OSTEOMARK® NTx Urine

An Enzyme-linked Immunosorbent Assay (EIA) for the Measurement of Cross-linked N-telopeptides of Type I Collagen (NTx) in Human Urine

For in vitro diagnostic use only

Intended Use of Osteomark®

Osteomark® is a urinary assay that provides a quantitative measure of the excretion of cross-linked N-telopeptides of type I collagen (NTx) as an indicator of human bone resorption. Elevated levels of urinary NTx indicate elevated human bone resorption. Measurement of NTx is intended for use in:

A. Predicting skeletal response (bone mineral density) to hormonal antiresorptive therapy in postmenopausal women

B. Therapeutic monitoring of:
   1. antiresorptive therapies in postmenopausal women
   2. antiresorptive therapies in individuals diagnosed with osteoporosis
   3. antiresorptive therapies in individuals diagnosed with Paget’s disease of bone
   4. estrogen-suppressing therapies

C. Identifying the probability for a decrease in bone mineral density after one year in postmenopausal women receiving calcium supplement relative to those treated with hormonal antiresorptive therapy.

The measurement range of Osteomark® is 20 to 3000 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 provide analytical data of 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 urinary cross-linked N-telopeptides of type I collagen (NTx) has provided a specific biochemical marker of human bone resorption which can be analyzed by an 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 is found in the urine as a stable end-product of degradation.

Osteomark® provides a quantitative measure of the excretion of NTx as an indicator of human bone resorption. 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.1, 2 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.3

A randomized, prospective clinical trial of postmenopausal women treated with either hormone-replacement therapy (HRT) plus calcium supplements (500 mg daily) or calcium supplements alone, supported the use of Osteomark® to predict response and monitor the antiresorptive effect of the therapy.4

The ability of Osteomark® to monitor the effect of antiresorptive therapy has been demonstrated in a study of patients diagnosed with osteoporosis treated with a bisphosphonate (alendronate sodium, 10 mg).5 Results demonstrate that the osteoporotic patient should be managed to an Osteomark® value of ≥ 40% change from baseline after 3 months of treatment.

A study was conducted to determine the ability of Osteomark® to monitor the effect of antiresorptive therapy in patients with Paget’s disease of bone. Osteomark® values at baseline and the percent change from baseline after therapy initiation correlated to serum total alkaline phosphatase. Osteomark® provided an earlier assessment than alkaline phosphatase of therapeutic response, defined by normalization of values. Paget’s patients treated with antiresorptive therapy should have a ≥30% change in Osteomark® from baseline after 3 months of treatment.

Assay Principles

The Osteomark® assay is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA) that utilizes microwells as the solid phase onto which NTx has been adsorbed. NTx in the specimen competes with the solid phase NTx for binding sites of a monoclonal antibody labeled with horseradish peroxidase. The amount of antibody bound to the solid phase is therefore inversely proportional to the amount of NTx in the specimen. Quantification of the NTx concentration in the specimen is determined spectrophotometrically and calculated from a standard calibration curve. Assay values are corrected for urinary dilution by urinary creatinine analysis and expressed in nanomoles bone collagen equivalents per liter (nM BCE) per millimole creatinine per liter (mM creatinine).

Kit Components

<table>
<thead>
<tr>
<th>Instructions for Use (1 booklet)</th>
<th>1 booklet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Antigen Coated 96-well Plate, 12 x8-well strips</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Antibody Conjugate Concentrate</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Antibody Conjugate Diluent</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>30X Wash Concentrate</td>
</tr>
<tr>
<td><strong>E</strong></td>
<td>Chromogen Reagent</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>Buffered Substrate</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td>Stopping Reagent</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>1 nM BCE Calibrator</td>
</tr>
<tr>
<td><strong>I</strong></td>
<td>30 nM BCE Calibrator</td>
</tr>
<tr>
<td><strong>J</strong></td>
<td>300 nM BCE Calibrator</td>
</tr>
<tr>
<td><strong>K</strong></td>
<td>1000 nM BCE Calibrator</td>
</tr>
<tr>
<td><strong>L</strong></td>
<td>3000 nM BCE Calibrator</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td>Level I Urine Control</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>Level II Urine Control</td>
</tr>
<tr>
<td><strong>O</strong></td>
<td>Plate Seals</td>
</tr>
</tbody>
</table>

