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Changes: § 5
Deletions: §

LIAISON® Osteocalcin (REF 310950)

1. INTENDED USE

The DiaSorin LIAISON® Osteocalcin assay is a chemiluminescence immunoassay (CLIA) intended for the quantitative determination of osteocalcin in human serum. Assay results should be used in conjunction with other clinical and laboratory data to assist in the diagnosis of postmenopausal osteoporosis and in the management of conditions involving an excess or deficiency of osteocalcin. This assay is intended for *in vitro* diagnostic use.

The test has to be performed on the LIAISON® Analyzer Family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Osteocalcin, a vitamin K-dependent protein, has been described as one of the most abundant non-collagenous proteins in bone. Osteocalcin may account for up to 3% of total bone protein and contains 3 amino acid residues of gamma carboxyglutamic acid (GLA), hence the designation, bone GLA protein (BGP). BGP or osteocalcin, is found only in bone tissue and is produced by the osteoblast. It is believed to play a role in the mineralization process and may be under the influence of the other calcium-regulating hormones: calcitonin, parathyroid hormone, and vitamin D. Because the levels of osteocalcin are a direct reflection of bone turnover, its measurement closely correlates with the actual status of bone metabolism in the patient.[2,3]

While such measurements as alkaline phosphatase and hydroxyproline have been widely used as biochemical markers for bone metabolism, there have been significant limitations in their usefulness as specific markers for bone disease. Generally, osteocalcin correlates with alkaline phosphatase; however, because the concentration of alkaline phosphatase is contributed by other areas of the body (i.e. liver, gastro-intestinal tract, placenta, and tumors), it is not specific for bone turnover.[1,4] Increased levels of osteocalcin are found in bone diseases characterized by increased bone turnover. Osteocalcin has been found to be elevated in the following disorders: Paget's disease of the bone, cancer accompanied by bone metastases, primary hyperparathyroidism and renal osteodystrophy. Osteocalcin has the important advantage of being a specific marker for bone disease.[6] More importantly, osteocalcin levels may serve as a useful index in evaluating the therapeutic management of the patient.[5,7,8]

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of osteocalcin is a direct, 2 site, sandwich type chemiluminescence immunoassay (CLIA). Affinity-purified mouse antibody to synthetic human osteocalcin is coated to the solid phase. The second affinity-purified mouse antibody is conjugated to an isoluminol derivative. During the incubation, osteocalcin binds to the solid phase and is subsequently bound by isoluminol conjugated antibody. 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 osteocalcin present in calibrators, controls, or samples.

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.4 mL)	SORB	Magnetic particles coated with mouse monoclonal antibody against osteocalcin, BSA, phosphate buffer, surfactant and 0.1% ProClin® 300.
Conjugate (20 mL)	CONJ	Mouse monoclonal antibody against osteocalcin is conjugated to an isoluminol derivative in a phosphate buffer with BSA, surfactant, EDTA,and 0.09% Sodium Azide.
Assay Buffer (20 mL)	BUF AS	Phosphate buffer with BSA, surfactant, EDTA, mouse IgG and 0.09% Sodium Azide.
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® and LIAISON® XL)

Included with Integral

Calibrator 1 (2 x 1 mL) Lyophilized	CAL 1	Human serum, EDTA, 0.1% ProClin® 300 and osteocalcin. Reconstitute in 1 mL distilled or deionized water. Aliquot and store reconstituted calibrator at –20°C for up to 12 weeks provided the calibrator has not exceeded its expiration date.
Calibrator 2 (2 x 1 mL) Lyophilized	CAL 2	Human serum, EDTA, 0.1% ProClin® 300 and osteocalcin. Reconstitute in 1 mL distilled or deionized water. Aliquot and store reconstituted calibrator at –20°C for up to 12 weeks provided the calibrator has not exceeded its expiration date.

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

Standardization: The calibrator concentrations (ng/mL) 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® Osteocalcin Control Set (REF 310951)

Additional recommended material

LIAISON® Osteocalcin Specimen Diluent (REF 310952)

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 pipette 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 an 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.

GHS/CLP:

	ProClin®	Sodium Azide
CAS No.:	55965-84-9	26628-22-8
Reagents:	SORB CAL 1 CAL 2	CONJ BUF AS
Classification:	Skin sensitization, Category 1 Aquatic Chronic, Category 3	None required
Signal Word:	Warning	None required
Pictogram:	 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

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.

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 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 resuspension 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, the Reagent Integral is stable for 4 weeks on board the analyzer or when stored at 2-8°C.

