

Read entire protocol before use.

Calcitonin-IRMA

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

I. INTENDED USE

Immunoradiometric assay kit for the in vitro quantitative measurement of human Calcitonin in serum and plasma.

II. GENERAL INFORMATION

A. Name: Bio-Line Calcitonin (CT) -Irma Kit
B. Catalogue number: BL-31-CT: 100 tests
C. Manufactured by: Bio-Line S.A.
Rue André Fauchille.17 - B-1150 Bruxelles - Belgium
For technical assistance or ordering information contact:
Tel: +32-2-736.62.18. Fax: +32-2-742.13.15.

III. CLINICAL BACKGROUND

Calcitonin (CT) is a 32 amino acid peptide hormone secreted by the para-follicular C-cells of the thyroid gland under serum calcium control. After acute administration this peptide acts as a potent hypocalcemic and hypophosphatemic hormone by increasing renal calcium clearance and reducing bone resorption. However its precise physiological role in bone metabolism is not yet fully understood.

Various forms of CT may be detected in blood samples, including a CT monomer, an oxidized monomer, a dimer, higher molecular weight forms, and possibly precursor of CT. The concentrations of these peptides vary with clinical status, renal function and tissular origin of CT (normal or ectopic production).

Medullary thyroid carcinoma (MTC) is a malignant tumor, developed from the C-cells, secreting calcitonin in large excess. This disease occurs either as a sporadic (80%) or a familial (20%) form, which is transmitted as an autosomal dominant gene or as a component of multiple endocrine neoplasia (IIb).

Moderate hypercalcitoninemia is also observed in pregnancy, pernicious anemia, renal failure and during the neonatal period. Preferably, monomeric form of CT is detected in this assay.


The measurement of CT by the present IRMA is used for :

- diagnosis of medullary thyroid carcinoma (MTC)
- follow up of malignant tumors, to check the success of surgery and to monitor for recurrence
- diagnosis of the preclinical cases of the familial forms of MTC (MEN II or Sipple syndrome) by the use of stimulation tests (calcium or pentagastrin)
- study of the pathophysiology of the calcium-phosphate and bone metabolism.

IV. PRINCIPLES OF THE METHOD

The Bio-Line Calcitonin-U.S. IRMA is an immunoradiometric assay based on coated tube separation. It uses monoclonal antibodies directed against distinct epitopes of human Calcitonin. The capture antibody is attached to the lower inner surface of a plastic tube. Calibrators and samples are dispensed into the tubes and bind to the capture antibody. The signal antibody (radiolabelled) is added immediately and after an incubation which allows the immunologic reaction, the tubes are emptied and washed to remove the excess unbound antibody. The radioactivity bound to the tubes is directly proportional to the initial antigen concentration in the calibrators and samples.

V. REAGENTS PROVIDED

Reagents	Quantity	Colour Code	Reconstitution		
 Tubes coated with anti CT (monoclonal antibodies)	2 x 50	blue	Ready for use		
<table border="1" data-bbox="76 689 197 734"> <tr> <td>Ab</td> <td>¹²⁵I</td> </tr> </table> Anti-CT- ¹²⁵ I (monoclonal antibodies) in TRIS Buffer with bovine serum albumin, thymol and inert red dye	Ab	¹²⁵ I	1 vial 5.5 ml 720 kBq	red	Ready for use
Ab	¹²⁵ I				
<table border="1" data-bbox="76 869 210 900"> <tr> <td>CAL</td> <td>N</td> </tr> </table> Calibrators 0-5 in Calcitonin free human serum with gentamycin and thymol (see exact values on vial labels)	CAL	N	6 vials lyophil.	yellow	Add 1 ml distilled water
CAL	N				
SERUM CT free human serum (to be used for samples dilution) with gentamycin and thymol	1 vial lyophil.	black	Add distilled water (see the volume on the label)		
WASH SOLN 70x Wash solution (TRIS-HCl)	1 vial 10 ml	brown	Dilute 70x with distilled water (use a magnetic stirrer).		
<table border="1" data-bbox="76 1438 274 1473"> <tr> <td>CONTROL</td> <td>N</td> </tr> </table> Controls 1 and 2 in human serum with gentamycin and thymol	CONTROL	N	2 vials lyophil.	silver	Add 1 ml distilled water
CONTROL	N				

