SUMMARY AND EXPLANATION

Bone is a dynamic tissue in which bone formation and bone removal (also referred to as resorption) continue throughout life in a process called remodeling. The remodeling process is a function of complex interactions between two types of bone cells: osteoblasts for the formation of bone, and osteoclasts for the resorption of bone (1-3). Bone formation and resorption are interdependent processes that are, under normal circumstances, tightly coupled (2,4). This coupled relationship is integral to maintaining the biochemical competence of the skeleton, thereby preserving the organization of bone structure, form, and strength (2,3,5).

Serum levels of BAP are believed to reflect the metabolic status of osteoblasts (6,7). An accurate assessment of bone metabolism is critical for determining the severity of metabolic bone disease and responses to therapy. Measurement of serum levels of BAP has been shown to be useful in evaluating patients with Paget's disease, osteomalacia, primary hyperparathyroidism, renal osteodystrophy, and metastases to bone (6-10). Total alkaline phosphatase determinations have been the accepted method for the diagnosis and monitoring of patients with Paget's disease.

Paget's disease of bone is a common skeletal disorder in which there is a focal proliferation of the normal cellular components of bone. Paget's disease is more prevalent than once thought with the incidence rate in certain populations at 3%-4% in middle-aged patients and 10%-15% in the elderly (11). This disease does not affect young individuals. The majority of patients with Paget's disease have no symptoms and often go undiagnosed unless an abnormal X-ray or serum alkaline phosphatase level is found in the course of a medical evaluation for unrelated reasons. The most common complaints in symptomatic patients are pain and deformity.

The risk of osteoporosis, another bone remodeling disorder, depends in part upon skeletal development, the attainment of peak bone mass, and, in later life, the amount of bone lost. In healthy children, bone formation is favored over bone resorption, which results in bone development and normal skeletal growth (3). In healthy young adults, bone formation and bone resorption are balanced, resulting in no net increase or decrease in skeletal mass. With advancing age, men and women experience an imbalance in bone remodeling in which resorption is slightly greater than formation, resulting in a continuous net loss of bone mass with time (1,2,4,12). If this imbalance persists, bone mass may decline until the skeleton is insufficient to withstand normal mechanical stresses, and it becomes abnormally susceptible to fractures. The excessive loss of bone mass with an increased susceptibility to fractures is a disorder known as osteoporosis (5).

The most common form of osteoporosis occurs in postmenopausal women and is the result of estrogen deficiency (2,12,13). Rapid bone loss accompanies the decline of estrogen levels at the onset of menopause or as a result of surgical removal of the ovaries. Rapid bone loss occurs as a result of the combined effects of an imbalance in bone remodeling and an increase in bone turnover (5,14-16). In the United States, estrogen deficiency affects some 25 million postmenopausal women and is the cause of approximately 1.5 million fractures annually, including approximately 500,000 vertebral crush fractures, 250,000 hip fractures, and 200,000 distal radius fractures (2,5,17).

Hormone replacement therapy is currently the most widely prescribed therapy for the prevention of osteoporotic fractures in postmenopausal women (4,5,18-20). However, many women cannot or will not avail themselves of hormone replacement therapy because of the potential for the increased risk of cancer and the resumption of menstrual bleeding. For this reason, other compounds such as bisphosphonates, a standard treatment for Paget's disease of bone, have been developed to treat osteoporosis. The anti-resorptive properties of bisphosphonates decrease bone remodeling and, consequently, decrease the overall loss of bone.

Biochemical markers are useful in monitoring metabolic bone disease. Urinary hydroxyproline and total serum alkaline phosphatase have been used for monitoring the treatment of Paget's disease. Osteoporosis, however, represents a more subtle modification of the bone remodeling process; therefore, more specific and sensitive markers are needed.

The Ostase® BAP assay is an in vitro device for the quantitative measurement of bone-specific alkaline phosphatase (BAP) in human serum. Changes in BAP have been shown to be useful in patients undergoing therapy for metabolic bone disorders (6,7,10,21,22).

