

# METRA™ BAP EIA kit

96 Assays for Bone-specific Alkaline Phosphatase

For *In Vitro* Diagnostic Use

Store at 2-8°C

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Made in USA

0461F (5/01)

Catalog number 8012

**QUIDEL®**

Read the entire product insert thoroughly before beginning the assay. The Metra™ BAP kit should be stored at 2-8°C until use. Do not subject the kit to freezing.

## INTENDED USE

The Metra™ BAP immunoassay provides a quantitative measure of bone-specific alkaline phosphatase (BAP) activity in serum as an indicator of osteoblastic activity. Measurement of BAP is intended for use as an aid in the:

- management of postmenopausal osteoporosis and Paget's disease;
- monitoring of postmenopausal women on hormonal or bisphosphonate therapy;
- prediction of skeletal response to hormonal therapy in postmenopausal women.

## SUMMARY AND EXPLANATION

The skeletal, or bone-specific, isoform of alkaline phosphatase is a tetrameric glycoprotein found on the cell surface of osteoblasts.<sup>1</sup> Osteoblasts are the cells responsible for synthesis of new bone matrix and its mineralization. The function of BAP has not been fully elucidated, though its role in skeletal mineralization has been confirmed.<sup>1,2,3</sup>

Bone is constantly undergoing a metabolic process called remodeling.<sup>3,4</sup> This includes a degradation process, bone resorption, mediated by the action of osteoclasts, and a building process, bone formation, mediated by the action of osteoblasts.<sup>3,4</sup> Remodeling is required for the maintenance and overall health of bone and is tightly coupled; that is, resorption and formation are in balance.<sup>3,4</sup> In abnormal states of bone metabolism this process becomes uncoupled and, when resorption exceeds formation, this results in a net loss of bone which can lead to osteoporosis,<sup>3,4</sup> or to the disordered bone tissue of pagetic lesions.<sup>5</sup> The measurement of specific biochemical markers of these remodeling events provides analytical data regarding the rate of bone metabolism or "turnover".<sup>3,4</sup>

## SUMMARY AND EXPLANATION (CONT.)

Osteoporosis is a metabolic bone disease characterized by abnormal bone remodeling. It is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in susceptibility to fractures.<sup>6</sup> The most common type of osteoporosis occurs in postmenopausal women as a result of the estrogen deficiency produced by the cessation of ovarian function.<sup>3</sup> Restoration of premenopausal estrogen levels by replacement therapy prevents bone loss and osteoporosis.<sup>3,6</sup> Estrogens and a class of compounds known as bisphosphonates are antiresorptive therapies which can be used to prevent bone loss or treat osteoporosis.<sup>3,6,7</sup>

Osteoporosis can also result from attaining an inadequate peak bone mass during the growing years, an age-related imbalance of bone remodeling with a net excess of resorption, and a number of clinical conditions and therapies which induce bone loss or bone remodeling imbalances.<sup>3</sup> These include endocrine diseases such as hypogonadism, hyperthyroidism, hyperparathyroidism, and hypercortisolism; renal failure; cancers metastatic to bone; gastrointestinal diseases related to nutrition and mineral metabolism; connective tissue diseases; multiple myeloma; chronic immobilization, alcoholism, or tobacco use; and chronic therapy with heparin or corticosteroids.<sup>3</sup>

Paget's disease of bone is a focal disorder resulting in pain and skeletal deformity in symptomatic patients.<sup>5</sup> Pagetic lesions are characterized by bone matrix of highly abnormal structure arising from excessive rates of remodeling activity. The lesions occur predominantly in the skull, spine, pelvis and long bones, and can result in fractures and neurological impairment.<sup>5</sup> The etiology of Paget's disease is unknown but hypotheses involving genetic and viral factors are compelling.<sup>5</sup> Bisphosphonates and calcitonin are currently used to suppress the high rate of biochemical activity to normal levels, enabling restoration of normal bone structure.<sup>9</sup>

As a quantitative measure of a marker of bone turnover, BAP provides useful information on bone remodeling in osteoporosis and Paget's disease, and changes in disease activity produced by antiresorptive therapy.<sup>10-12</sup> For the Metra™ BAP assay, antibody technology was employed to produce a monoclonal antibody that demonstrates specificity for BAP.<sup>10</sup> The specificity of the monoclonal antibody used in the assay allows for simple, convenient, reproducible and direct quantitation of BAP activity in serum.

## PRINCIPLE OF THE PROCEDURE

Metra BAP is an immunoassay in a microtiter strip format utilizing a monoclonal anti-BAP antibody coated on the strip to capture BAP in the sample. The enzyme activity of the captured BAP is detected with a pNPP substrate.

## REAGENTS AND MATERIALS

Metra BAP EIA kit part number 8012 contains the following:

- 1. Substrate Tablets** Part 0012 **3 each**  
p-Nitrophenyl phosphate (20 mg each).
- 2. BAP Standards A - F** Parts 4395 through 4400 **0.3 mL each**  
(0, 2, 20, 50, 80, 140 U/L BAP)  
BAP purified from osteosarcoma SAOS-2 cells in a buffered solution containing magnesium chloride, zinc sulfate, surfactant, carrier protein, blue dye, and sodium azide (0.05%) as a preservative.
- 3. Low/High Controls** Parts 4401, 4402 **0.3 mL each**  
BAP purified from osteosarcoma SAOS-2 cells in a buffered solution containing magnesium chloride, zinc sulfate, surfactant, carrier protein, blue dye, and sodium azide (0.05%) as a preservative.
- 4. Assay Buffer** Part 4403 **27 mL**  
A buffered solution containing magnesium chloride, zinc sulfate, surfactant, and sodium azide (0.05%) as a preservative.
- 5. Substrate Buffer** Part 4404 **3 each, 10 mL**  
A 2-amino-2-methyl-1-propanol solution containing HEDTA, magnesium chloride, zinc sulfate, and sodium azide (0.05%) as a preservative.
- 6. Coated Strips** Part 4660 **12 each**  
Purified murine monoclonal Anti-BAP IgG antibody adsorbed onto stripwells.
- 7. Stop Solution** Part 4702 **15 mL**  
1N NaOH
- 8. 10X Wash Buffer** Part 4703 **55 mL**  
Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative.

## WARNINGS

1. For *In Vitro* Diagnostic Use.
2. Serum samples should be treated as potentially biohazardous material.
3. 1N NaOH is poisonous and can cause severe burns. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
4. Sodium azide is used as a preservative. It may be fatal if swallowed or absorbed through the skin. Do not mix with acids. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
5. Test kits and components should be disposed of in a manner consistent with relevant regulations.

## PRECAUTIONS

1. Reagents supplied should be stored as indicated and used as an integral unit prior to the expiration date indicated on the package label.
2. Do not use Coated Strips if pouch is punctured.
3. Each sample should be tested in duplicate.
4. A standard curve must be performed with each assay.
5. A quadratic calibration curve fit must be used for accurate results. Equation:  $y = A + Bx + Cx^2$
6. Samples greater than 140 U/L should be diluted in Assay Buffer and retested.
7. The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation. The OD values are provided and are to be used as a guideline only. The results obtained by your laboratory may differ.

Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable and the samples should be repeated.

8. If the OD of the Metra BAP Standard F is less than 1.0, the results should be considered questionable and the samples should be repeated.
9. Use of multichannel pipets or repeat pipetors is recommended to ensure timely delivery of reagents.
10. For accurate measurement of samples, the addition of samples and standards must be precise. Pipet carefully using only calibrated equipment.
11. This assay may be performed with any validated washing method.

## REAGENT PREPARATION AND STORAGE

**Equilibrate reagents to room temperature (20-28°C) before use.**

1. **Coated Strips**  
Remove Stripwell Frame and the required number of Coated Strips from the pouch (See table in Assay Procedure Section). Ensure that the pouch containing any unused strips is completely resealed.
2. **Wash Buffer**  
Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer concentrate 1:10 with deionized water. Store at room temperature (20-28°C). Use 1X Wash Buffer within 24 hours of preparation.
3. **Working Substrate Solution**  
Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of room temperature Substrate Buffer (see table). Allow 30-60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix. Discard remaining Working Substrate Solution after use.

## SPECIMEN COLLECTION AND STORAGE

Collect serum using standard venipuncture technique. Specimens should be collected without anticoagulants and in such a way to avoid hemolysis. Allow the blood to clot and separate the serum by centrifugation. Serum can be stored for 5 days at 2-8°C, or store at  $\leq -40^\circ\text{C}$  for no longer than 12 months. Do not subject samples to more than 3 freeze/thaw cycles.

