ANTI- ISLET CELL ANTIBODY IFA TEST SYSTEM

48 Tests
Store kit at +2 to +8°C

INTENDED USE
These reagents are intended for use in the detection and quantitation of IgG antibody in human sera to monkey pancreas islet cells by the indirect fluorescent antibody (IFA) procedure. The Anti-islet Cell Antibody IFA Test System is not to be used for diagnostic purposes and is intended to be used only when the actual diagnosis is based on an established method or procedure including clinical findings. For Export Only. The test system is for Professional Use Only.

SUMMARY AND PRINCIPLES
Demonstration of islet cell antibody (ICA) by utilizing the indirect fluorescent antibody method enables serologic assessment or possible detection of pancreatic disease. The presence of a (histologically defined) circulating antibody to one or more of the islet cell antigens can aid in patient diagnosis and prognosis. The substrate utilized in this kit is sections of monkey pancreas.

Islet cell antibodies have been associated with a group of “autoimmune” endocrine disorders, more specifically with insulin dependent diabetes. Organ-specific autoimmunity is characterized by the presence of antibodies in patients that can be detected years before the onset of the clinical symptoms. These antibodies are useful monitors to detect well before metabolic tests can detect hemoglobin deficiencies.

Patients with autoimmune thyroiditis, adrenitis or gastritis have an increased risk of developing insulin dependent diabetes at any age. Overlapping of antibodies is one of the most important features in this group of disorders. The extreme situation is the “polyendocrine” syndrome where all the endocrine glands may be involved in the same patient. Since the discovery of the islet-cell antibodies in insulin dependent diabetes there has been growing interest as to their significance. Overlapping between disorders has been recognized clinically for over 60 years, with the need to screen for these antibodies gaining more attention.

So far, islet-cell antibodies have only been detected in association with overt autoimmunity, almost exclusively in insulin dependent diabetes, sometimes before onset as well as after the patient has been diagnosed. In these cases single or polyglandular autoimmune disorders coexist. This discovery lends strong credence to the concept of a true form of autoimmune diabetes mellitus. These islet cell antibodies may prove to be a marker for identifying autoimmune diabetes.

The indirect fluorescent antibody test is used for the detection of human IgG antibody to the antigens of monkey pancreas islet cells. Tissue is placed in the wells of specially prepared microscope slides. Dilutions of patient sera are placed on the wells where antibody, if present, binds to the antigen. The reaction is visualized through the use of a conjugate. The conjugate is fluorescein isothiocyanate (FITC) labeled, anti-human IgG (gamma chain specific). Excitation of the FITC by ultraviolet (UV) light causes the conjugate to bind with human IgG antibodies attached to the antigens causing fluorescence when viewed through a microscope equipped with a UV light source.

PRECAUTIONS
1. Follow the procedure instructions exactly as they appear in this insert to ensure valid results.
2. Thimerosal (Mercurochrome), used as a preservative in some of the reagents, may be toxic if ingested, inhaled or absorbed through skin and is a reproductive hazard.
3. Some components contain less than 0.1% sodium azide, which is toxic if ingested and forms potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.
4. The phosphate buffered saline and mounting medium found in this kit are irritating to the eyes, respiratory system and skin.
5. Some components in this kit contains 0.1% Proclin 300. At full strength Proclin 300 is corrosive and will cause burns and possibly sensitization by skin contact.
6. The conjugate in this kit contains 0.0015% Evan’s Blue. Evan’s Blue is a possible carcinogen and may cause reproductive harm.
7. WARNING - POTENTIAL BIOHAZARDOUS MATERIAL. Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of antibody to HIV, as well as HBsAg, and found to be negative (were not repeatedly reactive). Because no test method can offer complete assurance that human immunodeficiency virus (HIV), hepatitis B virus, or other infectious agents are absent, these human control reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimens in the CDC/NICH manual “Biosafety in Microbiological and Biomedical Laboratories”, 1999 (3).
8. Slides and reagents should be stored at +2 to +8°C until used.
9. Do not use components beyond their expiration date.
10. Handle slides by the edge since direct pressure on the antigen wells may damage the antigen.
11. Once the procedure has started, do not allow the wells to dry.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Prod #</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-5504</td>
<td>Monkey Pancreas 4 Well Slides</td>
<td>12 ea</td>
</tr>
<tr>
<td>10-5502</td>
<td>Islet Cell Positive Control</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>10-1201</td>
<td>Autoimmune Negative Control</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>10-502</td>
<td>FITC IgG Conjugate, Primate Ads w/ Evan’s Blue</td>
<td>4.0 mL</td>
</tr>
<tr>
<td>90-1610</td>
<td>FITC Mounting Medium (pH 7.5)</td>
<td>3.0 mL</td>
</tr>
<tr>
<td>90-1607</td>
<td>Phosphate Buffered Saline (pH 7.5)</td>
<td>2x10 gm</td>
</tr>
<tr>
<td>90-1700</td>
<td>Coverslips, 70x22 mm</td>
<td>12 ea</td>
</tr>
<tr>
<td>90-1704</td>
<td>Blotters, 4 well</td>
<td>12 ea</td>
</tr>
</tbody>
</table>