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. Do not pipette by mouth.
3. Do not eat, drink or smoke in designated work areas.
4. Wash hands thoroughly after handling specimens and kit reagents.
5. Some components in this kit contain material of human origin which has been tested using FDA-approved methods and has been found negative for antibody to human immunodeficiency virus (HIV-1 and HIV-2), antibody to Hepatitis C virus and for Hepatitis B surface antigen (HBsAg). No known test method can offer total assurance that HIV-1, HIV-2, Hepatitis B virus, Hepatitis C virus or other infectious agents are absent. Handle these reagents as if they were potentially infectious (23).
6. 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 (24).
7. The Quench Reagent for this kit contains 1 N NaOH, a corrosive which causes burns. Avoid contact with skin and eyes. Do not ingest or inhale. Safety glasses, gloves and lab coat should be worn when working in the laboratory.
8. Avoid microbial contamination of the reagents when dispensing aliquots.
PROCEDURAL COMMENTS

1. Note on plate washing: Immunoenzymetric assays require efficient washing to remove the unbound, biotin-labeled antibody. Therefore, it is very important to wash each well efficiently, removing the last drips of Wash solution to achieve optimal results.

2. If a specimen was found to contain BAP at a concentration higher than the highest calibrator, the specimen should be diluted with the Zero Calibrator/Diluent and assayed according to the assay Procedure. The dilution factor must be incorporated in the calculation of results. Each diluted specimen should be mixed thoroughly prior to testing. The recommended dilutions for specimens containing BAP greater than the highest calibrator are 1:3, 1:5, or 1:10.

3. Because absorbance is a function of temperature and duration of the Substrate reagent incubation, it is very important that this incubation be the same for all wells/plates. This can be accomplished by ensuring that the elapsed time for pipetting reagents from beginning to end – without interruption – is exactly the same for both the Substrate reagent addition step and the Quench Reagent addition step. To ensure best results, the addition of these reagents should not exceed 90 seconds and total substrate incubation should not exceed 15 minutes.

4. For convenience, repeating or multiphase pipettors may be used for dispensing assay conjugate, Wash solution, Substrate and Quench reagents. Pipettors with disposable tips are recommended for pipetting calibrators, controls and specimens. The pipette tips should be changed after each sample is pipetted to avoid potential sample carryover and contamination of the reagents or specimens.

5. Do not mix materials from different kit lots.

INSTRUMENTATION AND CALCULATION OF RESULTS

Results may be calculated by using computer-assisted methods or manually on linear graph paper.

Computer-Assisted Method

A point-to-point curve fit is recommended. Point-to-point software that connects a straight line between the means of calibrator reproducibility – including the 0 µg BAPL calibrator – provides good results with the procedure and calibration method described. For additional information on computer-assisted data reduction, consult your local sales representative.

Manual Method

The Ostase BAP calibration curve may be constructed manually on linear graph paper by plotting the average absorbance for each calibrator on the y-axis versus the concentration of BAP in the calibrator on the x-axis. A point-to-point curve should be drawn through the calibration points. Do not force the curve to a straight line.

To determine the concentration of BAP in the controls and patient specimens, extend a horizontal line from the absorbance value for the test sample to the calibration curve. At the point of intersection of the horizontal line and the curve, drop a vertical line to the x-axis and read the concentration of BAP. If the absorbance for any replicate of the sample is greater than the absorbance of the highest Calibrator the specimen must be diluted and re-assayed. The observed concentration of the diluted sample must be multiplied by the dilution factor.

