Read entire protocol before use.

INS-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 Insulin (INS) in serum and plasma.

II. GENERAL INFORMATION

A. Name: Bio-Line **INS-Irma** Kit
B. Catalogue number: **BL-28-CT**: 100 tests
C. Manufactured by: Bio-Line S.A.
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III. CLINICAL BACKGROUND

A Biological activities of insulin

Insulin, a polypeptide hormone with a molecular weight of 5800, is secreted by the beta cells of the islets of Langerhans from the pancreas. Insulin possesses a wide spectrum of biological actions. It stimulates cellular glucose uptake, glucose oxydation, glycogenesis, lipogenesis, proteogenesis and the formation of DNA and RNA. Insulin plays a key role in the regulation of plasma glucose levels (hepatic output inhibition, stimulation of peripheral glucose utilisation). The resulting hypoglycemic effects of insulin are counterbalanced by hormones with hyperglycemic effects (glucagon, growth hormone, cortisol, epinephrine). Insulin secretion is mainly controlled by the plasma glucose levels : hyperglycemia induces a prompt and important increase in circulating insulin levels. Neural influences, as well as various metabolic and hormonal factors (amino acids, glucagon, gastro intestinal hormone) also participate to the control of insulin secretion. Type I (insulin dependent : "juvenile") diabetes is due to a destruction of the beta cells, with a consequence of absolute lack of insulin. In type II (non-insulin-dependent : "maturity onset") diabetes, insulin resistance may play an important role; however after several years of evolution, beta-cells failure may occur, leading to a relative insulinopenia requiring, in some cases, insulin administration. Insulin resistance is associated with high circulation levels of the hormone. The most common case of insulin resistance is represented by obesity. Various endocrinopathies (acromegaly, Cushing syndrome) as well as rare cases of insulin receptor defects or cases with anti-insulin receptor antibodies are associated with glucose intolerance or even diabetes due to insulin resistance. The determination of plasma insulin levels is an important parameter in the diagnosis of hypoglycemia. Insulin levels are high in cases of insulinoma (beta-cell tumor). Functional postprandial hypoglycemia may also be associated with inappropriate insulin release to carbohydrate intake. Insulin levels are determined either in the fasting state or during dynamic test :

a) stimulation test : carbohydrate rich meal, oral glucose tolerance test (OGTT), arginin infusion, tolbutamide or other sulfonylureas administration.
b) inhibition test : fasting, somatostatine infusion

B. Clinical application of insulin determination

. Determination of the beta-cell reserve during glucose tolerance test or after a carbohydrate rich meal, as a guide for the instauration of insulin therapy;
. Contribution to the diagnosis of insulin and non-insulin-dependent diabetes;
. Characterisation and follow-up of states of glucose intolerance;
. Diagnosis and study of cases of insulin resistance;
. Diagnosis of insulinoma and other causes of hypoglycemia.

IV. PRINCIPLES OF THE METHOD

The Bio-Line INS-Irma is an immunoradiometric assay based on coated-tube separation. Mabs1, the capture antibodies, are attached to the lower and inner surface of the plastic tube. Calibrators or samples added to the tubes will at first show low affinity for Mabs1. Addition of Mab2, the signal antibody labelled with ^{125}I, will complete the system and trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration. The use of several distinct Mabs avoids hyperspecificity, common to two-site IRMA, as well as a need of a shaker or incubation at 37°C.

V. REAGENTS PROVIDED

Reagents	100 tests Kit	4x100 tests Kit	Colour Code	Reconstitution
Tubes coated with anti INS (monoclonal antibodies)	2 x 50	8 x 50	dark red	**Ready** for use
Ab ^{125}I TRACER: ^{125}Iodine labelled anti-INS (monoclonal antibodies) in HEPES buffer with bovine serum albumin, azide (<0.1%), EDTA and an inert red dye	1 vial 5.5 ml 350 kBq	4 vials 5.5 ml 350 kBq	red	**Ready** for use
CAL 0 Zero calibrator in human serum and thymol	1 vial lyophilized	2 vials lyophilized	yellow	**Add** 2.0 ml distilled water
CAL N Calibrator N = 1 to 5 (see exact values on vial labels) in human serum and thymol	5 vials lyophilized	2 x 5 vials lyophilized	yellow	**Add** 0.5 ml distilled water
WASH SOLN CONC Wash Solution (Tris-HCl)	1 vial 10 ml	4 vials 10 ml	brown	**Dilute** 70 x with distilled water (use a magnetic stirrer).
CONTROL N Controls - N = 1 or 2 in human serum with thymol	2 vials lyophilized	2 x 2 vials lyophilized	silver	**Add** 0.5 ml distilled water

Note: 1. Use the zero calibrator for sample dilutions.
2. 1 µIU of the calibrator preparation is equivalent to 1 µIU of WHO 66/304.