PREPARATION OF REAGENTS
1. Allow all reagents to come to room temperature before use.
2. Reconstitute each 10 gram vial of PBS (Prod #1607) with 1.0 L distilled water.
3. Slides (Prod #10-5044), should be brought to room temperature prior to breaking the package seal. Peel back the top portion of the package and remove the slide without touching the antigen wells. The slide is now ready to use.
4. FITC IgG conjugate (Prod #10-1502) is provided at the recommended working dilution. Note: The conjugate may require retitration. Variations in absolute fluorescence between microscopes can be expected due to the variation in the optical sensitivity of the microscope components including light source, objective lenses, ocular lenses, total magnification, etc. If the controls consistently yield results higher or lower than expected, the conjugate may be require retitration. This is accomplished by retesting the controls at appropriate two-fold dilutions of the conjugate using PBS as a conjugate diluent. If retitration of conjugate is required, please call the MarDx technical support department for assistance.
5. The mounting medium (Prod #90-1610) is used at the concentration provided.

ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED
1. Test tubes, test tube rack, pipettes, or a microtiter system for preparing dilutions.
2. Volumetric flask (1 liter) for PBS.
3. Moist incubation chamber.
4. Slide washing chamber.
5. Fluorescence microscope with 40x objective lens and 10X ocular lenses. FITC filter assemblies at an excitation of 490 nm and emission of 520 nm.
7. Distilled water.

STORAGE AND STABILITY
1. Store kit at +2 to +8°C.
2. Islet Cell Positive Control (Prod #10-5502): Store at +2 to +8°C. Refer to expiration date on label.
3. Autoimmune Negative Control (Prod #10-1201): Store at +2 to +8°C. Refer to expiration date on label.
4. FITC Labeled Anti-Human IgG Conjugate with Evan’s Blue, Primate Adsorbed (Prod #10-1502): Store at +2 to +8°C. Refer to expiration date on label.
5. Phosphate Buffered Saline, pH 7.5 (Prod #90-1607): PBS is stable at room temperature in its non-reconstituted form. Refer to label for expiration date. PBS contains no preservative and should be stored at +2 to +8°C after it is reconstituted. Discard if turbidity develops.
6. FITC Mounting Medium, pH 7.5 (Prod #90-1610): Store at +2 to +8°C. Refer to the expiration date on label.

SPECIMEN COLLECTION
Seraological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2°C to 8°C if it is to be analyzed within 4-7 days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, additions of a preservative such as 0.01% thimerosal (merthiolate) or 0.1% sodium azide is strongly recommended. The CLSI provides recommendations for storing blood specimens (Approved Standard Procedure for the Handling and Processing of Blood Specimens, H18-A2 2005) (4).
PREPARATION OF CONTROLS
Include the positive, negative, and PBS controls in each run.
1. The Positive Control (Prod #10-5502) is supplied as a ready to use liquid. No reconstitution is necessary. Mix the control by vortex or inversion prior to use. Prepare dilutions using PBS. The positive control serum is standardized to give end point positive fluorescence at the dilution stated on the vial. Include in the test procedure the positive control at this dilution and one two-fold dilution above and below the expected end-point dilution. Refer to the vial label for the specified dilution end-point of each lot.
2. The negative control serum (Prod #1-1201) is supplied as a ready to use liquid. No reconstitution is necessary. Mix the control by vortex or inversion prior to use. The negative control is standardized to demonstrate a negative reaction when used undiluted. Include in the test undiluted negative control.
3. A PBS control may be run to establish that the conjugate is free from nonspecific staining of the antigen substrate.