Example Data

<table>
<thead>
<tr>
<th>Well #</th>
<th>Description</th>
<th>Abs. 405 nm</th>
<th>Mean Abs.</th>
<th>BAP µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calibrator (0)</td>
<td>0.193</td>
<td>0.190</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>Calibrator (1)</td>
<td>0.401</td>
<td>0.402</td>
<td>7.2</td>
</tr>
<tr>
<td>3</td>
<td>Calibrator (2)</td>
<td>0.595</td>
<td>0.584</td>
<td>15.5</td>
</tr>
<tr>
<td>4</td>
<td>Calibrator (3)</td>
<td>0.908</td>
<td>0.887</td>
<td>31.9</td>
</tr>
<tr>
<td>5</td>
<td>Calibrator (4)</td>
<td>1.499</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Calibrator (5)</td>
<td>1.460</td>
<td>1.479</td>
<td>58.4</td>
</tr>
<tr>
<td>7</td>
<td>Calibrator (6)</td>
<td>2.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Calibrator (7)</td>
<td>2.051</td>
<td>2.026</td>
<td>85.8</td>
</tr>
<tr>
<td>9</td>
<td>Calibrator (8)</td>
<td>0.911</td>
<td>0.980</td>
<td>33.0</td>
</tr>
</tbody>
</table>

Example Curve

Graph showing relationship between absorbance at 405 nm and concentration of BAP in patient samples.
QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Good laboratory practices include the use of control specimens within an assay run to ensure that all reagents and protocols are performing properly. The Ostase BAP antibody set contains controls which can be used to verify assay performance.

1. Recovery of the control concentrations should fall within the stated ranges.
2. The coefficient of variation (CV) of the 405 nm absorbance readings for each calibrator and control sample should be less than 10%.

LIMITATIONS OF THE PROCEDURE

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 in such assays. Therefore, Ostase 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.

The immunoreactivity of liver ALP has been determined in the Ostase BAP assay. 100 U/L of liver ALP activity gives a result of 2.8 to 6.2 µL/L in the Ostase BAP assay. Serum samples with significant elevations of liver ALP activity may yield elevated results in the Ostase BAP assay. Patients with metabolic bone disorders who have low levels of disease activity may have bone-specific ALP levels that fall within the Ostase BAP assay expected values.

Ostase BAP results should be used only in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures. Therefore, the Ostase BAP assay is not recommended for use as a screening procedure to detect the presence of osteoporosis in the general population. Furthermore, the Ostase BAP assay cannot be used to assess the rate of bone formation or bone remodeling.

EXPECTED VALUES

The BAP results in the Expected Values and Clinical Studies sections were generated with the Tandem-R Ostase assay. However, a correlation study of the Ostase BAP and Tandem-R Ostase assays demonstrated good agreement between the assays (y = 1.02x + 0.29, r = 0.97, n = 136).

The Tandem-R Ostase assay was evaluated in studies involving apparently healthy adults (20 to 89 years of age) at 6 test sites. The mean BAP concentration, standard deviation (SD), median, and 95th percentiles for males (N = 217), premenopausal females (N = 228), and postmenopausal females (N = 529) are presented in the following table.

### Summary of BAP Concentrations in Apparently Healthy Adults*

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>BAP Median µg/L</th>
<th>SD</th>
<th>BAP 95th Percentile µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>217</td>
<td>12.3</td>
<td>4.3</td>
<td>11.6</td>
</tr>
<tr>
<td>Pre-menopausal Females</td>
<td>226</td>
<td>8.7</td>
<td>2.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Post-menopausal Females</td>
<td>529</td>
<td>13.2</td>
<td>4.7</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* Results generated with the Tandem-R Ostase Immunoradiometric Assay

The results presented above show that mean BAP concentrations in a population of postmenopausal women are elevated over mean BAP concentrations in premenopausal women (p ≤ 0.0001). This increase in mean BAP reflects the increase in bone remodeling associated with estrogen deficiency in a postmenopausal population compared to a premenopausal population (2,8,12,13). However, there is considerable overlap in BAP concentrations in the populations shown in the distribution below.

For each of the populations shown in the plot: 1) The horizontal lines in each box represent median concentrations. 2) The upper and lower margins of each box represent interquartile ranges — i.e., the upper margin delimits 25% of the values above the median while the lower margin delimits 25% of the values below the median. 3) The tip of each lower whisker represents the 5th percentile concentration, while the tip of each upper whisker represents the 95th percentile concentration.

