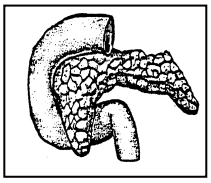
EXPORT ONLY VERSION

Isletest™-ICA

REF **7010**



Qualitative ELISA Test for the Detection of Circulating Autoantibodies Against Islet Cell Antigens

February 2018

BIOMERICA

I. INTRODUCTION AND INTENDED USE

Insulin-dependent diabetes mellitus (IDDM) or Type I Diabetes is a debilitating chronic disease that impairs production and secretion of the key hormone insulin and alters blood sugar metabolism. Insulin is synthesized and secreted by pancreatic islet cells or Islets of Langarhans[1]. The disruption of insulin synthesis is caused by immunological destruction of the islet cells by autoantibodies in IDDM patients[2-4]. Such abnormalities (autoimmunity) may be genetically inherited and/or triggered by exposure to toxic chemicals, viral infections and various forms of stress[5].

IDDM has a characteristic asymptomatic prediabetic phase that may last up to several years. During this period, the affected individuals exhibit the diminishing early-phase release of insulin in response to an intravenous/oral glucose challenge. In the majority of cases, these individuals carry circulating islet cell autoantibodies (ICA) and/or insulin autoantibodies (IAA). ICA can be detected as early as eight years prior to the clinical onset of IDDM[6] and thus may serve as an early indicator of the disease or predisposition to it. Individuals who are ICA-positive may show a progressive loss of the islet cell function as indicated by disruption of the early-phase insulin release. When this early phase insulin release completely stops, clinically overt IDDM develops[6].

Islet Cell Autoantibodies are present in 70% of patients with a recent onset of IDDM [13,14] compared with 0.1 - 0.5% of the control non-diabetic population [11,15]. ICA are also detected in first degree relatives of IDDM patients. These individuals comprise the segment of human population who are at a high risk of developing IDDM. Several studies reported that the ICA-positive first degree relatives of IDDM patients subsequently developed diabetes [16-19]. Other studies also suggested that the presence of serum ICA and IAA is an indicator of the enhanced likelihood to develop IDDM [3,6-12]. Therefore, serological detection of ICA may be a powerful tool for early diagnosis of IDDM. The significance of these autoantibodies as markers of IDDM is also illustrated by their presence in nondiabetic individuals who ultimately develop IDDM. Riley, et al. recently reported that determination of ICA in Type 2 Diabetes patients could identify IDDM prior to the onset of clinical symptoms and predict the need for insulin therapy [20]. Thus, those patients who are initially diagnosed with Type 2 Diabetes and carry serum ICA may deteriorate to insulin dependence.

An early detection of circulating ICA is important in order to identify the individuals in the general population, the siblings, and families of IDDM patients, who are at a higher risk of developing this disease because of their genetic predisposition to diabetes. At an international workshop on ICA, the imminent need for an ELISA test for the determination of islet cell autoimmunity was emphasized [21].

Currently, serum ICA are determined by indirect immuno-flourescence and histochemical methods employing frozen unfixed human/primate or rodent pancreatic sections as substrates. Despite various attempts to improve and modify this procedure since its original description in 1974 [22,23], the indirect immunoflourescence/histochemical technique suffers from inherent methodological problems. Standardization of the technique has proven to be very difficult. The reliability of this "frozen-section" technique is limited by factors such as the variation from one pancreas to another, the inevitable need for unfixed pancreatic tissue and infrequent availability of the suitable tissue.

Biomerica, Inc. has successfully identified a group of islet cell specific antigens that are recognized by serum ICA. Biomerica further purified this group of antigens and used them in a microwell-ELISA procedure to detect the presence of serum ICA.

Isletest™-ICA is a qualitative ELISA test for in vitro detection of circulating IgG antibodies against pancreatic islet cell antigens.

II. PRINCIPLE OF THE TEST

A purified mixture of pancreatic antigens is immobilized onto microwells. During an incubation period, antibodies in the serum sample are allowed to react at room temperature with antigen molecules on the microwells. After washing off excess/unbound serum materials, an enzyme (alkaline phosphatase) labeled goat antibody, specific to human IgG, is added to the antigen-antibody complex. After another thorough washing, a substrate (PNPP) is added and the color generated is measured spectro-photometrically. The intensity of the color is directly proportional to the concentration of ICA in the sample. An ICA-positive control serves as an internal quality control and ensures valid results.

III. WARNING AND PRECAUTIONS

All reagents provided with the kit are for *in vitro* diagnostic use only.

