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Changes: § 5
Deletions: §

LIAISON® Calcitonin II-Gen (REF 310650)

1. INTENDED USE

The DiaSorin LIAISON® Calcitonin II-Gen Assay is a one-step sandwich chemiluminescence immunoassay (CLIA) intended for the quantitative determination of calcitonin in human serum. Assay results should be used in conjunction with other clinical and laboratory data to assist in the diagnosis and management of conditions involving an excess or deficiency of calcitonin. The test has to be performed on the LIAISON® Analyzer family.

2. SUMMARY AND EXPLANATION OF THE TEST

Calcitonin (CT) is a 32 amino acid peptide hormone secreted by thyroidal C-cells. It is regulated by calcium levels and is metabolized by the kidney and liver. The CT molecule is cleaved from a larger pro-hormone. It contains a single disulfide bond, which causes the amino terminus to assume the shape of a ring (1).

Calcitonin participates in calcium and phosphorus metabolism. The major source of CT is from the parafollicular or C cells in the thyroid gland, but it is also synthesized in a wide variety of other tissues, including the lung and intestinal tract. Calcitonin circulates in multiple forms with molecular weights ranging from 3,500 to 60,000 Daltons. Levels tend to be elevated in children and be higher in men than in women. Serum CT is the most specific and sensitive marker of medullary thyroid carcinoma (MTC) for both the primary diagnosis and the post-surgical follow-up (2).

Medullary thyroid carcinoma (MTC) is a tumor of the CT-producing C-cells, although a rare tumor, it can occur in a familial pattern as part of MEN (Multiple Endocrine Neoplasia) type II syndrome. Calcitonin is produced in abnormally high concentration by almost the 100% of primary and metastatic MTCs (2). Patients with micro or macro forms of MTC (sporadic or familial form) have elevated CT levels that correlate with the tumor mass (3).

In a small percentage of patients bearing MTC, basal hormone levels are indistinguishable from normal. Many of these subjects represent the early stages of C-cell neoplasia or hyperplasia most amenable to surgical cure. To identify these patients with early disease, provocative tests for CT secretion (pentagastrin or Ca stimulation test) have been developed. Post operative follow-up of MTC should be performed just prior to and 6 months after surgery. The first post-operative CT measurement should not be made until 2 weeks after surgery (3).

Elevated CT levels can be found in other pathologies beside MTC and neuroendocrine tumors. Increased CT release occurs with autoimmune thyroid diseases (Hashimoto thyroiditis or Graves' diseases). Non-thyroidal conditions where elevated levels of CT were found are severe renal insufficiency, hypercalcemia, hypergastrinemia, acute pulmonary inflammatory conditions (3).

Calcitonin inhibits bone resorption and is administered as a drug for this purpose in cases of osteoporosis, Paget's disease and hypercalcemia of malignancy (1). The metabolism is a complex process and CT disappears from plasma in a multiexponential manner that includes an early half-life measured in minutes.

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of calcitonin is a direct, two-site, sandwich type chemiluminescence immunoassay (CLIA). Affinity-purified mouse antibody to the synthetic human calcitonin is coated to the solid phase. The second affinity-purified mouse antibody is conjugated to an isoluminol derivative. During the incubation, calcitonin 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 calcitonin 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 calcitonin, 0.1% BSA, phosphate buffer, surfactant and 0.2% ProClin® 300.
Conjugate (7 mL)	CONJ	Antibody against calcitonin is conjugated to an isoluminol derivative, in a 0.05% BSA and phosphate buffer with surfactant, EDTA, and 0.09% NaN3.
Assay Buffer (7 mL)	BUF AS	Phosphate buffer with surfactant, mouse IgG, and 0.09% NaN3.
Number of Tests		100

All reagents are supplied ready to use.

Included with Integral (2 vials each)

Calibrator 1 Lyophilized	CAL 1	Phosphate buffer, surfactants, EDTA, 3.2% BSA, 0.2% ProClin® 300 and calcitonin. Reconstitute in 2 mL distilled or deionized water. Store and aliquot reconstituted calibrator at –20°C.
Calibrator 2 Lyophilized	CAL 2	Phosphate buffer, surfactants, EDTA, 3.2% BSA, 0.2% ProClin® 300 and calcitonin. Reconstitute in 2 mL distilled or deionized water. Store and aliquot reconstituted calibrator at –20°C.

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

10 Barcode labels for each level of Calibrator.

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® Calcitonin II-Gen Control Set (REF 310651)

Additional recommended materials
LIAISON® Calcitonin II-Gen Specimen Diluent (REF 310652)

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 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 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.

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.

Alternatively, the LIAISON® Xcelerator (REF A0090) may be used to aid in re-suspension.

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 use, the reagent integral is stable for 6 weeks and should be stored on the LIAISON® Analyzer or returned to storage at 2-8°C.

8. SPECIMEN COLLECTION AND PREPARATION

Human serum should be 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 19 hours at 2-8°C, or for 2 hours at room temperature on board the instrument; otherwise, they should be stored frozen (-20°C or below). Specimens may be stored in glass or plastic vials. The minimum volume required is 400 µL. If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freeze-thaw cycles.
For shipping, use sterile containers and pack specimens in compliance with government regulations covering the transportation of etiologic agents.

Ensure that specimens reach their destination within the following:

1) Serum separated from the clot can be frozen at -20°C or below and shipped with dry ice.
2) Temperature level during entire shipment should be no greater (warmer) than -20°C.

9. CALIBRATORS 1 and 2

The LIAISON® Calcitonin II-Gen calibrators are supplied lyophilized. Reconstitute each vial with 2.0 mL of distilled or deionized water. Allow the vials to stand for 5minutes, at room temperature, then mix gently by inversion. Transfer a minimum of 800 µL (triplicate calibrators) to a glass or plastic sample tube. Affix the appropriate bar code label to the tube and place onto the LIAISON® Analyzer. Calibrate the assay as described in the LIAISON® Operator's Manual.

Calcitonin is a labile peptide and calibrators should be placed on ice after reconstitution if not immediately assayed. Calcitonin calibrators have been shown to be stable for 24 hours when stored at 2-8°C or on ice or 8 hour when stored at room temperature. Remaining reconstituted calibrators should be aliquoted into a minimum of 800 µL and frozen immediately. These are stable up to 6 weeks and can be used after 1 freeze-thaw cycle. When thawing frozen aliquot, mixing is required.

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

Individual Calcitonin II-Gen 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 allows 2 calibrations to be performed. Recalibration in triplicate is mandatory whenever at least 1 of the following conditions occurs:

- With each new lot of reagents (Reagent Integral or Starter Reagents).
- Every 21 days.
- After each servicing of the Analyzer.
- If quality control results are out of your acceptable range.

Measuring Range. The DiaSorin LIAISON® II-Gen Calcitonin Assay measures between 1 pg/mL and 2000 pg/mL. The lowest reportable value is 1 pg/mL. Values below 1 pg/mL should be reported as < 1 pg/mL. The highest reportable value without dilution is 2000 pg/mL. Any samples higher than the reportable range should be diluted in the DiaSorin LIAISON® Calcitonin II-Gen Specimen Diluent (310652), re-assayed and recalculated. See section 16.10.

11. ASSAY PROCEDURE

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

LIAISON® Analyzer. Each test parameter is identified via the 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 instructionl.
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 analyzer operations are as follows:

LIAISON® Analyzer:

1. Dispense assay buffer and magnetic particle into reaction module
2. Dispense sample, calibrator, or control into reaction module.
3. Incubate 10 minutes.
4. Dispense conjugate into reaction module.
5. Incubate 20 minutes.
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 particle into reaction module
2. Dispense sample, calibrator, or control into reaction module.
3. Incubate 7 minutes.
4. Dispense conjugate into reaction module.
5. Incubate 14 minutes.
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® Calcitonin II-Gen Control Set (REF 310651) is well suited for the determination of quality control requirements for this assay.

