ICA (Islet Cell Antibody) ELISA

For the qualitative determination of circulating IgG antibodies against pancreatic islet cell antigens

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 21-ICAHU-E01
Size: 96 wells
Version: February 2018 - ALPCO November 9, 2018

I. INTENDED USE AND INTRODUCTION

The ICA (Islet Cell Antibody) ELISA is a qualitative ELISA test for detection of circulating IgG antibodies against pancreatic islet cell antigens. *This kit is for research use only. It is not for use in diagnostic procedures.*

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 Langerhans [1]. The disruption of insulin synthesis is caused by immunological destruction of the islet cells by autoantibodies in IDDM [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].

Research has shown IDDM has a characteristic asymptomatic prediabetic phase that may last up to several years. During this period, studies have shown 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). Research indicates that ICA can be detected as early as eight years prior to the 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. Studies suggest that when this early phase insulin release completely stops, overt IDDM develops [6].

Islet Cell Autoantibodies are present in 70% of individuals 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 individuals. 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 individuals 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 researching IDDM development. The research 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 Diabetics could identify IDDM prior to the onset and predict the need for insulin therapy [20]. The research suggests that individuals with Type 2 Diabetes that carry serum ICA may deteriorate to insulin dependence.

Currently, serum ICA are determined by indirect immunoflourescence 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.

The ICA (Islet Cell Antibody) ELISA uses a purified group of islet cell specific antigens in a microwell-ELISA procedure to detect the presence of serum ICA.

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 spectrophotometrically. 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. WARNINGS AND PRECAUTIONS

All reagents provided with the kit are for research use only.

1. **Potentially Biohazardous Material**
   The matrix of the Calibrators and Controls is human serum. The human serum used has been found to be 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, Hepatitis C, 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 in plumbing to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.

3. **Stop Solution**
   Stop Solution consists of 1 N 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 spills with water before absorbing with paper towels.

4. **Substrate Solution**
   Substrate Solution consists of para-Nitrophenylphosphate (PNPP), a non-proteinaceous chromogenic substrate used in this ELISA. On occasion the substrate may display a yellowish color. This color will not interfere with the assay 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 assay is performed.
3. Use only clear serum as test samples. 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 (concentrate) — 2 x 1.0 mL
3. **DIL SPE 5X** = Sample Diluent (concentrate) — 1 x 25.0 mL
4. **CONJ ENZ DIL** = Conjugate Diluent — 1 x 10.0 mL
5. **CTRL REF ICA** = Reference Control — 1 x 1.5 mL
6. **CTRL + ICA** = Positive Control (human serum) — 1 x 1.5 mL
7. **CTRL – ICA** = Negative Control (human serum) — 1 x 1.5 mL
8. **SUBS PNPP** = Substrate Solution (PNPP) — 1 x 15.0 mL
9. **BUF WASH 25X** = Wash Buffer (concentrate) — 1 x 20.0 mL
10. **SOLN STP** = Stop Solution (1 N NaOH) — 1 x 6.0 mL

V. ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Absorbent paper towels to blot dry the strips after washing and parafilm/plastic wraps to cover strips during incubations
- Suitable sized glass tubes for serum dilution
- Micropipet with disposable tips to deliver 10 µL, 50 µL, and 100 µL
- Microtiter plate washer or a squeeze bottle for washing
- 5 mL pipettes for conjugate diluent delivery
- 500 mL graduated cylinder
- Microtiter plate reader with 405 nm absorbance capability
- Plastic label tape, to tape unused wells before assay
- Timer

VI. SAMPLE 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 sample may interfere with the performance of the assay. Freeze the serum sample at -20°C if it cannot be analyzed within 24 hours.

VII. REAGENT PREPARATION AND STORAGE

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

2. **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 (25 mL) into 100 mL of distilled/deionized water in a suitable container. Mix thoroughly; label the container as Sample Diluent, and store at 2-8°C. The diluted reagent is stable until the expiry shown on the vial. Please note that the precipitate seen in the concentrate has no effect on the performance of the assay and will not be present in the 1X working solution.

3. **Wash Solution:**
   If crystals are present in the Wash Buffer concrete 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 into 480 mL of distilled/deionized water in a suitable container. Mix thoroughly; label the container as wash, and store at 2-8°C. The diluted reagent is stable until the expiry shown on the vial.

4. **Serum Sample Preparation:**
   Accurately pipet 10 µL (0.010 mL) of serum sample into 1.0 mL of the Working Sample Diluent in an already labeled glass tube. Mix thoroughly.

VIII. ASSAY PROCEDURE

The 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 assayed. 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 affect the 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. Get familiarized with the indexing system of wells, e.g., wells 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 A2 and B2. For more samples, use additional strips and add diluted 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 ziplock bag provided 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 a 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 onto 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 (60 minutes).
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 the assay 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 Stop 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 ICA Sample Data example below.

1. Calculate the average OD reading from the duplicates of the Reference Control, Negative Control, Positive Control, and samples.

   Average OD: Reference (R_{AVE}), Negative (N_{AVE}), Positive (P_{AVE}), Samples (S_{AVE})

2. Divide the average OD of the Samples and the Positive and Negative Controls by the (R_{AVE}) value. This yields a Ratio Value for each.

Interpretation:

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, Ratio Values > 1.05 show a high level of ICA antibodies. Samples with Ratio Values between 0.95 and 1.05 are considered indeterminate. It is recommended to assay indeterminate samples again or to run them in parallel with a new sample taken at a later date.

ICA SAMPLE DATA

Section A: Control Results

Data				
Controls	**OD**	**Avg. OD**	**Ratio Value**	**Result**
Reference Control	1.072 1.092	R_{AVE} = 1.082	1.00	
Negative Control	0.290 0.303	N_{AVE} = 0.297	0.27	Negative
Positive Control	1.413 1.406	P_{AVE} = 1.409	1.30	Positive

Note: For a valid assay, the Ratio Value of the N_{AVE} should be <0.95 and the Ratio Value of the P_{AVE} should be >1.05. Repeat the assay if the results are not valid.

Section B: Sample Results

Data				
Controls	**OD**	**Avg. OD**	**Ratio Value**	**Result**
Reference Control	1.072 1.092	R_{AVE} = 1.082	1.00	
1	1.44 1.472	$S1_{AVE}$ = 1.458	1.35	Positive
2	0.549 0.534	$S2_{AVE}$ = 0.541	0.50	Negative
3	1.036 1.051	$S3_{AVE}$ = 1.043	0.96	Indeterminate

X. QUALITY CONTROL

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

XI.   PERFORMANCE CHARACTERISTICS

The analytical specificity of the kit's antigen coated microwell strips was established by Western blot analysis that used confirmed positive samples for IgG to Islet Cell Antigens. Samples with thyroid autoantibodies and rheumatoid factors read negative on this ICA ELISA.

XII. LIMITATIONS AND SOURCES OF ERROR

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

2. Poor test reproducibility may result from:
   a) Inconsistent delivery of reagents
   b) Improper storage of reagents
   c) Improper dilution of reagents
   d) Incomplete washing of microwells
   e) Substrate reagent that is old or exposed to light
   f) Unstable/defective spectrophotometer
   g) Error in following the assay procedure

XIII. REFERENCES

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