Note : 1. CT free human serum is to be used for samples dilution.
2. 1 pg of our reference preparation is equivalent to 0.19 μ IU MRC 89/620.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

- Distilled water
- Pipettes for delivery of: 50 μ l, 200 μ l and 1 ml. (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- 5 ml automatic syringe (Cornwall type) for washing
- Aspiration system (optional).
- Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- Calibrators :** Reconstitute the calibrators with 1 ml distilled water.
- Controls :** Reconstitute the controls with 1 ml distilled water.
- CT free serum :** Reconstitute the CT free serum with distilled water. (see the volume on the label)

- Working Wash solution :** Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators, controls and CT free serum should be frozen immediately after use and kept at -20°C for 3 months. Only one freeze thawing cycle is allowed, discard the calibrators, controls and CT free serum after the second use.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

Serum samples and EDTA plasma are recommended for this assay.

- Do not use hemolyzed samples.
- Do not use lipemic samples.
- If a specimen is expected or known to have a concentration above the highest calibrator, it has to be diluted with the CT free serum to fall within the measuring interval.
- If samples are not assayed the same day as the blood collection, then it is advisable to freeze them until the assay.
- Samples can only be thawed once.
- For repeat testing, freeze them in aliquots and discard each sample after first thawing.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use. Thoroughly mix all reagents and samples by gentle agitation or swirling. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times. Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

- Label coated tubes in duplicate for each calibrator, sample, control. For the determination of total counts, label 2 normal tubes
- Homogenize calibrators, controls, specimens and dispense 200 μ l of each into the respective tubes.
- Add 50 μ l of anti-CT-¹²⁵I (tracer) to all tubes, including the uncoated tubes for total counts.
- Shake the tube rack gently by hand.
- Incubate for 18 ± 1 hours at 2-8°C.
- Take the tubes for the total counts apart and aspirate the contents of the coated tubes. Be sure to remove all the liquid, remaining droplets will increase the background c.p.m..
- Wash tubes twice with 2 ml Wash Solution and aspirate. Avoid foaming during the addition of the wash solution. For manual washing procedure first aspirate the foam layer and then the liquid. For automated wash cycles continuous aspiration is recommended. The precision is improved by aspirating the tubes a second time two minutes after emptying the last tube.
- Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

- Calculate the mean of duplicate determinations.
- On semilogarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of CT (abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.
- Read the concentration for each control and sample by interpolation on the calibration curve. Computer assisted data reduction will simplify these calculations. If automatic result processing is to be used, a 4 parameter logistic function curve fitting is recommended.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

CT-U.S.-IRMA		cpm	B/T x 100 (%)
Total count		312150	100
Calibrator	0 pg/ml	351	0.11
	10 pg/ml	1760	0.56
	30 pg/ml	4523	1.44
	100 pg/ml	14756	4.72
	300 pg/ml	44509	14.25
	1000 pg/ml	131686	42.18

XIII. PERFORMANCE AND LIMITATIONS

A. Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations above the average counts at zero binding, was 0.8 pg/ml.

B. Specificity

Some potentially interfering hormones have been tested in this assay. At concentrations up to 100 ng/ml, none of the following hormones showed significant interference :

- CGRP
- Salmon-calcitonin
- PDN 21
- Procalcitonin N terminal.