## METRA BAP ASSAY PROCEDURE

<b>Kit Contents</b>	<b>Qty/Vol</b>	<b>Part</b>
Substrate Tablets	3 each	0012
Standard A (BAP 0 U/L)	0.3 mL	4395
Standard B (BAP 2 U/L)	0.3 mL	4396
Standard C (BAP 20 U/L)	0.3 mL	4397
Standard D (BAP 50 U/L)	0.3 mL	4398
Standard E (BAP 80 U/L)	0.3 mL	4399
Standard F (BAP 140 U/L)	0.3 mL	4400
Control, Low	0.3 mL	4401
Control, High	0.3 mL	4402
Assay Buffer	27 mL	4403
Substrate Buffer (3)	10 mL	4404
Coated Strips (12)	1 each	4660
Stop Solution	15 mL	4702
10X Wash Buffer	55 mL	4703

### Materials Required BUT NOT Provided

Micropipettes to deliver 20  $\mu\text{L}$  and 100-300  $\mu\text{L}$   
Items suitable for liquid measurement of 100-300 mL  
Container for wash buffer dilution  
Deionized or distilled water  
Plate reader capable of OD readings  $> 2.0$   
Quadratic calibration curve fitting software

**Determine amount of each reagent required for the number of strips to be used.**

<b># of Strips</b>	<b>4</b>	<b>6</b>	<b>8</b>	<b>12</b>
<b># of Samples</b>	<b>8</b>	<b>16</b>	<b>24</b>	<b>40</b>
<small>(tested in duplicate)</small>				
Substrate (bottle)	1	1	2*	2*
1X Wash Buffer (mL)	100	150	200	300

\*When more than one bottle or vial is to be used, combine the contents and mix prior to use.

### Sample Incubation

1. Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table) just prior to use. Ensure that the foil pouch containing any unused strips is completely resealed.
2. Place desired number of Coated Strips in the Stripwell Frame. Label strips to prevent mix-up in case of accidental removal from Stripwell Frame.
3. Add 125  $\mu\text{L}$  Assay Buffer to each well.
4. Add 20  $\mu\text{L}$  of Standard, Control or sample to each well. Do **not** mix with Assay Buffer by repeat pipeting. This step should be completed within 30 minutes. **Gently swirl strips to ensure mixing of sample and buffer.**
5. Incubate for 3 hours ( $\pm 10$  minutes) at room temperature (20-28°C).
6. Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of room temperature Substrate Buffer (see table). Allow 30-60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

### Wash Step

1. Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Store at room temperature (20-28°C). Use 1X Wash Buffer within 24 hours of preparation.
2. Manually invert/empty strips. Add at least 250  $\mu\text{L}$  of 1X Wash Buffer to each well and manually invert/empty strips. Repeat three more times for a total of four washes. Vigorously blot the strips dry on paper towels after the last wash.

### Substrate Incubation

1. Add 150  $\mu\text{L}$  of Working Substrate Solution to each well. Discard remaining Working Substrate Solution after use.
2. Incubate for 30 minutes ( $\pm 5$  minutes) at room temperature (20-28°C).

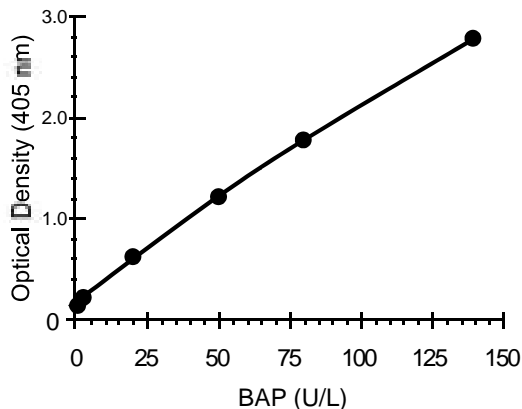
## METRA BAP ASSAY PROCEDURE (CONT.)

### Stop/Read

1. Add 100  $\mu\text{L}$  of Stop Solution to each well. Add Stop Solution in the same pattern and time intervals as the Substrate Solution addition.
2. Read the Optical Density (OD) at 405 nm. Assure that no large bubbles are present in the wells and that the bottom of the strips are clean. Strips should be read within **15 minutes** of Stop Solution addition.
3. Quantitation software with a quadratic calibration curve fitting equation **must** be used to analyze the Metra™ BAP assay results.

