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Changes: § 5
Deletions: §

LIAISON® BAP OSTASE® (REF 310970)

1. INTENDED USE

The DiaSorin LIAISON® BAP OSTASE® assay is a one step delayed addition sandwich chemiluminescence immunoassay (CLIA) intended for the quantitative determination of Bone Alkaline Phosphatase (BAP) in human serum. This device is intended to be used as an aid in the management of osteoporosis and Paget's disease. The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Bone Alkaline Phosphatase (BAP) is a serum marker for osteoblastic bone formation. The concentration of BAP in human serum correlates with the rate of skeletal osteoblastic bone formation. Measurement of BAP is useful in diagnosing Paget's disease and osteoporosis, and in monitoring the response to antiresorptive therapy in these patients.

Alkaline phosphatase (ALP) (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC3.1.3.1) is a highly conserved enzyme distributed widely in nature. In humans, four genetic loci encode ALP isozymes: TNALP (tissue non-specific), intestinal, germ line and placental.[1] BAP and liver alkaline phosphatase (LAP) are derived from a common TNALP locus and therefore are 100% homologous with regard to primary structure. Differences in immunoreactivity to LAP and BAP accrue from differential degrees of post translational modification at five N-glycosylation sites (N123, N213, N254, N286 & N413)[2] common to both isozymes and at O-glycosylation sites unique to BAP. In normal human sera, BAP and LAP isoforms constitute approximately 95% of total serum ALP activity with near equivalent amounts of each present.

BAP functions as an ectoenzyme that is attached to the cell membrane of osteoblasts through a hydrophobic glycosylphosphatidylinositol (GPI) anchor. *In vitro* studies demonstrate the participation of BAP in the initiation of bone matrix mineralization where it is released as an insoluble, matrix vesicle bound, tetrameric form. In serum, pursuant to the combined actions of two endogenous, circulating phospholipases, GPI-PLC &/or GPI-PLD, BAP is present as a homodimer.

Studies of hypophosphatasia (induced by missense mutations of the TNALP gene) indicate that BAP plays a significant role in the development and mineralization of the skeleton by acting as a pyrophosphatase[3]. Cleavage of pyrophosphate, a potent inhibitor of mineralization, promotes mineral deposition *in vivo*.[4] BAP is used to monitor bone formation in patients with kidney disease since it is one of the few markers that are not influenced by variations in kidney function.[5] BAP is finding an increased clinical utility in the discrimination of adynamic bone disease (low bone turnover) from osteitis fibrosa (high bone turnover) where current second generation PTH, parathyroid hormone, testing is non-diagnostic. BAP is also useful in monitoring the efficacy of therapeutic interventions monitoring Paget patients treated with bisphosphonates or post-menopausal osteoporotic women treated with bisphosphonates or estrogen replacement.

3. PRINCIPLE OF THE PROCEDURE

The LIAISON® BAP OSTASE® immunoassay is a direct two-site sandwich assay that utilizes two affinity purified mouse monoclonal antibodies for capture and detection of BAP. The assay uses 50 µL of human serum sample incubated with paramagnetic particles coated with a BAP monoclonal antibody. Following incubation, a second isoluminol conjugated monoclonal antibody is added to the reaction for a short incubation. After the incubation, the unbound material is removed with a wash cycle. The starter reagents are then added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units (RLU) and is proportional to the concentration of BAP present in the calibrators, controls, or samples.

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.4 mL)	SORB	Magnetic particles coated with mouse monoclonal antibody against BAP, BSA, phosphate buffer, surfactant, and < 0.1% sodium azide.
Conjugate (23.0 mL)	CONJ	Mouse monoclonal antibody conjugated to an isoluminol derivative, in MES (2-(N-morpholino)ethane-sulfonic acid monohydrate) buffer, BSA, surfactant, and 0.1% Proclin® 300.
Assay Buffer (21.0 mL)	BUF AS	MES buffer, BSA, surfactant, sodium chloride, mouse IgG, and 0.1% Proclin® 300.
Number of Tests		100

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the Reagent Integral.

