

An enzyme immunoassay for the quantitation of bone-specific alkaline phosphatase (BAP) in human serum

MicroVue™ BAP EIA Summary

Reagents and Samples Preparation

- Dilute 10X Wash Buffer 1:10 with DI Water

Assay Procedure

Pipette 125 µL of Assay Buffer into assay wells

Pipette 20 µL of the Standards, Controls, and Specimens into assay wells (*Gently swirl plate to ensure mixing of sample and buffer.*)

Incubate **3 hours ± 10 min** at 20 – 28°C

Wash **4 times** with
1X Wash Buffer

- Prepare Substrate Solution (*30 – 60 min before use*)
Add one Substrate tablet per bottle of Substrate Buffer (*Shake vigorously*)

Pipette 150 µL Substrate Solution

Incubate **30 ± 5 min** at 20 – 28°C

Pipette 100 µL Stop Solution

Read the Optical Density at 405 nm.
Analyze the assay results using a quadratic curve fit
 $y = A + Bx + Cx^2$

SUMMARY AND EXPLANATION

The skeletal, or bone-specific, isoform of alkaline phosphatase is a tetrameric glycoprotein found on the cell surface of osteoblasts.¹ 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.^{1,2,3}

Bone is constantly undergoing a metabolic process called remodeling.^{3,4} 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.^{3,4} Remodeling is required for the maintenance and overall health of bone and is tightly coupled; that is, resorption and formation are in balance.^{3,4} 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,^{3,4} or to the disordered bone tissue of pagetic lesions.⁵ The measurement of specific biochemical markers of these remodeling events provides analytical data regarding the rate of bone metabolism or "turnover."^{3,4}

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

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.³ 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.³

INTENDED USE

The MicroVue 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.

Paget's disease of bone is a focal disorder resulting in pain and skeletal deformity in symptomatic patients.⁵ 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.⁵ The etiology of Paget's disease is unknown but hypotheses involving genetic and viral factors are compelling.⁵ Bisphosphonates and calcitonin are currently used to suppress the high rate of biochemical activity to normal levels, enabling restoration of normal bone structure.⁹

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.¹⁰⁻¹² For the MicroVue BAP assay, antibody technology was employed to produce a monoclonal antibody that demonstrates specificity for BAP.¹⁰ 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

MicroVue 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 PROVIDED

96 Assays for Bone-specific Alkaline Phosphatase

MicroVue BAP EIA contains the following:

- A Standards:** Parts 4395 – 4400 0.4 mL, 1 each
(A = 0, B = 2, C = 20, D = 50, E = 80, F = 140 U/L BAP)
- B** 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
- C** 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
- D** 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
- E** 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
- F** 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
- L Low/High Controls** Parts 4401, 4402 0.4 mL, 1 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
- H** 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
- 1 Coated Strips** Part 4660 12 each
Purified murine monoclonal Anti-BAP IgG antibody adsorbed onto stripwells
- 2 Stop Solution** Part 4702 15 mL
0.5N NaOH
- 3 10X Wash Buffer** Part 4703 55 mL
Nonionic detergent in a buffered solution containing 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 x 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 Substrate Tablets** Part 0012 3 x 20 mg
p-Nitrophenyl phosphate

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes to deliver 20 µL and 100–300 µL
- Items suitable for liquid measurement of 100–300 mL
- Container for wash buffer dilution
- Deionized or distilled water
- Plate reader capable of Optical Density readings at $A_{405} > 2.0$
- Quadratic calibration curve fitting software

WARNINGS AND PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
3. Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Store assay reagents as indicated.
6. Do not use Coated Strips if pouch is punctured.
7. Test each sample in duplicate.
8. Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
9. 0.5N NaOH is considered corrosive and can cause irritation. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
10. Sodium azide is used as a preservative. Incidental contact with or ingestion of buffers containing sodium azide may cause irritation to the skin, eyes, or mouth. Only use buffers for intended purposes and avoid contact with acids. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build-up.
11. The substrate buffer contains 2-amino-2-methyl-1-propanol and may cause irritation to the eyes and/or skin with prolonged contact. Contacted areas should be immediately washed with soap and water.
12. Use of multichannel pipets or repeat pipetors is recommended to ensure timely delivery of reagents.
13. For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
14. Dilute samples greater than 140 U/L in Assay Buffer and retest. Include the dilution factor in the final calculation.
15. This assay may be performed with any validated washing method.

