

Read entire protocol before use.

SM-C-RIA-CT

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

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of human Somatomedin-C (SM-C) in serum and plasma.

II. GENERAL INFORMATION

- A. Name : Bio-Line **Somatomedin-C (SM-C)-RIA-CT** Kit
- B. Catalogue number : **BL-46-CT**: 100 tests
- C. Manufactured by : Bio-Line S.A.
Rue André Fauchille.17 - B-1150 Bruxelles - Belgium
- D. For technical assistance or ordering information contact :
Tel : +32-2-736.62.18. Fax : +32-2-742.13.15.

III. CLINICAL BACKGROUND

A. Biological activities

Somatomedin-C (SM-C) or Insulin-like growth factor I (IGF-I) is a basic 70 amino acid single chain polypeptide (MW : 7649 Da) similar to proinsulin (50% sequence homology), and to the other well-characterized member of the somatomedin family : IGF II (67AA, 70 % sequence homology with IGF-I). SM-C is the most important factor, which mediates the growth promoting actions of growth hormone, a pituitary hormone with highly fluctuating blood levels due to pulsatile release. The blood concentration of SM-C is more stable due to the binding to carrier proteins. The concentration of the predominant binding protein (MW 53000) as well as the production of SM-C, are regulated by growth hormone. SM-C is produced by the liver, and other tissues, and it has endocrine, paracrine and autocrine activities. It stimulates growth and regulates differentiation of various tissues, displays insulin-like activities and promotes cartilage growth. Although GH is the most important factor controlling SM-C secretion and concentration, other factors are also determinant: the age (with a peak at adolescence), the sex, the nutritional status, and other hormones (oestrogen, thyroxin, prolactin, ...). Specific trophic stimuli mainly control SM-C secretion in the local microenvironment of a particular organ (paracrine activities), while blood SM-C concentration is the most important variable for balanced systemic growth (endocrine activities).

B. Clinical applications



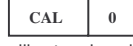
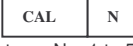

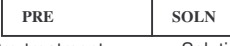
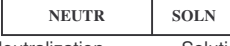
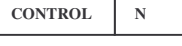
- **Growth retardation:** Growth retardation may be due to several causes, among which deficient GH production (hypopituitarism), which is associated with low SM-C blood levels. Because of the difficulties to get interpretable results from GH measurements (by dynamic multiple or stimulation tests), the determination of the stable SM-C concentration in plasma is often considered as a simple screening test to evaluation "GH impregnation" of the patient before deciding more extensive investigations. In several clinical situations with impaired growth, low SM-C levels may be observed despite normal or high GH production (i.e. malnutrition, chronic diseases states, some genetic dwarfs like Pygmies, ...). Interestingly, children with discrete GH neurosecretory dysfunction may display low SM-C values despite normal GH levels by conventional testing. The results of SM-C assay must be interpreted cautiously by considering the normal variations of SM-C during childhood and adolescence (see Rosenfeld et al).
- **Acromegaly:** SM-C levels are elevated in acromegaly (excess production of GH) and may serve as an indicator of disease severity. Results are more readily interpreted because the normal values are more easily defined in adults. SM-C measurements are also useful to monitor treatment.
- **Research:** The SM-C RIA kit is an invaluable tool to study the modifications of this growth factor during physiologic (i.e. pregnancy) or pathologic (i.e. diabetes) situations, and the local regulation of SM-C production in relation to its paracrine and autocrine activities (wound healing, organ regeneration, neoplastic growth, foetal development, gonadal regulation, etc).

IV. PRINCIPLES OF THE METHOD

In the present kit, Bio-Line has introduced a pre-treatment step in order to improve the clinical performance of the assay. It is well established that the binding proteins interfere with the radioimmunoassay for SM-C. The pre-treatment step used by Bio-Line is the acid-ethanol procedure of Daughaday et al. (8).