8. SPECIMEN COLLECTION AND PREPARATION

Human serum should be used. EDTA and lithium heparin plasma show a bias when compared to serum, however, these samples can be assayed in this kit provided a separate reference range is established for each sample type used. Fasting samples are recommended, but not required. 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 (-20°C or below). Specimens may be stored in glass or plastic vials. The minimum volume required is 225 µL. If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freeze-thaw cycles. When thawing frozen aliquot, mixing is required.

9. CALIBRATORS 1 and 2

The LIAISON® Osteocalcin calibrators are supplied lyophilized. Reconstitute each vial with 1.0 mL of distilled or deionized water. Allow the vials to stand for 10 minutes at room temperature, mix gently by inversion until completely dissolved. Transfer a minimum of 275 µL (triplicate calibrators) to a glass or plastic sample tube. Affix the appropriate bar code label to the tube and place in appropriate sized rack and load onto the Analyzer. Calibrate the assay as described in the Operator's Manual.

Osteocalcin is a labile peptide and calibrators should be placed on ice after reconstitution if not immediately assayed. If necessary, osteocalcin calibrators have been shown to be stable for 4 hours when stored at room temperature. Remaining reconstituted calibrators should be aliquoted into a minimum of 275 µL and frozen immediately (-20°C or below). Frozen calibrators are stable for up to 12 weeks, provided they have not exceeded their expiration date. Avoid repeated freeze-thaw cycles.

Once calibrators are mixed, remove caps and place calibrators into LIAISON® Analyzer sample rack with the barcode showing outward and slide rack into LIAISON® Analyzer sample area. Follow the analyzer operator's manual to load the specimens and start the assay.

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

10. CALIBRATION

Assay of calibrators allows the analyzer to recalibrate the stored master curve, as indicated via the bar codes or RFID tags on the Reagent Integral. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows 3 (per vial-2 vials per integral) calibrations to be performed. Recalibration in triplicate is mandatory whenever at least 1 of the following conditions occurs:

- A new lot of Reagent Integral or Starter kit is used
- The previous calibration was performed more than 14 days before.
- The analyzer has been serviced
- If quality control results are out of the acceptable range.

Refer to the analyzer operator's manual for calibration instructions.

Measuring Range. The DiaSorin LIAISON® Osteocalcin Assay measures between 0.5 ng/mL and 300 ng/mL. The lowest reportable value is 0.5 ng/mL. Values below 0.5 ng/mL should be reported as < 0.5 ng/mL. The highest reportable value without dilution is 300 ng/mL. Any samples higher than the reportable range should be diluted using DiaSorin LIAISON® Osteocalcin Specimen Diluent (REF 310952), re-assayed and recalculated. See section 16.9.

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 barcode on the Reagent Integral. 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 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.

The analyzer operations are as follows:

LIAISON® Analyzer

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

LIAISON® XL Analyzer

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

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.

The LIAISON® Osteocalcin Control Set (REF 310951) is well suited for the determination of quality control requirements for this assay. LIAISON® controls are intended to monitor for substantial reagent failure. Whenever controls lie outside the expected ranges calibration should be repeated and controls and samples retested. Do not report patient results until control results are within expected ranges.

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. Whenever controls lie outside the expected ranges, calibration should be repeated and controls re-tested.

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 LIAISON® Analyzer automatically calculates the concentration of osteocalcin in the sample. This concentration is expressed in ng/mL. To convert results to SI units: ng/mL $\times$ 0.171 = nmol/L.

14. LIMITATIONS OF THE PROCEDURE

- Skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination of samples or repeated freeze-thaw cycles may affect the test results.
- 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 1 particular analyzer type (either LIAISON® or LIAISON® XL).

15. EXPECTED VALUES

Serum samples from 66 male, 73 pre-menopausal female, and 66 post-menopausal female subjects in apparent good health and with 25 OH Vitamin D levels ≥ 20 ng/mL were analyzed by the LIAISON® Osteocalcin assay.

Population (N)	Median Osteocalcin	Observed 95% Range
Males (66)	18.4 ng/mL	4.6 – 65.4 ng/mL
Females		
Pre-Menopausal (73)	17.6 ng/mL	6.5 – 42.3 ng/mL
Post-Menopausal (66)	21.4 ng/mL	5.4 – 59.1 ng/mL

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

16. SPECIFIC PERFORMANCE CHARACTERISTICS

16.1 Analytical Sensitivity: The analytical sensitivity, defined as the minimum detectable dose distinguishable from 0 by 2 Standard Deviations is ≤ 0.5 ng/mL.

