OSTEOMARK® NTx Serum

The Osteomark® NTx Serum assay provides a quantitative measure of cross-linked N-telopeptides of type I collagen (NTx) in human serum as an indicator of bone resorption.

REF 9021

For *in vitro* diagnostic use only

Indications for Use

A Serum NTx level is used to aid in predicting skeletal response (bone mineral density) to antiresorptive therapy and in monitoring bone resorption changes following initiation of antiresorptive therapy. Prior to initiating antiresorptive therapy, a serum NTx level is used to determine the probability for a decrease in bone mineral density (BMD) after one year in postmenopausal women treated with hormonal antiresorptive therapy relative to those treated with calcium supplementation.

The measurement range of the Osteomark® NTx Serum assay is 3.2 to 40.0 nM Bone Collagen Equivalents (BCE).

Summary and Explanation of the Test

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

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

The Osteomark® NTx Serum assay provides a quantitative measure of NTx in serum as an indicator of human bone resorption. Elevated levels of serum NTx indicate elevated bone resorption.[1,2,3,4] 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.[5,6] Osteoporotic fractures are reported to be the major source of increased morbidity and mortality in older women.

A randomized trial of postmenopausal women was conducted at eight clinical sites across the US. Subjects were randomized to either hormone replacement therapy (HRT) plus calcium supplements (500 mg daily) or calcium supplements alone.[7] Serum samples collected during the study were tested using the the Osteomark® NTx Serum assay. Results of the testing support the use of the Osteomark® NTx Serum assay to monitor the antiresorptive effect of the therapy and to determine the probability for a decrease in BMD after one year if hormone therapy is not initiated.

A randomized, double-blind clinical study was conducted at a regional specialty hospital in postmenopausal women with low bone mass or diagnosed osteoporosis. Subjects were randomized to receive either placebo or 5-10 mg alendronate sodium[8]. Serum samples collected during the study were tested using the Osteomark® NTx Serum assay. Results obtained from this study support the utility of the Osteomark® NTx Serum assay to monitor the effect of antiresorptive therapy and to predict BMD response to therapy using early changes in the NTx serum value.

Assay Principles

The Osteomark® NTx Serum assay is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA/EIA) for quantitative determination of NTx in human serum.

NTx epitope is adsorbed onto a 96-well microplate. Diluted samples are added to the microplate wells, followed by a horseradish peroxidase labeled monoclonal antibody. NTx in the patient sample competes with the NTx epitope in the microplate well for antibody binding sites. Following a wash step, the amount of labeled antibody bound is measured by colorimetric generation of a peroxide substrate. Absorbance is determined spectrophotometrically and NTx concentration calculated using a standard calibration curve. Assay values are reported in nanomoles Bone Collagen Equivalents per liter (nM BCE).

Kit Components

Supplied materials sufficient for 96 wells

	Instructions for Use	1 booklet
A	Antigen Coated 96-well Plate, 12 1x8-well strips	1 plate
B	Specimen Diluent	40 mL bottle
C	Antibody Conjugate Concentrate	0.4 mL vial
D	Antibody Conjugate Diluent	25 mL bottle
E	Chromogen Reagent	0.9 mL bottle
F	Buffered Substrate	30 mL bottle
G	Stopping Reagent	25 mL bottle
H	30X Wash Concentrate	125 mL bottle
0	0 nM BCE Calibrator	20 mL vial
5	5 nM BCE Calibrator	0.4 mL vial
10	10 nM BCE Calibrator	0.4 mL vial
20	20 nM BCE Calibrator	0.4 mL vial
40	40 nM BCE Calibrator	0.4 mL vial
I	Level I Serum Control	0.4 mL vial
II	Level II Serum Control	0.4 mL vial
	Plate Sealers	1 pad

Component Description

PLATE — **Antigen Coated 96-Well Plate,** 12 1x8 well strips. Synthetic NTx antigen adsorbed onto microwell strips (1 plate)

DILSPE — **Specimen Diluent,** 1 bottle
Buffered reagent into which calibrators, controls and specimens are diluted. ProClin™ 300 (0.05%) included as a preservative

CONJ — **Antibody Conjugate Concentrate,** 1 vial
Purified murine monoclonal antibody directed against NTx and conjugated to horseradish peroxidase. ProClin™ 300 (0.05%) is included as a preservative. Supplied as a 100x concentrate.

