



Read entire protocol before use.

AIA-100

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

I. INTENDED USE

Radioimmunoassay for the *in vitro* semi-quantitative measurement of human free anti-insulin antibodies (AIA) in serum and plasma.

II. GENERAL INFORMATION

A. Name: Bio-Line **AIA**
B. Catalogue number : **BL-43-CT**: 100 tests
C. Manufactured by : Bio-Line S.A.
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III. CLINICAL BACKGROUND

A. Biological activity

The presence of circulating anti-insulin antibodies (AIA) in diabetics treated with insulin has been recognized as early as 1955. The highly purified insulin preparations, presently available, are less immunogenic than some of the previously used, less pure, preparations. Bovine insulin is more immunogenic than the porcine hormone. Also, it has recently been recognized that AIA may develop, in patients treated with human insulin.

The determination of circulating anti-insulin antibodies is of clinical importance for the following reasons :

- The presence of free anti-insulin antibodies in plasma interferes with the determination of insulin by radioimmunoassay;
- At very high titers, the anti-insulin antibodies may induce a state of insulin resistance;
- Anti-insulin antibodies may influence the quality of the glycemic control, in diabetic patients, by prolonging the half life of insulin.

B. Clinical applications

- Evaluation of the presence of free anti-insulin antibodies prior to the determination of insulin levels, by radioimmunoassay, in patients having received previous insulin therapy ;
- Evaluation of states of insulin resistance;
- Adjunct to the diagnosis of surreptitious insulin injections (factitious insulin-induced hypoglycaemia);
- Monitoring of the evaluation of anti-insulin antibodies, in patients receiving the newly formulated human insulin preparations (new cases; patients in whom a previous treatment with porcine or bovine insulin is replaced by the administration of human insulin).

IV. PRINCIPLES OF THE METHOD

The presence of circulating anti-insulin antibodies in insulin treated diabetics is estimated on a semi-quantitative basis, by the determination of the binding of ¹²⁵I-Tyr-A14-insulin to the serum fraction precipitated by the polyethylene glycol (PEG) (gamma globulins).

V. REAGENTS PROVIDED

Reagents	100 Test Kit	Colour Code	Reconstitution		
<table border="1" style="margin-left: 20px;"> <tr> <td style="text-align: center;">Ag</td> <td style="text-align: center;">¹²⁵I</td> </tr> </table> <p>TRACER: ¹²⁵Iodine labelled INSULINE in phosphate buffer with bovine serum albumin and merthiolate</p>	Ag	¹²⁵ I	1 vial 1 ml 74 kBq	red	Add 10 ml distilled water
Ag	¹²⁵ I				
<table border="1" style="margin-left: 20px;"> <tr> <td style="text-align: center;">CONTROL</td> <td style="text-align: center;">N</td> </tr> </table> <p>Controls - N = 1 to 3 (see exact values on vial labels) in human serum with merthiolate</p>	CONTROL	N	3 vials lyophilised	yellow	Add 1ml distilled water
CONTROL	N				
<table border="1" style="margin-left: 20px;"> <tr> <td style="text-align: center;">PEG</td> </tr> </table> <p>PEG: Polyethylene glycol (16%) in phosphate buffer with bovine serum albumin, Tween 20 and sodium azide (<0.1%)</p>	PEG	1 vial 105 ml	green	Ready for use	
PEG					

Negative Control: The first control contains no anti-insulin antibodies. It allows the determination of the non-specific tracer binding.

Positive Controls: The two other controls respectively contain low and high levels of bovine free anti-insulin antibodies.

VI. SUPPLIES NOT PROVIDED

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

- Distilled water
- Pipettes for delivery of: 100 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
- Disposable polystyrene tubes (12 x 75 mm)
- Plastic or aluminium foil
- Incubator at 37 °C
- Vortex mixer
- Magnetic stirrer
- Centrifuge operating at 1500 g
- Aspiration system (optional)
- Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- Tracers:** Reconstitute the tracer with 10 ml distilled water.
- Controls:** Reconstitute the controls with 1 ml distilled water.

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, tracer and controls are stable for 8 days at 2-8 °C.
For longer storage periods, aliquots should be made and kept at -20 °C for maximum 3 months. Avoid subsequent freeze-thaw cycles.
- 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 24 hrs, storage at -20 °C is recommended.
- Avoid subsequent freeze-thaw cycles.
- After thawing, the samples should be mixed and centrifuged.

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.
- Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. 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 polystyrene tubes in duplicate for each control, sample and total counts.
- Briefly vortex controls and samples and dispense 100µl of each into the respective tubes.
- Dispense 100 µl of ¹²⁵Iodine labelled Insuline into each tube, including the tubes for total counts.
- Shake the tube rack gently by hand to liberate any trapped air bubbles.
- Cover the tubes (with plastic or aluminium foil) and incubate for 2 hours at 37 °C.
- Add 1 ml of the PEG solution (at room temperature) into each tube, except the total counts. Briefly vortex the tubes.
- Incubate for 15 minutes at room temperature.
- Centrifuge for 15 minutes at 1500 g. The use of a refrigerated centrifuge is not necessary, provided that the temperature does not rise up to 25 °C.
- Immediately aspirate (or decant) the supernatants carefully from each tube (except total counts). Be careful not to disturb the precipitate.
- 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 total counts according to the following formula :

$$B/T (\%) = \frac{\text{Counts (Control or sample)}}{\text{Total Counts}} \times 100$$

XII. TYPICAL DATA

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

AIA	cpm	B/T (%)
Total count	33780	
Controls:		
Negative Control	1265	3.7
Low Positive Control	6877	20.4
High Positive Control	19435	57.5
Sample 1	4054	12.0
Sample 2	7904	23.4
Sample 3	12363	36.3

XIII. PERFORMANCE AND LIMITATIONS

A. Precision

INTRA-ASSAY PRECISION

INTER-ASSAY PRECISION

Serum	N	<X> ± SD (B/T x 100)	CV (%)	Serum	N	<X> ± SD (B/T x 100)	CV (%)
A	15	3.2 ± 0.4	12.5	A	38	3.5 ± 0.7	20
B	15	20.4 ± 0.6	2.9	B	38	20.8 ± 1.0	4.8
C	15	59.6 ± 1.0	1.6		38	60.0 ± 2.0	3.3

SD: Standard Deviation; CV: Coefficient of variation

XIV. INTERNAL QUALITY CONTROL

- 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.

The assay was performed on 110 sera of patients who never received insulin therapy. The observed binding percentage of ¹²⁵I-Tyr-A14-insulin was the following: 3.1 ± 0.5 (mean value ± 1 standard deviation). Consequently, one can consider that a binding percentage of ¹²⁵I-Tyr-A14-insulin higher than the mean value + 3 standard deviations (5 %) corresponds to the presence of circulating anti-insulin antibodies.

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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Diabetes 32:592-599.
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Immunogenicity of recombinant DNA human insulin.
Diabetologia 25: 465-469.

XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	SAMPLE(S) CONTROLS µl
Samples, controls Tracer	- 100	100 100
Incubation	2 hours at 37°C	
PEG	-	1000
Incubation Centrifugation Separation	15 minutes at room temperature 15 minutes at 1500 g Aspirate supernates	
Counting	Count tubes for 60 seconds	

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