A fixed amount of ¹²⁵I labelled SM-C competes with the SM-C to be measured present in the sample or in the calibrator for a fixed amount of antibody sites being immobilized to the wall of a polystyrene tube. After an overnight incubation at 2-8°C, an aspiration step stops the competition reaction. The tubes are then washed with 3 ml of wash solution and aspirated again. A calibration curve is plotted and the SM-C concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

Reagents	100 Test Kit	Colour Code	Reconstitution
 Tubes coated with anti SM-C	2 x 50	green	Ready for use
 Ag ¹²⁵ I TRACER: ¹²⁵ Iodine labelled SM-C (HPLC grade) in phosphate buffer with bovine casein and azide (<0.1%)	1 vial 50 ml 130 kBq	red	Ready for use
 CAL 0 Zero calibrator in phosphate buffer with ovalbumin and azide (<0.1%)	1 vial lyophilised	yellow	Add 3 ml reconstitution solution
 CAL N Calibrators - N = 1 to 5 (see exact values on vial labels) in phosphate buffer with ovalbumin and azide (<0.1%)	5 vials lyophilised	yellow	Add 1 ml reconstitution solution
 REC SOLN Reconstitution Solution containing ethanol	1 vial 10 ml	blue	Ready for use
 PRE SOLN Pre-treatment Solution containing ethanol	1 vial 20 ml	black	Ready for use
 NEUTR SOLN Neutralization Solution containing phosphate buffer with bovine casein and azide (<0.1%)	1 vial 30 ml	green	Ready for use
WASH SOLN CONC Wash solution (TRIS-HCl)	1 vial 10 ml	brown	Dilute 70 x with distilled water (use a magnetic stirrer).
 CONTROL N Controls - N = 1 or 3 in human serum with thymol	3 vials lyophilised	silver	Add 0.5 ml distilled water

Note : 1. Use the zero calibrator for sample dilutions.
2. 1 ng of the calibrator preparation is equivalent to 1 ng of the 1st IS 91/554.

VI. SUPPLIES NOT PROVIDED

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

1. Distilled water
2. Pipettes for delivery of: 100 µl, 400 µl, 500 µl, 600 µl, 1 ml and 3 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. 5 ml automatic syringe (Cornwall type) for washing

6. Aspiration system (optional)
7. Tube shaker (1200 rpm)
8. Centrifuge (3000 g)
9. Incubator at 2-8°C
10. Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- A. Calibrators:** Reconstitute the zero calibrator with 3 ml reconstitution solution and other calibrators with 1 ml reconstitution solution.
- B. Controls:** Reconstitute the controls with 0.5 ml distilled water.
- C. 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 kit components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for one week at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months.
- Avoid successive freezing and thawing.
- 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 or plasma samples must be kept at 2-8°C.
- If the test is not run within 48 hrs, storage in aliquots at -20°C is recommended.
- Avoid successive freezing and thawing.
- Serum and heparinized plasma provide similar results.
Y (serum) = 0.95x (hep. plasma) + 28 r = 0.91 n = 28
Y (serum) = 0.89x (EDTA plasma) + 32 r = 0.88 n = 28
- After extraction, the samples can be stored at 2-8°C for 7 days.

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. Pre-treatment step

1. Label two plastic tubes for each sample and control.
2. Dispense 100 µl of each sample and control into the first tube.
3. Add 400 µl of pre-treatment solution into this tube.
4. Shake all the tubes at 1200 rpm during 30 minutes.
5. Centrifuge for 10 minutes at 1500 g.
6. Take 100 µl of the supernatant and transfer it into the second labelled tube.
7. Add 600 µl of the neutralisation solution to the second tube.
8. Vortex each tube.

C. Modified pre-treatment procedure

In case of renal failure we recommend a modified extraction procedure.

- 1-8. See pre-treatment step.
9. Store the neutralized extract at -20°C for 1 h, then centrifuge immediately at 3000 g for 30 min at 4°C.
10. Decant the supernatant into fresh tubes and assay it as described below.

D. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes.

- Briefly vortex calibrators, controls and samples and dispense 100µl of each into respective tubes.
- Dispense 500 µl of ¹²⁵Iodine labelled SM-C into each tube, including the uncoated tubes for total counts.
- Shake the tube rack gently by hand to liberate any trapped air bubbles.
- Incubate overnight at 2-8 °C.
- Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
- Wash tubes with 3 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
- Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
- Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

- Calculate the mean of duplicate determinations.
- Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

- Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B₀(%)) values for each calibrator point as a function of the SM-C concentration of each calibrator point. Reject obvious outliers.
- Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
- By interpolation of the sample (B/B₀(%)) values, determine the SM-C concentrations of the samples from the calibration curve.
- The concentrations read on the calibration curve must be multiplied by 35 (dilution factor during the pre-treatment step).
- For each assay, the percentage of total tracer bound in the absence of unlabelled SM-C (B₀/T) must be checked.

