For technical assistance please contact:

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: (716) 691-0091
Fax: (716) 691-0466
Toll Free USA/Canada: 1-800-537-TEST
E-Mail: info@immcodiagnostics.com

or your local product distributor

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ImmuGlo™

ISLET CELL ANTIBODY (ICAb) TEST SYSTEM

For Research Use Only

PRODUCT INSERT

Catalog No. 1123 40 Determinations

INTENDED USE

An indirect immunofluorescence test for the detection and semi-quantitation of islet cell antibodies in human serum to aid in the diagnosis of *insulin dependent diabetes mellitus* (IDDM).

SUMMARY AND EXPLANATION

Diabetes is a chronic and complex metabolic disease influenced by various hereditary and environmental factors that result in the inability of the body to maintain the use of carbohydrates, fats and proteins. The condition, characterized by high blood glucose levels, is caused by a deficiency in insulin production or an impairment of insulin utilization. Most cases of diabetes fall into two clinical categories: *insulin-dependent diabetes mellitus* (IDDM or Type I diabetes) and *non-insulin dependent diabetes mellitus* (NIDDM or Type II diabetes).

Prognosis, treatment and disease management are different for each type. It is well accepted that IDDM is an autoimmune disease targeting ß-cells of the islets of Langerhans in the pancreas. The autoimmune response to islet cell antigens elicits antibody responses to antigens such as glutamic acid decarboxylase (GAD), ICA-512 and insulin. They have been found to be highly predictive markers, particularly if present in high titer[1-15]. Detection of these ICAb's by indirect immunofluorescence (IF) on pancreas substrate is considered the gold standard for diagnosis of IDDM[16,17]. These cytoplasmic ICAb's are currently used for the prediction of type I diabetes[18-20].

ICAb's are detected in up to 90% of newly diagnosed diabetic patients. In the Bart's-Windsor family study, 100% of the first degree relatives of IDDM patients with ICAb's >80 JDF units progressed to IDDM within 10 years. The level of ICAb's appears to be highest prior to the onset of Type I diabetes and diminishes progressively thereafter[12,18]. ICAb's have several distinct specificities and show two major patterns of reactivity[4]. The first pattern is restricted predominantly to the ß-cells. The second stains all cells within the islet, and is the classical staining pattern for cytoplasmic ICAb's.

PRINCIPLES OF PROCEDURE

Islet cell antibodies are detected by indirect immunofluorescence (IF). In this method, patients' sera are incubated on sections of monkey pancreas to allow binding of antibodies to the tissue substrate. Any antibodies not bound are removed by rinsing. Bound antibodies of the IgG class are detected by incubation of these sections with fluorescein-labeled conjugates. Reactions are observed under a fluorescence microscope equipped with appropriate filters.

The presence of islet cell antibodies is demonstrated by an apple green fluorescence of the cytoplasm of the islets of Langerhans. The titer (the reciprocal of the highest dilution giving a positive reaction) is then determined by testing serial dilutions of the positive control[21].

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C. Ready for use after equilibration to room temperature.

Precautions

All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. All human serum specimens and human derived products should be treated as potentially hazardous, regardless of their origin. Follow good laboratory practices in storing, dispensing, and disposing of these materials[22].

* WARNING - Sodium azide (NaN_3) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this insert to ensure valid results. Do not interchange kit components with those from sources other than the same catalog number from IMMCO Diagnostics. Do not use beyond expiration date.

Materials Provided

Anti-Islet Cell Antibody Test *Catalog No. 1123*

Kit contains sufficient reagents to perform 40 determinations.

10 x	4-well Monkey Pancreas Substrate Slides
1 x 0.5 ml	*Islet Cell Antibody Positive Control. Human serum containing islet cell antibodies, standardized against the JDF (Juvenile Diabetes Foundation) positive reference serum. **JDF units are stated on the vial label.**
1 x 0.5 ml	*Negative Control.
1 x 5.0 ml	*Goat anti-human IgG FITC conjugate, heavy chain specific. **Protect from light**.
1 x 5.0 ml	*Conjugate B. **Protect from light**.
1 x 60 ml	*Buffered Diluent. Ready to use.
2 vials	Phosphate Buffered Saline. Dissolve each vial to 1 liter.
1 x 5.0 ml	*Mounting Medium. **Do not freeze**.
1 x 1.0 ml	Evans Blue Counterstain
1 x 12	Coverslips
	*Contains <0.1% NaN_3

Materials Required but not Provided

- Fluorescence microscope
- Micropipette or Pasteur pipette
- Serological pipettes
- Staining dish (e.g. Coplin jar)
- Small test tubes (e.g. 13 x 75 mm) and test tube rack
- Distilled or deionized water
- 1 liter container
- Wash bottle
- Absorbent paper towels
- Incubation chamber

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LIMITATIONS OF THE PROCEDURE

Occasionally sera may exhibit strong staining for ANA or other autoantibodies. These may interfere with the ability to detect islet cell antibodies. In such cases, titrating the serum may permit the visualization of the islet cell antibodies. In other cases their titer may be lower than the ANA or other antibodies and hence may not be detected. The ImmuGlo™ islet cell antibody test should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. This method should be used for testing human serum samples only.

