COD 44609 48 Tests
COD 44572 12 x OOOO
STORE AT 2-8ºC
Reagents for the qualitative determination of anti- islet cell antibodies Only for *in vitro* use in the clinical laboratory

ANTI-ISLET CELL ANTIBODIES (AICA)

CE

BioSystems
REAGENTS & INSTRUMENTS

ANTI-ISLET CELL ANTIBODIES (AICA)
Indirect Immunofluorescence
MONKEY PANCREAS

PRINCIPLE OF THE METHOD

Serum anti-islet cell antibodies (AICA) bind to the corresponding antigens present in a monkey pancreas section. The antigen-antibody complexes are detected by means of a fluorescein labeled anti-human immunoglobulin, and visualized with the aid of a fluorescence microscope[1].

CONTENTS

	COD 44609
A. Slides	12 x 4 wells
B. PBS (10x)	1 x 100 mL
C–. Negative Control	1 x 0.3 mL
D. IgG FITC/Evans (M)	1 x 3.5 mL
E. Mounting Medium	1 x 3 mL
F. Blotting Paper	1 x 12

	COD 44572
A. Slides	12 x 4 wells

COMPOSITION

A. Slides. Monkey pancreas sections (MP) in each well.

B. PBS (10x). Sodium phosphate 112.5 mmol/L, potassium phosphate 30 mmol/L, sodium chloride 1.15 mol/L, sodium azide 0.95 g/L, pH 7.2.

C–. Negative Control: Human serum, sodium azide 0.95 g/L.

D. IgG FITC/Evans (M): Goat anti-human IgG conjugated with fluorescein isothiocyanate (FITC) and adsorbed with monkey serum. Evans blue 0.01g/L and sodium azide 0.95 g/L.

E. Mounting Medium: Mowiol 12%, Glicerol 30%, