Février 2019 – Modèle 014

Cisbio Bioassays
Parc Marcel Boiteux – BP 84175 – 30200 Codolet / France - Tél. 33 (0)4.66.79.67.00

ANTI-INSULIN ANTIBODIES

REF *AAI* Σ 100 IVD CE 2°C 8°C

Trousse pour le dosage radioimmunologique des anticorps anti-insuline libres et totaux dans le sérum humain

La trousse contient :

Traceur ≤ **52 kBq**	2 x 5 mL
Solution précipitante	1 x 100 mL
Réactif d'extraction	1 x 10 mL
Tampon de neutralisation	1 x 10 mL
Contrôle négatif (C1)	1 x qsp 1 mL
Contrôle positif (C2)	1 x qsp 1 mL
Mode d'emploi	1

Attention : Certains réactifs contiennent de l'azoture de sodium

Kit for radioimmunoassay for determination of free and total anti-insulin antibodies in human serum

Kit content :

Tracer ≤ **52 kBq**	2 x 5 mL
Precipitating solution	1 x 100 mL
Extraction reagent	1 x 10 mL
Neutralization buffer	1 x 10 mL
Negative control	1 x qs 1 mL
Positive control	1 x qs 1 mL
Instruction for use	1

Warning : Some reagents contain sodium azide

Kit zur radioimmunologischen Bestimmung von freien und totalen Anti-Insulin Antikörpern in Humanserum

Inhalt des Kits :

Tracer ≤ **52 kBq**	2 x 5 mL
Präzipitationslösung	1 x 100 mL
Extraktionsreagenz	1 x 10 mL
Neutralisationspuffer	1 x 10 mL
Negativkontrolle	1 x qs 1 mL
Positivkontrolle	1 x qs 1 mL
Gebrauchsinformation	1

Achtung : Einige Reagenzien enthalten Natriumazid

Kit per il dosaggio radioimmunologico degli anticorpi anti-insulina liberi e totali nel siero umano

Contenuto del kit :

Tracciante ≤ **52 kBq**	2 x 5 mL
Reagente immunoprecipitante	1 x 100 mL
Reagente di estrazione	1 x 10 mL
Tampone di neutralizzazione	1 x 10 mL
Controllo negativo (C1)	1x q.b a 1 mL
Controllo positivo (C2)	1x q.b a 1 mL
Istruzioni per l'uso	1

Attenzione : Alcuni reagenti contengono sodio azide

Equipo radioinmunológica para la determinación de los anticuerpos anti-insulina libres y totales en suero humano

Contenido del equipo :

Trazador ≤ **52 kBq**	2 x 5 mL
Solución precipitante	1 x 100 mL
Reactivo de extracción	1 x 10 mL
Tampón de neutralización	1 x 10 mL
Control negativo (C1)	1 x csp 1 mL
Control positivo (C2)	1 x csp 1 mL
Instrucciones de uso	1

Precauciones : Algunos reactivos contienen azida sódica

Δοκιμασία για τον ραδιοανοσολογικό προσδιορισμό των ελεύθερων και συνολικών αντισωμάτων έναντι της ινσουλίνης στον ανθρώπινο ορό.

Περιεχόμενα της τυποποιημένης συσκευασίας:

Ιχνηθέτης ≤ **52 kBq**	2 x 5 mL
Διάλυμα κατακρήμνισης	1 x 100 mL
Αντιδραστήριο εκχύλισης	1 x 10 mL
Ρυθμιστικό διάλυμα εξουδετέρωσης	1 x 10 mL
Αρνητικός μάρτυρας (C1)	1 x sq 1 mL
Θετικός μάρτυρας (C2)	1 x sq 1 mL
Οδηγίες χρήσης	1

Προσοχή: Ορισμένα αντιδραστήρια περιέχουν αζίδιο του νατρίου.