VI. SUPPLIES NOT PROVIDED

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

1. Distilled water
2. Pipettes for delivery of: 50 µl, 500 µl and 2 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. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. **Calibrators** : Reconstitute the zero calibrator with 2.0 ml distilled water and other calibrators with 0.5 ml distilled water.

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 kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for 3 days at 2 to 8°C. For longer storage periods, aliquots should be made and kept at –20°C.
- 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 and plasma must be kept at 2 - 8°C.
- If the test is not run within 24 hours, storage at -20°C is recommended
- Avoid subsequent freeze thaw cycles.
- Serum or EDTA plasma provide similar results.
- Do not use haemolysed samples.

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

1. Label coated tubes in duplicate for each calibrator, sample, control. For the determination of total counts, label 2 normal tubes
2. Briefly vortex calibrators, samples and controls and dispense 50 µl of each into respective tubes.
3. Dispense 50 µl of tracer into each tube.
4. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
5. Incubate for 2 hours at room temperature.
6. 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.
7. Wash tubes with 2 ml Working Wash solution (except total counts). Avoid foaming during the addition of the Working Wash solution.
8. Aspirate (or decant) the content of each tube (except total counts).
9. Wash tubes again with 2 ml Wash solution (except total counts) and aspirate (or decant).
10. After the last washing, let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
11. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. On semilogarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of INS

(abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.

3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is 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.

INS-IRMA		cpm	B/T (%)
Total count		129481	100
Calibrator	0.0 µIU/ml	197	0.15
	5.7 µIU/ml	529	0.41
	13.5 µIU/ml	1277	0.99
	46.0 µIU/ml	4796	3.70
	144.0 µIU/ml	16436	12.69
	440.0 µIU/ml	44090	34.05

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 1 µIU/ml.

B. Specificity

Cross-reactive hormones were added to a high value calibrator (100 µIU/ml or 4 ng/ml). The apparent INS response was measured.

As shown hereafter, animal insulins (except rat insulin) cross-react whereas human, pork and beef proinsulins present no cross-reaction.

Added analyte to a high value serum		Theoretical INS values (ng/ml)	Observed INS values (ng/ml)	Cross-reaction (%)
Porcine insulin	8 ng/ml	4.2	17.4	> 100
Bovine insulin	8 ng/ml	3.8	17.8	> 100
Dog insulin	16 ng/ml	4.2	17.2	81
Rabbit insulin	16 ng/ml	4.2	14.1	62
Rat insulin	16 ng/ml	3.8	3.7	0
Human proinsulin	32 ng/ml	4.3	4.4	0.3
Porcine proinsulin	16 ng/ml	4.3	4.7	2.5
Bovine proinsulin	16 ng/ml	4.3	4.4	0.6

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	N	<X> ± SD (µIUml)	CV (%)	Serum	N	<X> ± SD (µIUml)	CV (%)
A	10	6.6 ± 0.1	2.2	A	20	14.4 ± 0.9	6.5
B	10	53.3 ± 0.8	1.6	B	20	100.4 ± 6.1	6.1

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Sample	Added INS (µIU/ml)	Recovered INS (µIU/ml)	Recovery (%)
1	245.0	245	100
2	9.3	9	97
3	100.5	101	101
4	296.0	330	112
5	327.0	337	103

DILUTION TEST

Sample	Dilution	Theoretical Concent. (µIU/ml)	Measured Concent. (µIU/ml)
C	1/1	-	32.6
	1/5	6.5	6.1
	1/10	3.3	2.7
D	1/1	-	130.9
	1/5	26.2	25
	1/10	13.1	12.5
	1/20	6.5	5.9
	1/40	3.3	2.2
	1/80	1.6	1.8

Samples were diluted with zero calibrator.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrators have been added to the coated tubes.

TIME DELAY		
	0' (µIU/ml)	30' (µIU/ml)
Serum 1	8	7
Serum 2	16	17
Serum 3	37	42
Serum 4	81	82

F. Hook effect

A sample spiked with INS up to 125000 µIU/ml gives higher counts than the last calibrator point.

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. Do not freeze-thaw more than twice.
- Acceptance criteria for the difference between the duplo results of the samples should rely on Good Laboratory Practises

XV. REFERENCE INTERVALS

The range of insulin levels in 55 subjects with normal oral glucose tolerance tests, was 4 to 16 µIU/ml

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

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 radiosafety 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)	CALIBRA-TORS (ml)	SAMPLE(S) (ml)
Calibrators (0-5)	-	0.05	-
Samples	-	-	0.05
Tracer	0.05	0.05	0.05
Incubation	2 hours at room temperature		
Separation	-	aspirate (or decant)	
Working wash solution	-	2.0	
Separation	-	aspirate (or decant)	
Working wash solution	-	2.0	
Separation	-	aspirate (or decant)	
Counting	Count tubes for 60 seconds		