16.2 Functional Sensitivity: The functional sensitivity is defined as the concentration at which the %CV exceeds 20%. The derived functional sensitivity from the regression analysis of the precision profile is ≤ 3.0 ng/mL.

16.3 Precision with LIAISON® Analyzer: Precision was evaluated following CLSI EP5-A2. 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
Number of determinations	80	80	80	80	80	80	80	80
Mean (ng/mL)	19.7	106	6.9	12.4	41.9	70.9	154	238
Standard Deviation (ng/mL)	0.80	4.7	0.2	0.6	1.4	3.9	7.1	18.5
Coefficient of variation (%)	4%	4%	4%	5%	3%	5%	5%	8%

Reproducibility	1	2	3	4	5	6*	7	8
Number of determinations	80	80	80	80	80	80	80	80
Mean (ng/mL)	19.7	106	6.9	12.4	41.9	70.9	154	238
Standard Deviation (ng/mL)	1.1	7.2	0.3	0.7	1.7	5.1	14.1	22.4
Coefficient of variation (%)	6%	7%	5%	6%	4%	7%	9%	9%

*diluted 1:10 in specimen diluent

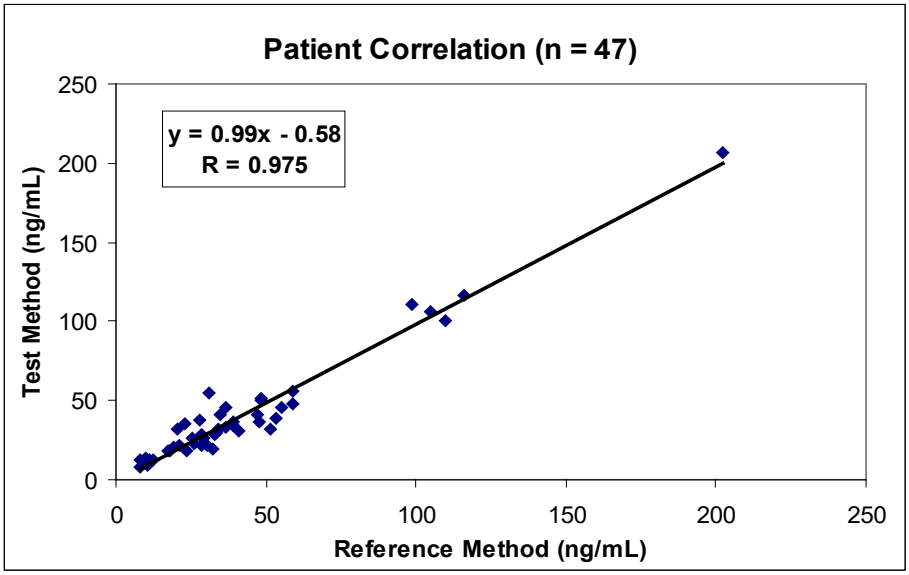
16.4 Precision with LIAISON® XL Analyzer: Precision was evaluated following CLSI EP5-A2. Samples containing different concentrations of analyte were assayed in duplicate, 2 runs per day, over 20 operating days, to determine the repeatability and reproducibility of the assay (i.e. within- and between-assay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

Repeatability	KC 1	KC 2	1	2	3	4	5	6*
Number of determinations	80	80	80	80	80	80	80	80
Mean (ng/mL)	29.3	140	10.4	19.7	63.1	168	238	59.8
Standard Deviation (ng/mL)	0.64	2.7	1.3	0.70	2.3	5.0	9.8	1.1
Coefficient of variation (%)	2%	2%	12%	4%	4%	3%	4%	2%

