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Changes: § 5
Deletions: §

LIAISON® 1-84 PTH Assay (REF 310630)

1. INTENDED USE

The DiaSorin LIAISON® 1-84 PTH assay is a chemiluminescent immunoassay (CLIA) intended for the quantitative determination of 1-84 PTH without cross-reaction to 7-84* PTH fragment in human serum and EDTA plasma. Measurements of parathyroid hormone levels are used in the differential diagnosis of hypercalcemia and hypocalcemia resulting from disorders of phosphorus and calcium metabolism. The test has to be performed on the LIAISON® Analyzer family**.

2. SUMMARY AND EXPLANATION OF THE TEST[1]

Parathyroid hormone (PTH), an 84 amino acid polypeptide, plays a critical role in the regulation of mineral homeostasis and bone metabolism[2]. PTH affects mineral homeostasis by regulating the rate of kidney mediated reabsorption of calcium and phosphorus and by stimulating the synthesis of calcitriol in the kidney. The direct effect of PTH on bone is to stimulate osteoclastic bone resorption, which is coupled with an increase in bone formation. Plasma ionized calcium level is the primary regulator of PTH secretion from the parathyroid gland. Vitamin D and phosphorus also directly affect PTH synthesis and secretion.

Primary and secondary hyperparathyroidism, kidney insufficiency, malabsorption-syndrome and pseudo-hypoparathyroidism result in elevated concentrations of PTH[3]. Decreased concentrations of PTH coincide with high doses of vitamin-D fortified milk, milk-alkali-syndrome, Morbus Boeck (sarcoidosis), hyper-thyreosis, ingestion of thiazide and hypercalcemia of malignancy. PTH concentration is also decreased with absorptive hypercalciuria and hypoparathyroidsm.

A succession of increasingly sensitive tests has been developed over the past few decades for measuring PTH[4]. Initial assays with specificity for the C-terminal portion of PTH were of limited utility since patients with chronic kidney disease (CKD) retained increased levels of inactive fragments that weren't cleared from the body due to impaired renal function.

Second generation or 'intact' PTH two-site immuno-radiometric assays were developed and initially believed to detect only active 1-84 PTH molecules. Generically, these 'sandwich' assays utilized capture antibodies specific to the 39-84 C-terminal portion and tracer antibodies specific to the 1-34 N-terminal portion of the 1-84 PTH protein. Over a period of twelve years from 1987 to 1998 it was progressively realized that intact PTH assays were also compromised: from 30% to 90% of their reported values accrued from 7-84* PTH fragment which also accumulates in uremic patients. The broad non-uniformity of commercial intact PTH assays has compromised the ability to achieve comparable clinical inference[1,5].

More recently, third generation assays have become available. Since 7-84* PTH fragments are not detected, these assays bring greater relative uniformity to diagnostic testing for 1-84 PTH[5]. With the disappearance of the Bio-Intact assay in early 2005[6], automated testing for 1-84 PTH was no longer available commercially. With the introduction of the LIAISON® 1-84 PTH assay, the same superior performance for true 1-84 PTH measurement[7] is now available on a reliable automated platform.

3. PRINCIPLE OF THE PROCEDURE

The LIAISON® 1-84 PTH assay is a modified two-step, two-site sandwich assay that uses two polyclonal antibodies for capture and detection of the 1-84 PTH molecule. The assay uses 150 µL of human serum or EDTA plasma incubated with an isoluminol conjugated polyclonal antibody with high specificity for the N-terminus of the 1-84 peptide. Following incubation, paramagnetic particles coated with a second polyclonal antibody that binds to the C-terminal region of the 1-84 molecule are added to the reaction and incubated. The use of these antibodies guarantees that only 1-84 PTH is detected with no cross reactivity to fragments such as the 7-84* PTH fragment. After the second 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 1-84 PTH present in the calibrators, controls or samples.

