Trinity Biotech

ANTI- ISLET CELL ANTIBODY IFA TEST SYSTEM

REF 10-5548 **48 Tests**

Store kit at +2 to +8°C

Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue
Dla innych języków

Para outras línguas
Για τις άλλες λώσσες
För andra språk
For andre språk

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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 humoral deficiencies.

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. 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 coexists. 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 this dye to emit longer, visible, wavelengths of light in the yellow-green portion of the color spectrum. The conjugate will 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 (Merthiolate), 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 contain 0.1% Proclin 300. At full strength Proclin 300 is corrosive and will cause burns and possibly sensitisation 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 specimen in the CDC/NIH 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.

The safety data sheet is available upon request.

WARNING
Some components of this kit contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
H317: May cause an allergic skin reaction.
H335: May cause respiratory irritation.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING
Some components of this kit contain < 0.1% sodium azide.
H302: Harmful if swallowed.
P264: Wash thoroughly with plenty of soap and water after handling.
P270: Do not eat, drink or smoke when using this product.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330: If swallowed, rinse mouth.
P501: Dispose of contents/container in accordance to local, regional, national and international regulations.

MATERIALS PROVIDED

Prod #	Description	Quantity
10-5504	Monkey Pancreas 4 Well Slides	12 ea.
10-5502	Islet Cell Positive Control	0.5 mL
10-1201	Autoimmune Negative Control	0.5 mL
10-1502	FITC IgG Conj., Primate Ads w/ Evan's Blue	4.0 mL
90-1610	FITC Mounting Medium (pH 7.5)	3.0 mL
90-1607	Phosphate Buffered Saline (pH 7.5)	2x10 gm
90-1700	Coverslips, 70x22 mm	12 ea.
90-1704	Blotters, 4 well	12 ea.

PREPARATION OF REAGENTS

1. Allow all reagents to come to room temperature before use.
2. Reconstitute each 10 gram vial of PBS (Prod #90-1607) with 1.0 L distilled water.
3. Slides (Prod #10-5504), 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 titrations.
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.
6. Microscope slide roller.
7. Distilled water.

STORAGE AND STABILITY

1. Monkey Pancreas 4 well Slides (Prod #10-5504): Store at +2 to +8°C. Slides are stable until their expiration date on the product label.
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

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°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 #10-1201) 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 titered 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 toweling 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 (#3).
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 titered to endpoint and reported as positive.

LIMITATIONS OF PROCEDURE

1. Light sources, total magnification, objective lenses, and ocular lenses influence intensity of staining. Variations in intensities may be observed when different microscope assemblies are used. Testing of sera should not be attempted unless the positive control serum gives the expected titer within one two-fold dilution and the negative control yields negative results.
2. The accuracy in the test often depends on the competency of the operator.
3. The patient clinical data and other laboratory tests should be carefully reviewed by a medical authority before a diagnosis is made.

REFERENCES

1. Doniach, D. 1983. Autoimmune Endocrine disorders: Hospital Update. Vol. 9, No 10.
2. MacCuish, AC, Irvine, WJ, Barnes, EW, Ducan, LJP: 1974 Antibodies to Pancreatic Islet Cells in Insulin Dependent Diabetes with Coexistent Autoimmune Disease. The Lancet.
3. Centers for Disease Control/National Institutes of Health (CDC-NIH) Manual. 1999. In: *Biosafety in Microbiological and Biomedical Laboratories,* 4th Edition, U.S. Dept. of Health and Human Services, Public Health Service.
4. Clinical Laboratory Standards Institute (CLSI). 2005. Procedures for the Handling and Processing of Blood Specimens; Approved Guideline - Second Edition, CLSI Publication H18-A2.

Consult Instructions for Use

REF	Product Number
LOT	Lot Number
IVD	*In Vitro* Diagnostic Medical Device
EC REP	Authorized Representative in the European Community
	Use By
	Caution, consult accompanying documents
	Temperature limitation
	Manufacturer
 or 	WARNING
CONTROL -	Negative Control
CONTROL IC +	Islet Cell Positive Control
CONJ	Conjugate
PBS	Phosphate Buffered Saline
MTMED	Mounting Medium
CVRSLP	Coverslips
BLTRS 4W	Blotters, 4 Well
SLD 4W	Slide, 4 Well

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10-5548-29-Rev. 4
05/2015