REAGENT PREPARATION

Bring reagents and materials for the assay to 20–28°C before use. After removing the needed reagents and materials, return unused items to 2–8°C.

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.

Wash Buffer

Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer concentrate 1:10 with deionized water. Store at 20–28°C. Use 1X Wash Buffer within 21 days of preparation.

Working Substrate Solution

Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of 20–28°C 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.

STORAGE

Store kit at 2–8°C. Do not freeze. Store unused reagents at 2–8°C. Equilibrate reagents to 20–28°C before use. Store 1X Wash Buffer (10X diluted) at 20–28°C.

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, at ≤ -40°C for 12 months, or at ≤ -80°C for 36 months. Do not subject samples to more than 3 freeze/thaw cycles.

“Off the clot” serum, serum separator tube serum, Na heparin plasma, and Li heparin plasma yield substantially equivalent results. It is recommended that plasma samples not be prepared using chelating agents such as EDTA or citrate.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See REAGENT PREPARATION before proceeding.

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

# of Strips	4	6	8	12
# of Samples (tested in duplicate)	8	16	24	40
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 µL Assay Buffer to each well.
4. Add 20 µL of Standards, Controls, and Specimens to assay wells. **Do not** mix with Assay Buffer by repeat pipetting. This step should be completed within 30 minutes. **Gently swirl plate to ensure mixing of sample and buffer.**
5. Incubate for 3 hours (± 10 minutes) at 20–28°C.
6. Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of 20–28°C Substrate Buffer (see table). Allow 30–60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

Washing Step

7. Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Store at 20–28°C. Use 1X Wash Buffer within 21 days of preparation.
8. Manually invert/empty strips. Add at least 250 µL 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

9. Add 150 µL of Working Substrate Solution to each well. Discard remaining Working Substrate Solution after use.
10. Incubate for 30 minutes (± 5 minutes) at 20–28°C.

Stop/Read

11. Add 100 µL of Stop Solution to each well. Add Stop Solution in the same pattern and time intervals as the Substrate Solution addition.
12. Read the optical density at 405 nm. Assure that no large bubbles are present in the wells and that the bottoms of the strips are clean. Strips should be read within **15 minutes** of Stop Solution addition.
13. Quantitation software with a quadratic calibration curve fitting equation **must** be used to analyze the MicroVue BAP assay results.
Equation: $y = A + Bx + Cx^2$

QUALITY CONTROL

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. The optical density 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.

If the optical density of the MicroVue BAP Standard F is less than 1.0, the results should be considered questionable and the samples should be repeated.

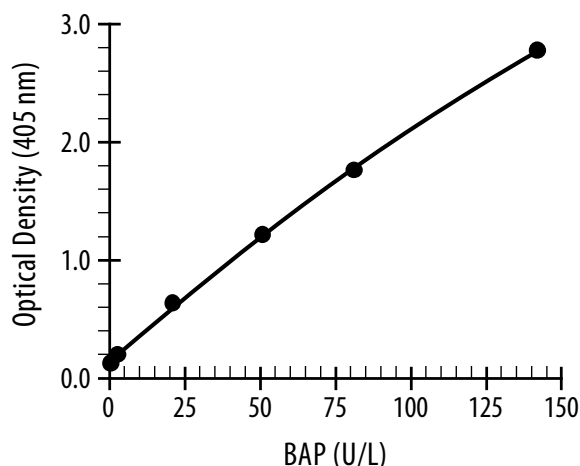
INTERPRETATION OF RESULTS

Sample results are expressed as U/L and **do not** need to be corrected for dilution (unless sample was diluted prior to testing).

In the MicroVue BAP assay, 1 Unit represents 1 μmol of pNPP hydrolyzed per minute at 25°C in 2-amino-2-methyl-1-propanol buffer.

Representative Standard Curve

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



LIMITATIONS OF THE PROCEDURE

HAMA Interference

Some individuals have antibodies to mouse antibodies (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, MicroVue BAP 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.

Samples with significant elevations of liver alkaline phosphatase activity may cause aberrantly elevated results in the MicroVue BAP assay.