CONJ | **DIL** — **Antibody Conjugate Diluent,** 1 bottle
Buffered reagent into which Antibody Conjugate Concentrate is diluted. ProClin™ 300 (0.05%) included as a preservative.

WASHBUF | **30x** — **30x Wash Concentrate,** 1 bottle
Ionic detergent solution. Supplied as a 30x concentrate. ProClin™ 300 (<0.1%) is included as a preservative.

CHROMOGEN — **Chromogen Reagent,** 1 vial
3.3' , 5.5' – tetramethylbenzidine in dimethyl-sulfoxide. Supplied as a 100x concentrated reagent.

BUFFER — **Buffered Substrate,** 1 bottle
Buffered hydrogen peroxide.

SOLN | **STOP** — **Stopping Reagent,** 1 bottle
1N sulfuric acid.

CAL | **xx nM** — **Assay Calibrators 0, 5, 10, 20, 40 nM BCE,** 1 vial each
Purified NTx antigen in stabilized protein diluent. ProClin™ 300 (0.05%) included as a preservative.

CONTROL | **xx** — **Level I and Level II Serum Controls,** 1 vial each
Human serum base with known NTx concentration. ProClin™ 300 (0.10%) included as a preservative.

PLATE | **SEALERS** — **Plastic Plate Sealer,** 1 pad

Storage of Reagents

Reagents must be stored at 2 – 8ºC when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater

than 25ºC. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required But Not Supplied

- Precision single and multichannel pipettes.
- Disposable pipette tips. (New pipette tips must be used for each addition of different specimens or reagents during the assay procedure).
- Microtubes or equivalent for preparing dilutions.
- Disposable plastic containers for preparing working conjugate and chromogen solutions.
- Reagent reservoirs.
- Automated microwell washer.
- Microwell or microstrip plate reader with 450nm and 630nm filters.
- Software capable of computing results using a 4-parameter logistic curve-fitting equation.
- Deionized water.

Specimen Collection and Storage

Human serum collected by standard venipuncture technique is used in the Osteomark® NTx Serum assay. The use of plasma samples has not been established. Allow blood to fully clot and remove the serum from the red blood cells promptly. Specimens collected in serum separation tubes should be removed from the gel. Store serum samples refrigerated (2 – 8ºC) for up to 24 hours, or store frozen
(-20ºC or below) for longer term storage. Specimens may undergo three freeze/ thaw cycles.

For monitoring therapy, baseline samples should be collected just prior to or on the day of therapy initiation. Subsequent specimens for comparison should be collected at approximately the same time of day as the baseline specimen.

Warnings and precautions

- **For *in vitro* diagnostic use only.**
- Do not interchange the Osteomark® NTx Serum assay values with the Osteomark® NTx Urine assay values, especially when monitoring therapy.
- The calibrators and controls contain processed antigen from human bone tissue or human serum. Although each lot has been documented to be non-reactive for HIV 1, HIV 2, HBsAg, HCV and RPR by FDA approved methods, these materials should be handled as potentially infectious and should be disposed of properly.
- The Stopping Reagent contains 1N sulfuric acid
  Signal word: Danger
  Hazard statements: H314 - Causes severe skin burns and eye damage, H318 - Causes serious eye damage
- The Chromogen Reagent contains 3,3',5,5'- tetramethylbenzidine and dimethylsulfoxide. Dimethylsulfoxide is readily absorbed through the skin.
  Signal word: Warning
  Hazard statements: H315 - Causes skin irritation, H319 - Causes serious eye irritation, H227 - Combustible liquid.
- ProClin is included as a preservative in most reagents, at concentrations listed in the reagent section.
  Signal word: Warning
  Hazard statements: H315 - Causes skin irritation, H317 - May cause an allergic skin reaction, H319 - Causes serious eye irritation.
- Assay Calibrators, Serum Controls: Contains materials of human origin.
- Serum specimens may contain infectious agents and should be disposed of properly. Decontamination is most effectively accomplished with a 0.5% solution of sodium hypochlorite (1:10 dilution of household bleach) or by autoclaving one hour at 121ºC. Do not autoclave solutions containing sodium hypochlorite. Do not combine sodium hypochlorite solution with acid.
- Never pipette reagents or clinical specimens by mouth.
- Wear protective gloves and clothing when handling specimens and reagents. Wash hands thoroughly after use.
- Do not use reagents beyond their expiration dates.
- Do not mix components from other lots of the Osteomark® NTx Serum assay.
- Microwell strips must be stored desiccated. Do not remove the desiccant pillow from the foil pouch, and reseal any unused strips in the pouch with the desiccant pillow.
- Do not re-use microwells. Dispose of properly after use.
- Perform the assay procedure in a controlled laboratory environment that adheres to the stated incubation requirements. Avoid extreme environmental conditions during the procedure.

