DIAGNOSTIKA GMBH

Instructions for Use

Insulin Antibody RIA

^{125}I-Radioimmunoassay for the Quantitative Determination of Antibodies against Insulin in Serum

CE

IVD

REF	RA107/50	RA108/100
Σ	50	100
+2 +8 °C	2 – 8 °C	

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Contents

Symbols

I V D	In-Vitro-Diagnostic Device	CE	EC Declaration of Conformity
CONT	Contents		Expiry Date
LOT	Lot Number	+2 +8 °C	Store
	Manufactured by	Σ	Sufficient for
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Hazard Pictograms

radioactive

1. Introduction and Principle of the Test

Insulin-dependent diabetes mellitus (IDDM) is characterised by the presence of several distinct circulating autoantibodies including autoantibodies to glutamic acid decarboxylase (GAD), autoantibodies to a protein tyrosine phosphatase, usually referred to as IA2, and autoantibodies to insulin.

In the assay, test serum samples are incubated, first with ^{125}I-labelled (A14)-monoiodinated insulin. This is followed by addition of anti-human IgG to precipitate any labelled insulin-anti-insulin complexes which have formed. After centrifugation, the precipitates are counted for ^{125}I and the amount of radioactivity in the precipitates is proportional to the concentration of insulin antibody in the test sample.

2. Precautions

- For in vitro use only.
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- This radioactive product assay only be received, stored as used by persons so authorized and by laboratories covered by such authorization. It must not be administered to humans or animals under any circumstances.
- Do not eat, drink or smoke where radioactive materials are being handled.
- Do not pipette by mouth.
- Wear disposable gloves when handling radioactive materials.
- The kit components "Negative and Positive Controls" are made with human serum. All sera used are tested for HIV I/II antibodies and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potentially biohazardous materials.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.

3. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the kit labels. Do not mix various lots of any kit component within an individual assay.

4. Contents of the Kit

4.1 **Tracer** **TRACER**  1 (2) vial(s)
^{125}I- labelled Insulin
1.5 ml, lyophilized, activity < 40 kBq per vial

4.2 **Assay Buffer** **ASSAY BUFFER** 1 (2) bottle(s)
250 ml, ready for use

4.3 **Positive Controls** **CONTROL A** **&** **CONTROL B** 2 vials
each 250 µl, lyophilized,
Concentrations see QC Certificate

4.4 **Standards** **CAL A** **–** **CAL E** 5 vials
Standard A, 0.5 ml normal human serum
Standard B - E, each 250 µl, lyophilized,

Concentrations:

Standard	A	B	C	D	E
U/ml	0	0.4	1	10	50

4.5 **Anti-human-IgG** **ANTI-IGG** 1 (2) vial(s)
5.5 ml, ready for use

Additional materials and equipment required but not provided:

- Pipettes for 20, 25, 100 µl, and 1 ml
- Plastic round bottom tubes and suitable rack
- Centrifuge (preferable refrigerated) capable of at least 3,000 x g
- Suitable device for aspirating or decanting the tubes.
- Vortex mixer
- Gamma counter

5. Sample Collection and Storage

Serum should be used in the assay. Do not use lipemic or grossly haemolized specimen. Repeated freezing and thawing should be avoided. Samples which appear turbid should be centrifuged before assay to remove any particulate material.

Samples can be stored up to one week at 2 - 8 °C or at - 20 °C for longer periods.

6. Preparation of Samples and Reagents

6.1 Patient Samples

Allow the patient sera to reach room temperature, mix gently, and centrifuge to remove any particulate material.
Please do not omit this centrifugation step!

6.2 Tracer TRACER

Reconstitute the lyophilized tracer with 1.5 ml Assay Buffer (4.2) prior to use and mix carefully.
The reconstituted tracer can be stored at 2-8 °C for up to 4 weeks and must not be frozen.

6.2 Standards B - E, Positive Controls

Reconstitute each vial with 250 µl distilled water. Store at 2 - 8 °C after reconstitution for up to 2 months. Standard A is ready for use.