EXPECTED VALUES

Approximately 50-80% of new-onset diabetics are positive for islet cell antibodies. The prevalence of islet cell antibodies in non-diabetic first-degree relatives and in non-diabetic normal subjects has been reported to be 2-5% and 0.25-1.7%, respectively[5-16]. Approximately 11% of islet cell antibody positive first-degree relatives develop diabetes each year. Intervals of as long as 8 years between detection of islet cell antibodies and the onset of diabetes have been reported.

Incidence of Anti-Islet Cell Antibodies

Disease Group	Age (years)	No. Patients examined	% Positive
Type I Diabetes (IDDM)			
at onset	<1-10	19	63
	11-20	25	60
	21-40	8	25
long standing	<1-10	22	41
	11-20	71	39
	21-40	26	24
	41-70	13	0
	71-80	3	33
Type II Diabetes (NIDDM)			
at onset	<1-40	0	-
	41-80	39	3
long standing	<1-10	0	-
	11-20	5	20
	21-80	75	1
Non-diabetic first-degree relatives	<1-30	61	0
	31-50	119	2
	51-80	19	0
non-diabetic controls	>18	200	0

SPECIMEN COLLECTION AND PREPARATION

Only serum specimens should be used for this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of this test and should not be used. Store specimens at 2°- 8°C for no longer than one week. For longer storage, serum should be frozen at -20°C. Avoid repeated freezing and thawing of samples.

PROCEDURE

Test Method

A. Screening:

Step 1. Dilute each patient serum **1:5** with the Buffered Diluent provided (0.1 ml serum + 0.4 ml diluent). Screening at more than one dilution helps to avoid a "prozone phenomenon." For screening, **DO NOT dilute the Positive or Negative Controls**. Save the undiluted sera to determine antibody titers if screening tests are found to be positive.

Step 2 Allow pouches containing substrate slides to equilibrate to room temperature for **10-15 minutes**. Carefully remove the slides from their pouch without touching the substrate.

Step 3 Label the slides and place them in an incubation chamber lined with paper towels moistened with water to prevent drying.

Step 4 Using a micropipette or Pasteur pipette, apply **1 drop** (approximately 50 µl) of the Negative Control to well #1. Similarly apply 1 drop of Positive Control to well #2. Avoid overfilling the wells.

Step 5 Using a micropipette or Pasteur pipette, apply **1 drop** of patient's diluted serum (approximately 50 µl) to the other wells. Avoid overfilling the wells.

Step 6 Place the lid on the incubation chamber and incubate slides **18-24 hours at 2-8° C**.

Step 7 Remove a slide from the incubation chamber. Hold slide at tab end and rinse gently with approximately **10 ml** of PBS using a pipette, or rinse slide in a beaker filled with PBS. Do not use wash bottle. Transfer slide immediately into Coplin jar and wash **10 minutes**. Repeat process with all remaining slides.

Step 8 Remove slide(s) from Coplin jar. Blot the edge of the slide on a paper towel to remove excess PBS. Place the slide in the incubation chamber. Immediately invert the **goat anti-human IgG FITC conjugate** dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) to each well. Repeat process with all remaining slides.

Step 9 Replace the lid on the incubation chamber. Incubate **30 minutes** at room temperature.

Step 10 Repeat **Steps 7 through 9** except in **Step 8** use **Conjugate B**.

Step 11 Remove a slide from incubator. Hold the slide at the tab end and dip the slide in a beaker containing PBS to remove excess conjugate. Place slide(s) in a staining dish filled with PBS for **10 minutes**. Repeat process with all remaining slides. If desired, **2-3 drops** of Evans blue counterstain may be added to the final wash. **NOTE:** Improper washing may lead to increased background fluorescence.

Step 12 Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS. **While slide is still wet mount the coverslip**. Place **3 drops** of the Mounting Medium evenly spaced on a coverslip and invert the slide onto the coverslip. To remove any air bubbles gently apply pressure along the edge of the coverslip. Avoid any movement of the coverslip. Repeat process with all remaining slides.