Note: Safety data sheets are available upon request.

Assay Procedure

Preparatory Steps

1. Allow all specimens and kit components to equilibrate to room temperature (20 – 25ºC). Mix all reagents thoroughly. Avoid foaming.
2. Prepare working strength wash solution. Dilute 30X Wash Concentrate 1:30 with deionized water (1 part 30X Wash Concentrate with 29 parts deionized water; example dilution would be 30 mL Wash Concentrate plus 870 mL deionized water) and mix for a minimum of five (5) minutes. The diluted wash solution is stable for one (1) month at room temperature.
3. Plan the plate configuration, and create a plate map. It is recommended that each calibrator and control be run in duplicate. An example for 10 specimens is below:

	1	2	3
A	0 Calibrator	40 Calibrator	Specimen #3
B	0 Calibrator	40 Calibrator	Specimen #4
C	5 Calibrator	Level I Cont.	Specimen #5
D	5 Calibrator	Level I Cont.	Specimen #6
E	10 Calibrator	Level II Cont.	Specimen #7
F	10 Calibrator	Level II Cont.	Specimen #8
G	20 Calibrator	Specimen #1	Specimen #9
H	20 Calibrator	Specimen #2	Specimen #10

4. Prepare working strength conjugate solution. Using a clean disposable plastic container, dilute the Antibody Conjugate Concentrate to a 1:101 ratio using Antibody Conjugate Diluent. Mix gently by inversion only. Do not vortex or use a magnetic stir bar. Avoid foaming. Do not reuse the container. Use the following table as a guideline for reagent preparation.

Total Number of Strips	Conjugate Concentrate (µL)	Conjugate Diluent (mL)
3-4	40	4
5-8	80	8
9-12	120	12

Use the diluted conjugate solution within one hour of preparation.

5. Thoroughly mix the Calibrators, Controls and specimens.
6. Prepare 1:5 dilutions of all Calibrators, Controls and specimens with Specimen Diluent in microtubes, or equivalent (1 part sample and 4 parts Specimen Diluent). A minimum volume of 200 µL diluted sample is required for each sample. (e.g. 50 µL sample + 200 µL diluent). Vortex the diluted samples to mix thoroughly, avoid foaming.
7. Remove the appropriate number of microwell strips from the sealed foil pouch. Place any unused strips back in the pouch, resealing the pouch along the zipper. Do not remove the desiccant pillow from the foil pouch.

Specimen and Antibody Incubation

8. Pipette 100 µL of each diluted Calibrator, Control or sample into the microplate according to the plate configuration. It is recommended that calibrators and controls be run in duplicate. Use a calibrated pipettor and a new pipette tip for each Calibrator, Control, and sample. Immediately proceed to step 9.
9. Using a multichannel pipette, deliver 100 µL of working strength conjugate solution into each microwell. Apply a plate sealer and gently swirl the plate on a flat surface for 15-20 seconds to ensure mixing.
10. Incubate the plate at room temperature (20-25ºC) for 90 ± 5 minutes.
11. Prepare Chromogen Reagent/Buffered Substrate solution during the last 5 minutes of incubation. Dilute Chromogen Reagent into Buffered Substrate using a 1:101 ratio. Use a clean, disposable, plastic container. Do not re-use disposable container. **Mix well by inversion only. Do not vortex, shake vigorously or use a magnetic stir bar to mix.** (This solution should be colorless when mixed. A blue color indicates that the reagent may be contaminated and should be discarded.) As a guideline, prepare 2 mL of solution (20 µL Chromogen Reagent into 2 mL Buffered Substrate) per strip assayed.
12. At the end of the incubation period, carefully remove and discard the plate sealer. Wash microwells five (5) times with the working strength wash solution using an automated plate washer. Use a minimum wash volume of 350 µL per well per wash cycle. Blot on absorbent paper after the final wash. (Too few or too many washes may cause inaccurate results.) Immediately proceed to step 13. Do not allow strips to dry.