Component Description

**PLATE**

Antigen Coated 96-well Plate, 12 x8-well strips

**CONJ**

Antibody Conjugate Concentrate, 1 vial

**DIL**

Antibody Conjugate Diluent, 1 bottle

**WASHBUF**

30X Wash Concentrate, 1 bottle

**CHROM**

Chromogen Reagent, 1 vial

**BUFF**

Buffered Substrate, 1 bottle

**CAL**

1 nM sulfonic acid, (25 mL)

**CONTROL**

Level I and Level II Urine Controls, 1 vial each

**PLATE**

Plastic plate sealer, 1 pad

Storage of Reagents

Reagents must be stored at 2 - 8 °C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 30 °C or less than 2 °C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but Not Supplied

- Single and multichannel pipettes capable of delivering 25 μL, 100 μL and 200 μL volumes.
- Disposable pipet tips.

OSA6006
Urine Specimen Collection and Storage

- Collect a second void of the morning (spot) urine specimen or a 24 hour urine specimen in an appropriate collection device with a tight fitting lid.
- DO NOT ADD PRESERVATIVE TO 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.
- Store refrigerated (2 - 8 °C) for up to 72 hours or at room temperature for up to 24 hours. Store frozen (−20 °C or below) for longer term storage. Specimens may undergo three freeze/thaw cycles.
- When monitoring therapy, baseline samples should be collected prior to initiation of therapy. Subsequent specimens for comparison should be collected at the same time of day as the baseline specimen.

Preparatory Steps

- Do not reuse microwells. Dispose of properly after use.
- 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 121 °C. 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.
- mRNA is harmful by inhalation, contact with skin, or if swallowed. If exposed, flush area with water for 15 minutes. If eyes are exposed, obtain medical attention.
- Always adhere to the stated incubation requirements. Avoid extreme foaming. Use the working strength conjugate solution within one hour of preparation. Do not reuse the container.
- Do not add preservatives to urine specimens. A stored urine specimen in an appropriate collection device with a tight fitting lid.

Specimen and Antibody Incubation

- Specimens may undergo three freeze/thaw cycles.
- Allow all specimens and reagents to equilibrate to room temperature (18 - 28 °C) for at least one hour before performing the assay. To facilitate warming, remove reagents from the kit box. Frozen urine specimens may be thawed at 37 °C, in either a water bath or an incubator, then brought to room temperature prior to use in the assay. The Chromogen Reagent contains dimethylsulfoxide, which may solidify when reconstituted but is liquid at room temperature.
- Prepare the working strength wash solution. Dilute 30X Wash Concentrate 1:30 with deionized water (1 part 30X Wash Concentrate to 29 parts deionized water) and mix for a minimum of five (5) minutes. This solution is stable for one (1) month at room temperature.
- Create a plate map indicating location of calibrators, controls and urine specimens. It is recommended that calibrators, controls and urine specimens be run in duplicate microwells. An example of a plate map is provided below for an Osteomark® assay with 4 specimens:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1 nM BCE Calibrator</td>
<td>1000 nM BCE Calibrator</td>
<td>Specimen #1</td>
</tr>
<tr>
<td>B</td>
<td>1 nM BCE Calibrator</td>
<td>1000 nM BCE Calibrator</td>
<td>Specimen #1</td>
</tr>
<tr>
<td>C</td>
<td>30 nM BCE Calibrator</td>
<td>3000 nM BCE Calibrator</td>
<td>Specimen #2</td>
</tr>
<tr>
<td>D</td>
<td>30 nM BCE Calibrator</td>
<td>3000 nM BCE Calibrator</td>
<td>Specimen #2</td>
</tr>
<tr>
<td>E</td>
<td>100 nM BCE Calibrator</td>
<td>Level I Urine Control</td>
<td>Specimen #3</td>
</tr>
<tr>
<td>F</td>
<td>100 nM BCE Calibrator</td>
<td>Level I Urine Control</td>
<td>Specimen #3</td>
</tr>
<tr>
<td>G</td>
<td>300 nM BCE Calibrator</td>
<td>Level II Urine Control</td>
<td>Specimen #4</td>
</tr>
<tr>
<td>H</td>
<td>300 nM BCE Calibrator</td>
<td>Level II Urine Control</td>
<td>Specimen #4</td>
</tr>
</tbody>
</table>

- 3. Swirl the plate gently on a flat surface for 5 - 10 seconds to ensure mixing.