	FRA	ENG	DEU	ITA	SPA	ELL	CES	TUR	SRB
	Explication des symboles	**Explanation of symbols**	**Erläuterung der Sumbole**	**Spiegazione dei simboli**	**Significado de los simbolos**	**Επεξήγηση των συμβόλων**	**Vysvětlení symbolů**	**Sembollerin açıklaması**	**Objašnjenje simbola**
CE	Conforme aux normes européennes	European conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Σύμφωνο προς τα ευρωπαϊκά πρότυπα	Evropská shody	Avrupa'ya uyum	Evropska usaglašenost
	T° limite de stockage	Storage temperature limitation	Grenzwerte der Lagertemperatur	Limiti per la temperatura di conservazione	Limites de temperatura de almacenamiento	Όριο θερμοκρασίας αποθήκευσης	Mezní teplota skladování	Depolama sıcaklığı sınırlaması	Ograničenje temperature za čuvanje
LOT	N° de lot	Batch code	Chargencode	codice lotto	Código de lote	Αριθμός παρτίδας	Č. šarže	Parti kodu	Šifra serije
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Consumir antes de	Ημερομηνία λήξης	Použitelné do	Son kullanım tarihi	Upotrebiti do
	Consulter la notice d'utilisation	Consult operating instructions	Im Handbuch nachschlagen	consultare le istruzioni per l'USO	Consultar las instrucciones de manejo o funcionamiento	Συμβουλευτείτε τις οδηγίες χρήσης	Přečtěte si návod k použití	İşletim talimatlarına danışın	Pogledajte uputstvo za upotrebu
IVD	Diagnostic In Vitro	In Vitro Diagnostic device	In Vitro-Diagnostische Anwendung	Dispositivo Diagnostico In Vitro	Dispositivo de diagnóstico In Vitro	Διαγνωστική συσκευή In Vitro	Diagnostika in vitro	In Vitro Tanılama cihazı	Uređaj za dijagnostiku *in vitro*
	Fabriqué par	Manufactured by	Hergestellt von	Prodotto da	Fabricado por	Κατασκευάζεται από την	Vyrobil	Üretici	Proizveo
REF	Référence	Catalogue number	Katalog Nr.	N. catalogo	Número de catálogo	Αριθμός καταλόγου	Reference	Katalog numarası	Kataloški broj
Σ 100	Nombre de tubes	Number of determinations	Anzahl der Bestimmungen	Numero di determinazioni	Número de determinaciones	Αριθμός προσδιορισμών	Počet zkumavek	Saptama sayısı	Broj određivanja
TRACER	Traceur radioactif	Radioactive tracer	Radioaktiver Tracer	Tracciante radioattivo	Trazador radiactivo	Ραδιενεργός ιχνηθέτης	Tracer	Radyoaktif izleyici	Radioaktivni indikator
RIP	Solution précipitante	Precipitating solution	Präzipitationslösung	Reagente immunoprecipitante	Solución precipitante	Διάλυμα κατακρήμνισης	precipitační roztok	presipitasyon solüsyonu	Precipitirajući rastvor
CONTROL	Contrôle	Control	Kontrolle	Controllo	Control	Ορός ελέγχου	Kontrola	Kontrol	Kontrola
EXTRACT	Réactif d'extraction	Extraction reagent	Extraktionsreagenz	Reagente di estrazione	Reactivo de extracción	Αντιδραστήριο εκχύλισης	extrakční reagencie	ekstraksiyon reaktifi	Ekstrakcioni reagens
BUF	Tampon de neutralisation	Neutralization buffer	Neutralisationspuffer	Tampone di neutralizzazione	Tampón de neutralización	εξουδετέρωσης	neutralizační pufr	nötralizasyon tamponu	Neutralizacioni pufer

MISE A JOUR / UPDATING

CE

AAI
Cisbio Bioassays - Février 2019 - Modèle 014

FRA **Modifications par rapport à la version précédente :**
Mise à jour code langue Serbe.

ENG **Changes from the previous version:**
Updated Serbian language code.

DEU **Änderungen gegenüber der Vorgängerversion:**
Serbischer Sprachcode aktualisiert.

ITA **Modifiche rispetto alla versione precedente:**
Aggiornato il codice della lingua serba.

SPA **Cambios desde la versión anterior:**
Código de idioma serbio actualizado.

ELL **Αλλαγές από την προηγούμενη έκδοση:**
Ενημερώθηκε κώδικας σερβικής γλώσσας.

CES **Změny od předchozí verze:**
Aktualizovaný srbský kód jazyka.

TUR **Bir önceki sürüm üzerinde yapılan değişiklikler:**
Sırp dil kodu güncellendi..