7. Test Procedure

7.1 Pipette each 20 µl standards, controls and patient sera into the corresponding tubes.

7.2 Pipette each 25 µl reconstituted tracer into all tubes and to 2 empty tubes for total counts.

7.3 Mix thoroughly (vortex) and incubate for 16 - 24 hours (overnight) at room temperature (20-25 °C).

7.4 Mix the vials of anti-human-IgG (4.6) carefully on a vortex (preferably 5 times for 1 second each) and pipette each 100 µl of anti-human-IgG into all tubes (except T). Mix, cover and incubate for 1 hour at 2-8 °C.

7.7 After the 1-hour incubation, add 2 ml of ice-cold assay buffer to each tube (except T). Mix thoroughly (vortex) and centrifuge for 20 minutes at 3,000 x g, if possible in a refrigerated centrifuge.

7.8 Decant or aspirate the supernatant (except T) and add a further 2 ml of ice-cold assay buffer. Mix thoroughly (vortex) and centrifuge for 20 minutes at 3,000 x g, if possible in a refrigerated centrifuge. Remove the supernatants by aspiration or decantation.

7.9 Count all tubes for least 2 minutes in a gamma counter.

8. Calculation of Results

Construct a standard curve by plotting the mean cpm of each Standard versus its corresponding concentration.
The concentration of the Controls and patient samples can then be read off the standard curve.

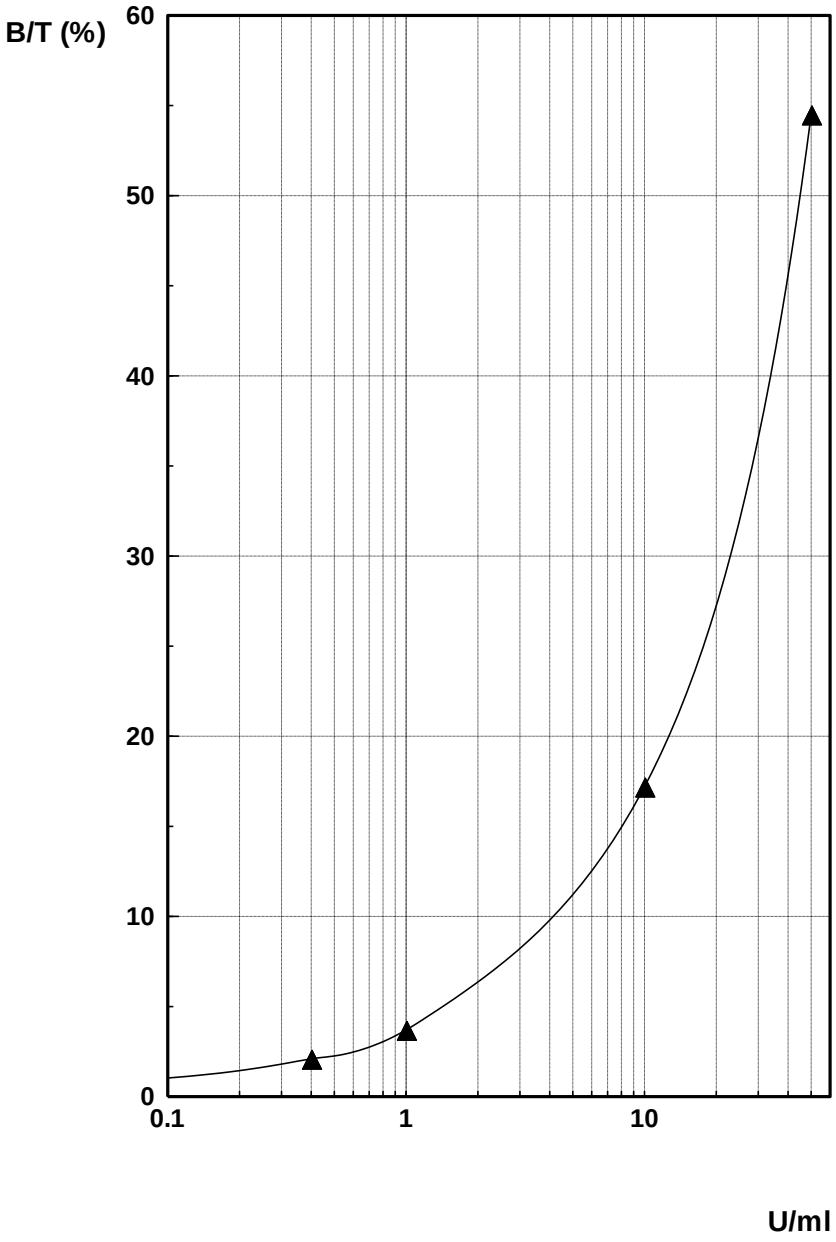
Alternatively, the cpm of the standards, controls, and patient samples can be related to the cpm of the Totals (B/T in %) and used on the y-axis for constructing the standard curve and for reading off the measured concentrations.

