RSR

RiaRSR™ IAA

Assay for antibodies to insulin from RSR – Instructions for use

CE

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INTENDED USE

The RSR Insulin Ab RIA Assay kit is intended for use by professional persons only, for the quantitative determination of insulin antibodies (IAb) in human serum. Autoantibodies to pancreatic beta cell antigens are important serological markers of type 1 diabetes mellitus (type 1 DM). The antigens recognised by these antibodies include insulin, glutamic acid decarboxylase (GAD_{65} kDa isoform), the islet cell antigen IA-2 or ICA-512 and zinc transporter 8 (ZnT8).

REFERENCES

M. Masuda et al. Autoantibodies to IA-2 in IDDM. Measurements with a new immunoprecipitation assay.
Clinica Chimica Acta 2000 **291**; 53 - 66

S Chen et al. Sensitive non-isotopic assays for autoantibodies to IA-2 and to a combination of both IA-2 and GAD_{65}.
Clinica Chimica Acta 2005 **357**; 74 – 83

ASSAY PRINCIPLE

In RSR's insulin antibody (IAb) assay test serum samples are incubated first with ^{125}I-(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 precipitate is proportional to the concentration of IAb in the test sample. The measuring range is 0.4 – 50 u/mL (arbitrary RSR units).

STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C. 40 µL is sufficient for one assay (duplicate 20 µL determinations). When required, thaw test samples at room temperature and mix gently to ensure homogeneity. Lipaemic or haemolysed sera should not be used. Remove any particulate matter by centrifugation prior to assay (preferably for 5 min at about 10,000rpm i.e. about 10,000g in a microfuge).

SYMBOLS

Symbol	Meaning
CE	EC Declaration of Conformity
IVD	In Vitro Diagnostic Device
REF	Catalogue Number
LOT	Lot Number
	Consult Instructions
	Manufactured by
Σ	Sufficient for
	Expiry Date
8°C 2°C	Store
CONTROL -	Negative Control
CONTROL +	Positive Control

MATERIALS SUPPLIED IN 50 and 100 TUBE KITS (Table 1)

MATERIAL	50 Tube	100 Tube
^{125}I-Labelled Insulin	1 x 1.5 mL	2 x 1.5 mL
Calibrators	4 x 0.25 mL	4 x 0.25 mL
Anti-Human IgG	1 x 5.5 mL	2 x 5.5 mL
Assay Buffer	1 x 250 mL	2 x 250 mL
Negative Control	1 x 0.5 mL	1 x 0.5 mL
Positive Controls	2 x 0.25 mL	2 x 0.25 mL

MATERIALS REQUIRED AND NOT SUPPLIED

3.5 mL Assay tubes
Pipettes capable of dispensing 20 µL, 25 µL, 100 µL, 250 µL, 1.5 mL and 2 mL.
Vortex mixer
Refrigerated centrifuge capable of 1500g
Gamma counter
Pure water

PREPARATION OF REAGENTS SUPPLIED

Store unopened kits and all kit components (A-F) at 2–8°C.

A	**^{125}I-Labelled Insulin** **40kBq/vial** (at manufacture) Reconstitute each vial by addition of 1.5 mL assay buffer (D) and mix gently to dissolve. Once reconstituted, store at 2–8°C for up to 4 weeks.
B1-4	**Calibrators** 0.4, 1, 10, and 50 u/mL; reconstitute each vial with 0.25 mL pure water. Once reconstituted, store at 2–8°C for up to 2 months. Units are arbitrary RSR units.

C	**Anti-Human IgG (containing precipitation enhancer)** Vortex before use. Store at 2–8°C (Variations in appearance may occur without influence on assay performance).
D	**Assay Buffer** Ready for use. Store at 2–8°C
E	**Negative Control** Ready for use. Store at 2–8°C
F1-2	**Positive Controls I & II** Reconstitute each vial with 0.25 mL pure water. Once reconstituted, store at 2–8°C for up to 2 months.