** 7-84 PTH is used to refer to the collection of inactive fragments associated with 7-84 PTH*

** LIAISON®, LIAISON® XL and LIAISON® XS

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.4 mL)	SORB	Magnetic particles coated with polyclonal antibody against 1-84 PTH, BSA, phosphate buffer, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.
Conjugate (12.0 mL)	CONJ	Antibody with high specificity for N-terminus of 1-84 PTH peptide conjugated to an isoluminol derivative, in phosphate buffer, BSA, surfactant, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.
Assay Buffer (25.0 mL)	BUF AS	Phosphate buffer, BSA, surfactant, blockers, 0.1% ProClin® 300 and 0.05% gentamicin sulfate.
Number of tests		100

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

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

Additional components not on the Reagent Integral

Calibrator 1 2 vials (Lyophilized)	CAL 1	Equine serum, 1-84 PTH, protease inhibitor, 0.09% sodium azide, and 0.2% ProClin® 300. Reconstitute in 2 mL distilled or deionized water. Aliquot and store reconstituted calibrator at -20ºC.
Calibrator 2 2 vials (Lyophilized)	CAL 2	Equine serum, 1-84 PTH, protease inhibitor, 0.09% sodium azide, and 0.2% ProClin® 300. Reconstitute in 2 mL distilled or deionized water. Aliquot and store reconstituted calibrator at -20ºC.

Standardization**:** The LIAISON® 1-84 PTH Calibrator concentrations (pg/mL) are referenced to an in-house preparation of synthetic human PTH (1-84).

Materials required but not provided (system related)

LIAISON® XL Analyzer	**LIAISON® Analyzer**	**LIAISON® XS Analyzer**
LIAISON® Wash/System Liquid (REF 319100)	LIAISON® Wash/System Liquid (REF 319100)	LIAISON® EASY Wash Buffer (REF 319301)
-	-	LIAISON® EASY System Liquid (REF 319302)
LIAISON® XL Waste Bags (REF X0025)	LIAISON® Waste Bags (REF 450003)	LIAISON® EASY Waste (REF X0054)
LIAISON® XL Cuvettes (REF X0016)	LIAISON® Module (REF 319130)	LIAISON® Cuvettes on Tray (REF X0053)
LIAISON® XL Starter Kit REF 319200) or	LIAISON® Starter Kit (REF 319102) or	LIAISON® EASY Starter Kit (REF 319300)
LIAISON® EASY Starter Kit (REF 319300)	LIAISON® XL Starter Kit (REF 319200) or	LIAISON® Disposable Tips (REF X0055)
LIAISON® XL Disposable Tips (REF X0015) or	LIAISON® EASY Starter Kit (REF 319300)	LIAISON® EASY Cleaning Tool (REF 310996)
LIAISON® Disposable Tips (REF X0055)	LIAISON® Cleaning Kit (REF 310990)	-
-	LIAISON® Light Check 12 (REF 319150)	-

Materials required but not provided

LIAISON® 1-84 PTH Control Set (REF 310631)

Additional Materials recommended

LIAISON® 1-84 PTH Specimen Diluent (REF 310632)

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).

GHS/CLP:

	ProClin®	Sodium Azide
CAS No.:	55965-84-9	26628-22-8
Reagents:	SORB CONJ CAL 1 CAL 2 BUF AS	CAL 1 CAL 2
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 Re-suspension of Magnetic Particles

Magnetic particles must be completely re-suspended 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 color 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 re-suspended.

- Repeat as necessary until the magnetic particles are completely re-suspended.
- 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, calibrators in particular 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 and LIAISON® XS Analyzer

- LIAISON® XL analyzer and LIAISON® XS 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 expiration date. Do not freeze. The Reagent Integral should not be used past the expiration date indicated on the kit and Reagent Integral labels. After opening, integrals may be returned to the kit box and stored upright at 2-8°C or stored on LIAISON® Analyzer, LIAISON® XL or LIAISON® XS. Integrals properly stored have an open use stability of 4 weeks. Refer to Section 10 for calibration intervals.

8. SPECIMEN COLLECTION AND PREPARATION

Human serum or EDTA-plasma may be used in this assay.
One hundred Eighty-eight matched patient sets of EDTA plasma and serum spanning the assay range were tested to determine sample equivalence. Comparison of serum to EDTA plasma yielded the following equation:
Serum = 1.0481x + 0.1438; R^2 = 0.9981.

Blood should be collected aseptically by venipuncture. Serum samples should be allowed to clot, and the serum separated from the clot as soon as possible. Plasma samples should be centrifuged and removed from the cells immediately after centrifugation. 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. Proper sample handling is crucial to ensuring the integrity of PTH.