### Representative Standard Curve

Standard BAP levels: 0, 2, 20, 50, 80, 140 U/L



## QUICK GUIDE TO ASSAY STEPS

1. Add 125  $\mu\text{L}$  Assay Buffer.
2. Add 20  $\mu\text{L}$  Standards, Controls, and samples; swirl.
3. Incubate 3 hours  $\pm$  10 minutes at room temperature.
4. Wash 4 times with 1X Wash Buffer.
5. Add 150  $\mu\text{L}$  room temperature Working Substrate Solution.
6. Incubate 30  $\pm$  5 minutes at room temperature.
7. Add 100  $\mu\text{L}$  Stop Solution and read OD at 405 nm.

## INTERPRETATION OF RESULTS

1. Sample results are expressed as U/L and **do not** need to be corrected for dilution (unless sample was diluted prior to testing).
2. In the Metra BAP assay, 1 Unit represents 1  $\mu\text{mol}$  of pNPP hydrolyzed per minute at 25°C in 2-amino-2-methyl-1-propanol buffer.

## LIMITATIONS

1. **HAMA Interference**  
Some individuals have antibodies to mouse protein (HAMA), which can cause interference in immunoassays that employ antibodies derived from mice. In particular, it has been reported that serum samples from patients who have undergone therapy or diagnostic procedures that include infusion of mouse monoclonal antibody may produce erroneous results. Therefore, Alkphase-B results for such patients should be used only in conjunction with results from some other diagnostic procedure and with information available from the clinical evaluation of the patient.
2. Samples with significant elevations of liver alkaline phosphatase activity may cause aberrantly elevated results in the Metra BAP assay.
3. Paget's patients who have low levels of disease may have bone-specific alkaline phosphatase levels that fall within the Metra BAP reference range.

## BAP EXPECTED VALUES

BAP reference ranges have been established for normal males over 25 years of age (n=126), normal premenopausal females between the ages of 25 and 44 (n=178), and normal postmenopausal females (n=107). For the purposes of establishing reference ranges, normal subjects were defined as:

- Basically healthy, no bone, endocrine or chronic disorders
- Regular menstrual cycles (premenopausal females)
- Not pregnant or breast feeding (females)
- Not currently taking any medication known to influence bone metabolism

Values may be influenced by such factors as low estrogen production, low calcium intake or low physical activity.<sup>8</sup> Estrogen deficiency in postmenopausal women can result in elevated bone turnover.<sup>3,4</sup> Each laboratory should establish its own normal reference range. The ranges are expressed as nonparametric reference intervals (90% CI).

	Age (yr)		Range (U/L)	Median
Females	25 - 44	premenopausal	11.6 - 29.6	18.3
Females	$\geq$ 45	postmenopausal	14.2 - 42.7	25.0
Males	$\geq$ 25		15.0 - 41.3	23.2

## PERFORMANCE CHARACTERISTICS

### Antibody Specifications

The bone-specific alkaline phosphatase antibody has selective, high affinity for the bone-specific alkaline phosphatase isoform, low cross-reactivity to the liver form of alkaline phosphatase, and negligible binding of intestinal and placental isoenzymes.

AP Isoenzyme	% Reactivity
Bone	100
Liver	3 - 8
Placental	0
Intestine	0.4

### Sensitivity

The minimum detection limit of the Metra BAP assay is 0.7 U/L, determined by the upper 3 SD limit in a zero standard precision study.

### Recovery - Spike Recovery

Spike recovery was determined by adding a known quantity of purified BAP to serum samples with different levels of endogenous BAP. Typical results are provided after spiking serum samples with low, medium, and high concentrations of BAP and assaying in triplicate.

Endogenous (U/L)	Added (U/L)	Observed (U/L)	Recovery (%)
13.4	15.7	29.1	99
17.6	37.5	55.3	99
27.2	57.2	88.1	106

### Recovery - Linearity

Linearity was determined by serially diluting samples and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Observed (U/L)	Expected (U/L)	Recovery (%)
1	neat	108.5	-	-
	1:2	51.1	54.2	94
	1:4	25.8	27.1	99
	1:6	18.0	18.1	99
2	neat	39.1	-	-
	1:2	20.1	19.5	103
	1:4	10.3	9.8	105
	1:6	6.7	6.5	103
3	neat	58.4	-	-
	1:2	29.9	29.2	102
	1:4	15.7	14.6	108
	1:6	9.7	9.7	100

## PERFORMANCE CHARACTERISTICS (CONT.)

### Precision

Within-run and between-run precision were determined by assaying 3 serum samples in 9 runs. Typical results are provided below.