- For in vitro diagnostic use only

- Use a calibrated pipettor and new pipette tips for each calibrator, control, or urine specimen.

DO NOT ASSUME THE SPECIMEN IS URINE.

- The Chromogen Reagent contains 3,3',5,5'- tetramethylbenzidine and dimethylsulfoxide. Dimethylsulfoxide is readily absorbed through the skin.

- Do not use reagents beyond their expiration dates.

- Always adhere to the stated incubation requirements. Avoid extreme foaming. Use the working strength conjugate solution within one hour of preparation. Do not reuse the container.

- Do not add preservatives to urine specimens. A stored urine specimen in an appropriate collection device with a tight fitting lid.

- The Stopping Reagent contains 1N sulfuric acid. Avoid contact with skin or eyes. If exposed, flush area with water for 15 minutes. If eyes are exposed, obtain medical attention.

- 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.

- Microwave wash strips must be kept desiccated. Reuse unused microwave strips in the pouch containing desiccant.

- Do not reuse 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.

- Do not mix components from different lots of Osteomark® kits.

- Allow the plate to sit at room temperature for 5 minutes before reading absorbance values.

- Swirl the plate gently on a flat surface for 5 - 10 seconds to ensure mixing. Allow the plate to sit at room temperature for 5 minutes before reading absorbance values.

- Within 30 minutes of adding the Stopping Reagent, read the absorbance of the Calibrators, Controls, and urine specimens. Use a microwell plate reader at 450 nm with a reference filter of 630 nm. The reader must have a maximum optical density reading of ≤3,000.

- 1. Determine concentration values (nM BCE) of Controls and urine specimens using a 4-parameter curve fitting equation.

- EXAMPLE: CALIBRATION CURVE

- 4. Using a clean disposable plastic container, dilute the Antibody Conjugate Concentrate 1:101 using the Antibody Conjugate Diluent. As a guideline, for each Antigen Coated microwell strip used, dilute 20 μL of Antibody Conjugate Concentrate into 2 mL of Antibody Conjugate Diluent. Mix gently by inversion only. Do not vortex or use a magnetic stir bar. Avoid foaming. Use the working strength conjugate solution within one hour of preparation. Do not reuse the container.

- 5. Do not pipette, gently mix the Calibrators, Controls and urine specimens.

- 6. Store refrigerated but is liquid at room temperature.

- 7. Following the plate map generated in Step 3, pipette 25 μL of each Calibrator, Control, or urine specimen into the bottom of the designated microwells. Use a calibrated pipettor and new pipette tips for each calibrator, control, or urine specimen.

- 8. Using a multichannel pipettor, deliver 200 μL of the working strength conjugate solution into each microwell. Apply a plate sealer and swirl the plate gently on a flat surface for 5 - 10 seconds to ensure mixing.

- 9. Inoculate the plate at room temperature (18 - 28 °C) for 90 ± 5 minutes.

- 10. Prepare the Chromogen/Buffered Substrate solution during the last 10 minutes of incubation by making a 1:101 dilution of the Chromogen Reagent into the Buffered Substrate. As a guideline, for each Antigen Coated microwell strip used, dilute 20 μL of the Chromogen Reagent into 2 mL of the Buffered Substrate. Place the Buffered Substrate into a clean plastic disposable container. Thoroughly mix the Chromogen Reagent prior to pipetting. Add the Chromogen Reagent to the Buffered Substrate Reagent and invert gently to mix. Do not vortex, shake vigorously or use a magnetic stir bar. Allow cloudy or turbid specimens to settle for 5 minutes prior to pipetting. Urine specimens containing particulates may be centrifuged before use.

- 11. At the end of the incubation period, carefully remove and discard the plate sealer. Wash the plate five (5) times with the working strength wash solution using an automated plate washer. The automated washer must dispense at least 350 μL of the working strength wash solution per well. Between wash cycles, wells should be filled with working strength wash solution. When wash procedure is complete, grasp the plate frame at the center of each side and invert, blotting on an absorbent paper towel. Immediately add the prepared Chromogen/Buffered Substrate solution as described below.