1. **Potential Biohazardous Material**

The matrix of the Calibrators and Controls is human serum. The human serum used has been found non-reactive to HbsAg, anti-HIV 1/2 and anti-HCV when tested with FDA licensed reagents. Because there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled as if potentially infectious.

2. **Sodium Azide**

Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper, or brass to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.

3. **Stopping Solution**

Stopping Solution consists of 1N NaOH. This is a strong base and should be handled with caution. It can cause burns and should be handled with gloves. Wear eye protection and appropriate protective clothing. Avoid inhalation. Dilute a spill with water before absorbing the spill with paper towels.

4. **Substrate Solution**

Substrate Solution consists of para-Nitrophenylphosphate (PNPP), a non-proteinaceous chromogenic substrate used in this ELISA test. On occasion substrate may display a yellowish color. This color will not interfere with test results.

Precautions

1. Do not freeze test reagents, store all kit components at 2-8°C at all times.
2. Positive and Negative Controls must be run each time the test is performed.
3. Use only clear serum as test specimens. The test sample should not have gross turbidity, hemolysis, or microbial contamination.
4. All samples should be analyzed in duplicate.
5. Do not mix reagents from different lots.
6. Do not use expired reagents.
7. Do not allow reagents to stand at room temperature for extended periods of time.
8. Do not expose substrate solution to light.
9. Careful pipetting technique is necessary for reproducible and accurate results.

IV. REAGENTS AND MATERIALS

Materials Supplied:

1. **PLA ICA** = ICA- Microwell Strips (with the holder) ..... 12 strips
2. **CONJ ENZ 6X** = ICA- IgG Enzyme Conjugate (conc.)..... 2 x 1.0 ml
3. **DIL SPE 5X** = Isletest Sample Diluent (concentrate)..... 1 x 25.0 ml
4. **CONJ ENZ DIL** = Isletest Conjugate Diluent ..... 1 x 10.0 ml
5. **CTRL REF ICA** = ICA – Reference Control ..... 1 x 1.5 ml
6. **CTRL + ICA** = ICA- Positive Control (human serum)..... 1 x 1.5 ml
7. **CTRL – ICA** = ICA- Negative Control (human serum)..... 1 x 1.5 ml
8. **SUBS PNPP** = Isletest Substrate Solution (PNPP) ..... 1 x 15.0 ml
9. **BUF WASH 25X** = Isletest Washing Buffer (concentrate) ..... 1 x 20.0 ml
10. **SOLN STP** = Isletest Stopping Solution (1N NaOH)..... 1 x 6.0 ml

V. ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Distilled or deionized water.
2. Absorbent paper towels to blot dry the strips after washing and parafilm/plastic wraps to cover strips during incubations.
3. Suitable sized glass tubes for serum dilution.
4. Micropipette with disposable tips to deliver 10 µl, 50 µl, and 100 µl.
5. A microtiter plate washer or a squeeze bottle for washing.
6. 5 ml pipettes for conjugate diluent delivery.
7. A 500 ml graduate cylinder.
8. Microtiter plate reader with 405 nm absorbance capability.
9. Plastic label tape, to tape unused wells before assay.

VI. SPECIMEN COLLECTION

Collect 5-10 ml of blood by venipuncture into a clot (red top) tube. Serum separators may be used. Separate serum by centrifugation. Serum samples may be stored at 2-8°C. Excessive hemolysis and the presence of large clots or microbial growth in the test specimen may interfere with the performance of the test. Freeze the serum sample at -20°C if it cannot be analyzed within 24 hours.

VII. REAGENT PREPARATION AND STORAGE

1. **ICA-IgG Enzyme Conjugate Reconstitution**:

Accurately transfer 5 ml of the Conjugate Diluent into one bottle containing the ICA-IgG Enzyme Conjugate (concentrate). Close the bottle and mix thoroughly by inversions. Store the diluted conjugate at 2-8°C when not in use. Record the date of reconstitution on the label**. This diluted reagent expires 30 days after reconstitution.** Two bottles containing the conjugate concentrate are provided. Each bottle contains enough conjugate for 6 strips. Reconstitute as needed.

2. **Isletest Sample Diluent Buffer:**

If precipitate is present in the sample diluent buffer concentrate due to storage at lower temperature such as 2-8°C, dissolve by placing the vial in a 37°C water bath for 30 minutes. Transfer the entire contents (25ml) into 100 ml of distilled/deionized water in a suitable container. Mix thoroughly; label the container as Isletest Sample Diluent, and store at 2-8°C. The diluted reagent is stable until the expiration shown on the vial. Please note that the precipitate seen in the concentrate has no affect on the performance of the test and will not be present in the 1X working solution.