*(LIAISON®, LIAISON® XL)

Additional components not on the Reagent Integral

Calibrator 1 (2 x 1.0 mL) (Lyophilized)	CAL 1	Human serum, 0.2% Proclin® 300 and BAP. Reconstitute in 1 mL of distilled or deionized water. Aliquot and store reconstituted calibrator at -20°C.
Calibrator 2 (2 x 1.0 mL) (Lyophilized)	CAL 2	Human serum, 0.2% Proclin® 300 and BAP. Reconstitute in 1 mL of distilled or deionized water. Aliquot and store reconstituted calibrator at -20°C.

ProClin is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

Standardization: The calibrator concentrations (µg/L) are referenced to an in-house standard preparation.

Materials required but not provided (system related)

LIAISON® XL Analyzer	**LIAISON® Analyzer**
LIAISON® Wash/System Liquid (REF 319100)	LIAISON® Wash/System Liquid (REF 319100)
LIAISON® XL Waste Bags (REF X0025)	LIAISON® Waste Bags (REF 450003)
LIAISON® XL Cuvettes (REF X0016)	LIAISON® Module (REF 319130)
LIAISON® XL Starter Kit REF 319200)	LIAISON® Starter Kit (REF 319102)
LIAISON® XL Disposable Tips (REF X0015)	LIAISON® XL Starter Kit (REF 319200)
	LIAISON® Cleaning Kit (REF 310990)
	LIAISON® Light Check 12 (REF 319150)

Additional required materials
LIAISON® BAP OSTASE® Control Set (REF 310971)

Additional recommended materials
LIAISON® BAP OSTASE® Specimen Diluent (REF 310972)

5. WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.

General Safety:

- All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Avoid contact with skin, eyes or mucous membranes. Follow good industrial hygiene practices during testing.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipet solutions by mouth.
- Avoid direct contact with all potentially infectious materials by wearing lab coat, protective eye/face wear and disposable gloves.
- Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach solution (containing 0.5% sodium hypochlorite) and disposed of as though potentially infectious.
- Waste materials should be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country.
- Do not use kits or components beyond the expiration date given on the label.

Chemical Hazard And Safety Information:
Reagents in this kit are classified in accordance with US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and applicable European Union directives (see Material Safety Data Sheet for additional information).

Reagents Containing Human Source Material:
Warning – Treat as potentially infectious.
Each serum/plasma donor unit used in the preparation of this product has been tested by a U.S. FDA approved method and found non-reactive for the presence of the antibody to Human Immunodeficiency Virus 1 and 2 (HIV 1/2), the Hepatitis B surface antigen (HBsAg), and the antibody to Hepatitis C (HCV). While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source diseases for which there is no approved test. Because no known test method can offer complete assurance that HIV, Hepatitis B Virus (HBV) and HCV or other infectious agents are absent, all products containing human source material should be handled following universal precautions; and as applicable in accordance with good laboratory practices as described in the Centers for Disease Control and the National Institutes of Health current manual, Biosafety in Microbiological and Biomedical Laboratories (BMBL); or the World Health Organization current edition, Laboratory Biosafety Manual.

Reagents Containing Sodium Azide: Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

GHS/CLP:

	ProClin®	Sodium Azide
CAS No.:	55965-84-9	26628-22-8
Reagents:	CONJ CAL 1 CAL 2 BUF AS	SORB
Classification:	Skin sensitization, Category 1 Aquatic Chronic, Category 3	None required
Signal Word:	Warning	None required
Pictograms:	 GHS07 – Exclamation mark	None required
Hazard Statements:	H317 – May cause an allergic skin reaction. H412 – Harmful to aquatic life with long lasting effects.	None required
Precautionary Statements:	P261 – Avoid breathing mist or spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P273 – Avoid release to the environment. P280 – Wear protective gloves and clothing, and eye protection.	None required

6. PREPARATION OF THE REAGENT INTEGRAL

Please note the following important reagent handling precautions:

6.1 Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:

- Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have been resuspended.
- Repeat as necessary until the magnetic particles are completely resuspended.
- After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

6.2 Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

- Visually inspect the reagents to ensure there is no foaming present before using the integral. If foam is present after re-suspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

6.3 Loading of integral into the reagent area

LIAISON® Analyzer

- Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL Analyzer

- LIAISON® XL Analyzer is equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
    a. Insert the reagent integral into the dedicated slot.
    b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
- Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

7. STORAGE AND STABILITY OF THE REAGENT INTEGRAL

Upon receipt, the Reagent Integral must be stored in an upright position to facilitate re-suspension of magnetic particles. When the Reagent Integral is stored unopened the reagents are stable at 2-8°C up to the expiry date. Do not freeze. The Reagent Integral should not be used past the expiry date indicated on the kit and Reagent Integral labels. After removing seals, integrals may be returned to the kit box and stored upright at 2-8°C or stored on board the Analyzer. Open use is 4 weeks when properly stored.

8. SPECIMEN COLLECTION AND PREPARATION

Human serum should be used. Blood should be collected aseptically by venipuncture, allowed to clot, and the serum separated from the clot as soon as possible. No additives or preservatives are required to maintain integrity of the sample. Samples having particulate matter, turbidity, lipemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying. Samples are stable for 72 hours at 2-8°C, otherwise they should be stored frozen at -20°C or below for short term storage or at -70°C for long term storage. Specimens may be stored in glass or plastic vials. If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freeze-thaw cycles.

The minimum volume required for a single determination is 250 µL [50 µL specimen for testing + 200 µL dead volume (volume left at the bottom of the aliquot tube which the instrument cannot aspirate)].

9. CALIBRATION

Individual LIAISON® BAP OSTASE® Reagent Integrals contain specific information for calibration of the particular Reagent Integral lot. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows 2 calibrations to be performed. Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:

- With each new lot of reagents (Reagent Integral or Starter Reagents).
- The previous calibration was performed more than 28 days prior.
- Quality control results are out of the acceptable range.
- The Analyzer has been serviced.

Measuring Range. The DiaSorin LIAISON® BAP OSTASE® assay measures between 1.5 µg/L and 120 µg/L. The lowest reportable value is 1.5 µg/L. Values below 1.5 µg/L should be reported as < 1.5 µg/L. The highest reportable value without dilution is 120 µg/L.

Any samples higher than the reportable range should be diluted in the DiaSorin LIAISON® BAP OSTASE® Specimen Diluent (REF 310972), re-assayed and recalculated. See section 16.10.

10. CALIBRATORS LEVEL 1 AND 2

The LIAISON® BAP OSTASE® calibrators are supplied lyophilized. Reconstitute each vial with 1.0 mL of distilled or deionized water. Allow the vials to stand for 5 minutes at room temperature, mix gently by inversion for 30 minutes prior to use.

Transfer a minimum of 350 µL to a glass or plastic sample tube. Affix the appropriate bar code label to the tube and place onto the analyzer. Calibrate the assay as described in the Operator's Manual.

LIAISON® BAP OSTASE® calibrators should be aliquoted after reconstitution if not immediately assayed**.** BAP calibrators have been shown to be stable for 8 hours when stored at room temperature. Remaining reconstituted calibrators should be aliquoted into a minimum of 350 µL and stored frozen at -20°C. Frozen calibrators are stable up to 2 months and can be used through 3 freeze thaw cycles. When thawing frozen aliquot, mixing is required.

Calibrator and reagent integral lot number are lot specific. Do not use calibrators matched with a different reagent lot in the same assay.

11. ASSAY PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Analyzer.

LIAISON® Analyzer: Each test parameter is identified via bar codes on the reagent integral label. In the event that the barcode label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

LIAISON® XL Analyzer: Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral: contact your local DiaSorin technical support for instruction.

For details, refer to the analyzer operator's manual.

The analyzer operations are as follows:

1. Dispense sample, calibrator, or control, magnetic particles and assay buffer into reaction module.
2. Incubate.
3. Dispense conjugate into reaction module.
4. Incubate.
5. Wash with Wash/System liquid.
6. Add the Starter Reagents and measure the light emitted.