XII. TYPICAL DATA

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

SM-C	cpm	B/Bo (%)
Total count	41020	
Calibrator		
0 ng/ml	12493	100.0
0.45 ng/ml	11441	91.6
1.6 ng/ml	9590	76.8
5.1 ng/ml	6288	50.3
15 ng/ml	3401	27.2
47 ng/ml	1486	11.9

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 below the average counts at zero binding, was 0.25 ng/ml (x 35 for neat sample).

B. Specificity

The percentages of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

Compound	Cross-Reactivity (%)
SM-C (IGF-1)	100
IGF-II	0.3
Insuline	< 0.01
GH	< 0.01
EGF	< 0.01
MSA	< 0.01

Note: this table shows the cross-reactivity for the anti SM-C.

C. Precision

INTRA-ASSAY PRECISION

INTER-ASSAY PRECISION

Serum	N	<X> ± SD (ng/ml)	CV (%)	Serum	N	<X> ± SD (ng/ml)	CV (%)
A	20	38.8 ± 3.8	9.8	A	20	172 ± 18	10.4
B	20	160.8 ± 15.4	9.6	B	20	621 ± 32	5.2
C	20	664.0 ± 53.5	8.1				

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/ml)	Measured Concent. (ng/ml)
Serum A	1/1	-	549
	1/2	275	292
	1/4	137	153
	1/8	69	81
	1/16	34	31
	1/32	17	13
Serum B	1/1	-	449
	1/2	225	237
	1/4	112	128
	1/8	56	64
	1/16	28	30
	1/32	14	7

Samples were diluted with the zero calibrator.

RECOVERY TEST

Sample	added SM-C (ng/ml)	Recovered SM-C (ng/ml)	Recovered (%)
C1	155	123	79.4
C2	243	217	89.3
C3	316	271	85.8

Conversion factor:

From ng/ml to nmol/L: x 0.1307
From nmol/L to ng/ml: x 7.649

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 its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

Normal subjects

Age Group	MALES (ng/ml)			FEMALES (ng/ml)		
	Mean	Range	N	Mean	Range	N
0 - 2 years	75	21 - 154	46	77	15 - 159	34
3 - 5 years	98	22 - 217	36	127	38 - 257	40
6 - 8 years	167	88 - 265	16	184	89 - 345	10
9 - 11 years	223	128 - 458	13	289	133 - 626	12
12 - 14 years	385	192 - 792	22	551	386 - 832	14
15 - 17 years	535	401 - 786	12	514	329 - 848	30
18 - 20 years	354	222 - 486	10	347	223 - 471	10
21 - 25 years	282	152 - 412	10	280	192 - 368	10
26 - 30 years	223	91 - 355	10	267	131 - 403	10
31 - 35 years	217	151 - 283	10	239	97 - 381	10
36 - 40 years	202	108 - 296	11	234	138 - 330	9
41 - 45 years	178	100 - 256	10	198	144 - 252	10
46 - 50 years	188	94 - 282	10	224	92 - 356	9
51 - 55 years	185	107 - 263	10	201	133 - 269	10
56 - 60 years	197	149 - 245	10	181	119 - 243	8
61 - 65 years	181	83 - 289	10	172	66 - 278	4

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 logbook 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 radiation safety 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.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	CALIBRATORS µl	SAMPLE(S) CONTROLS µl
PRE-TREATMENT			
Samples, controls	-	-	100
Pre-treatment solution	-	-	400
Incubation	30 minutes with continuous shaking at 1200 rpm		
Centrifugation	10 minutes at 1500 g		
Supernatant	-	-	100
Neutralization Solution	-	-	600
Shaking	Vortex		
INCUBATION			
Calibrators (0 to 5)	-	100	-
Pre-treated Samples, controls	-	-	100
Tracer	500	500	500
Incubation	Overnight at 2-8°C		
Separation	-	Aspirate (or decant)	
Working Wash solution		3.0 ml	
Separation		Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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