ASSAY PROCEDURE

Allow all reagents, except assay buffer (D), to stand at room temperature (20-25°C) for at least 30 minutes prior to start of the assay. Mix each thoroughly before use. A repeating type Eppendorf pipette is recommended for steps 2, 4 and 5.

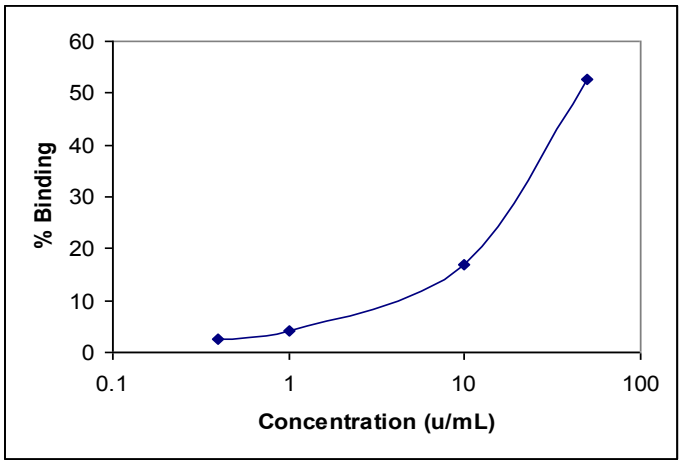
1.	Pipette 20 µL of patient sera, negative control (E), calibrators (B1-4) and positive controls (F1-2) into appropriately labelled assay tubes (in duplicate is recommended).
2.	Pipette 25 µL of ^{125}I-labelled insulin (A) into each tube and into two additional empty tubes for total counts.
3.	Mix the tubes briefly on a vortex mixer, cover and incubate overnight (16-24h) at room temperature (20-25 °C).
4.	Pipette 100 µL of anti-human IgG (C) into each tube, excluding the two total count tubes, mix briefly on a vortex mixer, cover and incubate at 2–8°C for 1 hour.
5.	Pipette 2 mL of cold (2–8°C) assay buffer (D) into each tube, excluding the two total count tubes, mix briefly on a vortex mixer and centrifuge each tube at 1500g for 20 minutes at 4°C.
6.	After centrifugation, carefully aspirate or decant the supernatants.
7.	Repeat steps 5 and 6.
8.	Count the tubes (including total count tubes) for 1 minute on a gamma counter.

RESULT ANALYSIS

A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the % ^{125}I-insulin bound for the calibrators on the y-axis (linear scale). IAb concentrations in test sera can then be read off the calibration curve [plotted at RSR as a spline log/lin curve (smoothing factor = 0)]. Other data reduction methods can be used. The negative control can be assigned a value of 0.04 u/mL to assist in computer processing of assay results. Samples with high IAb concentrations can be diluted in kit negative control (E). For example, 10 µL of sample plus 90 µL of negative control to give a 10x dilution. Other dilutions (e.g. 100x) can be prepared from a 10x dilution or otherwise as appropriate. Some sera will not dilute in a linear way.

TYPICAL RESULTS (Example only, not for use in calculation of actual results)

Calibrator u/mL	% ^{125}I–insulin bound	Concentration u/mL
B1	2.6	0.4
B2	4.9	1
B3	18.8	10
B4	56.9	50
Negative Control	0.8	
Positive Control I	3.2	0.51
Positive Control II	20.8	11.3
	Total cpm = 30,424	



ASSAY CUT OFF

	u/mL
Negative	<0.4 u/mL
Positive	≥0.4 u/mL

This cut off has been validated at RSR. However, each laboratory should establish its own normal and pathological reference ranges for IAb levels. It is also recommended that each laboratory include its own panel of control samples in the assay.