Immunoassay results can be affected by temperature fluctuations. Users should be aware of variations in their laboratory environment; more frequent use of controls and subsequent recalibration may be necessary.

12. QUALITY CONTROL

Quality control is required to be performed once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI C24-A3, and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® BAP OSTASE® controls are intended to monitor for substantial reagent failure. LIAISON® controls should be run in singlicate to monitor the assay performance. If control values lie within the expected ranges provided on the certificate of analysis, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and patient specimens must be repeated.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates the concentration of BAP in the sample. This concentration is expressed in µg/L.

14. LIMITATIONS OF THE PROCEDURE

1. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
2. Bacterial contamination of samples or repeated freeze-thaw cycles may affect the test results.
3. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

Integrals may not be exchanged between analyzer types (LIAISON® and LIAISON® XL). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON® or LIAISON® XL).

15. EXPECTED VALUES[6]

A panel of samples comprising men and women not suffering from osteoporosis, Paget's disease, chronic kidney disease or any other disease which may cause bone turnover related problems was evaluated. Samples with abnormal values for 25-OH Vitamin D and intact PTH (defined as <32 ng/mL and >58 pg/L respectively), with calcium and phosphate levels outside the laboratory reference range and estimated glomerular filtration rate < 60 mL/min/1.73 m^2 were excluded from this study. Based on the central 95% interval, the following values were established.

Population	n	Median BAP (µg/L)	Observed 95% Range (µg/L)
Males	120	10.8	5.5 – 22.9
Premenopausal Female	120	10.4	4.9 – 26.6
Postmenopausal Female	120	10.8	5.2 – 24.4
Considering no statistical difference was observed between the 3 populations, data was combined:			
Adults	360	10.7	5.5 – 24.6

Consider these limits as guidelines only. Each laboratory should establish its own reference ranges.

16. SPECIFIC PERFORMANCE CHARACTERISTICS

16.1 Analytical Sensitivity: Following a method adapted from CLSI EP17-A, the analytical sensitivity for the LIAISON® BAP OSTASE® assay, defined as the minimum detectable dose distinguishable from zero by 2 Standard Deviations, is ≤ 0.1 µg/L.

16.2 Functional Sensitivity: Following a method adapted from CLSI EP17-A, the functional sensitivity for the LIAISON® BAP OSTASE® assay is defined as the concentration at which the %CV exceeds 20%. The derived functional sensitivity from the regression analysis of the precision profile is 1.5 µg/L.

16.3 Precision with LIAISON® Analyzer: Precision for the LIAISON® BAP OSTASE® assay was evaluated following CLSI EP5-A2. 9 samples, containing different concentrations of analyte, were assayed in duplicate, 2 runs per day, over 20 operating days, to determine the repeatability and reproducibility of the assay (i.e. within- and between-assay variability).

Repeatability	1	2	3	4	5	6	7	8	9*
N	80	80	80	80	80	80	80	80	80
Mean (µg/L)	14.5	25.7	56.6	64.7	87.4	6.5	12.2	53.3	31.6
sd (µg/L)	0.47	1.01	1.85	2.77	3.46	0.22	0.52	2.06	1.26
%CV	3.3	3.9	3.3	4.3	4.0	3.4	4.3	3.9	4.0

Reproducibility	1	2	3	4	5	6	7	8	9*
N	80	80	80	80	80	80	80	80	80
Mean (µg/L)	14.5	25.7	56.6	64.7	87.4	6.5	12.2	53.3	31.6
sd (µg/L)	1.18	1.69	4.06	5.21	5.33	0.42	0.98	3.56	2.36
%CV	8.1	6.6	7.2	8.0	6.1	6.4	8.1	6.7	7.5