Color Development and Measurement

- Prepare the Chromogen/Buffered Substrate solution prepared in step 10 into each well using a multichannel pipettor. Cover the plate with a new plate sealer.

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

- Following incubation, carefully remove and discard the plate sealer. Using a multichannel pipettor, add 100 μL of Stopping Reagent to each well in the same order as addition of the Chromogen/Buffered Substrate Reagent. Wells which have developed a blue color will now turn yellow.

- Swirl the plate gently on a flat surface for 5 -10 seconds to ensure mixing. Allow the plate to sit at room temperature for 5 minutes before reading absorbance values.

- Within 30 minutes of adding the Stopping Reagent, read the absorbance of the Calibrators, Controls, and urine specimens. Use a microwell plate reader at 450 nm with a reference filter of 630 nm. The reader must have a maximum optical density reading of ≤3,000.

- Analysis of Results

- 1. Determine concentration values (nM BCE) of Controls and urine specimens using a 4-parameter curve fitting equation.

EXAMPLE: CALIBRATION CURVE

nM BCE
2. Assay results are valid if the following criteria are met:
   - The mean absorbance value of the 1 nM BCE Calibrator must be ≥1.500.
   - The span of the calibrator curve (difference between absorbance values of the 1 nM BCE and 3000 nM BCE Calibrators) must be ≥1.300.
   - The recommended coefficient of variation (% CV) between urine specimen concentration value (nM BCE) duplicates is ≤±20%.
   - Specimens with >20% CV should be rerun.
   - The lower limit of detection is 20 nM BCE (assay value, does not include creatinine correction).
   - Urine specimens that exceed 3000 nM BCE may be diluted 1:5 in a urine specimen or pool of known osteomalacia, and retested. When using urine as a diluent, the nM BCE of the urine diluent should be confirmed by testing as a specimen in the same plate as the diluted unknown specimen. The dilution factor and background (the diluent nM BCE) should be incorporated into the final calculation.

Example: 1040 nM BCE assay value derived from a 1:5 dilution of a 4000 nM BCE specimen using a urine diluent of known Osteomark® value (300 nM BCE)

1040 nM BCE - (0.8 x 300 nM BCE) = 800 nM BCE
800 nM BCE x 5 (dilution factor) = 4000 nM BCE

Note: 1:5 dilutions represent 80% diluent (0.8), 20% specimen contribution.

6. Report the concentration values for urine specimens as nM BCE/mM creatinine, as shown in the following example:

Assay value = 360 nM BCE
Urinary creatinine = 60 mg/dL creatinine

\[ \text{NTx} = \frac{360 \text{ nM BCE}}{60 \text{ mg/dL creatinine}} = 6 \text{ nM BCE/mM creatinine} \]

*Note: Conversion factor used to convert mg creatinine per dL to millimole creatinine per liter.

7. These Urine Control ranges have been established by the manufacturer. It is recommended that each laboratory establish its own control ranges.

Limitations of the Procedure
While Osteomark® 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 or hyperparathyroidism. When using Osteomark® to monitor therapy, results may be confounded in patients affected with clinical conditions known to affect bone resorption, e.g., metastases to bone. While an Osteomark® value provides a measure of the level of bone resorption, a single Osteomark® value cannot provide the rate of bone resorption as reported results do not contain a measure of time. Osteomark® results should be interpreted in conjunction with clinical findings and other diagnostic results.

Interfering Substances
Various urine components and microorganisms were evaluated for an 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 assay performance. Human albumin, bilirubin, glucose, and vitamin C did not interfere with assay performance. Urine specimens obviously contaminated with microorganisms, whole blood or that have visible hemolysis may interfere with assay performance. These specimens should be discarded, and a new urine specimen collected.

Expected Values
A multi-center, cross-sectional study was conducted to determine the reference range for normal premenopausal women (mean age 36 years, range 25-49) and men (mean age 56 years, range 31-87).

| Table 1: Expected Osteomark® Values for Men and Premenopausal Women Range |
|-----------------|-----------------|-----------------|
|                | Mean ± Std Dev  | Range (mean ± 2 std dev) | N |
| Women          | 35 ± 15         | 5-65             | 258 |
| Men            | 43 ± 15         | 3-63             | 206 |

When the premenopausal women expected value range is log-transformed, the range is 14-74 nM BCE/mM creatinine. The log-transformed reference range for men is 13-78 nM BCE/mM creatinine.