3. **Isletest Wash Solution:**

If crystals are present in the Wash Buffer concentrate due to storage at a lower temperature such as 2-8°C, dissolve by placing the vial in a 37°C water bath or incubator for 30 minutes. Transfer the entire contents into 480 ml of distilled/deionized water in a 500 ml container. Mix thoroughly; label the container as Isletest wash, and store at 2-8°C. The diluted reagent is stable until the expiration shown on the vial.

4. **Serum Sample Preparation:**

Accurately pipette 10 µl (0.010 ml) of serum sample into 1.0 ml of the Working Sample Diluent into an already labeled glass tube. Mix thoroughly.

VIII. ASSAY PROCEDURE

The test kit contains 12 microwell strips coated with purified islet cell antigens. The number of microwell strips used in each assay depends upon the number of serum samples to be tested. If 12 microwell strips are used, a total of 45 sample sera can be tested in duplicate with this kit.

IMPORTANT NOTE: Bring all the reagents, including serum samples, to room temperature (25°C) before starting the assay Incubation temperatures varying by greater than ± 1°C can definitely affect results.

1. Assemble the number of microwell strips needed for the test in the holder provided. The microwell strip must be snapped in place firmly or it may fall out and break.
2. Familiarize yourself with the indexing system of wells, e.g. well #A1, B1, C1, D1, etc.

3. Dispense 100 µl of Negative Control into microwells C1 and D1.
4. Dispense 100 µl of Positive Control into microwells E1 and F1.
5. Dispense 100 µl of Reference Control into microwells G1 and H1.
6. Add 100 µl of diluted sample serum (see #4, Section VII, Reagent Preparation) to microwells starting from A2 and B2. For more patient samples, use additional strips and add other diluted patient samples to microwells in duplicate. There should be 100 µl of solution in each microwell to be assayed except A1 and B1 which are empty at this point and will be used later.
7. Any strips not used should be properly stored with desiccant in the zip lock bag provided for the next run. Any wells not used on the strip should be properly covered and saved for the next run.
8. Cover the plate with a parafilm/plastic wrap (to prevent contamination) and leave for 1 hour at room temperature (25° ± 1°C).
9. After incubation, discard the solution into sink by quick decantation and blot the plate dry by tapping gently onto a paper towel. If an automatic plate washer is being used, wash each well 3 times with 300 µl (0.3 ml) of the Wash Solution. If a squeeze bottle is used, fill the wells with the Wash Solution carefully and decant the buffer from the microwells. Repeat the procedure two more times and blot the plate dry with a paper towel.
10. Add 100 µl of ICA-IgG Enzyme Conjugate reagent (see #1, Section VII, Reagent Preparation) to all microwells except wells A1 and B1.
11. Cover the plate with a parafilm/plastic wrap and let it stand at room temperature (25°±1°C) for one hour.
12. After incubation, repeat the washing step (step #9) and blot the microwells dry.
13. Add 0.1 ml (100 µl) of Substrate Solution to all microwells including wells A1 and B1. Be sure to dispense the substrate reagent at a rapid steady pace without any interruption. On occasion substrate may display a yellowish color. This color will not interfere with test results.
14. Cover the plate and leave it in the dark for 30 minutes at room temperature (25° ± 1°C).
15. After 30 minutes promptly add 50 µl of the Stopping Solution into each well at a rapid steady pace without any interruption.
16. Set up microplate reader to read the absorbance at 405 nm according to manufacturing instructions, and blank the plate reader with well A1 or B1.
17. Calculate the data according to Section IX.

IX. CALCULATION OF DATA

Record the spectrophotometric readings [optical density (OD) in absorbance units] as shown in the example Isletest ICA Sample Data. The actual OD reading from your Isletest-ICA may be different. This is only an example.

1. Calculate the average O.D. reading of the Reference, Negative and Positive Controls and Patient samples done in duplicate.
   Average OD: Reference $(\bar{R})$, Negative $(\bar{N})$, Positive $(\bar{P})$, Samples $(\bar{S})$
2. Divide the average O.D. of Samples and Controls by the $\bar{R}$ value. This gives a Ratio Value for each sample.

Interpretations:

ICA Ratio Value (U/mL)	Result
< 0.95	Negative
> 1.05	Positive
0.95 – 1.05	Indeterminate (Borderline)

Samples with Ratio values < 0.95 show a low level of ICA antibodies, value > 1.05 show a high level of ICA antibodies. Samples with values between 0.95 and 1.05 are considered as indeterminate. The suggestion is to repeat indeterminate samples or to run in parallel with a new sample taken at a later date.