Serum or EDTA plasma samples are stable at ambient temperature for up to **8 hours** and for up to **48 hours** at 2-8°C. If the assay is performed within 48 hours of sample collection, the samples may be kept at 2-8°C, otherwise they should be stored deep-frozen (-20°C or below). If samples are stored frozen, mix thawed samples well before testing. Samples may be frozen-thawed 3 times.
Ensure that specimens reach their destination within the following:

1) Do not ship serum on the clot or EDTA samples on the cells.
2) Serum and EDTA plasma should be separated from the cells/clot, frozen at -20°C or below and shipped with dry ice. Temperature level during entire shipment should be no greater (warmer) than -20°C.

The minimum sample volume is 350 µL per specimen [150 µL specimen for testing + 200 µL dead volume (volume left at the bottom of the aliquot tube which the instrument cannot aspirate)].

9. CALIBRATORS LEVEL 1 AND 2

The LIAISON® 1-84 PTH calibrators are supplied lyophilized. Reconstitute each vial with 2.0 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 650 µL (triplicate calibration) to a glass or plastic sample tube. Affix the appropriate bar code label to the tube. Place onto the LIAISON® , LIAISON® XL or LIAISON® XS analyzer. Calibrate the assay as described in the Analyzer Operator's Manual.

LIAISON® 1-84 PTH calibrators should be aliquoted after reconstitution if not immediately assayed. 1-84 PTH calibrators have been shown to be stable for 2 hours when stored at room temperature (18-22°C). Remaining liquid calibrators should be aliquoted to a minimum of 650 µL and frozen. These can be used through 1 freeze-thaw cycle and stored at -20°C for up to 2 months. 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.

10. CALIBRATION

Individual LIAISON® 1-84 PTH reagent integrals have a barcode label containing 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 allow 3 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).
- Every 28 days if stored at 2-8°C after each use.
- Every 7 days if stored On-Board the Analyzer:LIAISON®, LIAISON® XL or LIAISON® XS.
- After each servicing of the Analyzer.
- If Quality Control results are out of the acceptable range.

Measuring range: The DiaSorin LIAISON® 1-84 PTH assay measures between 4 and 1800 pg/mL. The lowest reportable value is 4.0 pg/mL. Values below 4.0 pg/mL should be reported as < 4.0 pg/mL. The highest reportable value with out dilution is 1800 pg/mL. Samples that read above the assay range may be diluted with the LIAISON® 1-84 PTH Specimen Diluent (REF 310632) and retested. See LIAISON® 1-84 PTH Specimen Diluent Instructions for Use for suggested dilutions.

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 barcodes 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 and LIAISON® XS 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.

In case external calibrator barcodes fail to be read, data present on the external calibrator labels (under the barcode) may be manually entered on the LIAISON® Analyzer family. For details, refer to the analyzer operator's manual.

The LIAISON® Analyzer operations are as follows:

1. Dispense calibrators, controls or specimens into the reaction module.
2. Dispense assay buffer and conjugate.
3. Incubate.
4. Dispense coated magnetic particles into the reaction module.
5. Incubate.
6. Wash with Wash/System liquid.
7. Add the Starter reagents and measure the light emitted.

The LIAISON® XL Analyzer and LIAISON® XS Analyzer operations are as follows:

1. Dispense assay buffer and conjugate.
2. Dispense calibrators, controls or specimens into the reaction module.
3. Incubate.
4. Dispense coated magnetic particles into the 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[8], and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® 1-84 PTH Kit Control Set (REF 310631) is 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 LIAISON® Analyzer automatically calculates the concentration of PTH in the samples. This concentration is expressed in pg/mL. To convert results to SI units: pg/mL x 0.106 = pmol/L.

Calibrators and controls may give different RLU or dose results on LIAISON®, LIAISON® XL and LIAISON® XS, but patient results are equivalent.

14. LIMITATIONS OF THE PROCEDURE

1. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
2. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
3. Grossly hemolyzed, icteric or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested.
4. Bacterial contamination of samples may affect the test results.
5. Do not heat inactivate serum or EDTA plasma.
6. Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays.
7. Patients routinely exposed to animals, animal serum products, or other immunogenic products that may elicit heterophilic antibody production against the assay's reagents can be prone to this interference and anomalous values may be observed.
8. Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.
9. 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®, LIAISON® XL or LIAISON® XS).

15. EXPECTED VALUES

Paired Serum and EDTA plasma samples from 125 apparently healthy adults from the Midwestern U.S. were used to determine the reference range for the LIAISON® 1-84 PTH assay.