Step 13 Examine for specific fluorescence under a fluorescence microscope at a magnification of **200x** or greater.

Slides may be read as soon as prepared. However, because of the presence of an antifading agent in the mounting medium, no significant loss of staining intensity occurs if reading is delayed. Slides should be stored in the dark at 2°- 8°C.

B: End Point Determination (Titration)

Serum found positive in the screening test may be further tested to determine the titer by following **Steps 5 through 13**. Each test run should include the undiluted Negative Control and **undiluted**, **1:2, 1:4, 1:8 and 1:16** dilutions of the Positive Control. Serial two-fold dilutions of the patient's serum should be made as well starting at **1:5**. The reciprocal of the highest dilution producing a positive reaction is the titer. If the Positive Control titer is within the limits defined by the enclosed QC specifications, the levels of the antibody in patient serum can then be reported in JDF units[16]. The JDF unit value of the Positive Control is printed on the vial label. To calculate the JDF unit value of the unknown serum, simply divide the titer of the unknown by the titer of the Positive Control and multiply this by the JDF units on the Positive Control label.

Example:

Positive Control titer is 1:4.
Unknown Sample titer 1:10.
Positive Control label reads 160 JDF units.

Calculation:

$$\text{Concentration of ICAb} = \frac{10}{4} \times 160 = 400 \text{ JDF units}$$

Preparation of Serial Dilutions

For each serum positive for islet cell antibodies upon screening, number four tubes 1 to 4 and add **0.4 ml** of Buffered Diluent to tube 1 and **0.2 ml** to subsequent tubes. Pipette **0.1 ml** of undiluted serum to tube 1 and mix thoroughly. Transfer **0.2 ml** from tube 1 to tube 2 and mix thoroughly. Continue transferring **0.2 ml** from one tube to the next after mixing to yield the expected dilutions as depicted in the following table:

Tubes	1	2	3	4
Serum + Buffered Diluent	0.1 ml + 0.4 ml	0.2 ml	0.2 ml	0.2 ml
Transfer	0.2 ml →	0.2 ml →	0.2 ml →	
Final dilution	1:5	1:10	1:20	1:40

Quality Control

Both a positive and negative control serum should be included with each test run. The negative control should show no significant fluorescence of the islet cells. The positive control should stain the cytoplasm of the islet cells. Upon titration of the positive control, an endpoint titer of ± doubling dilution from the indicated titer should be obtained. If expected results are not obtained, the run should be repeated. If inadequate results continue to occur with the controls, these may be due to:

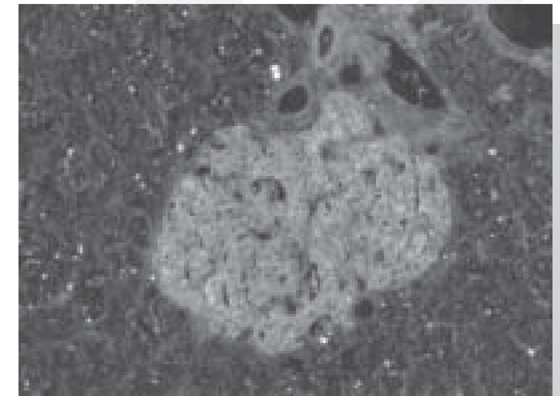
- Turbidity. Discard and use another control.
- Problems with the optical system of the fluorescence microscope. These may include: improper alignment, use of the bulb beyond the expected performance life, etc.
- Allowing the slide to dry during the procedure.
- Improper preparation of serial dilutions of control.

RESULTS

The results of the tests for islet cell antibodies should be reported as negative (<5) or positive with titer or JDF units[8].

Read for specific staining of the cytoplasm of the islet of Langerhans. Various other tissue antibodies such as antinuclear antibodies (ANA), mitochondrial antibodies, and smooth muscle antibodies may also be observed on pancreas sections. Sera giving any of these reactions should be reported as negative for antibodies to islet cells. Any sera giving nuclear staining reactions may be tested with the Antinuclear Antibody (ANA) Test (HEp-2 Cells) or the Antinuclear Antibody (ANA) Test (Mouse Liver Sections). Any sera giving smooth muscle or mitochondrial staining reactions may be tested with the Autoantibody Test System (Mouse Kidney/ Stomach Sections).

NOTE: Anti-Islet Cell Antibody reactions are, by their nature, much weaker than reactions for ANA or most other immunofluorescence autoantibody reactions.



Note specific staining of the cytoplasm of the islet of Langerhans.