*diluted 1:2 in specimen diluent

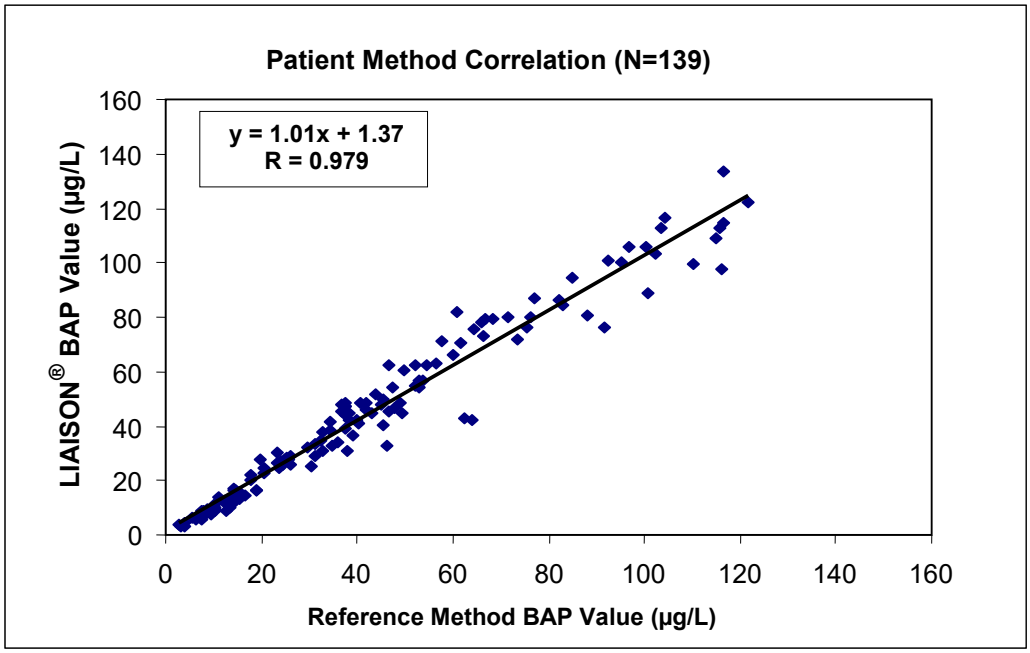
16.4 Precision with LIAISON® XL Analyzer: Precision for the LIAISON® BAP OSTASE® assay was evaluated following CLSI EP5-A2. 9 samples, containing different concentrations of analyte, were assayed in duplicate, 2 runs per day, over 20 operating days, to determine the repeatability and reproducibility of the assay (i.e. within- and between-assay variability).

Repeatability	1	2	3	4	5	6	7	8	9*
N	80	80	80	80	80	80	80	80	80
Mean (µg/L)	13.1	78.2	6.83	14.5	22.4	37.9	47.8	84.6	28.8
sd (µg/L)	0.61	1.92	0.23	0.73	0.53	0.73	1.19	2.08	0.76
%CV	4.6	2.5	3.4	5.0	2.4	1.9	2.5	2.5	2.6

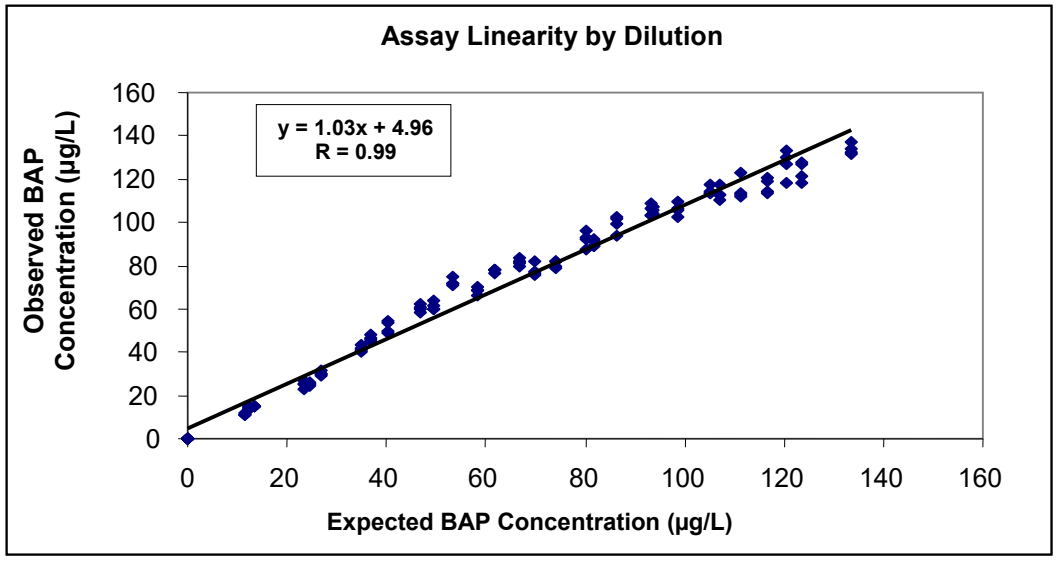
Reproducibility	1	2	3	4	5	6	7	8	9*
N	80	80	80	80	80	80	80	80	80
Mean (µg/L)	13.1	78.2	6.83	14.5	22.4	37.9	47.8	84.6	28.8
sd (µg/L)	0.73	2.79	0.27	0.92	0.72	1.25	1.66	2.31	0.79
%CV	5.6	3.6	3.9	6.4	3.2	3.3	3.5	2.7	2.7