LIAISON® 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. 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. STANDARDIZATION

The LIAISON® Calcitonin II-Gen is referenced to the World Health Organization Calcitonin International Standard (WHO 89/620).

The LIAISON® Analyzer automatically calculates the concentration of calcitonin in the sample. This concentration is expressed in pg/mL (1 pg/mL = 1 ng/L SI = 0.19 μIU 2nd IS 89/620).

If conversion to pmol/L is required, the following conversion factor is used: 1 pg/mL = 0.29 pmol/L

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

14. LIMITATIONS OF THE PROCEDURE

- A 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.
- If the assay results are not consistent with other clinical and laboratory data, the specimen should be repeated in duplicate on the LIAISON® Calcitonin II-Gen assay or additional confirmatory testing is recommended.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays.
- 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.
- Elevated levels of RF above 150 IU/mL may cause interference. Assay results from patients with elevated RF (heterophiles) should be interpreted with caution.

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

Serum samples from 267 apparently healthy subjects with normal TSH, FT4, and anti-Tg values were analyzed in the LIAISON® Calcitonin II-Gen assay. From this population 145 samples were from male subjects and 122 samples were from female subjects. For each population a median concentration and the central 95% interval, which was calculated using a nonparametric method according to CLSI C28A, were calculated. The median concentration for male samples was 2.9 pg/mL, with a interval of <1.0 pg/mL – 11.8 pg/mL, the median concentration for female samples was <1.0 pg/mL, with a reference interval of <1.0 pg/mL – 4.8 pg/mL.

From the total population analyzed 91% of all values obtained were below 5.0 pg/mL and 98% of all values obtained were below 10 pg/mL, results are shown in the following table:

Population (N)	Percentage of Samples Below Listed Concentration	Concentration of Samples
All Samples (267)	91% (244/267)	5.0 pg/mL
	98% (262/267)	10.0 pg/mL

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, the analytical sensitivity for the LIAISON® Calcitonin II-Gen assay, defined as the minimum detectable dose distinguishable from zero by 2 Standard Deviations, is ≤ 1.0 pg/mL.

16.2 Functional Sensitivity:

Following a method adapted from CLSI EP17, the functional sensitivity for the LIAISON® Calcitonin II-Gen assay, is defined as the concentration at which the inter-assay %CV exceeds 20%. The derived functional sensitivity from the regression analysis of the precision profile is ≤ 3.0 pg/mL.

16.3 Precision with LIAISON® Analyzer:

Assay precision performance was established at DiaSorin following protocol outlined in CLSI document, EP5. A coded panel comprised of 11 frozen repository samples was prepared by DiaSorin and tested in the LIAISON® Calcitonin II Gen assay. The panel contained samples prepared to span the range of the assay. All panel members were divided into aliquots and stored frozen prior to testing. The kit controls (REF 310651) and the coded panel were tested in 2 replicates per run for 20 runs on 2 LIAISON® Analyzers using 1 reagent lot. The results are summarized in the following table. These data are representative examples of the assay performance.

20 Day Precision: two LIAISON® Analyzers; one reagent lot

Repeatability

Sample	KC1	KC2	1	2	3	4	5*	6*	7	8	9*	10	11
Number of determinations	160	160	160	160	160	160	160	160	160	160	160	160	160
Mean (pg/mL)	15.3	602	4.39	7.90	17.9	29.8	30.7	54.2	91.9	312	431	1361	1986
Std. Deviation (pg/mL)	0.51	9.01	0.37	0.61	0.65	0.87	1.18	2.96	0.94	7.26	16.6	19.4	33.2
Coefficient of Variation (%)	3%	2%	8%	8%	4%	3%	4%	5%	1%	3%	4%	1%	2%

Reproducibility

Sample	KC1	KC2	1	2	3	4	5*	6*	7	8	9*	10	11
Number of determinations	160	160	160	160	160	160	160	160	160	160	160	160	160
Mean (pg/mL)	15.3	602	4.39	7.90	17.9	29.8	30.7	54.2	91.9	312	431	1361	1986
Std. Deviation (pg/mL)	0.69	19.0	0.59	0.92	0.91	1.18	1.68	5.23	1.96	16.4	28.0	50.1	94.9
Coefficient of Variation (%)	5%	3%	13%	12%	5%	4%	6%	10%	2%	5%	7%	4%	5%

* Samples were diluted 1:100 in specimen diluent prior to each run.