9. Typical Example

Typical results are shown in the following table

Sample	cpm1	cpm2	Mean	B/T (%)	U/ml
Total Act.	30,214	30,448	30,331		
Standard A	225	260	243	0.8	0
Standard B	650	624	637	2.1	0.4
Standard C	1,105	1,139	1,122	3.7	1
Standard D	5,224	5,210	5,217	17.2	10
Standard E	16,687	16,374	16,530	54.5	50

Typical Standard Curve



10. Reference Ranges

The normal range was determined by measuring the sera of 100 healthy adult blood donors and 68 patients with various autoimmune diseases. From these studies a upper normal limit of 0.4 U/ml is recommended. However, each individual laboratory should establish their own normal ranges.

Out of 62 IDDM patients who had never received insulin treatment, 21 (34%) were positive for IAb. However, in patients who had received insulin treatment, IAb prevalence is much higher suggesting that insulin antibodies were being induced by insulin treatment in many patients.

11. Assay Characteristics

Intra-Assay Variation

	Sample 1	Sample 2
N	24	24
Mean (U/ml)	3.1	9.1
Standard Deviation (U/ml)	0.18	1.8
Coefficient of Variation (%)	5.8	3.0

Inter-Assay Variation

	Sample 1	Sample 2
N	12	12
Mean (U/ml)	0.73	11.1
Standard Deviation (U/ml)	0.05	0.47
Coefficient of Variation (%)	6.7	4.2

Sensitivity

The lower limit of detection at 2 standard deviations from repeated measurements of the Negative Control is 0.03 U/ml.

Clinical Accuracy

18/18 Graves' disease sera, 10/10 Rheumatoid Arthritis sera, 10/10 SLE sera and 20/20 Hashimoto's disease sera gave values of less than 0.4 units per mL. 9/10 celiac disease sera also gave values of less than 0.4 units per mL and the remaining sample a value of 2.5 units per mL (value reduced to less than 0.4 units per mL after addition of unlabelled insulin). These results suggest an assay detection limit of 0.4 units per mL but individual laboratories should establish their own normal ranges.

Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 5mg/mL; intralipid up to 3000 mg/dL or bilirubin up to 20mg/dL

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Pipetting Scheme

	T	B_0	Standards	Control	Patient
Standard A µl		20			
Standard B-E µl			20		
Control A & B µl				20	
Patient Sample µl					20
^{125}I-Tracer µl	25	25	25	25	25

Mix thoroughly and incubate for 16 - 24 hours (overnight)
at room temperature

anti-human IgG µl		100	100	100	100

Mix thoroughly and incubate for 1 hour at 2 - 8 °C

Assay Buffer*) ml		2	2	2	2

*) 2 - 8 °C

Mix thoroughly, centrifuge (2 - 8 °C) for 20 minutes at min. 3,000 x g

Decant or aspirate the supernatant

Assay Buffer*) ml		2	2	2	2

*) 2 - 8 °C

Mix thoroughly, centrifuge (2 - 8 °C) for 20 minutes at min. 3,000 x g

Decant or aspirate the supernatant

Measure for 2 minutes in a gamma counter