*diluted 1:2 in specimen diluent

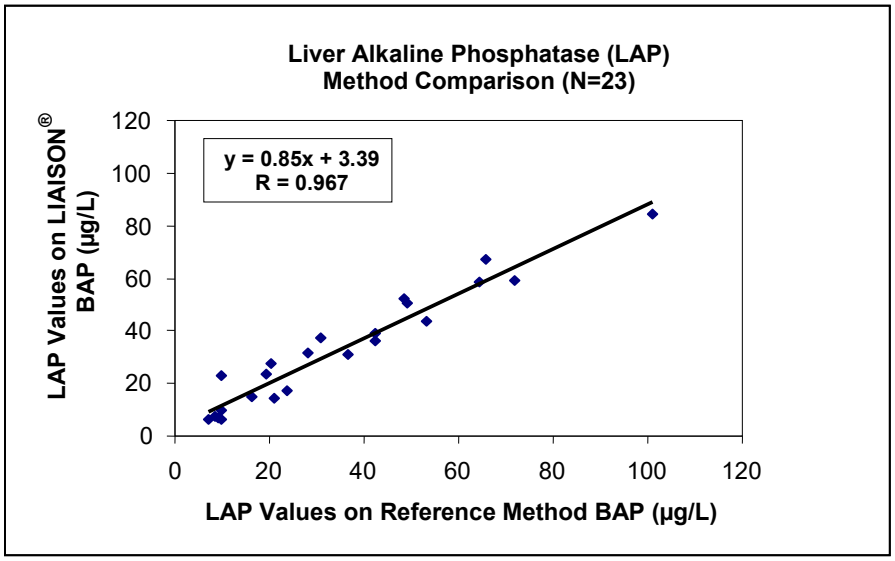
16.5 Patient Correlation: A total of 139 clinical samples were tested by LIAISON® BAP OSTASE® assay and by another automated method following CLSI EP9-A2, and yielded the following comparison; LIAISON® BAP = 1.01 (Reference Method) + 1.37; R = 0.979.



16.6 Assay Linearity: 3 patient samples were diluted and analyzed by the LIAISON® BAP OSTASE® assay following CLSI EP6-A. The results were analyzed as a linear regression of Observed BAP Concentration versus Expected BAP Concentration. The resulting regression equation is; Observed BAP = 1.03 (Expected BAP) + 4.96; R=0.99.



16.7 Specificity: The cross-reactivity of the LIAISON® BAP OSTASE® assay to Liver Alkaline Phosphatase (LAP) was compared to a commonly used reference method. The cross-reactivity to LAP for serum samples enriched in LAP (> 50% LAP enriched) is comparable to the reference method with the equation; LAP Values on LIAISON® BAP = 0.85 (Reference Method LAP Values) + 3.39; R = 0.967. Samples with high elevations of LAP may yield elevated results in the LIAISON® BAP OSTASE® assay.