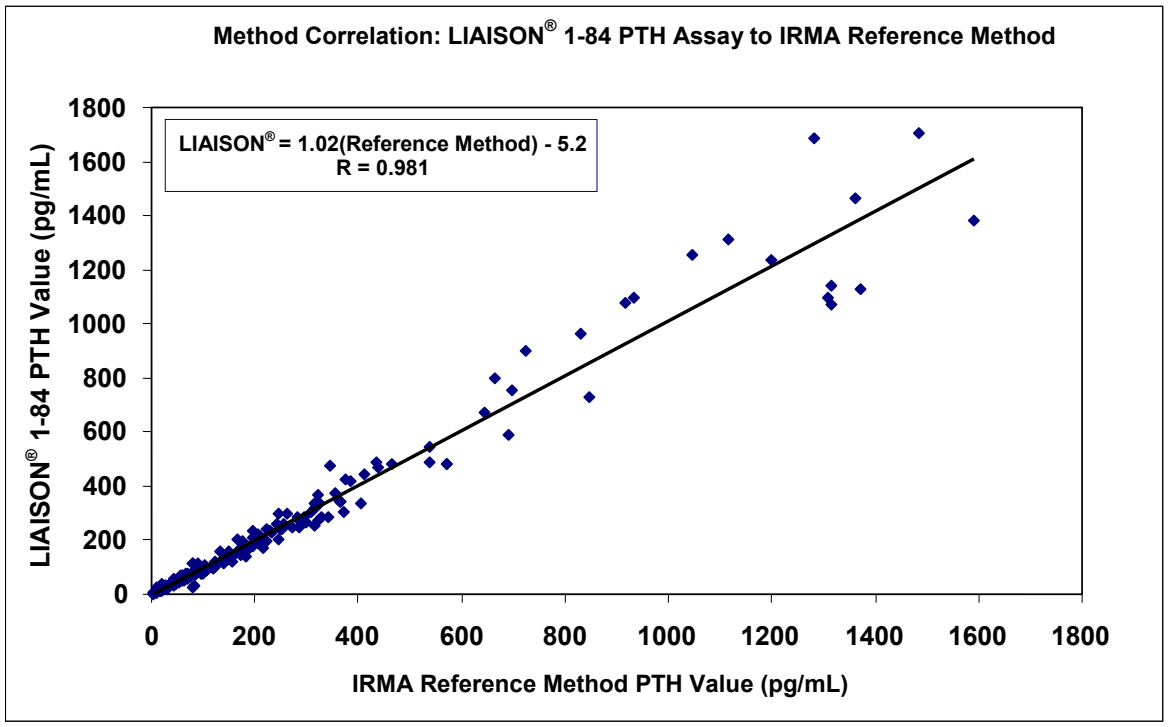
Sample	Median 1-84 PTH Concentration	Observed 95% Range (pg/mL)
Serum (125)	15.3 pg/mL	6.5 – 36.8 pg/mL
EDTA Plasma (125)	16.1 pg/mL	6.7 – 38.8 pg/mL
Sample	Absolute range	
Serum (125)	4.6 – 58.1 pg/mL	
EDTA Plasma (125)	4.0 – 57.6 pg/mL	
Sample	Absolute range with subject 25-OH Vitamin D levels >30 ng/mL	
Serum (82)	4.6 – 38.4 pg/mL	
EDTA Plasma (82)	4.0 – 39.4 pg/mL	
Sample	Absolute range with subject 25-OH Vitamin D levels >30 ng/mL & 8.5 mg/dL ≤ Total Calcium ≤10.1 mg/dL	
Serum (74)	5.5 – 38.4 pg/mL	
EDTA Plasma (74)	4.6 – 39.4 pg/mL	

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

16. SPECIFIC PERFORMANCE CHARACTERISTICS

16.1 Patient Correlation/Method Comparison:

A total of 206 EDTA Plasma samples (199 clinical samples + 7 spiked 1-84 PTH samples) were tested by the LIAISON® 1-84 PTH assay and by a manual IRMA method following CLSI EP9-A3[9], and yielded the following comparison; LIAISON® 1-84 PTH = 1.02 (Reference Method) – 5.2; R = 0.981.