Paget's patients who have low levels of disease may have bone-specific alkaline phosphatase levels that fall within the MicroVue BAP reference range.

SAMPLE 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.⁸ Estrogen deficiency in postmenopausal women can result in elevated bone turnover.^{3,4} 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	≥ 45	Postmenopausal	14.2 - 42.7	25.0
Males	≥ 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 MicroVue 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

Precision

Within-run precision was determined for ≥ 21 replicates of 3 samples on 3 plates from each of 3 kit lots (9 plates total). Between-run precision was determined for 3 samples run in 6 separate plates from each of 3 kit lots (18 plates total). Typical results are provided below.

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

¹ n=21

² 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 following drugs with the highest concentrations tested were found not to interfere with the assay:

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/ethynil estradiol mixture (oral contraceptive)	3 mg/mL
Vitamin D	400 IU/mL

Accuracy

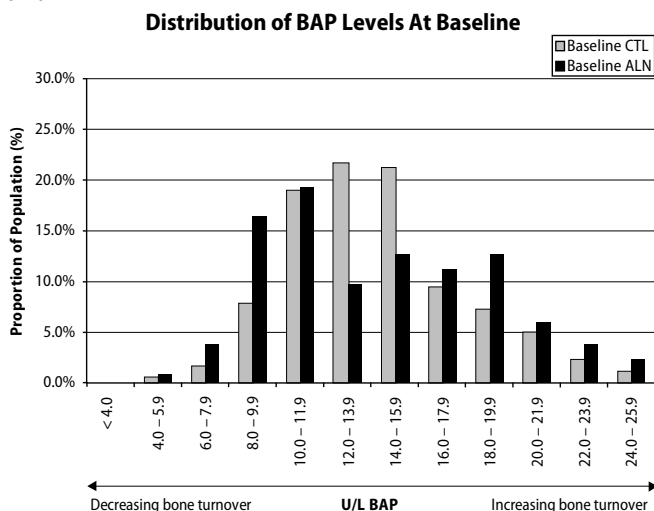
Comparative studies were performed to assess the correlations between measurements of serum bone-specific alkaline phosphatase (BAP) obtained using the MicroVue BAP assay to results obtained using three currently marketed methods for measuring total alkaline phosphatase (TAP) or BAP. The studies were 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 MicroVue 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 MicroVue BAP assay. Thirteen patients had values less than the upper limit of the reference ranges.

CLINICAL STUDIES

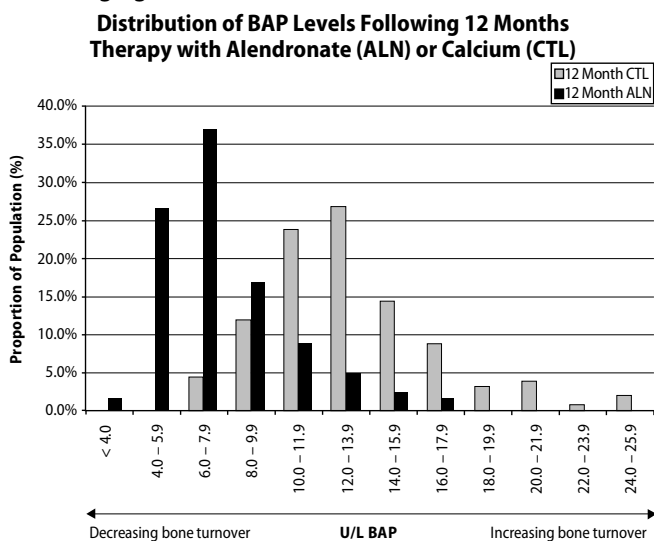
Use of MicroVue BAP for Monitoring the Efficacy of Antiresorptive Therapy in Osteoporosis

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the MicroVue 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,⁷ 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 (\pm 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.