SRB **Promene od prethodne verzije:**
Ažurirana šifra srpskog jezika

1. NAME AND INTENDED USE

ANTI-INSULIN RIA is a radioimmunometric assay for determination of free and total anti-insulin antibodies in human serum.
The kit is intended for professional use.

2. INTRODUCTION

The ANTI-INSULIN RIA kit allows to measure:
- **anti-insulin antibodies induced** during a treatment by human or porcine insulin.
- **anti-insulin autoantibodies**, present in prediabetes, before any treatment by insulin.

3. PRINCIPLE

The principle of anti-insulin antibody (AIA) detection is based on the demonstration of a specific binding with iodine 125-labeled insulin.
Two determinations can be performed with the kit:

1) Determination of free anti-insulin antibodies (free AIA)

The free AIA are antibodies not complexed with circulating insulin. They are determined by a radioimmunoprecipitation assay in liquid phase:
- serum containing anti-insulin antibodies incubates with iodine 125-labeled insulin.
- the ^{125}I-insulin bound to AIA fraction is separated from the free ^{125}I-insulin fraction by precipitation with polyethylene glycol (PEG).
- after centrifuging, radioactivity counting of the pellets (bound fraction) allows the calculation of the binding percentage of ^{125}I-insulin.

2) Determination of total anti-insulin antibodies (total AIA)

The total AIA are the free AIA plus the antibodies complexed with serum insulin. They are determined by the same protocol as the free AIA after dissociation of immune complexes in acid medium, insulin adsorption by active charcoal, neutralization then centrifuging.

The detection of free anti-insulin antibodies (AIA) and/or total AIA is carried out within 2 hours. This incubation time provides sufficient sensitivity for the monitoring of AIA induced by insulin treatment.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	SYMBOLS	QUANTITY	STORAGE
Human INSULIN ^{125}I: ready for use. Human insulin iodinated on A14 and purified by HPLC, in TRIS buffer containing phenol red. Contains 0.1% sodium azide (NaN_3). The 2 vials contain approximately **52 kBq** (1.4 µCi) ^{125}I-insulin with specific activity close to 300 µCi/µg (11.1 Mbq/µg) on the labeling day.	**TRACER**	2 vials of 5 mL	2-8°C until the expiry date.
PRECIPITATING SOLUTION, blue: ready for use. Polyethylene glycol solution in phosphate buffer. Contains 0.1% sodium azide (NaN_3).	**RIP**	1 vial of 100 mL	2-8°C until the expiry date.
EXTRACTION REAGENT, black: ready for use. Solution containing charcoal-dextran in glycine buffer at an acid pH. Contains 0.1% sodium azide (NaN_3). This reagent should be dispensed under shaking.	**EXTRACT**	1 vial of 10 mL	2-8°C until the expiry date.
NEUTRALIZATION BUFFER, colorless: ready for use. Barbital buffer. Contains 0.1 % sodium azide (NaN_3).	**BUF**	1 vial of 10 mL	2-8°C until the expiry date.
NEGATIVE CONTROL (C1): lyophilized. Normal human serum containing 0.1% sodium azide (NaN_3). Reconstitute with 1 mL demineralized or distilled water. Gently homogenize after complete dissolution. Avoid the formation of foam.	**CONTROL -**	1 vial qsp 1 mL	2-8°C until the expiry date. After reconstitution: the C1 and C2 controls should be promptly divided into aliquots and frozen at – 20°C. Under these conditions, the controls are stable for at least 6 months.
POSITIVE CONTROL (C2): lyophilized. (*) Human specimen containing a known amount of free and total AIA and 0.1% sodium azide (NaN_3). Reconstitute with 1 ml demineralized or distilled water. Gently homogenize after complete dissolution. Avoid the formation of foam.	**CONTROL +**	1 vial qsp 1 mL	

(*) The acceptance range true values are printed on the vial label.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious. Do not pipette by mouth. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.
Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.
The enforcement of the basic rules for handling radioactive products ensures adequate security.
A summary of these is given below:
Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date. Do not mix reagents from different batches. Avoid any microbic contamination of the reagents or of the water.
Fully respect the incubation conditions and the washing instructions indicated.
The human 125I Insulin reagent is sensitive to temperature variations (do not freeze, do not store at room temperature) and must be stored at 2-8 ° C until the expiry date.