The ranges of observations reported above are representative of this study only, and do not necessarily reflect the ranges that will be observed in a particular clinical laboratory. Each laboratory should establish its own reference interval.

CLINICAL STUDIES

Paget's Disease

Correlation studies were performed to compare serum BAP concentrations obtained using the Tandem-R Ostase assay to those results obtained using two currently marketed electrophoretic methods and two enzyme activity assays for total alkaline phosphatase (TAP). These studies were conducted at clinical investigation sites using 100 samples from patients with Paget's disease. The correlation coefficient (r) obtained between the Tandem-R Ostase assay and the electrophoretic methods is 0.9418. The correlation coefficient (r) obtained for the correlation between Tandem-R Ostase and the total alkaline phosphatase enzyme activity assays is 0.9459.

Postmenopausal Osteoporosis – Bisphosphonate Treatment

In order to demonstrate the ability of the Tandem-R Ostase assay to reflect alterations in bone remodeling in response to therapy in postmenopausal osteoporosis, a study was conducted in patients with clinically-defined osteoporosis who were treated with a bisphosphonate (alendronate sodium). This study was a multi-site, prospective, double-blind, placebo-controlled study (23). Data are available for subjects who were randomized to placebo (N = 146) or to alendronate 10 mg (N = 74). All subjects took 500 mg of supplemental calcium daily. Bone mineral density (BMD) measurements were made at the lumbar spine using dual-energy x-ray absorptiometry. Serial BAP determinations were made using the Tandem Ostase assay.

Mean percent changes from baseline and standard error bars for BMD in the placebo and treated groups are plotted at each timepoint in the figure below. A 7.8% increase in BMD over baseline is observed in the alendronate group at 24 months, indicating that the bisphosphonate therapy resulted in a positive effect on bone. As expected, a 0% decrease in BMD is observed in the calcium-supplemented placebo group at 24 months.
The within-subject variability of the Tandem Ostase assay was determined from serum samples from 24 healthy postmenopausal women collected every day over a five-day period. The average biological coefficient of variation was calculated as 4.2%.

A summary of two-year data consisting of serial BAP concentrations as measured by the Tandem Ostase assay for the placebo and the alendronate 10 mg groups is presented below. Mean percent decreases from baseline and standard error bars for BAP for the placebo (baseline BAP = 17.7 µg/L ± 4.6) and alendronate-treated (baseline BAP = 17.0 µg/L ± 4.8) groups are plotted at each timepoint. The data show a time-dependent decrease in BAP concentrations for the alendronate-treated group, reaching a nadir at 6 months. As expected a small, transient change in marker levels is observed in the calcium-supplemented placebo group (26,27). The decrease and subsequent stabilization of the BAP changes in the alendronate-treated group suggest that a new steady state in bone remodeling occurred as a result of the bisphosphonate treatment (24, 28, 29).

The observed decreases in BAP concentrations for the alendronate-treated group, as measured by the Tandem Ostase assay, are significantly different from baseline (p ≤ 0.0001) as early as 3 months and at all subsequent timepoints (6, 12, and 24 months). In contrast, BAP concentrations decreased by 11% at 3 and 6 months in the placebo group and returned to baseline by 24 months.

The BAP changes and BMD changes in this study are consistent with current knowledge of bone remodeling and the mechanism of action of anti-osteoporotic therapies (24). From these results, it may be concluded that the decrease in BAP concentrations as measured by the Tandem Ostase assay in alendronate-treated subjects reflects changes in bone remodeling that result from anti-osteoporotic therapy.