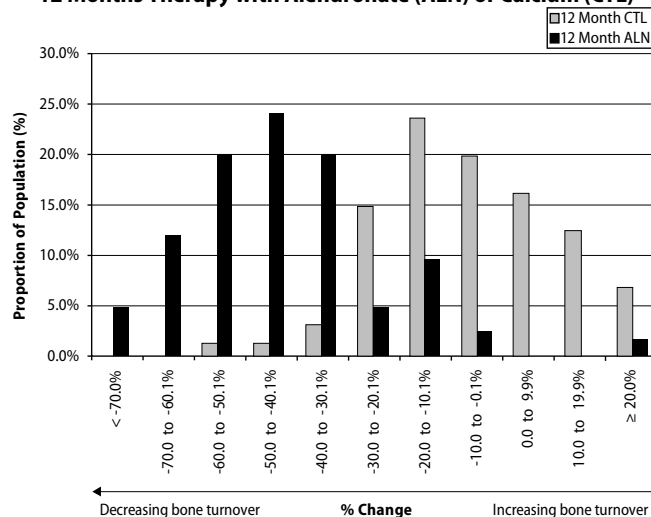
BAP was significantly lower for ALN than CTL at 3 (9.6 ± 3.5 vs. 13.4 ± 4.0 , $p < 0.00001$), 6 (8.0 ± 3.0 vs. 13.2 ± 3.8 , $p < 0.00001$), and 12 months (7.8 ± 2.6 vs. 13.3 ± 3.9 , $p < 0.00001$). Distributions of BAP values following 12 months in the ALN and CTL groups are depicted in the following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



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

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 change¹⁴ 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 ²)	12 months (g/cm ²)	Δ (%)
CTL	159	0.75 ± 0.09	0.74 ± 0.09	-0.6 ± 3.4
ALN	121	0.74 ± 0.10	0.79 ± 0.10	5.5 ± 4.1

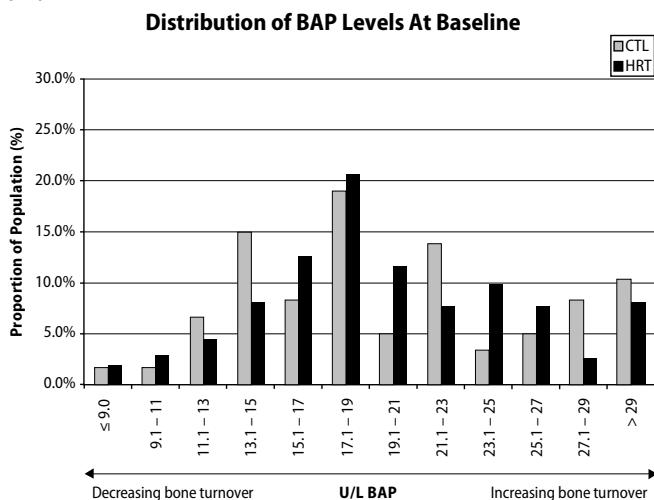
These results indicate that the MicroVue BAP assay is safe and effective for monitoring the antiresorptive effect of amino-bisphosphonate (alendronate) therapy among subjects diagnosed with osteoporosis.

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

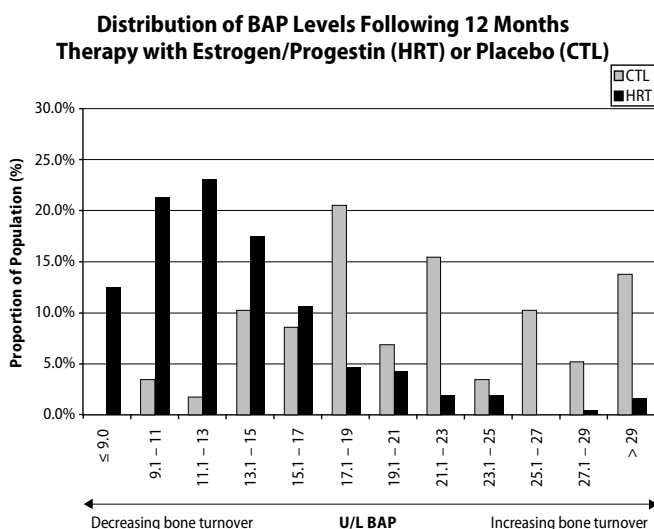
Monitoring Therapy:

A multicenter, randomized controlled trial was successfully conducted to establish the safety and efficacy of the MicroVue 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.^{3,6} Subjects were postmenopausal women, aged 45 to 64 years (mean 56 ± 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® (0.625 mg daily) with placebo progestin, Premarin (0.625 mg daily) and an active progestin (Provera® 2.5 mg/day continuous, Provera 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 ± 7.6 vs. 20.3 ± 6.8 U/L, $p = 0.704$) and LSBMD (0.97 ± 0.17 vs. 0.97 ± 0.15 g/cm², $p = 0.970$) 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.