6. SPECIMEN COLLECTION AND PREPARATION

- The determination of anti-insulin antibodies can be performed on serum or plasma obtained after collection of blood on EDTA and immediate centrifuging of the sample.
- The samples must be stored at + 2 / 8 °C. If the test is not run within 24 hours following sampling, freeze at - 20 °C.
- Avoid using a sample which has been thawed several times.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar with disposable tips, capable of dispensing 50 µL, 100 µL, 200 µL and 1000 µL. Their calibration should be checked regularly.
Disposable plastic tubes. Distilled or demineralized water. Vortex-type mixer. Appropriate racks. Parafilm®. Multitubes centrifuge (3000 g). Gamma scintillation counter calibrated for 125 iodine measurement.

7.2 Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use.
Dispensing of the reagents into the tubes is also carried out at room temperature.
The assay requires the following groups of tubes:
T group for the determination of total activity,
Control group for the control,
Sx groups for the samples to be assayed.
The controls (C1-C2) and specimens should be assayed during the same run.

- It is recommended to perform the assay in duplicate for control and samples.
- **The aspiration of the supernatant after precipitation and centrifuging should be carried out thoroughly, so that a minimum of supernatant remains in the tube, avoiding any aspiration of the precipitate.**

A - Free AIA assay

Respect the order in which reagents are to be added:

- Dispense 50 µL of sample to be tested, negative and positive control (C1 and C2) into the corresponding groups of tubes.
- Add 100 µL ^{125}I-insulin to each tube.
- Manually shake the tube holder.
- Incubate for 2 hours at room temperature (+18-25°C).
- Add 1000 µL precipitating solution into each tube (except T tubes).
- Vortex **tube after tube** then incubate 10 minutes at room temperature (+18-25 °C).
- Centrifuge all the tubes (except T tubes) at 3000 g at + 2 / 8 °C for 10 minutes.
- Aspirate the supernatant.
- Count the precipitate radioactivity for 1 minute.

Free AAI (Assay flow chart)

Tubes	**Samples Controls (C1-C2) µL**	**^{125}I Insulin µL**		**Precipitating solution µL**		
T	-	100	Manually shake the tube holder - Incubate for 2 hours at 18-25°C	-	Vortex **tube after tube** - Incubate for 10 minutes at + 18-25°C - Centrifuge 10 minutes (3000 g) at + 2-8°C - Aspirate the supernatant	Count
Negative Control Positive Control	50	100		1000		
Samples	50	100		1000		

B – Total AIA assay

The determination of total AIA is performed in two steps:

1) <u>Extraction by active charcoal</u>

- To 200 µL sample, add 100 µL extraction reagent under shaking.
- Manually shake the tube holder.
- Incubate for 10 minutes at room temperature (+ 18 / 25 °C).
- Add 100 µL neutralization buffer.
- Manually shake the tube holder.
- Centrifuge for 10 minutes at 3000 g and at + 2 / 8 °C.

2) <u>Immunoprecipitation</u>

- Remove 100 µL supernatant after extraction, add 100 µL ^{125}I-Insulin to each tube (perform the test with duplicates for each extract).
- Manually shake the tube holder.
- Incubate for 2 hours at room temperature (+ 18 / 25 °C).
- Add to each tube (1 and 2 excluded): 1000 µL precipitating solution.
- Vortex **tube after tube** then incubate for 10 minutes at room temperature (+18 / 25°C).
- Centrifuge all the tubes (1 and 2 excluded) at 3000 g and at + 2 / 8 °C for 10 minutes.
- Aspirate the supernatant.
- Count the precipitate radioactivity for 1 minute.