The expected short- and long-term intra-subject variability was determined from prospective studies conducted in healthy postmenopausal women (n=276, 268) and men greater than 30 years of age (n=36, 35). Short-term variability was determined from sequential urine specimens collected over 3-4 days and long-term variability was determined from 2-3 monthly collections. Short-term variability was 15% for women and 18% for men. Long-term variability was 18% for women and 19% for men.

Performance Characteristics
Reproducibility and Precision
Intra-assay variability was assessed using eight urine specimens tested in replicates of 10 by each of four operators. Results are provided below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (nM BCE)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>79</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>412</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>1167</td>
<td>4</td>
</tr>
</tbody>
</table>

Inter-assay variability was assessed using three urine specimens tested in duplicate by one operator over 20 separate assay runs. Results are provided below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (nM BCE)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>26</td>
<td>19</td>
</tr>
<tr>
<td>B</td>
<td>111</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>417</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>694</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td>1113</td>
<td>5</td>
</tr>
<tr>
<td>F</td>
<td>1768</td>
<td>6</td>
</tr>
<tr>
<td>G</td>
<td>2640</td>
<td>5</td>
</tr>
</tbody>
</table>

Total assay precision was evaluated by testing the Level I Urine Control and the Level II Urine Control at three clinical laboratory sites over a 30 day period. The Level I Urine Control had a mean value of 438 nM BCE, with a 10% CV. The Level II Urine Control had a mean value of 1537 nM BCE, with a 7% CV.

Antigen Recovery
Antigen recovery was evaluated by adding known amounts of NTx to each of three urine specimens of known NTx concentration. Recovery represented the observed assay value of the “spiked” specimens, calculated as a percent of the expected urine value (baseline urine value plus added antigen (NTx) value). The results demonstrated an average antigen recovery of 100% across the assay range.

Dilutional Linearity
Dilutional linearity was evaluated by performing serial dilutions of four urine specimens with high nM BCE values into a urine specimen with a 200-500 nM BCE value. Results demonstrated correlation coefficients of r = 0.999 to r = 1.000 across the assay range.

Clinical Studies
Use of Osteomark® in Postmenopausal Women
A clinical trial was conducted to determine the ability of the Osteomark® assay to monitor the effect of hormonal replacement therapy (HRT) on bone resorption in early postmenopausal women, and to predict response to HRT. Results support the clinical utility of Osteomark® to monitor hormonal antiresorptive therapy in early postmenopausal women and to predict changes in bone mineral density (BMD) measured by dual energy x-ray absorptiometry (DEXA) in response to HRT, thereby identifying who will receive the greatest benefit from such therapy, and to identify those at risk for bone loss as measured by BMD. Examination of the contrast between treated and control groups provides information regarding risk of BMD loss (Table 2): In the lowest NTx quartile (<38 nM BCE/mM creatinine), there was no statistically significant difference between the HRT and control groups in the likelihood of bone loss over 1 year. A high baseline NTx (≥67 nM BCE/mM creatinine) indicated a 17.3 times higher risk of BMD loss if not treated with HRT.

| Table 2: Relative Risk of Loss of BMD Comparing Calcium and HRT Groups |
|-----------------|-----------------|-----------------|
| Baseline NTx (nM BCE/mM creatinine) | Relative Risk | 95% C.I |
| 18-38           | 1.4            | 0.8 – 2.5     |
| 38-51           | 2.5            | 1.0 – 6.1     |
| 51-67           | 17.3           | 2.5 – 118.5   |

*N = 54, 53, 57, and 55 for each quartile respectively

Use of Osteomark® in Patients Diagnosed with Osteoporosis
A study was conducted to establish the safety and efficacy of a new amino-bisphosphonate (alendronate) in the treatment of osteoporosis. The following data support the clinical utility of Osteomark® to monitor levels of bone resorption in osteoporotic women treated with antiresorptive therapy (alendronate 10 mg).
• Three months after initiating treatment 80% (71/89) of the subjects had an Osteomark® value < 35 nM BCE/mM creatinine and 87% (76/87) had a > 40% decrease from baseline.
• After 1 year of therapy, 90% (77/86) of the subjects had an Osteomark® value < 35 nM BCE/mM creatinine and 92% (78/84) of the subjects had a > 40% decrease from baseline.
• Therefore, Osteomark® values should be monitored in osteoporotic women treated with antiresorptive therapy to a value of < 35 nM BCE/mM creatinine, or a > 40% decrease from baseline after 3 months.