BAP (U/L BAP)	Within-run <sup>1</sup> CV%	Between-run <sup>2</sup> CV%
12	5.8	5.2
35	3.9	7.6
100	5.2	5.0

<sup>1</sup>n=21    <sup>2</sup>n=6 runs

### Interfering Substances

The following substances were tested at the specified concentrations, and were found not to interfere with the assay.

Substance	Concentration
Hemoglobin	500 mg/dL
Bilirubin	25 mg/dL
Triglycerides	1420 mg/dL
Total Protein	6.0 g/dL †
Total Protein	15.6 g/dL †
Total Protein	6.0 g/dL ‡
Total Protein	15.6 g/dL ‡

† Protein with water

‡ Protein with BAP (BAP concentration = 43.6 U/L)

### Drug Interferences

Various concentrations of drugs were added to three separate serum pools containing approximately 35, 70, and 105 U/L BAP and assayed in triplicate. The drugs and the highest concentrations tested were:

Substance	Highest Concentration
Etidronate	350 µg/mL
Estrogen	100 µg/mL
Ibuprofen	150 µg/mL
Acetaminophen	350 µg/mL
Aspirin	350 µg/mL
Calcitonin - Human	80 µg/mL
Calcitonin - Salmon	80 µg/mL
Calcium	500 µg/mL
Norethindrone/ethinyl estradiol mixture (oral contraceptive)	3 mg/mL
Vitamin D	400 IU/mL

### Accuracy

Comparative studies were performed to assess the correlations between measurements of serum bone-specific alkaline phosphatase (BAP) obtained using the Metra™ BAP assay to results obtained using three currently marketed methods for measuring total alkaline phosphatase (TAP) or BAP. The studies were conducted at an independent clinical investigational site, utilizing sera from 114 patients with Paget's disease and 464 healthy subjects. The first comparative method was a colorimetric technique for the measurement of TAP. The correlation coefficient (r) obtained between this colorimetric method and the Metra BAP assay was 0.99. The second comparative method was an electrophoresis method for the determination of BAP isoenzyme levels (r=0.99). The third comparative method was an immunoradiometric assay for the measurement of BAP (r=0.99). Of the 114 patients diagnosed with Paget's disease, 101 patients had values greater than the upper limit of the reference ranges for the Metra BAP assay. Thirteen patients had values less than the upper limit of the reference ranges.

## ASSISTANCE

If you have any questions regarding the use of this product, please call Quidel's Technical Support Number, 800-524-6318 (toll free). If outside the United States, contact your local Quidel office or distributor.

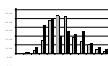
## CLINICAL STUDIES

### Use of Metra BAP for Monitoring the Efficacy of Antiresorptive Therapy in Osteoporosis

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the Metra BAP assay to monitor changes in serum BAP concentrations associated with amino-bisphosphonate (alendronate) antiresorptive therapy. Subjects, drawn from a larger study of the efficacy of alendronate for treating osteoporosis,<sup>7</sup> were postmenopausal women, aged 45 to 84 years (mean 64 ± 7 years), diagnosed with osteoporosis (based on clinical presentation or baseline lumbar spine bone mineral density [LSBMD] more than 2.5 standard deviations below the mean for mature premenopausal women). At baseline, eligible subjects were randomized to receive either 10 mg alendronate and 500 mg calcium per day (ALN) or placebo and 500 mg calcium per day (CTL). Serum specimens were obtained at baseline, 3, 6 and 12 months from all subjects.

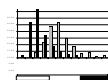
Mean (± 1SD) baseline BAP concentration (14.6 ± 5.4 vs. 14.6 ± 4.6, p=0.900) and LSBMD (0.74 ± 0.10 vs. 0.75 ± 0.09, p=0.751) were similar values for ALN and CTL. Distributions of baseline BAP values in ALN and CTL are depicted in the following figure by proportion of the study population.