Color Development and Measurement

13. Using a multichannel pipettor, add 200 µL diluted Chromogen Reagent/Buffered Substrate to each microwell. Apply a new plate sealer.
14. Incubate at room temperature (20-25ºC) for 30 ± 2 minutes. A blue color will develop in wells containing bound antibody-horseradish peroxidase conjugate.
15. At the end of the incubation, carefully remove and discard the plate sealer.
16. Using the multichannel pipettor, add 100 µL of Stopping Reagent to each well. Wells that have developed a blue color will turn yellow. Swirl the plate gently on a flat surface for 15-20 seconds to ensure mixing. Allow the plate to sit at room temperature (20-25ºC) for 5 minutes before reading absorbance values.
17. Within 30 minutes of adding the Stopping Reagent, read the absorbance of the Calibrators, Controls, and specimens using a microwell plate reader (read at 450 nm with a 630 nm reference filter).

Analysis of Results

1. The Osteomark® NTx Serum assay values are expressed in nanomoles BCE/L (nM BCE).
2. Determine the concentration values (nM BCE) of Controls and specimens from the calibration curve. The most accurate results are obtained using a 4-parameter logistic curve fitting equation. [NOTE: Some 4-parameter logistic curve fitting equation software packages do not accept a calibrator value of 0, requiring entry of a nominal concentration (such as 0.001) for the 0 nM BCE calibrator.]
3. Assay results are valid if the following criteria are met:
    - mean absorbance of the 0 nM BCE Calibrator is greater than or equal to 1.300
    - the span of the calibrator curve (absorbance difference between 0 nM BCE Calibrator and the 40 nM BCE Calibrator) is greater than or equal to 0.900.
4. If specimens are run in duplicate, the recommended coefficient of variation (% CV) between concentration value (nM BCE) duplicates is ≤ 20% CV. Those with > 20% CV should be rerun.
5. Patient specimens giving absorbance values below the 40 nM BCE calibrator should be diluted 1:2 in the 0 nM BCE Calibrator (1 part specimen and 1 part 0 nM BCE Calibrator) before diluting 1:5 in Specimen Diluent, and retested. Calculate final result by multiplying the concentration determined from the diluted sample by a factor of 2.
6. These Serum Control ranges have been established by the manufacturer. It is recommended that each laboratory establish its own ranges.

Limitations of the Procedure

While the Osteomark® NTx Serum assay is used as an indicator of bone resorption, use of this test has not been established to predict development of osteoporosis or future fracture risk. Use of this test has not been established in primary hyperparathyroidism, hyperthyroidism, or Paget's disease of bone. When using the Osteomark® NTx Serum assay to monitor therapy, results may be confounded in patients afflicted with other clinical conditions known to affect bone resorption, e.g. metastases to bone. While the Osteomark® NTx Serum assay value provides a measure of the level of bone resorption, a single Osteomark® NTx Serum assay value cannot provide the rate of bone resorption as reported results do not contain a measure of time. The Osteomark® NTx Serum assay results should be interpreted in conjunction with clinical findings and other diagnostic results.

Interfering Substances

Various serum components were evaluated for an interfering effect on the Osteomark® NTx Serum assay. These components, including total and direct bilirubin, glucose, cholesterol, triglycerides, total protein, albumin and hemoglobin, were tested at levels elevated from physiological norm and did not interfere with assay performance.

Expected Values

A multi-center, cross-sectional study was conducted at five regional sites to determine the reference range for normal premenopausal women (mean age 36 years, range 25-49). The male reference range was determined from a multi-center, cross-sectional study conducted at three regional sites (mean age 51 years, range 31-80).[9]

	Mean*	Std Dev	Range (mean ± 2 Std Dev)	N
Women	12.6	3.2	6.2 – 19.0	257
Men	14.8	4.7	5.4 – 24.2	176

When the expected value range for premenopausal women is log-transformed, the range is 7.7 - 19.3 nM BCE. The log-transformed male range is 8.1 - 24.8 nM BCE. These ranges are provided as guidelines only. Each laboratory should establish its own reference ranges.