CLINICAL EVALUATION

Clinical Specificity and Sensitivity

100% (n=100) of healthy blood donor sera samples gave values of less than 0.4 units per mL. Out of 62 type 1 DM patients who had never received insulin treatment, 21 (34%) were positive for IAb. However, in patients who have received insulin treatment, IAb prevalence is much higher suggesting that insulin antibodies are being induced by insulin treatment in many patients.

Lower Detection Limit

The negative control was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at +2 standard deviations was 0.03 u/mL.

Inter Assay Precision

Sample	u/mL (n=20)	CV (%)
1	6.3	8.0
2	18.7	6.5

Intra Assay Precision

Sample	u/mL (n = 25)	CV (%)
A	0.57	3.3
B	7.0	1.9
C	8.9	1.6

Clinical Accuracy

Sera from patients with Graves' Disease (n = 17), Hashimoto's Thyroiditis (n = 20), Coeliac Disease (n = 10), Systemic Lupus Erythematosus (n = 10) or Rheumatoid Arthritis (n = 10) gave values for IAb of less than 0.4 u/mL. This indicated no interference from autoantibodies to the TSH receptor, thyroglobulin, thyroid peroxidase, tissue transglutaminase, dsDNA or from rheumatoid factor. These results suggest an assay detection limit of 0.4 u/mL but values between 0.3 and 0.5 u/mL should be checked after addition of unlabelled insulin (details available from RSR on request).

Interference

No interference was observed when samples were spiked with intralipid up to 3000 mg/dL or bilirubin at 20 mg/dL. Interference was observed from haemoglobin at 5 mg/mL.

SAFETY CONSIDERATIONS

This kit is intended for use by professional persons only. Follow the instructions carefully. Observe expiry dates stated on the labels and the specified stability for reconstituted reagents. Refer to Safety Data Sheet for more detailed safety information.

The kit contains radioactive material. Users should make themselves aware of, and observe, any national and local legislation and codes of practice governing the use, storage, transportation and disposal of radioactive materials. Avoid all actions likely to lead to ingestion. Avoid contact with skin and clothing. Wear protective clothing and, where appropriate, personal dosimeters. Radioactive materials should only be used by authorised personnel and in designated areas. Wash hands thoroughly after handling. Monitor hands and clothing before leaving the designated area.
Materials of human origin used in the preparation of the kit have been tested and found non-reactive for HIV1 and 2 and HCV antibodies and HBsAg but should, none-the-less, be handled as potentially infectious. Wash hands thoroughly if contamination has occurred and before leaving the laboratory. Sterilise all potentially contaminated waste, including test specimens, before disposal.
Materials of animal origin used in the preparation of the kit have been obtained from animals certified as healthy but these materials should be handled as potentially infectious. Some components contain small quantities of sodium azide as preservative. With all kit components, avoid ingestion, inhalation, injection or contact with skin, eyes or clothing. Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.

ASSAY PLAN

Allow all reagents and samples to reach room temperature (20-25°C) before use (excluding assay buffer)	
Pipette:	20 µL Calibrators, controls and patient sera
Pipette:	25 µL ^{125}I-labelled insulin into all tubes (plus two extra empty tubes for total counts)
Tubes:	Mix on vortex mixer and cover
Incubate:	Overnight (16 - 24 hours) at 20-25 °C
Pipette:	100 µL Anti-human IgG (excluding tubes containing total counts)
Tubes:	Mix on vortex mixer and cover
Incubate:	1 Hour at 2-8 °C
Pipette:	2 mL Cold assay buffer (2-8 °C)
Tubes:	Mix on vortex mixer and centrifuge at 1500 x g for 20 minutes at 4°C
Tubes:	Aspirate supernatant
Pipette:	2 mL Cold assay buffer (2-8 °C)
Tubes:	Mix on vortex mixer and centrifuge at 1500 x g for 20 minutes at 4°C
Tubes:	Aspirate supernatant
Count tubes using gamma counter for 1 minute	