Distribution of BAP Levels At Baseline



BAP was significantly lower for ALN than CTL at 3 (9.6 ± 3.5 vs. 13.4 ± 4.0, p<0.00001), 6 (8.0 ± 3.0 vs. 13.2 ± 3.8, p<0.00001), and 12 months (7.8 ± 2.6 vs. 13.3 ± 3.9, p<0.00001). Distributions of BAP values following 12 months in the ALN and CTL groups are depicted in the following figure.

Distribution of BAP Levels Following 12 Months Therapy with Alendronate (ALN) or Calcium (CTL)

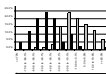


The mean (± 1SD) BAP concentration in CTL subjects decreased modestly from baseline to -5.4% (± 19.1%) at 12 months (p=0.00004) which may reflect the limited bone-sparing effect of calcium.<sup>13</sup>

## CLINICAL STUDIES (CONT.)

Mean BAP concentrations in ALN subjects decreased  $30.5 \pm 24.6\%$  at 3 months,  $42.8 \pm 17.3\%$  at 6 months, and  $42.2 \pm 19.2\%$  at 12 months. Subjects in ALN were more likely than CTL subjects to demonstrate BAP losses exceeding minimum percent change<sup>14</sup> with 68.5%, 83.9%, and 86.1% of ALN and 9.5%, 15.9% and 9.0% of CTL individuals decreasing by  $\geq 25\%$  at the 3, 6, and 12 month timepoints. Distributions of the percent change from baseline in BAP values following 12 months in the ALN or CTL groups are depicted in the following figure.

**Distribution of Percent Change in BAP Levels Following 12 Months Therapy with Alendronate (ALN) or Calcium (CTL)**



At 12 months, subjects in ALN had gained LSBMD compared to CTL ( $p < 0.00001$ ) as shown in the following table

### Changes in LSBMD (Mean $\pm$ SD)

	n	Baseline (g/cm <sup>2</sup> )	12 months (g/cm <sup>2</sup> )	$\Delta$ (%)
CTL	159	$0.75 \pm 0.09$	$0.74 \pm 0.09$	$-0.6 \pm 3.4$
ALN	121	$0.74 \pm 0.10$	$0.79 \pm 0.10$	$5.5 \pm 4.1$

These results indicate that the Metra BAP assay is safe and effective for monitoring the antiresorptive effect of amino-bisphosphonate (alendronate) therapy among subjects diagnosed with osteoporosis.

### Use of Metra BAP for Monitoring Hormonal Antiresorptive Therapy and Predicting Skeletal Response (Bone Mineral Density) in Postmenopausal Women

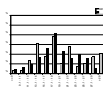
#### Monitoring Therapy:

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the Metra BAP assay to monitor the changes in serum BAP concentrations associated with estrogen/progestin antiresorptive therapy. Increased bone turnover and significant loss of bone are often associated with postmenopausal estrogen deficiency. Estrogen replacement has been shown to effectively decrease bone turnover and protect existing bone mass.<sup>3,6</sup> Subjects were postmenopausal women, aged 45 to 64 years (mean  $56 \pm 4$  years), who had undergone natural or surgical menopause within the last 10 years. At baseline, eligible subjects were randomized to either an active treatment group (HRT): Premarin<sup>®</sup> (0.625 mg daily) with placebo progestin, Premarin<sup>®</sup> (0.625 mg daily) and an active progestin (Provera<sup>®</sup> 2.5 mg/day continuous, Provera<sup>®</sup> 10 mg/day cyclical, or micronized progesterone 200 mg/day cyclical); or to the control group (CTL): placebo estrogen and placebo progestin. Serum specimens were obtained at baseline and 12 months from all subjects.

Mean ( $\pm$  1SD) baseline BAP concentration ( $20.7 \pm 7.6$  vs.  $20.3 \pm 6.8$  U/L,  $p=0.704$ ) and LSBMD ( $0.97 \pm 0.17$  vs.  $0.97 \pm 0.15$  g/cm<sup>2</sup>,  $p=0.970$ ) were similar for CTL and HRT. Distributions of baseline BAP values in HRT and CTL are depicted in the following figure by proportion of the study population.

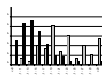
## CLINICAL STUDIES (CONT.)