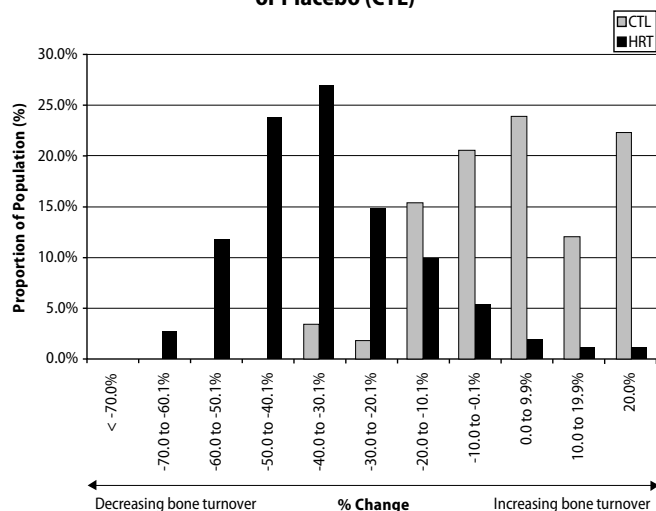


BAP was significantly lower for HRT than CTL at 12 months (13.3 ± 5.0 vs. 21.9 ± 7.9 U/L, $p < 0.00001$). Distributions of BAP values following 12 months in the HRT and CTL groups are depicted in the following figure.



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 change¹² 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.

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)

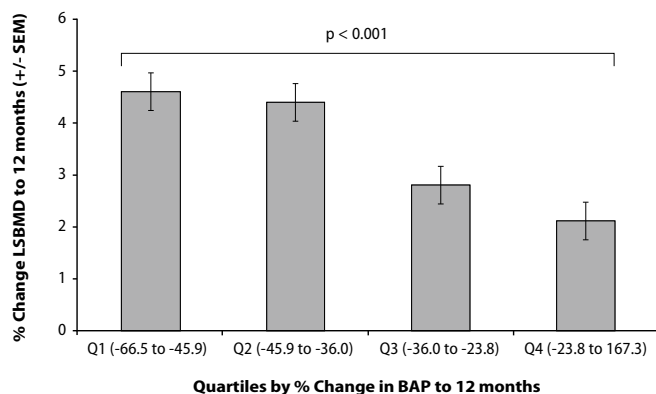
	n	Baseline (g/cm ²)	12 months (g/cm ²)	Δ (%)
CTL	58	0.97 ± 0.17	0.95 ± 0.16	-1.6 ± 2.8
HRT	262	0.97 ± 0.15	1.00 ± 0.15	$+3.5 \pm 2.8$

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

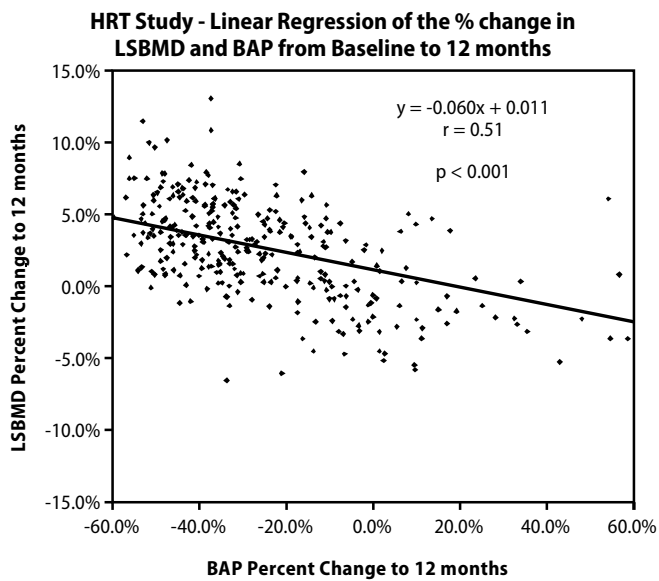
Predicting Skeletal Response:

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 month



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).



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.

ASSISTANCE

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GLOSSARY



Consult Instructions for Use on CDROM



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