into the slot needs to be done within ten seconds once the green light starts to flash.
2. Touch **Sample Programming**
3. Touch **Tray**, enter a tray number and touch **Return**
4. At **Sample ID** enter the barcode number of Calibrator 1
5. Touch **Cal**, **Urine** and **NTx**
6. Repeat for the second and third calibrators and put the calibrators into their assigned positions on the sample tray.
7. Put the tray on the instrument.
8. Touch the Sample Processing icon.
9. Remove the tray with the Calibrators from the instrument after they are sampled, or stop the tray line from rotating by touching the Sample Processing icon.
10. Recap and return Calibrators to the refrigerator as soon as possible,

C. Verification

Two different levels of controls are tested whenever patient samples are run. If, within a testing series, these controls do not conform to specifications as defined in the quality control manual and the problem is not resolved by repeating the testing on new control aliquots, recalibration should be performed.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

1. Preparing the samples:
   Remove all samples to be tested from the freezer, thaw and mix. (Specimens can be thawed in the 37 degree C water bath or incubator.) Pour off clinical samples into 12x75 aliquot tubes which have been labeled appropriately. Specimens should then be centrifuged for five minutes at 1500 RPM. Remove all samples to be tested from the freezer, thaw and mix. (Specimens can be thawed in the 37 degree C water bath or incubator.) Pour off clinical samples into 12x75 aliquot tubes which have been labeled appropriately. Specimens should then be centrifuged for five minutes at 1500 RPM.
2. Starting the instrument:
   a. Touch the Sample Processing icon in the upper right corner of the computer monitor screen
   b. If the system has not been used lately, it may stop. Touch the red square that will light up at the top of the screen. Read the message: if it says **Reagent metering performance test outside acceptable limits**, Press the initialize button at the bottom of the monitor screen. Once the Sample Processing icon at the upper right of the screen has ceased to be grayed-out (i.e. looks fuzzy), press the Sample Processing icon again to begin the run.

B. Assay procedure:

Loading an Individual Specimen:

1. At the Main Menu touch the **SPECIMEN PROGRAMMING** icon.
2. Touch the word **TRAY** in the center of the tray mock-up
3. Enter the number of the tray to be used, then return
4. Enter the name/number of the specimen/control, then return
5. Identify the specimen, **PATIENT** or **CONTROL** by touching the appropriate word
6. Identify the specimen as urine by touching the word **URINE**.
7. Touch the **NTx** symbol
8. At the bottom of the screen touch **SAVE/NEXT**
9. Repeat as needed for number of tests to be performed.
10. Place the specimen in the appropriate positions of the sample tray.
11. Place the sample tray on a spindle in the Sample Supply Subsystem.

Test Request through Batch Mode:

1. At the Main Menu touch **SPECIMEN PROGRAMMING**.
2. Touch **TRAY** enter the number of the tray to be used
3. Touch **URINE**
4. Touch **NTX**
5. Touch **BATCH SAVE**
6. Inactivate the positions that are not going to be used by touching the appropriate positions on the tray mock up.
7. Touch **OK**
8. If the samples are to be diluted, Touch **Assay Dil** and enter dilution desired before touch Batch Save.

Reflexive Testing:

1. The Vitros is programmed to do reflexive testing on samples that have an NTx value >3000.
2. After the samples have been processed by the Vitros, remove the trays from the sample subsystem and cover with parafilm to prevent evaporation.
3. Once the Vitros has finished all the scheduled assays, determine which specimens require reflex test by touching **Sample Programming**, touch **Tray** and enter the number of a tray. If any of the samples assigned to that tray require reflexive testing, the patient icon will still be present. Cap and remove all completed samples and reintroduce the tray to the Vitros subsystem and push the Sample Processing icon. **The Vitros is programmed to perform a one to ten dilution of that sample as long as the sample remains in the same tray and the same cup position.**

C. Calculations

Results are automatically calculated by the Vitros Eci and are reported to NHANES as nM BCE without correction based on urine creatinine concentration.

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) based on the following formulas:

Example:

Unknown Assay value = 360 nM BCE

$$\text{Unknown Urinary Creatinine} = \frac{60\text{mg/dl creatinine}}{11.3} = 5.3 \text{ mM creatinine}$$

NOTE: 11.3 = conversion factor used to convert mg/dl to mM for creatinine.

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

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 Westagard 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:

C. Verify that the controls have not exceeded the expiration date.

D. Check the control ranges for accuracy.

E. Recalibrate the assay prior to testing samples.

F. Control results are evaluated by Westgard rules for each run by entry into Levey Jennings plot tables. Levey Jennings graphs are evaluated prior to reporting of any patient samples. Any violations of control specifications should be referred to supervisor.

G. 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 judgements 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.