16.2 Precision with LIAISON® Analyzer:

2 kit controls and 7 samples containing concentrations of analyte prepared to span the range of the assay were assayed twice per day in duplicate, over 20 operating days on 2 LIAISON® instruments using 1 reagent lot, to determine repeatability and reproducibility of the LIAISON® 1-84 PTH assay.

Repeatability

Sample	KC 1	KC 2	1	2	3	4	5	6	7
Number of determinations	160	160	160	160	160	160	160	160	160
Mean (pg/mL)	25.3	248	12.2	21.2	39.1	197	416	1126	1854
Standard Deviation (pg/mL)	1.22	9.50	0.73	1.14	1.34	5.97	15.55	39.56	70.48
Coefficient of Variation (%CV)	4.7%	3.8%	5.9%	5.4%	3.4%	3.0%	3.8%	3.5%	3.8%

Reproducibility

Sample	KC 1	KC 2	1	2	3	4	5	6	7
Number of determinations	160	160	160	160	160	160	160	160	160
Mean (pg/mL)	25.3	248	12.2	21.2	39.1	197	416	1126	1854
Standard Deviation (pg/mL)	2.14	15.28	1.10	1.76	2.54	10.67	29.7	61.9	104.3
Coefficient of Variation (%CV)	8.5%	6.2%	9.0%	8.3%	6.5%	5.4%	7.1%	5.5%	5.6%

16.3 Precision with LIAISON® XL Analyzer:

2 kit controls and 7 samples containing concentrations of analyte prepared to span the range of the assay were assayed twice per day in duplicate, over 20 operating days on 2 LIAISON® XL instruments using 1 reagent lot, to determine repeatability and reproducibility of the LIAISON® 1-84 PTH assay.

Repeatability

Sample	KC 1	KC 2	1	2	3	4	5	6	7
Number of determinations	160	160	160	160	160	160	160	160	160
Mean (pg/mL)	28.5	288.5	10.6	16.6	33.5	171.3	377.7	876.6	1662
Standard Deviation (pg/mL)	1.4	13.8	0.46	0.60	1.0	6.5	12.4	32.4	51.6
Coefficient of Variation (%CV)	4.9%	4.8%	4.3%	3.6%	3.1%	3.8%	3.3%	3.7%	3.1%

Reproducibility

Sample	KC 1	KC 2	1	2	3	4	5	6	7
Number of determinations	160	160	160	160	160	160	160	160	160
Mean (pg/mL)	28.5	288.5	10.6	16.6	33.5	171.3	377.7	876.6	1662
Standard Deviation (pg/mL)	2.0	16.2	0.59	0.79	1.4	8.6	15.3	38.9	78.0
Coefficient of Variation (%CV)	7.2%	5.6%	5.5%	4.7%	4.1%	5.0%	4.0%	4.4%	4.7%

16.4 Precision with LIAISON® XS Analyzer:

2 kit controls and 7 samples containing concentrations of analyte prepared to span the range of the assay were prepared and tested at DiaSorin Inc. once per day in replicates of 6, over 5 operating days on 3 LIAISON® XS Analyzers, using 1 reagent lot of the LIAISON® 1-84 PTH assay. The testing was performed according to CLSI EP15-A3.

Sample ID	Mean	Intra-Run		Total	
		SD	%CV	SD	%CV
Kit Control 1	25.37	1.77	7.0%	2.28	9.0%
Kit Control 2	244.1	5.29	2.2%	14.68	6.0%
1	9.72	0.26	2.6%	0.63	6.5%
2	18.36	0.52	2.8%	1.19	6.5%
3	34.00	0.73	2.2%	1.31	3.8%
4	220.0	4.63	2.1%	7.51	3.4%
5	447.2	7.36	1.6%	15.46	3.5%
6	1049.7	28.15	2.7%	61.42	5.9%
7	1591	44.94	2.8%	127.84	8.0%

The Table below summarizes the precision that can be expected when testing in fewer runs and/or on fewer instruments.

Level	Expected Repeatability	Expected Reproducibility
4 – 20 pg/mL	≤10%	≤12%
20 – 1800 pg/mL	≤6%	≤9%

16.5 Limit of Blank
Following the method from CLSI EP17-A2[10], the limit of blank for serum and EDTA plasma on the LIAISON® 1-84 PTH is ≤ 0.50 pg/mL.