TOTAL AIA (Assay flow chart)

<table>
<tr><td colspan="8">1) EXTRACTION</td></tr>
<tr><td>Tubes</td><td>Samples Controls (C1-C2)

µL</td><td>Extraction Reagent
µL</td><td rowspan="4">Manually shake the tube holder
-
Incubate for 10 minutes at 18-25°C</td><td>Neutralization buffer
µL</td><td colspan="3" rowspan="4">Manually shake the tube holder
-
Centrifuge for 10 minutes (3000 g) at + 2-8°C</td></tr>
<tr><td>T</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Control negative
Control positive</td><td>200</td><td>100</td><td>100</td></tr>
<tr><td>Samples</td><td>200</td><td>100</td><td>100</td></tr>
<tr><td colspan="8">2) IMMUNOPRECIPITATING</td></tr>
<tr><td>Tubes</td><td>Sample supernatant Control (C1-C2) surpernatant
µL</td><td>^{125}I-Insulin

µL</td><td rowspan="4">Manually shake the tube holder
-
Incubate for 2 hours at 18-25°C</td><td>Precipitating solution
µL</td><td rowspan="4">Vortex
tube after tube
-
Incubate for 10 minutes at + 18-25°C
-
Centrifuge 10 minutes (3000 g) at + 2-8°C
-
Aspirate the supernatant</td><td rowspan="4">Count</td></tr>
<tr><td>T</td><td>-</td><td>100</td><td>-</td></tr>
<tr><td>Control negative
Control positive</td><td>100</td><td>100</td><td>1000</td></tr>
<tr><td>Samples</td><td>100</td><td>100</td><td>1000</td></tr>
</table>

8. QUALITY CONTROL

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

- Determine the cpm mean values for each duplicate.
- Calculate the binding capacity of each sample in percentage of total radioactivity, as follows:

$$B/T\ (\%) = \frac{\text{Tube radioactivity (cpm)}}{\text{Total radioactivity (cpm)}} \times 100$$

Example only: this data must under no circumstances be substituted for results obtained in the laboratory.

. Free AIA

	Mean cpm	B/T (%)
Total activity	17827	
Negative control (C1)	715	4.0
Positive control (C2)	6254	35.1
Sample 1	647	3.6
Sample 2	6678	37.5
Sample 3	9836	55.2

. Total AIA

	Mean cpm	B/T (%)
Total activity	17539	
Negative control (C1)	657	3.7
Positive control (C2)	8224	46.9
Sample 1	634	3.6
Sample 2	9060	51.7
Sample 3	10616	60.5

10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.

11. EXPECTED VALUE

Each laboratory should establish its own range of normal values. The value given below is only indicative.
A positivity threshold (P.T.) was determined from 150 normal subjects, as follows :

$$P.T. = \text{mean value} + 3\,\sigma = 3.6 + (3 \times 0.6) = 5.5\ \%\ B/T$$

Therefore it is possible to consider that a binding percentage greater than this positivity threshold indicates the presence of anti-insulin antibodies.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

The **test precision** is provided by the within-run and between-run reproducibility.

A - Free Anti-Insulin Antibodies

1. Within-Run Reproducibility

3 sera were each tested 10 times during the same run.

	Mean (% B/T)	Standard Deviation (% B/T)	CV (%)
Serum 1 (negative)	3.9	0.2	5.1
Serum 2	29.5	0.7	2.4
Serum 3	54.4	1.1	2.0

2. Between-Run Reproducibility

3 sera were each tested in duplicate during 5 different runs, using three batches of tracer with different ages, by three technicians.

	Mean (% B/T)	Standard Deviation (% B/T)	CV (%)
Serum 1 (negative)	3.5	0.5	14.3
Serum 2	36	1.1	3.1
Serum 3	54	0.7	1.3

B - Total Anti-Insulin Antibodies

1. Within-Run Reproducibility

3 sera were each tested 10 times during the same run after extraction.

	Mean (% B/T)	Standard Deviation (% B/T)	CV (%)
Serum 1 (negative)	3.5	0.6	17.1
Serum 2	29.7	0.5	1.7
Serum 3	58.5	0.6	1.0

2. Between-Run Reproducibility

3 sera were each tested in duplicate during 5 different runs, using three batches of tracer with different ages, by three technicians.

	Mean (% B/T)	Standard Deviation (% B/T)	CV (%)
Serum 1 (negative)	3.3	0.4	12.1
Serum 2	51.2	1.6	3.1
Serum 3	58.9	1.8	3.1

13. INTERFERENCES

No interference with bilirubin, haemoglobin and triglycerides, measured up to respective concentrations of equal to 250 mg/L, 10 g/L and 20 g/L has been observed.