ISLETEST™ - ICA SAMPLE DATA

Section A: Control Results

Data			Ratio Value	Result
Controls	**O.D.**	**Ave. O.D.**		
Reference Ctrl	1.072	$\bar{R}$ = 1.082	1.00	
	1.092			
Negative Ctrl	0.290	$\bar{N}$ = 0.297	0.27	Negative
	0.303			
Positive Ctrl	1.413	$\bar{P}$ = 1.409	1.30	Positive
	1.406			

Note: For a valid test, Ratio Value, $\bar{N}$ < 0.95 and $\bar{P}$ > 1.05
Repeat the test if results are not valid.

Section B: Patient Sample Results

Data				
Sample	**O.D.**	**Ave. O.D.**	**Ratio Value**	**Result**
Reference Ctrl	1.072	$\bar{R}$ = 1.082	1.00	
	1.092			
1	1.444	$\bar{S}_1$ = 1.458	1.35	Positive
	1.472			
2	0.549	$\bar{S}_2$ = 0.541	0.50	Negative
	0.534			
3	1.036	$\bar{S}_3$ = 1.043	0.96	Indeterminate
	1.051			

X. QUALITY CONTROL

Negative and Positive Controls must be run along with unknown samples each time in order for the results to be valid. The Negative Control should show a ratio value < 0.95 Units/ml and the Positive Control should show a value > 1.05 Units/ml.

XI. PERFORMANCE CHARACTERISTICS

The specificity of antigen coated Isletest microwell strips was established by Western blot analysis using confirmed positive samples for IgG to Islet Cell Antigens. Samples with thyroid autoantibodies and rheumatoid factors read negative on Isletest ICA.

XII. CLINICAL SIGNIFICANCE

This *in vitro* test procedure detects the presence of ICA antibodies in patient sera. Results obtained by using this procedure alone must not be used for the diagnosis of IDDM.

Save the weak positive and borderline samples (within 5 % of the Reference Control O.D.) and store at -20°C. Fresh samples from

these patients should be tested again every six months together with the previous serum samples.

THIS IS A SCREENING TEST ONLY. THE DIAGNOSIS OF IDDM SHOULD BE MADE WITH DATA FROM THE PATIENT'S MEDICAL HISTORY, CLINICAL SYMPTOMS, AND RESULTS OF OTHER TESTS.

XIII. LIMITATIONS AND SOURCES OF ERROR

1. Although a higher ICA titer will produce a higher O.D. reading, the test is designed for qualitative determination of ICA only.

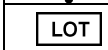
2. Poor test reproducibility may result from:

   a. Inconsistent delivery of reagents;
   b. Improper storage of reagents;
   c. Improper reconstitution of reagents;
   d. Incomplete washing of microwells;
   e. Substrate reagent old or exposed to light;
   f. Unstable/defective spectrophotometer;
   g. Error in following the assay procedure.

XIV. LITERATURE

1. Orci, L., J. Vassali, and A. Parrelet (1988). The insulin factory. *Sci. Amer*., **261**:85-94.
2. Eisenbarth, G.S. (1985). Type I diabetes: A chronic autoimmune disease. *New Engl. J. Med*., **314**:1360-1368.
3. Eisenbarth, G.S., J. Connelly, and J.S. Soeldner (1987). The natural history of Type I diabetes. *Diabet./Metab. Rev*., **3**:873-891.
4. Etzioni, A. (1987). Immunological concepts in insulin-dependent (Type I) Diabetes Mellitus *Immunol Res*., **6**:224-251.
5. Rossini, A.A., J.P. Mordes, and E.S. Handler (1989). A "Tumbler" hypotheses: The autoimmunity of insulin-dependent diabetes mellitus. *Diabet. Spect*., **2**:195-201.
6. Soeldner, J.S. (1985). The prodermal phase of insulin-dependent diabetes. In: Current Medical Literature - *Diabetes*, **2**:66-70.
7. Colman, P.G., R.C. Nayak, J. Connelly, A. Rabizadeh, J.S. Soeldner, and G.S. Eisenbarth (1986). Islet cell antibodies: Clinical utility and target antigens. In: *The Immunology of Diabetes Mellitus* (Jaworski, M.A. ed.). Elsevier, NY, 345-350.
8. Palmer, J.P. (1987). Insulin autoantibodies: Their role in pathogenesis of IDDM. *Diabet./Metab. Rev*., **3**:1005-1015.
9. Srikanta, S., A.T. Ricker, D.K. McCulloch, J.S. Soeldner, G.S. Eisenbarth, and J.P. Palmer (1986). Autoimmunity to insulin, beta cell dysfunction, and development of insulin-dependent diabetes mellitus. *Diabete*s, **35**:139-142.
10. Dean, B.M., F. Becker, J.M. McNally, A.C. Tarn, G. Schwart, E.A.M. Gale, and Bottazo, G.F. (1986). Insulin autoantibodies in the pre-diabetic period: correlation with islet cell antibodies and development of diabetes. *Diabetologi*a, **29**:339-342.
11. Irvine, W.J., R.S. Gray, and J.M. Steel (1980). Islet cell antibody as a marker for early stage Type I diabetes. In: *The immunology of diabetes* (Irvine, W.J., ed.), Teviot, Edinburgh, pp. 117-154.
12. Kamalesh, M., A. Karasik, and S. Srikanta (1986). Islet cell antibodies as predictors of insulin-dependent diabetes mellitus. *Pract. Cardiol*., **12**:79-91.
13. Irvine, W.J., C.J. McCallum, R.S. Gray, C.J. Campbell, J.J.P. Duncan, J.W. Faraquar, H. Vaughan, and P.J. Morris (1977). Pancreatic islet cell antibodies in diabetes mellitus correlated with duration and type of diabetes, coexistant autoimmune disease and HLA type. *Diabetes*, **26**:138-147.
14. Landrum, R., G. Walker, A.G. Cudworth, C. Theophanides, D.A. Pyke, A.J. Bloom, and D.R. Gambler (1976). Islet cell antibodies in diabetes mellitus. *Lancet*, **2**:1273-1276.
15. Riley, W. and N. MacLaren (1984). Islet cell antibodies are seldom transient. *Lancet*, **1**:1351-1352.
16. Soeldner, J.S., M. Tuttleman, S. Srikanta, O.P. Ganda, and G.S. Eisenbarth (1985). Insulin-dependent diabetes and initiation of autoimmunity: Islet cell autoantibodies, insulin autoantibodies and beta cell function. *New Engl. J. Med*., **313**:893-900.
17. Battazo, G.F., R. Pujol-Barrell, and E. Gale. In: *The Diabetes Annual* (K.G.M.M. Alberti and L.P. Krall, eds.), Elsevier, NY, Oxford, **1**:16-52.
18. Srikanta, S., O.P. Ganda, R.A. Jackson, R.E. Gleason, M.E. Kaldany, M.R. Garovoy, E.L. Milford, C.B. Carpenter, J.S. Soeldner, and G.S. Eisenbarth (1983). Type I diabetes mellitus in monozygotic twins: chronic progressive beta cell dysfunction. *Annals Int. Med*., **99**:320-326.
19. Srikanta, S., O.P. Ganda, A. Rabizadeh, J.S. Soeldner, and G.S. Eisenbarth (1985). First-degree relatives of patients with Type I diabetes mellitus: Islet cell antibodies and abnormal insulin secretion. *New Engl. J. Med*., **313**:461-464.
20. Riley, W.J., N.K. MacLaren, and J.H. Silverstein (1988). The predictability of insulin-dependent diabetes mellitus. Adv. Pediatr., **35**:167-187.
21. Boitard, C., E. Bonifacio, G.F. Bottazo, H. Gleichman, and J. Molenaar (1988). Immunology and diabetes workshop: Report on the third international (stage 3) workshop on standardization of cytoplasmic islet cell antibodies. *Diabetologi*a, **31**:451-452.
22. Bottazo, G.F., A. Florin-Christensen, and D. Doniach (1974). Islet cell antibodies in diabetes mellitus with polyendocrine deficiencies. *Lancet*, **II**:1279-1283.
23. Srikanta, S., A. Rabizadeh, M.A.K. Omar, and G.S. Eisenbarth (1980). Assay for islet cell antibodies: Protein A-monoclonal antibody method. *Diabetes*, **34**:300-305.

XV. SYMBOLS

	Storage Temperature
	Lot Code
	Expiration
	Manufacturer
	Authorized Representative
	Caution, see instructions
	For in vitro diagnostic use
	Catalog No.

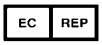
XVI. ORDERING INFORMATION

Contact:

BIOMERICA, INC.
17571 Von Karman Avenue
Irvine, California 92614

Toll Free (800) 854-3002 Within the U.S.
Phone: (949) 645-2111
FAX: (949) 553-1231
E-Mail: bmra@biomerica.com

67010-07_eng.doc February 2018



according to IVDD 98/79/ EC
MDSS GmbH
Schiffgraben 41
D-30175 Hannover
Germany