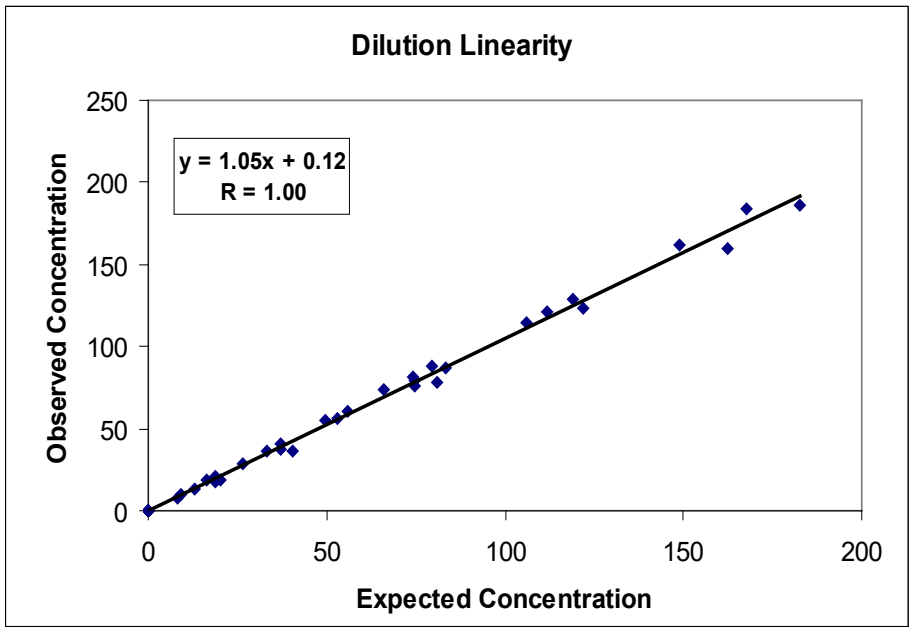
Reproducibility	KC 1	KC 2	1	2	3	4	5	6*
Number of determinations	80	80	80	80	80	80	80	80
Mean (ng/mL)	29.3	140	10.4	19.7	63.1	168	238	59.8
Standard Deviation (ng/mL)	1.4	7.1	1.4	0.99	3.9	9.2	13.7	4.4
Coefficient of variation (%)	5%	5%	14%	5%	6%	6%	6%	7%

*diluted 1:10 in specimen diluent

16.5 Patient Correlation:
A total of 47 clinical samples and spiked samples were tested by LIAISON® Osteocalcin and by another automated method which yielded the following comparison. LIAISON® = 0.99 x -0.58; r = 0.975.



16.6 Trueness Dilution Test:
The assay trueness has been verified by the dilution test. 5 patient samples were diluted and analyzed. The results were analyzed as a linear regression of the Expected vs. Observed values. The resulting regression equation is: Observed = 1.05(Expected) + 0.12; r = 1.00



16.7 Recovery

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

	Defined	Expected	Observed	
High Sample 1 (HS1)	88.0			
2 HS1 : 1 LS1		64.0	65.8	103%
1 HS1 : 1 LS1		51.6	55.0	107%
1 HS1 : 2 LS1		39.3	45.2	115%
Low Sample 1 (LS1)	15.3			
High Sample 2 (HS2)	95.6			
2 HS2 : 1 LS2		70.4	66.3	94%
1 HS2 : 1 LS2		57.4	50.5	88%
1 HS2 : 2 LS2		44.4	39.0	88%
Low Sample 2 (LS2)	19.2			
High Sample 3 (HS3)	110			
2 HS3 : 1 LS3		78.3	71.3	91%
1 HS3 : 1 LS3		61.9	52.6	85%
1 HS3 : 2 LS3		45.5	38.4	84%
Low Sample 3 (LS3)	13.7			
High Sample 4 (HS4)	204			
2 HS4 : 1 LS4		145	134	92%
1 HS4 : 1 LS4		114	105	92%
1 HS4 : 2 LS4		84.0	73.5	88%
Low Sample 4 (LS4)	25.1			
High Sample 5 (HS5)	219			
2 HS5 : 1 LS5		157	141	90%
1 HS5 : 1 LS5		125	114	91%
1 HS5 : 2 LS5		93.4	85.8	92%
Low Sample 5 (LS5)	31.5			
			Mean	**93%**
			SD	**0.085**

16.8 High Dose Hook Effect:

No High dose hook effect was observed for osteocalcin concentrations up to 1500 ng/mL

16.9 Dilution of high sample (> 300 ng/mL)

Samples that read greater than 300 ng/mL should be diluted using LIAISON® Osteocalcin Specimen Diluent 1:10. A recommended dilution would be 270 µL diluent + 30 µL patient sample. Mix the dilution well, re-assay, and calculate the final concentration by multiplying by the dilution factor.

16.10 Specificity:

Data on the cross-reactivity of the antiserum used in this assay were obtained by spiking the potential cross-reactant and assaying. The observed cross-reactivity is listed below.

Cross reactant	Concentration Tested (ng/mL)	% Cross reactivity
Osteocalcin 1-43 Fragment	100 ng/mL	100%
Calcitonin	100 ng/mL	0%
Bone Specific Alkaline Phosphatase	500 ng/mL	0%
Parathyroid Hormone	100 ng/mL	0%

16.11 Interfering substances:

Controlled studies of potentially interfering substances showed that the assay performance was not affected by cholesterol (at 500 mg/dL), hemolysis (at 500 mg/dL), bilirubin (at 20 mg/dL), and triglyceride (at 3000 mg/dL) and rheumatoid factor (RF) (at 455 IU/mL).

17. References

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