Use of Osteomark® in Patients Diagnosed with Paget’s Disease of Bone
A study was conducted to determine the ability of Osteomark® to monitor the effect of bisphosphonate therapy on bone resorption in patients diagnosed with Paget’s disease of bone. The following data support the clinical utility of Osteomark® to monitor the effect of bisphosphonate therapy on bone resorption levels in patients diagnosed with Paget’s disease of bone.

<table>
<thead>
<tr>
<th>Visit</th>
<th>Alendronate</th>
<th>Etidronate</th>
<th>Pamidronate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month 1*</td>
<td>-48%</td>
<td>-39%</td>
<td>-71%</td>
</tr>
<tr>
<td>Month 3**</td>
<td>-77%</td>
<td>-58%</td>
<td>-67%</td>
</tr>
<tr>
<td>Month 6***</td>
<td>-87%</td>
<td>-72%</td>
<td>-71%</td>
</tr>
</tbody>
</table>

*Osteomark® values should be monitored in Paget’s disease of bone patients treated with antiresorptive therapy to a > 30% change or into the normal range.

Table 4. Percent Responders as Defined by Normalization of Marker (Osteomark® 5-65 nM BCE/mM creatinine, total alkaline phosphatase 39-115 IU/L; number of patients with normalized marker value/total number of patients)

<table>
<thead>
<tr>
<th>Visit</th>
<th>Osteomark® Responders</th>
<th>Total Alkaline</th>
<th>Phosphatase Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month 1</td>
<td>19%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>Month 3</td>
<td>34%</td>
<td>29%</td>
<td>29%</td>
</tr>
<tr>
<td>Month 6</td>
<td>42%</td>
<td>42%</td>
<td>42%</td>
</tr>
</tbody>
</table>

A decrease in total alkaline phosphatase into its normal range is used as an indicator of therapeutic response. Osteomark® values also measure the response and provide an earlier assessment than total alkaline phosphatase.

Use of Osteomark® to Monitor Estrogen Suppressing Therapy
A clinical trial was conducted to determine the ability of the Osteomark® assay to monitor the effect of estrogen suppressing therapy on bone resorption in premenopausal women. The results support the clinical utility of Osteomark® to monitor the effect of estrogen suppressing therapy on bone resorption levels.

References

Osteomark® NTx Urine Quick Reference
1. Thoroughly read the Assay Procedure before you begin.
2. Bring kit components and specimens to room temperature.
3. Dilute the Antibody Conjugate Concentrate into the Antibody Conjugate Diluent, using a 1:101 ratio. Mix gently by inversion only. Use the working strength conjugate solution within one hour of preparation.
4. Pipette 25 μL samples of each Calibrator, Control, and urine specimen into designated microwells.
5. Pipette 200 μL of the working strength conjugate solution into each microwell. Gently swirl to mix. Incubate the plate at room temperature for 90 ± 5 minutes.
6. Prepare Chromogen Reagent/Buffered Substrate solution during the last 10 minutes of incubation. Dilute the Chromogen Reagent into the Buffered Substrate using a 1:101 ratio. Mix gently BY INVERSION ONLY. Do not vortex, shake vigorously, or mix with a magnetic stir bar. Use the Chromogen/Buffered Substrate within 30 minutes of preparation.
7. Wash microwells five (5) times with the working strength wash solution at 350μL per well, and blot on absorbent paper towel.
8. Add 200 μL of Chromogen/Buffered Substrate to each microwell, and incubate at room temperature for 15 ± 1 minutes.
9. Add 100 μL of Stopping Reagent to each microwell. Gently swirl the plate to mix.
10. Incubate at room temperature for 5 minutes and read the absorbance of each microwell at 450 nm - 630 nm. Calculate the results using a 4-parameter curve fitting equation.

Ordering and Contact Information:
U.S. Toll Free 1-877-441-7440
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Technical Support:
Advice Line
Further information can be obtained from your distributor, or by contacting Alere Technical Support on:

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