16.8 Recovery: 5 high concentration spiked clinical samples and 5 low concentration clinical samples were analyzed neat. Recovery samples are prepared by mixing defined ratios of the high and low samples and analyzing these in replicates of 5.

	Defined (µg/L)	Expected (µg/L)	Observed (µg/L)	% Recovery
High Sample 1 (HS1)	128.6			
2 HS1 : 1 LS1		91.1	93.7	103
1 HS1 : 1 LS1		71.8	74.6	104
1 HS1 : 2 LS1		52.5	53.9	103
Low Sample 1 (LS1)	15.0			
High Sample 2 (HS2)	97.2			
2 HS2 : 1 LS2		70.2	69.8	99
1 HS2 : 1 LS2		56.3	58.2	103
1 HS2 : 2 LS2		42.3	44.2	104
Low Sample 2 (LS2)	15.3			
High Sample 3 (HS3)	99.8			
2 HS3 : 1 LS3		71.6	71.4	100
1 HS3 : 1 LS3		57.1	56.9	100
1 HS3 : 2 LS3		42.5	44.3	104
Low Sample 3 (LS3)	14.3			
High Sample 4 (HS4)	112.9			
2 HS4 : 1 LS4		79	80.2	102
1 HS4 : 1 LS4		61.6	65	106
1 HS4 : 2 LS4		44.1	45.7	103
Low Sample 4 (LS4)	10.3			
High Sample 5 (HS5)	111.9			
HS5 : 1 LS5		78.5	82.3	105
1 HS5 : 1 LS5		61.3	64.5	105
1 HS5 : 2 LS5		44.1	47.3	107
Low Sample 5 (LS5)	10.7			
			Mean	**103**
			SD	**2.2**

16.9 High Dose Hook Effect: No High dose hook effect was observed for BAP concentrations up to 2,000 µg/L.

16.10 Dilution of high sample (> 120 µg/L): Samples that read greater than 120 µg/L should be diluted using the LIAISON® BAP OSTASE® Specimen Diluent 1:2. A recommended dilution would be 125 µL diluent + 125 µL patient sample. Mix the dilution well, re-assay, and calculate the final concentration by multiplying by the dilution factor.

Precision: Precision was evaluated with a diluted sample* following CLSI EP5-A2 (§ 16.3 – 16.4).

16.11 Interfering substances: Controlled studies of potentially interfering substances at BAP levels showed no interference at the highest concentration for each substance listed below in the LIAISON® BAP OSTASE® assay. The testing was based on CLSI EP7-A2.

Substance	Concentration Tested
	mg/dL
Hemoglobin	200
Bilirubin	20
Triglycerides	3,000
Cholesterol	500
Acetaminophen	35
Alendronate	8
Aspirin	50
Calcitonin (salmon)	112 IU/dL
Calcium	40
Estradiol	10
Etidronate	105
Ibuprofen	40
Pamidronate	18
Progesterone	25
Risedronate	6
Raloxifene	12
Vitamin D2	80,500 IU/dL
Vitamin D3	80,500 IU/dL
HAMA	30 ng/mL
Rheumatoid Factor (RF)	455 IU/mL

17. References

1. Millan JL, Fishman WH 1995 *Biology of Human Alkaline Phosphatases With Special Reference to Cancer* Critical Reviews Clinical Laboratory Science 21, 1-39.
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4. Johnson KA, Hessle L, Vaingankar S, Wennberg C, Mauro S, Narisawa S, Goding JW, Sano K, Millan JL, Terkeltaub R 2000 *Osteoblast Tissue-Nonspecific Alkaline Phosphatase Antagonizes and Regulates PC-1* Am J Physiol Regul Integr Comp Physiol 279, R1365 – R1377.
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6. Cavalier E, Rozet E, Carlisi A, Bekaert CA, Rousselle O, Hubert P, Chapelle JP, Delanaye P 2010 Analytical validation of serum bone alkaline phosphatase (BAP OSTASE) on Liaison Clin Chem Lab;48:67–72.

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