16.4 Precision with LIAISON® XL Analyzer:

Assay precision performance was established at DiaSorin following protocol outlined in CLSI document, EP5. A coded panel comprised of 11 frozen repository samples was prepared by DiaSorin and tested in the LIAISON® Calcitonin II Gen assay. The panel contained samples prepared to span the range of the assay. All panel members were divided into aliquots and stored frozen prior to testing. The kit controls (REF 310651) and the coded panel were tested in 2 replicates per run for 20 runs on 2 LIAISON® Analyzers using 1 reagent lot. The results are summarized in the following table. These data are representative examples of the assay performance.

20 Day Precision: two LIAISON® XL Analyzers; one reagent lot

Repeatability

Sample	KC1	KC2	1	2	3	4	5	6	7	8	9*	10*	11*
Number of determinations	160	160	160	160	160	160	160	160	160	160	160	160	160
Mean (pg/mL)	12.8	444	5.22	6.32	23.5	31.3	91.9	353	1663	2326	32.6	53.8	422
Std. Deviation (pg/mL)	0.40	5.0	0.21	0.22	0.48	0.54	1.06	5.74	17.2	23.6	0.66	1.17	6.6
Coefficient of Variation (%)	2.9%	1.1%	4.0%	3.4%	2.1%	1.7%	1.2%	1.6%	1.0%	1.0%	2.0%	2.2%	1.6%

Reproducibility

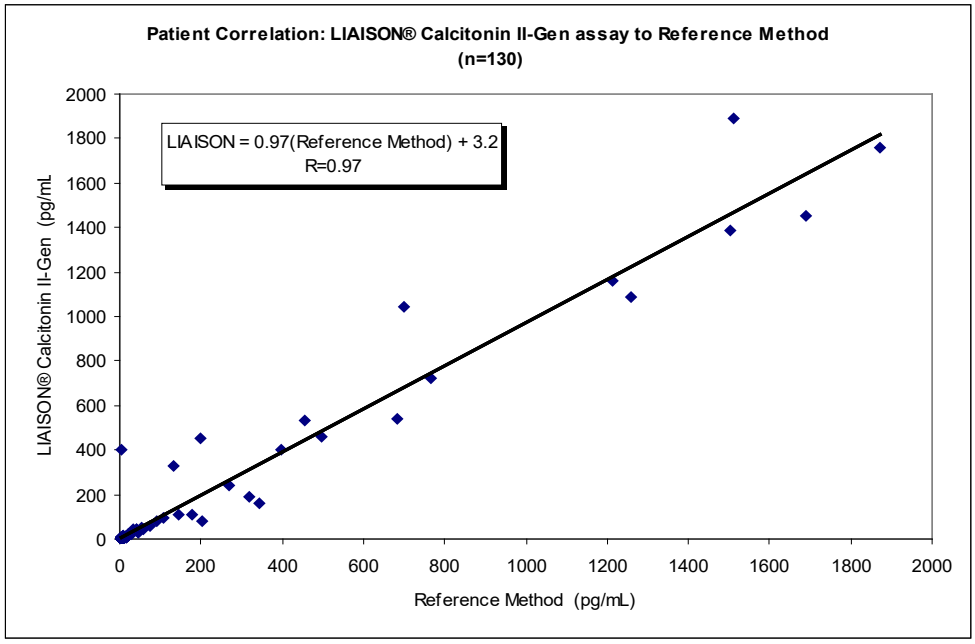
Sample	KC1	KC2	1	2	3	4	5	6	7	8	9*	10*	11*
Number of determinations	160	160	160	160	160	160	160	160	160	160	160	160	160
Mean (pg/mL)	12.8	444	5.22	6.32	23.5	31.3	91.9	353	1663	2326	32.6	53.8	422
Std. Deviation (pg/mL)	0.58	13.8	0.33	0.36	1.31	0.97	1.88	10.6	64.1	84.9	2.39	3.62	23.2
Coefficient of Variation (%)	4.6%	3.1%	6.3%	5.7%	5.6%	3.1%	2.0%	3.0%	3.9%	3.7%	7.3%	6.7%	5.5%

* Samples were diluted 1:100 in specimen diluent prior to each run.

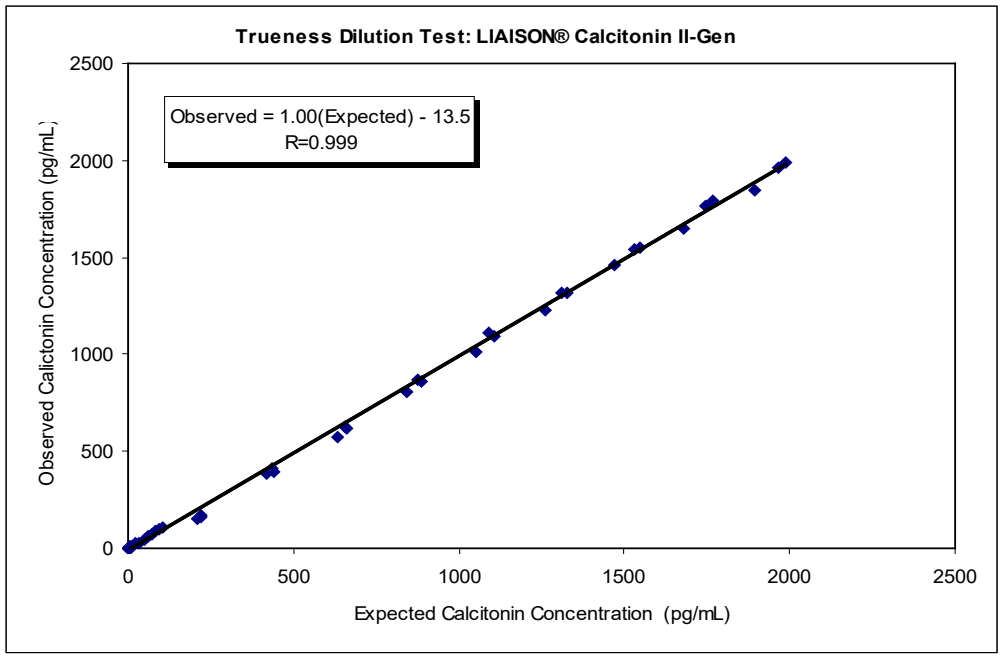
The table below summarizes the precision that can be expected when testing in fewer runs and/or fewer instruments.

Level	Expected Repeatability	Expected Reproducibility
> 20 pg/mL	≤ 8%	≤ 10%
11 – 20 pg/mL	≤ 9%	≤ 11%
9 – <11 pg/mL	≤ 10%	≤ 12%
4 – <9 pg/mL	≤ 15%	≤ 18%

16.5 Patient Correlation:
A total of 130 serum samples were tested by the LIAISON® Calcitonin II-Gen assay and by another automated method following CLSI EP7 and yielded the following comparison: LIAISON® Calcitonin II-Gen = 0.97 (Reference Method) + 3.2; R = 0.97



16.6 Trueness Dilution Test:
The assay trueness has been verified by the dilution test.
5 patient samples were diluted and analyzed by the LIAISON® Calcitonin II-Gen assay following CLSI EP6. The results were analyzed as a linear regression of observed calcitonin concentration versus expected calcitonin concentration.
The resulting regression equation is: Observed calcitonin = 1.00 (expected calcitonin) -13.5; R = 0.999.



16.7 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.

