095%Introduction

Islet Cell antibodies have been associated with a group of "Autoimmune" endocrine disorders, more specifically with insulin dependent diabetes. Organ-specific autoimmunity (AI) is characterized by the presence of antibodies in patients that can be detected years before the onset of the clinical symptoms.[1] These antibodies are useful monitors to detect well before metabolic test can detect hormonal deficiencies. The situation becomes far more complex in the case of "stimulating" antibodies that produce hormonal excess, and hormonal receptor antibodies.

Patients with autoimmune thyroiditis, adrenalitis 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 (AI).[1] The extreme being "polyendocrine" syndromes where all the endocrine glands may be involved in the same patient. Since the discovery of the islet-cell antibodies in insulin dependent diabetes (IDDM) 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 disease coexists.[2] 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.[1,2]

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1502L (3.0 ml) is to be stored at 2-8 C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label.
2. The antigen slides of primate pancreas sections must be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.

3. Islet Cell positive control No. 5502L (0.5 ml) should be stored at 2-8 C upon receipt. Check label for specific expiration date.
4. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.
5. Buffer Pack No. 1601 - Phosphate Buffered Saline is stable at room temperature storage for 5 years. The reconstituted Buffer does not contain preservatives and should be stored at 2-8 C. Care should be taken to avoid contamination.
6. Mounting Medium No. 1610 is stable when stored at 2-8 C. Check label for specific expiration date.

Note: All kit components are available separately. Please see the current SCIMEDX Corporation Catalog for more details.

Additional Materials Required but not Provided:

Test tubes and rack or microtiter system
Disposable pipettes
Staining Dish and Slide Forceps
Moisture Chamber
Volumetric Flask (500 ml)
Distilled H_20
Fluorescence Microscope
Paper Towels - lint free

Reagent Preparation:

1. Buffer Pack No. 1601. Rehydrate buffer with 1 liter of sterile distilled water.

Specimen Collection:

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8 C if it is to be analyzed within a few 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, addition of a preservative such as 0.01% (thimerosal) or 0.095% sodium azide is strongly recommended.

Test Instruction:

Screening: dilute test serums 1:4 in PBS.

Titration: set up doubling dilutions of serum starting at 1:4, 1:8, 1:16, 1:32, etc.

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluted serum (20 to 30 µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24 C).
4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the well.
5. Wash in PBS for five minutes. Repeat using fresh PBS.
6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. **Do not allow tissue to dry.** Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.
7. Deliver 1 drop (25-30 µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

Quality Control:

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.
3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

Results:

Cytoplasmic immunofluorescence of the Islet Cells can be observed. The staining of the Islet cells may become more visible on titration. Due to overlapping autoimmune responses, the islet cell may appear masked at the lower screening dilution.

Limitations of Procedure

1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.
2. This test is for In Vitro Diagnostic Use.

Precautions

1. All human components have been tested by radioimmunoassay for (HB_sA_g) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB_sA_g or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to insure valid results.
5. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
6. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

BIBLIOGRAPHY:

1. Doniach, D.: Autoimmune Endocrine disorders: Hospital Update, Volume 9, No. 10 October 1983.
2. MacCuish, A.C., Irvine, W.J., Barnes, E.W., Ducan, L.J.P.: Antibodies to Pancreatic Islet Cells in Insulin-Dependent Diabetes with Coexistent Autoimmune Disease. The Lancet, Saturday, December 28th 1974.

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