A study was conducted to determine the intra-subject variability of serum NTx in postmenopausal women.[10] Subjects provided blood specimens for three consecutive days to assess short-term variability, and for two consecutive months to assess long-term variability. The mean % CV in the short-term specimen set (n=271) was 7.3%. The mean % CV in the long-term specimen set (n=261) was 8.7%.

Intra-subject variability in men was assessed in a subset of the above male reference range study population. The short-term (4 days) intra-subject variability (n=32) was 9.1%, and the long-term (3 months) intra-subject variability (n=27) was 9.5%.

Performance Characteristics

Assay Reproducibility and Precision

Intra-assay variability was determined by testing four human serum specimens with BCE values distributed throughout the calibration range of the assay and following NCCLS Precision Performance Guideline EP5-T2. From these test results the the Osteomark® NTx Serum assay intra-assay variability is established as 4.6%.

Inter-assay variability was determined by testing eight human serum specimens with BCE values distributed throughout the calibration range of the assay. From these test results the Osteomark® NTx Serum assay inter-assay variability is established as 6.9%.

Total assay precision was evaluated by testing the Level I Serum Control (9.4 nM BCE) and the Level II Serum Control (30.0 nM BCE) at four clinical laboratories. The estimate for the total precision % CV for the Level I Serum Control was 13.99%, and for the Level II Serum Control was 11.92%.

Antigen Recovery was evaluated by adding known amounts of NTx to each of nine serum specimens of known NTx concentration. Recovery represented the observed assay value of the "spiked" specimens, calculated as a percent of the expected serum value. Results demonstrated an antigen recovery of 94 – 105% across the assay range.

Dilutional linearity was evaluated by performing serial dilutions of five serum specimens with high nM BCE values into a serum specimen with a known low nM BCE value. Percent linearity was determined as the measured value divided by the expected value multiplied by 100. Results demonstrated an average recovery of linear diluted samples of 98%.

Clinical Studies

Use of the Osteomark® NTx Serum Assay in Postmenopausal Women Treated with HRT

A clinical trial was conducted to determine the ability of the Osteomark® NTx Serum assay to monitor the effect of HRT on bone resorption and to determine the probability for a decrease in BMD after one year if treated with only calcium supplements relative to those treated with supplements and HRT.[7] Results of the study supported these clinical uses. Figures 1a and 1b provide the Osteomark® NTx Serum assay values throughout the study for each of the treatment groups. Prior to HRT[7] initiation, the Osteomark® NTx Serum assay mean baseline value in this group was 15.9 nM BCE, which was significantly higher than the premenopausal mean of 12.6 nM BCE. In the HRT group, NTx values fell significantly after 6 months of therapy to 11.9 nM BCE; a mean 24.4% decrease was observed (Figure 2).

Mean values in the calcium group remained constant throughout the 12 month study; 15.4 nM BCE at baseline and 15.8 nM BCE after 12 months. From the baseline NTx value, the relative risk for loss of BMD was compared between the HRT and calcium only groups. In the lowest NTx quartile at baseline (<12.5 nM BCE), there was no statistically significant difference in the likelihood of bone loss over 1 year between the HRT and calcium groups. A high baseline NTx (>18.1 nM BCE) indicated a 6 times higher risk of BMD loss if not treated with HRT.

Figure 1a. Calcium Group – The Osteomark® NTx Serum Assay Values throughout the Study

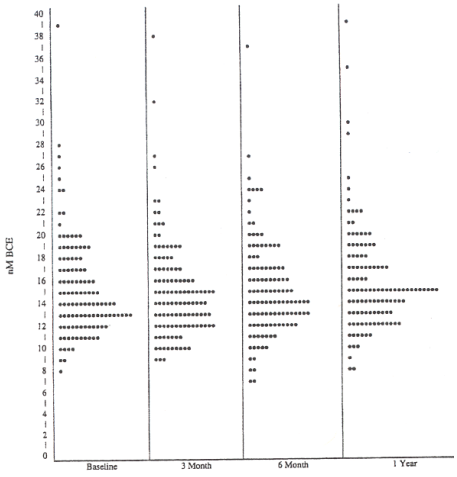


Figure 1b. HRT Group – The Osteomark® NTx Serum Assay Values throughout the Study