The percent change between two serum marker values must exceed both the biological (within-subject) variation of the marker and the analytical variation of the assay in order to have clinical significance (30). This threshold is referred to as the minimum percent change. Percent changes in a marker that exceed the minimum percent change may be attributed to a change in the clinical status of the subject, such as response to therapy (30). The minimum percent change for a biochemical marker has been described by Soletormos (30) and others (31) with the formula

\[
\text{Minimum Percent Change} = \frac{Z \times CV_{\text{w}}}{} + CV_{\text{a}}^2
\]

in which \(CV_w\) is within-subject variability, \(CV_a\) is assay imprecision, and Z is the Z-statistic, which depends on the probability selected for significance and on whether the change expected is unidirectional (Z = 1.645) or bidirectional (Z = 1.96).

The within-subject variability of the Tandem Ostase assay was determined from serum samples from 17 healthy postmenopausal women collected every day over a five-day period. The average biological coefficient of variation was calculated as 4.2%.

Analytical variation was based on between-run precision data found in the SPECIFIC PERFORMANCE CHARACTERISTICS section of this insert.

The minimum percent change for BAP as measured by the Tandem Ostase assay was calculated as 25%. Therefore, changes in BAP concentrations in postmenopausal women that exceed 25% may be attributed to changes in bone remodeling.

Percent changes of BAP relative to baseline for individual subjects are presented below. Data are presented for both the placebo and the alendronate 10 mg subjects at the 3-month and 6-month timepoints. The horizontal line at zero represents baseline, and the horizontal line at ~25% represents the minimum percent change for BAP as measured by the Tandem Ostase assay. For the alendronate 10 mg group, 77.0% (37/48) of the subjects had an BAP decrease from baseline (time 0) of 25% or greater at 3 months, and 85.1% (63/74) of the subjects had an BAP decrease from baseline of 25% or greater at the nadir (6 months). By 24 months, 90.3% (65/72) had a decrease in BAP of 25% or more. For the calcium-supplemented placebo group, 15.7% (112/748) of the subjects had an BAP increase from baseline (time 0) that was less than 25% (at 6 months). By 24 months, 88.1% (129/143) of the placebo group had BAP decreases of less than 25%. These data are further summarized in the table provided below. This table shows the number of treated and placebo subjects (and the percent of subjects) who had BAP decreases from baseline < 25% or > 25% (at 3 and 6 months, respectively).

### Summary of Individual BAP Percent Changes from Baseline for Alendronate 10 mg and Placebo Subjects Number of Subjects/Total Number of Subjects

<table>
<thead>
<tr>
<th>Time</th>
<th>Placebo 10 mg</th>
<th>Placebo Placebo</th>
<th>Alendronate 10 mg 3 Months</th>
<th>Placebo Placebo</th>
<th>Alendronate 10 mg 6 Months</th>
<th>Placebo Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Months</td>
<td>N = 148</td>
<td>33/148 (22.3%)</td>
<td>57/74 (77.0%)</td>
<td>N = 148</td>
<td>36/148 (24.3%)</td>
<td>N = 148</td>
</tr>
<tr>
<td>6 Months</td>
<td>N = 74</td>
<td>115/148 (77.7%)</td>
<td>17/74 (23.0%)</td>
<td>N = 74</td>
<td>112/148 (75.7%)</td>
<td>N = 74</td>
</tr>
</tbody>
</table>

In the alendronate 10 mg group, a mean BAP decrease of 45.7% from baseline to 6 months was followed by a mean increase in BMD of 7.8% at 24 months. Conversely, the small change from baseline for BAP at 6 months for the calcium-supplemented placebo group (~11.3%) was accompanied by a small decrease in BMD of ~0.8% at 24 months.