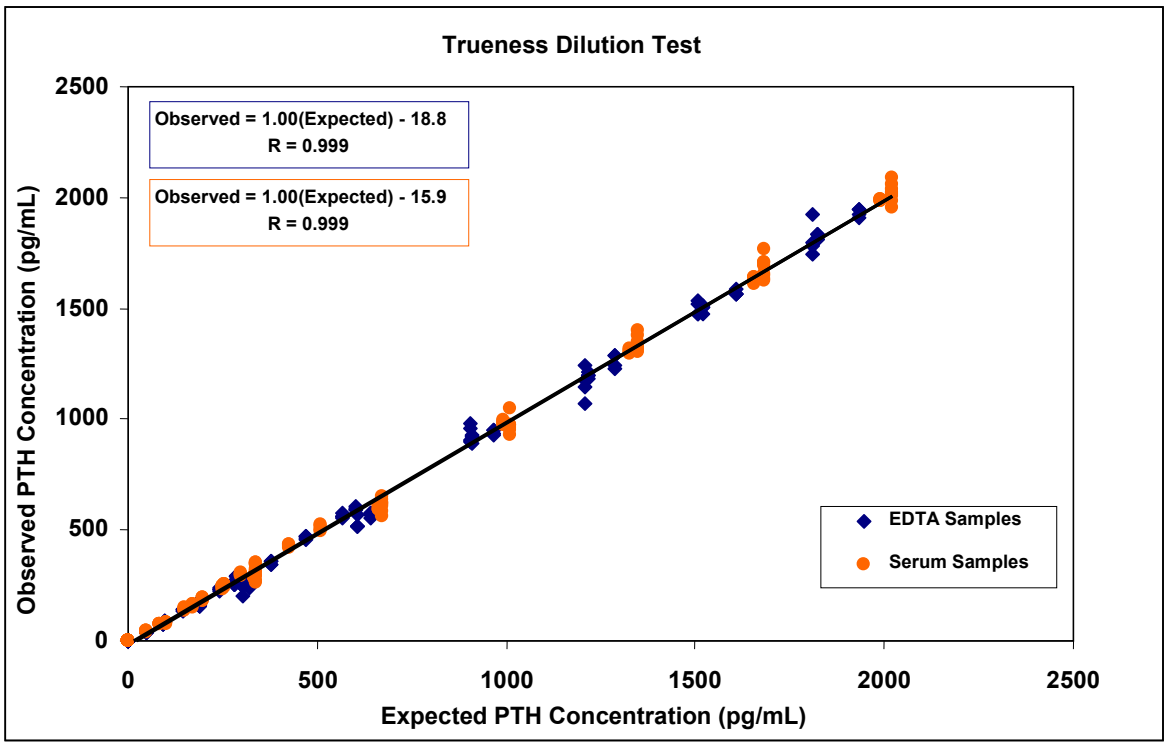
Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term "analytical sensitivity".

16.6 Limit of Detection
Following the method from CLSI EP17-A2, the limit of detection for serum and EDTA plasma on the LIAISON® 1-84 PTH is 1.7 pg/mL.

16.7 Limit of Quantitation (or Functional Sensitivity):
Following the method from CLSI EP17-A2, the limit of quantitation for serum and EDTA plasma on the LIAISON® 1-84 PTH is 4.0 pg/mL.

16.8 Trueness Dilution Test:
5 samples of each sample type, EDTA plasma and serum, were diluted and analyzed by the LIAISON® 1-84 PTH assay following CLSI EP6-A[11]. The results for each sample type were analyzed by a linear regression of Observed PTH Concentration versus Expected PTH Concentration. The resulting equation for EDTA plasma samples is; Observed PTH = 1.00 (Expected PTH) – 18.8, R = 0.999.

The resulting equation for serum samples is; Observed PTH = 1.00 (Expected PTH) – 15.9, R = 0.999.



16.9 Recovery

3 high concentration spiked serum and spiked EDTA samples and 3 low concentration serum and EDTA samples were analyzed neat. Recovery samples were then prepared by mixing defined ratios of the high and low samples and analyzing these in replicates of five.