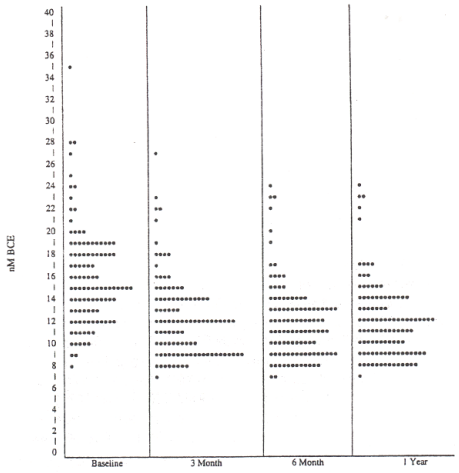
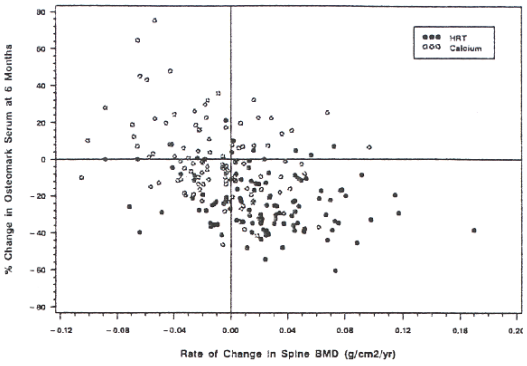


Figure 2. Rate of Change in BMD vs. % Change in The Osteomark® NTx Serum Test



Use of the Osteomark® NTx Serum Assay in Postmenopausal Women Treated with
Bisphosphonate Therapy

A study was conducted at a regional specialty hospital in the northeastern United States to determine if early changes in the Osteomark® NTx Serum assay following treatment with the bisphosphonate alendronate sodium predicts an increase in BMD.[8] In this double-blind clinical study, women were randomized to either placebo or 5-10 mg alendronate sodium. In the alendronate treated group, the mean Osteomark® NTx Serum assay value of 11.0 nM BCE after 6 months of treatment, was significantly lower than the baseline mean of 16.1 nM BCE (Figures 3a and 3b).

Figure 3a. Placebo Group – The Osteomark® NTx Serum Assay Values throughout the Study

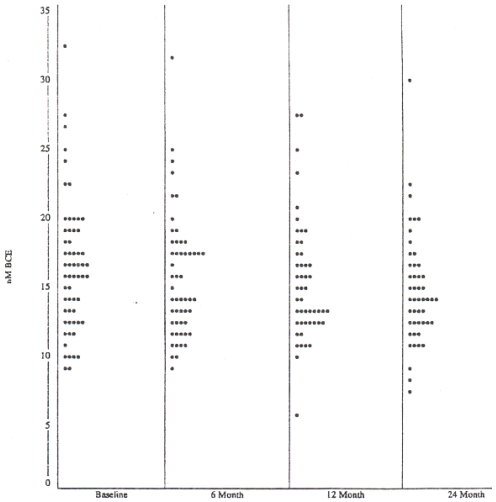
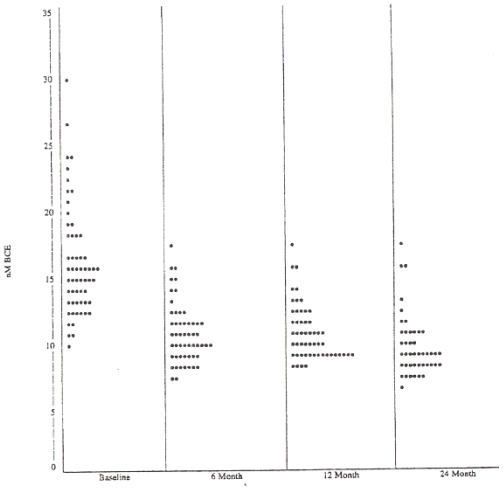
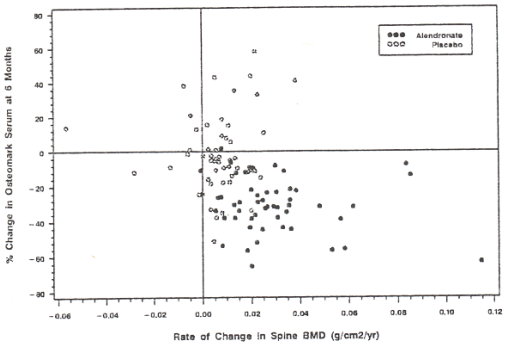


Figure 3b. Alendronate Group – The Osteomark® NTx Serum Assay Values throughout the Study



Stratification by tertile of baseline Osteomark® NTx Serum assay value demonstrates that subjects in the highest tertile baseline value (>16.6 nM BCE) had a significantly greater gain in spine BMD than those in the lowest tertile (10.1 – 13.8 nM BCE), p=0.003. Figure 4 provides the rate of change in spine BMD vs. the percent change in Osteomark® NTx Serum assay after 6 months of therapy.