### Distribution of BAP Levels At Baseline



BAP was significantly lower for HRT than CTL at 12 months ( $13.3 \pm 5.0$  vs.  $21.9 \pm 7.9$  U/L,  $p < 0.00001$ ). Distributions of BAP values following 12 months in the HRT and CTL groups are depicted in the following figure.

**Distribution of BAP Levels Following 12 Months Therapy with Estrogen/Progestin (HRT) or Placebo (CTL)**



The mean ( $\pm$  1SD) BAP concentration in CTL subjects increased slightly from baseline to  $+9.8\%$  ( $\pm 33.2\%$ ) at 12 months ( $p=0.08$ ) whereas BAP concentrations in HRT subjects decreased from baseline to  $-32.4$  ( $\pm 21.5\%$ ) at 12 months ( $p < 0.00001$ ). Subjects in HRT were more likely than CTL subjects to demonstrate BAP losses exceeding minimum percent change<sup>12</sup> with 73.3% of HRT and 3.4% of CTL individuals decreasing by  $\geq 25\%$  at the 12 month timepoint. Distributions of the percent change from baseline in BAP values following 12 months in the HRT and CTL groups are depicted in the following figure.

## CLINICAL STUDIES (CONT.)

### Distribution of Percent Change in BAP Levels Following 12 Months Therapy with Estrogen/Progestin (HRT) or Placebo (CTL)

At 12 months, subjects in HRT had gained LSBMD compared to CTL ( $p < 0.00001$ ) as shown in the following table

#### Changes in LSBMD (Mean $\pm$ SD)

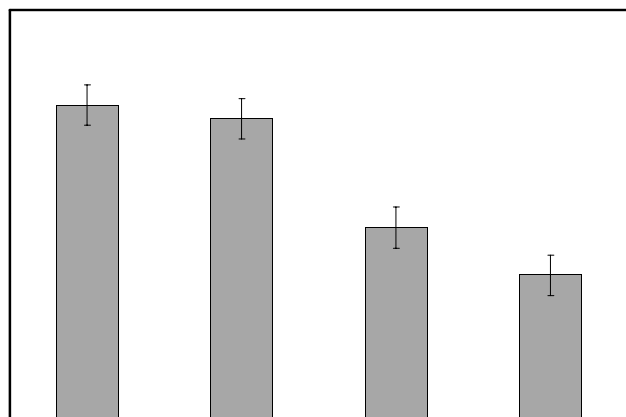
	n	Baseline (g/cm <sup>2</sup> )	12 months (g/cm <sup>2</sup> )	$\Delta$ (%)
CTL	58	0.97 $\pm$ 0.17	0.95 $\pm$ 0.16	-1.6 $\pm$ 2.8
HRT	262	0.97 $\pm$ 0.15	1.00 $\pm$ 0.15	+3.5 $\pm$ 2.8

These results indicate that the Metra BAP assay is safe and effective for monitoring the antiresorptive effect of hormone replacement therapy in postmenopausal women.

#### Predicting Skeletal Response:

The following figure depicts the % decrease in BAP values from baseline to 12 months by quartile for the HRT-treated group. Subjects in the highest quartile (Q1: greatest % decrease) showed the greatest gain in LSBMD in response to HRT.

#### HRT Group - Values of % Change in BAP to 12 months stratified by Quartile and Corresponding % Change in LSBMD at 12 months



## CLINICAL STUDIES (CONT.)

The following figure provides the linear regression analysis ( $y = -0.060x + 0.011$ ,  $r = -0.51$ ,  $p < 0.001$ ) of the percent change from baseline to 12 months BAP and percent change from baseline to 12 months BMD for all subjects in the study (placebo and treated).

#### HRT Study - Linear Regression of the % change in LSBMD and BAP from Baseline to 12 months



Contingency table analysis showed that a  $\geq 25\%$  decrease in BAP at 12 months was significantly associated ( $p < 0.0001$ ) with a positive skeletal response to HRT (gain in BMD) at 12 months. The binomial (second order approximation) 85% confidence intervals for the sensitivity and specificity of using a 25% decrease in BAP for predicting a response to HRT are:  
Sensitivity = 77% (95% CI 75%, 82%); Specificity = 61% (95% CI 41%, 78%).

These results indicate that the % change in BAP concentration can be used to predict the degree of skeletal response (BMD) to HRT treatment.

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