### Alendronate 10 mg and Placebo Group Data

#### Mean Percent Change in BAP (± 1 SE) vs. Time

<table>
<thead>
<tr>
<th>Months</th>
<th>-50</th>
<th>-40</th>
<th>-30</th>
<th>-20</th>
<th>-10</th>
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#### Alendronate 10 mg and Placebo Group Data

#### Mean Percent Change in BAP (± 1 SE) vs. Time

<table>
<thead>
<tr>
<th>Months</th>
<th>-100</th>
<th>-80</th>
<th>-60</th>
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</tbody>
</table>

### Key Points

- **Within-subject variability** of the Tandem Ostase assay was determined.
- **Analytical variation** was based on between-run precision data.
- The minimum percent change for BAP was calculated as 25%.
- Changes in BAP concentrations in postmenopausal women that exceed 25% may be attributed to changes in bone remodeling.
- Percent changes of BAP relative to baseline for individual subjects are presented.
- Data are presented for both the placebo and the alendronate 10 mg groups at 3-month and 6-month timepoints.
- The within-subject variability of the Tandem Ostase assay was determined from serum samples from 24 healthy postmenopausal women.
In summary, it has been demonstrated that:

1. BAP concentrations reflect the increase in bone remodeling associated with estrogen deficiency in apparently healthy postmenopausal females.

2. BAP concentrations reflect changes in bone remodeling that occur as a result of anti-osteoporotic therapy with alendronate in postmenopausal women.

3. Clinically significant decreases in BAP concentrations at 3 and 6 months are indicators of changes in bone remodeling. Effective therapy with alendronate 10 mg in postmenopausal osteoporotic women is indicated by increases in BMD at 24 months.

4. A decrease in BAP of 25% or more from baseline, as measured by the Tandem Ostase assay, may indicate changes in bone remodeling resulting from anti-osteoporotic therapy.

Subjects undergoing anti-osteoporotic therapy with alendronate whose BAP levels do not decrease by 25% from baseline at early timepoints should be re-tested at later timepoints. Of the 11 subjects who demonstrated less than 25% decrease in BAP from baseline at 6 months, 9 of them eventually demonstrated greater than 25% decrease in BAP from baseline by 24 months. All of these subjects responded to alendronate 10 mg as determined by increases in BMD. Treated subjects whose BAP levels do not decrease by 25% from baseline should be evaluated by other clinical means to determine treatment efficacy.

Postmenopausal Women — Hormone Replacement Therapy (HRT)

In addition to the bisphosphonate study described above, other studies were initiated in order to demonstrate the ability of the Tandem Ostase assay to reflect alterations in bone remodeling in response to estrogen therapy (29,32). Data are presented below from one of the studies. In this study, healthy non-hysterectomized postmenopausal women (n = 12), aged 65-75 years, received daily estrogen/progestin therapy (Premarin 0.625 mg and Provera 2.5 mg) for two years. In addition, 6 healthy hysterectomized women received estrogen alone for a total study population of 18.

Skeletal ALP was measured by the Tandem Ostase assay at baseline and at 6, 12, and 24 months. BMD at the lumbar spine was evaluated at baseline and at 24 months.

Mean percent changes from baseline and standard error bars for BAP (left y-axis) and for BMD (right y-axis) are plotted at each timepoint in the figure below. A 6.5% increase in BMD at the lumbar spine is observed for the estrogen-treated subjects, indicating that the estrogen therapy resulted in a positive effect on bone. The data presented in the figure below also show a time-dependent decrease in BAP concentrations for the treated group, reaching a nadir at 12 months. The observed decreases in BAP concentrations for the estrogen treated group, as measured by the Tandem Ostase assay, are significantly different from baseline (p ≤ 0.0001) at 6 months and at all subsequent timepoints (12 and 24 months).

![Graph showing Mean Percent Change from Baseline (± 1 SE) in BAP and L-Spine BMD vs. Time]

The BAP and BMD changes in this study are consistent with current knowledge of bone remodeling and the mechanism of action of anti-osteoporotic therapies (5,33). From these results, it may be concluded that estrogen therapy with alendronate results in a decrease in BAP and increase in BMD.