H. 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.

I. 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:

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

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

L. 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 ug 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. Samples containing triolein (33.9 mmol/l), bilirubin (0.21 mmol/L), glucose (13.9mmol/L), vitamin C (1.14 mmol/L or human albumin (5g/L interfere with this assay by less than 10%. Samples containing the microorganisms P. aeruginosa (ATCC 27853) (1×10^5 CFU/mL), E. coli (ATCC 25922)($1x\ 10^5$CFU/mL) or C. albicans (ATCC 14053) (1×10^5 CFU/mL) interfere with this assay by less than 5%. Biotin levels in urine remained elevated for up to 24 hours after oral or intravenous biotin administration. Samples containing 100,000 ng biotin/mL interfere by less than 10%.

D. Hemoglobin interferes with the Vitros NTx assay. At a certain concentration of approximately 50 mg of hemoglobin/L the measured concentration of NTx in urine was up to 17% higher than in the absence of hemoglobin.

E. Do not use turbid samples.

13. REFERENCE RANGES (NORMAL VALUES)

The reference range is based on the NTx/creatinine ratio. 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).

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

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

From Vitros package insert:

Adult males: 21-83 nM BCE/mM Creat
Premenopausal females: 17-94 nM BCE/mM Creat
Postmenopausal females: 26-124 nM BCE/mM Creat

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 ^{o}C during testing. After testing, the samples are stored at $\leq$ -70 ^{o}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 ^{o}C for no longer than 72 hours. Otherwise, specimens should be stored at $\leq$ -70 ^{o}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

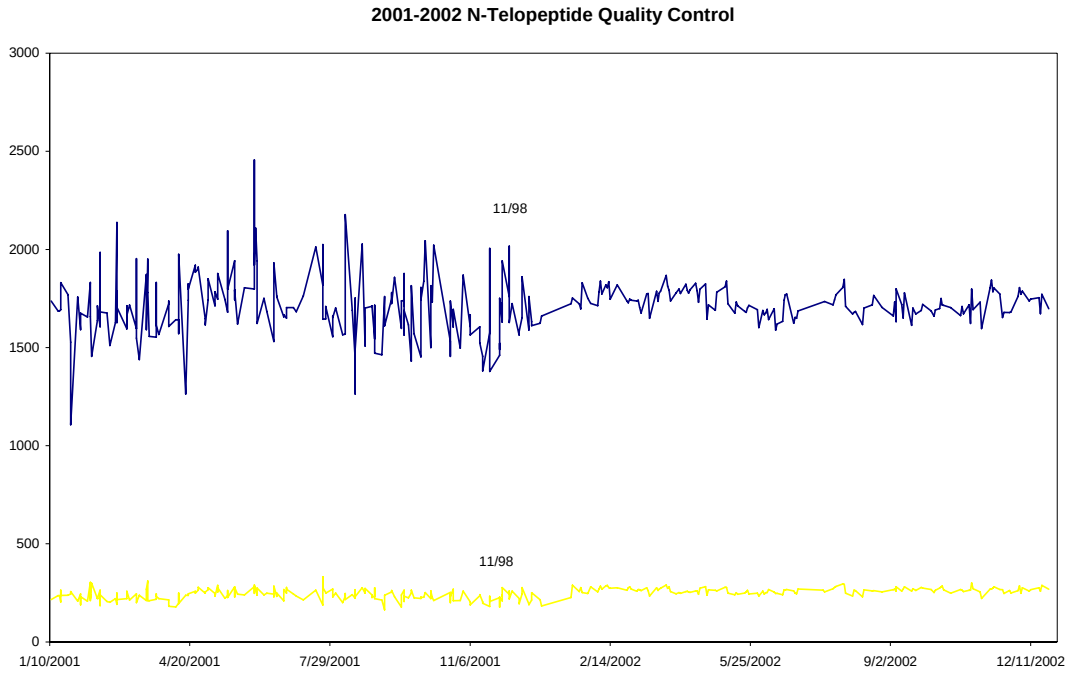
Standard record keeping should be used for tracking specimens. The primary results include daily test results as well as stored quality control results.

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 result files are transferred onto a CD along copies of original ship files and QC information.

The residual serum is stored at $\leq$ -70 ^{o}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

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



REFERENCES

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." Journal of Bone and Mineral Research, Vol. 7; November 11, 1992. pp. 1251-1258.
2. Wilson, JD, Foster DW, Kronenberg HM, Larson PR: Williams Textbook of Endocrinology, 9th Ed., WB Saunders Co., Philadelphia, 1998, p.1220.

Other Sources:

1. Vitros Immunodiagnostic Product NTx Reagent Pack package insert.
Copyright 2000 Ortho-Clinical Diagnostics.

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