Serum Samples	Defined Concentration	Expected Pg/mL	Observed Pg/mL	% Recovery
Serum High Sample 1 (HS1)	953			
2 HS1 : 1LS1		646	651	101%
1 HS1 : 1LS1		487	444	91%
1 HS1 : 2LS1		329	310	94%
Serum Low Sample 1 (LS1)	21.3			
Serum High Sample 2 (HS2)	1493			
2 HS2 : 1LS2		1007	989	98%
1 HS2 : 1LS2		756	720	95%
1 HS2 : 2LS2		505	492	97%
Serum Low Sample 2 (LS2)	18.9			
Serum High Sample 3 (HS3)	1048			
2 HS3 : 1LS3		710	734	103%
1 HS3 : 1LS3		536	518	97%
1 HS3 : 2LS3		361	364	101%
Serum Low Sample 3 (LS3)	22.9			
			Mean Recovery	**98%**
			SD	**0.037**

EDTA Plasma samples	Defined Concentration	Expected Pg/mL	Observed Pg/mL	% Recovery
EDTA High Sample 1 (HS1)	1107			
2 HS1 : 1LS1		746	699	94%
1 HS1 : 1LS1		560	511	91%
1 HS1 : 2LS1		374	345	92%
EDTA Low Sample 1 (LS1)	13.54			
EDTA High Sample 2 (HS2)	1072			
2 HS2 : 1LS2		724	677	94%
1 HS2 : 1LS2		545	511	94%
1 HS2 : 2LS2		366	340	93%
EDTA Low Sample 2 (LS2)	18.04			
EDTA High Sample 3 (HS3)	1044			
2 HS3 : 1LS3		703	662	94%
1 HS3 : 1LS3		527	463	88%
1 HS3 : 2LS3		351	329	94%
EDTA Low Sample 3 (LS3)	9.52			
			Mean Recovery	**93%**
			SD	**0.019**

16.10 Specificity

Controlled studies of potentially cross-reacting substances were performed on the LIAISON® 1-84 PTH Assay. LIAISON® 1-84 PTH Specimen Diluent was spiked with the following human PTH fragments and structurally similar proteins at the concentrations listed below. Testing was based on CLSI EP-7A2[12].

Cross-Reactant	Spiked concentration	% Cross Reactivity
7 - 84	200,000 pg/mL	0.00105%
1 - 34	200,000 pg/mL	0.00005%
13 - 34	200,000 pg/mL	0.00020%
39 - 68	200,000 pg/mL	0.00090%
44 - 68	200,000 pg/mL	0.00055%
39 - 84	200,000 pg/mL	0.00050%
53 - 84	200,000 pg/mL	0.00015%
Calcitonin	200,000 pg/mL	0.00008%
Osteocalcin	200,000 pg/mL	0.00005%
C-Telopeptide (β-crosslaps)	200,000 pg/mL	0.00001%

16.11 High Dose Hook Effect:

Testing was conducted to determine if the LIAISON® 1-84 PTH Assay is susceptible to artificially low results in the presence of very high levels of PTH (Hook Effect). 3 serum and 3 EDTA plasma samples were spiked with 1-84 PTH to equal several concentrations above the assay measuring range of 1800 pg/mL. No high dose hook effect was observed for 1-84 PTH concentrations measured up to 60,000 pg/mL.

16.12 Interfering substances

Controlled studies of potentially interfering substances at 2 PTH levels in EDTA Plasma (40 and 70 pg/mL) showed no interference in the LIAISON® 1-84 PTH at the highest spiked concentration for each substance listed below.
The testing was based on CLSI-EP7-A2[12].

Drug/Substance	Concentration Tested
Hemoglobin	500 mg/dL
Bilirubin (conjugated)	40 mg/dL
Bilirubin (unconjugated)	20 mg/dL
Triglycerides	3,000 mg/dL
Cholesterol	500 mg/dL
Albumin	12 g/dL
HAMA	4,088 ng/mL
Rheumatoid Factor	5,380 IU/mL
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	65 mg/dL
Salicylic Acid	60 mg/dL
Ibuprofen	50 mg/dL
Alendronate	8 mg/dL
Etidronate	105 mg/dL
Pamidronate	18 mg/dL
Risedronate	6 mg/dL
Vitamin D2	240 ng/mL
Vitamin D3	240 ng/mL
Calcitriol	1 ng/mL
Alfacalcidol	2.5 µg/mL
Biotin	1 µg/mL
Calcium Acetate	40 mg/dL
Calcium Citrate	40 mg/dL
Magnesium Chloride	40 mg/dL
Aluminum Sulfate	40 mg/dL
Lanthanum Chloride	40 mg/dL

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