Tandem Ostase results should be used only in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

**Within-Run Precision**

Within-run precision was determined by assaying four serum pools containing various concentrations of BAP. Each serum pool was assayed in replicates of 20. The data are presented below:

<table>
<thead>
<tr>
<th>Serum Pool</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean µg/L</td>
<td>7.4</td>
<td>27.3</td>
<td>52.7</td>
<td>79.5</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.48</td>
<td>0.78</td>
<td>1.35</td>
<td>3.54</td>
</tr>
<tr>
<td>% CV</td>
<td>6.5</td>
<td>2.9</td>
<td>2.6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

**Between-Run Precision**

Between-run precision was determined by duplicate measurements of four serum pools over a series of 20 individually calibrated runs:

<table>
<thead>
<tr>
<th>Serum Pool</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Assays</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean µg/L</td>
<td>8.4</td>
<td>29.2</td>
<td>55.6</td>
<td>81.1</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.47</td>
<td>1.88</td>
<td>2.03</td>
<td>4.92</td>
</tr>
<tr>
<td>% CV</td>
<td>5.8</td>
<td>6.4</td>
<td>3.7</td>
<td>6.1</td>
</tr>
</tbody>
</table>

**Recovery and Dilution**

Various quantities of a serum sample containing elevated levels of BAP were added to human sera containing endogenous BAP and the samples were assayed in triplicate.

A serum sample containing an elevated BAP concentration was diluted with the Zero Calibration/Diluent and assayed at multiple dilutions.

**Interfering Substances**

- Hemoglobin, which was tested at concentrations up to 500 mg/dL, does not interfere with the Ostase BAP assay.
- Unconjugated and conjugated bilirubin, which were tested at concentrations up to 40 mg/dL and 20 mg/dL, respectively, do not interfere with the Ostase BAP assay.
- Triglycerides, which were tested at concentrations up to 2000 mg/dL, do not interfere with the Ostase BAP assay.
- Total protein, which was tested at concentrations between 3 and 14 g/dL, does not interfere with the Ostase BAP assay.
- 100 µL Intestinal ALP produced a result of 1.0 µg/L in the Ostase BAP assay.
- 100 µL Placental ALP did not produce a detectable result in the Ostase BAP assay.

**Liver ALP Reactivity**

The liver ALP reactivity in the Ostase BAP assay was determined using serum samples from liver disease patients and serum samples from patients with Paget's disease. Samples were screened by electrophoresis and shown to contain > 95% liver ALP or BAP.

Two methods were used to evaluate liver ALP reactivity. The first procedure, described by Moss and Whitby (35), used a heat inactivation method that minimizes the contribution of endogenous BAP in the samples with elevated liver ALP. Using this method, 100 µL liver ALP activity produced a result of 2.8 to 3.4 µg/L in the Ostase BAP assay.

The second procedure, described by Price, et al. (36), used the slope method (Ostase BAP assay vs. total ALP), where no pretreatment of the samples occurred. From the slope values of the liver ALP and BAP samples, it was determined that:

- 100 µL of liver ALP activity produced a result of 6.2 µg/L in the Ostase BAP assay; and
- 100 µL of liver ALP activity produced a result of 36.9 µg/L in the Ostase BAP assay.
Interference by Drugs

Various concentrations of drugs were added to three separate serum pools containing BAP and assayed in quadruplicate. The drugs and highest concentrations tested are listed below. The testing was based on NCCLS Guideline EP-7P (Interference Testing in Clinical Testing).

- acetaminophen: 20 mg/dL
- alendronate: 5 mg/dL
- aspirin: 50 mg/dL
- calcitonin-salmon: 112 IU/dL
- calcium: 20 mg/dL
- estrogen: 400 ng/dL
- etidronate: 105 mg/dL
- ibuprofen: 40 mg/dL
- pamidronate: 18 mg/dL
- progesterone: 25 mg/dL
- vitamin D: 80,500 IU/dL

These drugs did not interfere with the recovery of BAP from the serum pools in the Ostase BAP assay.

Minimum Detectable Concentration

The minimum detectable concentration of BAP in the Ostase BAP assay is estimated to be 0.7 µg BAP/L. The minimum detectable concentration is defined as the concentration of BAP that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 20 replicate determinations of the Zero Calibrator/Diluent.
REFERENCES/BIBLIOGRAPHY/LITERATURE