PREPARATION OF SPECIMENS
Prepare neat and 1:4 (0.2 mL of serum into 0.6 mL of PBS) screening dilutions of patient sera. Note: Samples screening positive undiluted or at 1:4 should be titrated to end point by preparing two-fold serial dilutions. Mix equal volumes of diluted serum and PBS for subsequent two-fold dilutions.

TEST PROCEDURE
1. Remove the number of slides needed from the sealed pouches and mark them with a marking pen as necessary.
2. Add controls and diluted serum (approximately 25 µL) to wells.
3. Incubate slides in a moist chamber at room temperature for 30 minutes.
4. After incubation with sera the slides should be tapped onto a piece of paper towel in such a way as to prevent the serum of one well coming into contact with any of the other wells. Direct a gentle stream of PBS over the slide using a wash bottle. Do not aim the stream of PBS directly onto the wells.
5. Place the slides in a wash chamber filled with PBS for 5 minutes. Replace wash chamber with fresh PBS and wash slides for another 5 minutes.
6. Remove the slides from the PBS and place, antigen side up, on a dry paper towel. Carefully place the 4 well blotter over the slide, positioned so as not to come into contact with the reaction wells. Hold one edge of the blotter with one hand to keep the blotter in place and apply sufficient gentle pressure with the microscope slide roller to remove the moisture surrounding antigen wells. DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
7. Using dispenser provided, deliver 1 drop of conjugate per antigen well. The conjugate dispenser is provided with a calibrated tip and allows quantitative delivery of reagents from the storage bottle. To use, wipe the tip with a paper towel, invert the bottle and squeeze gently to release one drop. If the tip contains an air bubble, tap the bottle gently to remove air bubble which will ensure precise drop delivery.
8. Incubate slides as described above (#5).
9. Rinse, wash and blot slides as described above (#4, #5, #6). DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
10. Place 2 to 3 drops of mounting medium on slide and cover with a coverslip avoiding air bubbles.
11. Read slides with a fluorescence microscope.

READING SLIDES
1. Do not attempt to read the slides before the microscope has been switched on for at least 5 minutes.
2. Read slides within one hour. Slides may be read within 24 hours if stored refrigerated in a moist chamber. Allow refrigerated slides to warm to room temperature before reading.
3. The slides should be examined at a total magnification of 400X.
4. Drying may disturb the most peripherally situated antigen in the well, therefore disregard these reactions.
5. The staining intensity may vary, however, the degree of staining is based on the overall appearance of the antigen.
6. Record reaction intensity at each dilution using the following criteria:
   2+ to 4+ = moderate to strong yellow-green fluorescence
   1+ = Weak but definite yellow-green fluorescence
   Negative = Vaguely visible or no fluorescence
7. The titer is the reciprocal of the highest dilution showing 1+ or greater fluorescence.
8. Read the controls before proceeding to the test sera.

QUALITY CONTROL
1. The positive control serum must demonstrate end point positive fluorescence within one dilution of the dilution stated on the vial or the test is invalid.
2. The negative control serum must demonstrate the absence of yellow-green specific fluorescence or the test is invalid.
3. Reading of test serum end-points with each microscope assembly must be made with reference to the reactivities of the control sera with the slides and conjugate provided.
4. The PBS control, if included, must demonstrate the absence of yellow-green specific fluorescence or the test is invalid.

INTERPRETATION OF RESULTS
Anti-islet cell antibody activity is interpreted as positive even in undiluted serum. Positive reacting sera should be titrated to endpoint and reported as positive.
Consult Instructions for Use

Product Number
Lot Number
In Vitro Diagnostic Medical Device
Authorized Representative in the European Community
Use By
Caution, consult accompanying documents

Temperature limitation

Manufacturer

Irritant - Precaution

Negative Control
Islet Cell Positive Control
Conjugate
Phosphate Buffered Saline
Mounting Medium
Coverslips
Blotters, 4 Well
Slide, 4 Well

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