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 controls should result in bright 3+ to 4+ fluorescence. If these controls show 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 non-specific 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.

TEST LIMITATIONS

1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.

LITERATURE REFERENCES

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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INSTRUCTIONS FOR USE

Anti-Islet Cell Antibody Test System

K4815 - 48 Tests
K9615 - 96 Tests
K5015 – 50 Tests
K0015 – 100 Tests

Also for: **Monkey pancreas slides**
S4211 - 4 well
S8211 - 8 well
S5211 - 5 well
S0211 - 10 well

INTENDED USE

The Bio-Diagnostics Anti-Islet Cell Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of antibodies to islet cells, in human serum.

SUMARY AND EXPLANATION

Islet Cell antibodies (ICA) have been associated with a group of "autoimmune" endocrine disorders, more specifically with insulin dependent diabetes. Organ-specific autoimmunity (Al) is characterised 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 tests 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 (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 recognised 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).

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PRINCIPLE OF THE TEST

The primary test reaction involves circulating anti-islet cell antibodies present in the patient's serum, which attach to their homologous antigens. This occurs during the incubation period whilst the serum covers the antigen surface. A secondary reaction then follows a rinsing period that removes the unbound human antibody. The reagent used in the secondary reaction is a fluorescein labelled antihuman globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope.

WARNINGS AND PRECAUTIONS

1. The human components of the controls have been screened and found to be negative for HBsAg and antibodies to HIV-1, HIV-2 and HCV. However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The controls and conjugate included in the kit 0.1% sodium azide, 0.01% thiomersal or 0.05% ProClin as preservatives. Although these are at low concentrations, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
5. For in vitro diagnostic use.
6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
7. 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 artefacts.

KIT CONTENTS

SLIDE	Monkey pancreas substrate antigen slides (S4211, S8211, S5211 or S0211)
CONJ **IgG**	FITC Conjugate (for use with Primate substrates) with Evans Blue Counterstain: J502.This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
CONTROL **+**	Islet cell antibody Positive Control no: C012N/C012N-0.5
CONTROL **-**	Universal Negative Control no: C000N/C000N-0.5
IFA/DFA **PBS**	Buffer Pack no: R002
MM	Mounting Medium no: R005

Note: All kit components are available separately.
Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

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

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

Anti-Islet Cell Antibody Test System

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

Manufacturer	Contains sufficient for <n> tests	**RFU**	Ready for use
Temperature limitation	**IVD**	In vitro diagnostic medical device	

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2ºC to +8ºC. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents.
Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

TEST INSTRUCTIONS

Screening: dilute test serums 1/4 (1 part patient sample to 3 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.
Titration: set up doubling dilutions of serum starting at 1/4, (i.e. 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 30µl) 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 18-24ºC).
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 onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Carefully dry the back and edges of the slide using a paper towel. **Do not allow tissue to dry.**
7. Deliver 1 drop (20-30µl) 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.