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	N	X ± S.D. (pg/ml)	CV %	Serum	N	X ± S.D. (pg/ml)	CV %
A	10	76.1 ± 2.1	2.7	A	20	62.3 ± 2.1	3.3
B	10	337.4 ± 6.5	1.9	B	20	216.9 ± 4.1	1.9

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Added Calcitonin (pg/ml)	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)	Recovery (%)
0	3.3	9.4	-
6	9.3	19.3	101.1
15.5	18.8	51.8	102.7
48	51.3	142	101
142.5	145.8	521	97.4
500	503.3		103.5
0	22.5	25.9	-
6	28.5	35.5	90.9
15.5	38	68.9	93.4
48	70.5	166	97.7
142.5	165	586	100.6
500	522.5		112.2

DILUTION TEST

Dilution	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)	Recovery (%)
1/1	396.0	396.0	-
1/2	198.0	193.6	97.8
1/4	99.0	92.3	93.2
1/8	49.5	49.3	99.6
1/16	24.7	23.6	95.2
1/32	12.3	12.1	97.6
1/64	6.2	6.4	103.2

E. Hook effect

A serum sample with a calcitonin concentration of 230.000 pg/ml gives a signal above the highest calibrator concentration.

XIV. INTERNAL QUALITY CONTROL

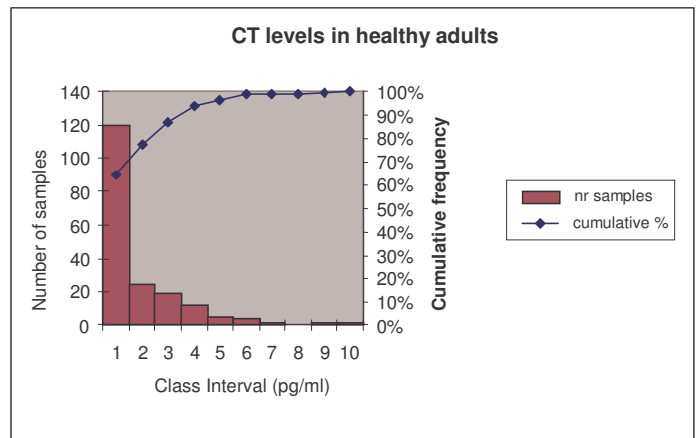
- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make their own pools of control samples, which should be kept frozen in aliquots. Do not freeze-thaw more than once.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises

XV. REFERENCE INTERVALS

Normal values

The graph below shows the CT levels in healthy adult population of both sexes (n = 187).

64% of the results were below the detection limit (0.8 pg/ml). All samples had values below 10 pg/ml.

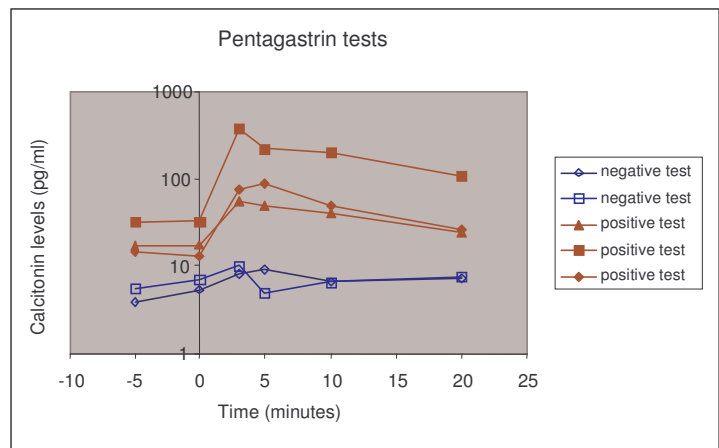


Pentagastrin test:

Patient with MTC with elevated base levels of CT:

Time after Pentagastrin Stimulation (min)	CT measured with CT-U.S.-IRMA (pg/ml)
0	7200
1	12200
2	50000
5	48000
10	34000
20	20000

Patients with low base levels of CT:



XVI. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiosity procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

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XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS ml	CALIBRATORS ml	SAMPLE(S) ml
Calibrators (0-5)	-	0.2	-
Samples	-	-	0.2
Tracer	0.05	0.05	0.05
Incubation	Overnight at 2-8°C		
Separation	-	aspirate	
Washing solution	-	2.0	
Separation	-	aspirate	
Washing solution	-	2.0	
Separation	-	aspirate	
Counting	Count tubes for 60 seconds		

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