Figure 4. Rate of Change in BMD vs. % Change in the Osteomark® NTx Serum Assay



References

1. Clemens J.D., et. al. Evidence that serum NTx (collagen-type I N-telopeptides) can act as immunochemical marker of bone resorption. *Clin Chem*. 43:2058-2063. 1997.
2. Gertz B.J., et. al. Application of a new serum assay for type I collagen crosslinked N-telopeptides: Assessment of diurnal changes in bone turnover with and without alendronate treatment. *Calcif Tissue Int.* 63:102-106. 1998.
3. Scariano J.K., et. al. Serum levels of cross-linked N-telopeptides in aminoterminal propeptides of type I collagen indicate low bone mineral density in elderly women. *Bone*. 23:471-477. 1998.
4. Prestwood K.M., et. al. Low dose estrogen and calcium have an additive effect on bone resorption in older women. *J Clin Endo and Met.* 84:179-183. 1999.
5. Garnero P., et. al. Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *J Clin Endo and Met*. 79:1963-1700. 1994.
6. Garnero P., et. al. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *J Bone Miner Res.* 11:337-349. 1996.
7. Chesnut C.H., et. al. Hormone replacement therapy in postmenopausal women: Urinary N-telopeptide of type I collagen monitors therapeutic effect and predicts response of bone mineral density. *Am J Med.* 102:29-37. 1997.
8. Greenspan S.L., et. al. Early changes in biochemical markers of bone turnover predict the long-term response to alendronate therapy in representative elderly women: A randomized clinical trial. *J Bone Miner Res.* 13:1431-1438. 1998.
9. Orwoll E.S., et. al. Collagen N-telopeptide excretion in men: The effects of age and intra-subject variability. *J Clin Endo and Met.* 83:3930-3935. 1998.
10. Weiss S., et. al. Determination of the intra-subject variability in NTx excretion in postmenopausal women. *J Bone Miner Res.* Vol. 12. Suppl. 1, pp. S506. 1997.

Osteomark® NTx Serum Assay Quick Reference Guide

1. Thoroughly read the Assay Procedure before you begin.
2. Allow all specimens and kit components to come to room temperature. Mix all reagents thoroughly.
3. Prepare working strength wash solution. Dilute 30X Wash Concentrate 1:30 with deionized water.
4. Plan the plate configuration and create a plate map.
5. Prepare working strength antibody conjugate solution at a 1:101 dilution. You will need approximately 1 mL per strip.
6. Prepare 1:5 dilutions of all Calibrators, Controls and specimens in Specimen Diluent using microtubes or equivalent.
7. Pipette 100 µL of each diluted Calibrator, Control, and specimen into the microplate according to the plate map.
8. Pipette 100 µL of working strength antibody conjugate solution into each microwell. Cover the plate with a plate sealer, gently swirl to mix and incubate the plate at room temperature for 90 ± 5 minutes.
9. Prepare Chromogen Reagent/Buffered Substrate solution at a 1:101 dilution during the last 5 minutes of incubation. You will need approximately 2 mL per strip.
10. Wash microwells five (5) times with working strength wash solution. Blot on absorbent paper after the final wash.
11. Add 200 µL diluted Chromogen Reagent/Buffered Substrate to each microwell. Cover the plate with a plate sealer and incubate at room temperature for 30 ±2 minutes.
12. Add 100 µL of Stopping Reagent to each microwell. Gently swirl the plate to mix.
13. Incubate at room temperature for five (5) minutes and read the absorbance of each microwell at 450 nm - 630 nm. Calculate the results using a 4-parameter logistic curve fitting equation.

				
Hazard pictograms. See precautions.	CE Mark	Authorized Representative in the European Community	Manufacturer	Catalog number

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