Compound	Conc. Added (pg/mL)	% Cross reactivity
ACTH	100,000	<0.01%
C-peptide	40,000,000	<0.01%
Salmon-CT	50,000	<0.01%
Porcine-CT	500,000	<0.01%
Chicken-CT	500,000	<0.01%
Gastrin I	2,000,000	<0.01%
PTH	150,000	<0.01%
Prolactin	1,000,000	<0.01%
Insulin	33,500,000	<0.01%
TSH	8,000,000	<0.01%
HGH	9,000,000	<0.01%
Human Big Gastrin	1,000,000	<0.01%
CCK	10,000,000	<0.01%
Elcatonin	100,000,000	<0.01%
CT Gene-related peptide	40,000,000	<0.01%
CT Gene-related peptide II	40,000,000	<0.01%
Katacalcin	40,000,000	<0.01%
N-Pro Calcitonin	1,000,000	<0.01%

16.8 Recovery:
5 high concentration spiked serum samples and 5 low concentration serum 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 concentration	Expected pg/mL	Observed pg/mL	%Recovery
High Sample 1 (HS1)	1889			
2 HS1 : 1 LS1		1266	1307	103%
1 HS1 : 1 LS1		946	1017	108%
1 HS1 : 2 LS1		625	700	112%
Low Sample 1 (LS1)	3.354			
High Sample 2 (HS2)	1345.8			
2 HS2 : 1 LS2		904	909	100%
1 HS2 : 1 LS2		677	682	101%
1 HS2 : 2 LS2		450	482	107%
Low Sample 2 (LS2)	8.31			
High Sample 3 (HS3)	681			
2 HS3 : 1 LS3		460	464	101%
1 HS3 : 1 LS3		346	351	101%
1 HS3 : 2 LS3		232	203	88%
Low Sample 3 (LS3)	10.94			
High Sample 4 (HS4)	453			
2 HS4 : 1 LS4		304	289	95%
1 HS4 : 1 LS4		228	212	93%
1 HS4 : 2 LS4		151.6	138.6	91%
Low Sample 4 (LS4)	3.292			
High Sample 5 (HS5)	211			
2 HS5 : 1 LS5		145.4	144.0	99%
1 HS5 : 1 LS5		111.5	116.0	104%
1 HS5 : 2 LS5		77.7	81.5	105%
Low Sample 5 (LS5)	11.88			
			Mean Recovery	101%
			SD	0.07

16.9 High Dose Hook Effect:
No high dose hook effect was observed for calcitonin concentrations up to 500,000 pg/mL.

16.10 Dilution of high sample (>2000 pg/mL):
Samples that read greater than 2000 pg/mL should be diluted 1:100 using the LIAISON® Calcitonin II-Gen Specimen Diluent. The recommended is 10 µL of sample + 990 µL of specimen diluent. Mix the dilution well, re-assay, and calculate the final concentration by multiplying by the dilution factor.

16.11 Interfering substances:
Controlled studies of potentially interfering substances at 2 calcitonin levels, 10 pg/mL and 100 pg/mL, showed no interference at the highest concentration for each substance listed below in the LIAISON® Calcitonin II-Gen assay. The testing was based on CLSI EP7.

Interfering Substance Tested	Concentration Tested
Hemoglobin	200 mg/dL
Bilirubin	20 mg/dL
Triglycerides	3000 mg/dL
Cholesterol	500 mg/dL
HAMA	450 ng/mL
Rheumatoid Factor (RF)	150 IU/mL

17. References

1. Williams Textbook of Endocrinology, ninth edition, 1998.

2. R Elisei et al. Impact of Routine Measurement of Serum Calcitonin on the Diagnosis and Outcome of Medullary Thyroid Cancer: Experience in 10,864 Patients with Nodular Thyroid Disorders, JCEM 89(1): 163-168, 2004.

3. Laurence M. Demers, Ph.D., F.A.C.B. and Carole A. Spencer Ph.D., F.A.C.B., NACB: Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease, Volume 58, 2003, pg 138-140.

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