BIBLIOGRAPHY

Bottazzo GF, Genovese F, Bosi E, Dean BM, Christie MR, and Bonifacio E. Novel considerations on the antibody/autoantigen system in type 1 (insulin-dependent) diabetes mellitus. Annals of medecine. 1991;23:453-61.

Bridel MP, Levy-Marchal C, and Porquet D. Les auto-anticorps anti-insuline : marqueur prédictif de survenue de diabète insulino-dépendant ? Immunoanal Biol Spéc. 1991;25:37-41.

Castano L, Ziegler AG, Ziegler R, Schoelson S, and Eisenbarth GS. Characterization of insulin autoantibodies in relatives of patients with type 1 diabetes. Diabetes 42. 1993;1202-9.

Freychet P. Mécanisme d'action de l'insuline. Médecine/Sciences 4. 1988;70-82.

Green A, Gale EAM, and Patterson CC. Incidence of childhood-onset insulin-dependent diabetes mellitus : the Eurodiab Ace Study. Lancet . 1992;339:905-9.

Greenbaum CJ, Wilkin TJ, and Palmer JP. Fifth international serum exchange workshop for insulin autoantibody (IAA) standardization. Diabetologia. 1992;35:798-800.

Kuglin B, Kolb H, Greenbaum C, Maclaren NK, Lenmark A, and Palmer JP. The fourth international workshop on the standardization of insulin autoantibody measurement. Diabetologia. 1990;33:638-9.

Levy-Marchal C, Bridel MP, Sodoyez-Goffaux F, Koch M, Tichet J, Czernichow P, and Sodoyez JC. Superiority of radiobinding assay over ELISA for detection of IAAs in newly diagnosed type 1 diabetic children. Diabetes Care. 1991; 14:61-3.

Muir A, Shatz DA, and Maclaren NK. The pathogenesis, prediction, and prevention of insulin-dependent diabetes mellitus. Endoc. Metab. Clin. North Am. 1992;21:199-219.

Nerup J, Mandrup-Poulsen T, Molvig J, Helqvist S, Wogensen L, and Egeberg J. Mechanisms of pancreatic β-cell destruction in type 1 diabetes. Diabetes Care. 1988;11:16-23.

Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, and Paquette TL. Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science. 1983;222:1337-9.

Sodoyez-Goffaux F, Koch M, Dozio M, Brandenburg D, and Sodoyez JC. Advantages and pitfalls of radioimmune and enzyme linked immunosorbent assays of insulin antibodies. Diabetologia. 1988;34:694-702.

Sodoyez JC, Koch M, Lemaire I, Sodoyez-Goffaux F, Rapaille A, François-Gérard C, and Sondag D. Influence of affinity of antibodies upon their detection by liquid phase radiobinding assay and solid phase enzyme linked immunosorbent assay. Demonstration using monoclonal antibodies raised against rDNA human proinsulin. Diabetologia. 1991;34:463-8.

Swenne I. Pancreatic Beta-cell growth and diabetes mellitus. Diabetologia. 1992;35:193-201.

Vandewalle CL, Decraene T, Schuit FC, De Leeuw IH, Pipellers DG, Gorus FK, and the Belgian Diabetes Registry. Insulin autoantibodies and high titre islet cell antibodies are preferentially associated with the HLA DQA1*0301-DQB1*0302 haplotype at clinical onset of type 1 (insulin-dependent) diabetes mellitus before age 10 years, but not at onset between age 10 and 40 years. Diabetologia. 1993;36:1155-62.

Vardi P, Brik R, and Barzilai D. Insulin autoantibodies : reflection of disturbed self-identification and their use in the prediction of type 1 diabetes. Diabetes/Metabolism Reviews. 1991;7:209-22.

Vardi P, Dib SA, Tuttleman M, Connely JE, Grinbergs M, Radizabeth A, and all. Competitive insulin autoantibody assay. Prospective evaluation of subjects at high risk for development of type 1 diabetes mellitus. Diabetes. 1987;36: 1286-91.

Yassin N, Seissler J, Glück M, Boehm BO, Heinze E, Pfeiffer EF, and Scherbaum WA. Insulin autoantibodies as determined by competitive radiobinding assay are positively correlated with impaired Beta-cell function-the Ulm Frankfurt population study. Klin Wochenschr. 1991;69:736-41