Insulin Autoantibody ELISA (IAA ELISA)

For the qualitative determination of IgG antibodies to insulin in serum

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

Catalog Number: 21-IAAHU-E01
Size: 96 wells
1. **INTRODUCTION AND INTENDED USE**

The IAA test detects the presence of specific human IgG to human insulin. It is a screening test which, together with other clinical information, may be useful as an aid to the research of Type I Diabetes. This kit is for research use only.

Insulin-dependent diabetes mellitus (IDDM) or Type I Diabetes is an autoimmune disease in which insulin deficiency is a consequence of immunological destruction of the pancreatic beta cells. In individuals who are genetically predisposed to IDDM, the immunological attack on the beta cells occurs during an asymptomatic period (1) which is referred to as the "Prediabetic Phase". This prediabetic phase usually begins several years before the clinical onset of IDDM. During this phase, autoantibodies directed against pancreatic islet cell antigens (ICA) and/or insulin (IAA) are detected in the blood of many prediabetic subjects.

The most sensitive method currently being used for determination of IAA in human serum employs a radiometric competitive assay (15). The IAA is an enzyme-linked immunosorbant assay (ELISA) for IAA determination. It is simple to use and does not require the use of radioactive materials. The IAA is intended for research use detection of circulating autoantibodies against human insulin.

2. **PRINCIPLE OF THE TEST**

Human insulin is immobilized onto microwells. The reference, positive, and negative controls, and diluted serum samples are added to the appropriate microwells. Human IgG specific antibodies to insulin in the serum sample and controls bind to the insulin molecules on the microwells. After washing off unreacted serum materials, an enzyme (alkaline phosphatase) labeled goat antibody specific to human IgG is added to the antigen-antibody complex. After thorough washing to remove the unbound enzyme, a substrate (PNPP) solution is added and the color development is measured spectrophotometrically. The intensity of the color is directly proportional to the concentration of IAA in the sample. Two quality controls (positive and negative) are provided to monitor and validate assay results.

3. **WARNINGS AND PRECAUTIONS**

All reagents provided with the kit are for research 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. **Stop Solution**

   Stop 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.
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.

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

5. 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 wrap 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.

6. 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.

7. REAGENT PREPARATION AND STORAGE
1. IAA-IgG Enzyme Conjugate Reconstitution:
Accurately transfer 5 ml of the Conjugate Diluent into one bottle containing IAA-IgG Enzyme Conjugate (concentrate). Close the bottle and mix thoroughly by inversion. Store the diluted conjugate at 2-8°C at all times. Record the date of reconstitution on the label.
This diluted reagent expires 30 days after reconstitution. Each of the two conjugate (concentrate) bottles is sufficient for 6 strips. Reconstitute as needed.

2. IAA-Sample Diluent Buffer:
Transfer the entire contents (25ml) into 100 ml of distilled/deionized water in a suitable container. Mix thoroughly; label the container as IAA-Sample Diluent, and store at 2-8°C. The diluted reagent is stable until the expiration shown on the vial.

3. Wash Solution:
If crystals are present in the Wash Buffer concentrate due to storage at a lower temperature such as 2-8°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 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 in an already labeled glass tube. Mix thoroughly.

8. ASSAY PROCEDURE
The test kit contains 12 microwell strips coated with human insulin. 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 samples 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 strips needed for a test run in the holder provided. The microwell strip must be snapped firmly in place or it may fall out and break.
2. Familiarize yourself with the indexing system of wells, e.g. well #A1, B1, C1, D1, etc. and label the strips used with a marking pen.
3. Dispense 100 µl of IAA-Reference Control into microwells C1 and D1
4. Dispense 100 µl of IAA-Negative Control into microwells E1 and F1.
5. Dispense 100 µl of IAA-Positive Control into microwells G1 and H1.
6. Add 100 µl of diluted sample (see #4, Section 7 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 wells not used on the strip should be properly covered and saved for the next run. Any well strips not used should be stored with the desiccant in the ziplock bag provided at 2-8°C for the next run.
8. Cover the plate with parafilm/plastic wrap (to prevent contamination) and leave at 2-8°C overnight (12-16 hrs.).
9. The next morning, discard the solution into sink by quick decantation. Blot the plate dry by tapping gently on a paper towel. If an automatic plate washer is being used, wash each well 3 times with 300 µl of the Wash Solution (prepared under Reagent Preparation Section 7, #3). If a squeeze bottle is used, fill the wells with 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 IAA-IgG Enzyme Conjugate reagent (see #1, Reagent Preparation Section 7) to all microwells except wells A1 and B1.
11. Cover the plate with parafilm/plastic wrap and let it stand at 25° ± 1°C for one hour.
12. After incubation, repeat the washing step (step #9) and blot dry the microwells.
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.
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 the Calculation of Data section 9.

9. CALCULATION OF DATA

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

1. Calculate the average O.D. reading of the Reference, Negative and Positive Controls and samples done in duplicate.
   Average OD: Reference \((R)\), Negative \((N)\), Positive \((P)\), Samples \((S)\)

2. Divide the average O.D. of Samples and Controls by the average Reference O.D. value \((R)\). This gives a Ratio Value for each sample.

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

IAA SAMPLE DATA

Section A: Control Results

<table>
<thead>
<tr>
<th>Data (Net O.D.)</th>
<th>O.D.</th>
<th>Avg. O.D.</th>
<th>Ratio Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference Control</td>
<td>1.224</td>
<td><strong>R = 1.247</strong></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.271</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.498</td>
<td><strong>N = 0.481</strong></td>
<td>0.39</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>0.464</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>1.855</td>
<td><strong>P = 1.809</strong></td>
<td>1.45</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>1.764</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Section B: Sample Results
<table>
<thead>
<tr>
<th>Sample</th>
<th>O.D.</th>
<th>Avg. O.D.</th>
<th>Ratio Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Control</td>
<td>1.224</td>
<td>$R = 1.247$</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.994</td>
<td>$S_1 = 2.002$</td>
<td>1.61</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>0.540</td>
<td>$S_2 = 0.541$</td>
<td>0.43</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>1.280</td>
<td>$S_3 = 1.270$</td>
<td>1.02</td>
<td>Indeterminate</td>
</tr>
</tbody>
</table>

10. 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.

11. PERFORMANCE CHARACTERISTICS

The IAA is a qualitative test designed to detect the presence of circulating autoantibodies to human insulin. The antigen coated on the wells does not react with other autoantibodies such as islet cell autoantibodies, anti-thyroglobulin and anti-rheumatoid factor.

12. SIGNIFICANCE

This research use only test procedure detects the presence of human insulin autoantibodies in sample sera.

13. LIMITATIONS AND SOURCES OF ERROR

1. This a qualitative screening test only.

2. Old substrate solution may give high background color.

3. Poor reproducibility may result from:
   
   a. Inconsistent delivery.
   b. Improper storage of reagents.
   c. Improper reconstitution of reagents.
   d. Faulty washing of microwells.
   e. Inconsistent/defective instrument.
   f. Using outdated reagents.

Therefore, it is very essential that the instructions are followed carefully and consistently. For better reproducibility, test conditions and test equipment should not vary extremely.
14. LITERATURE


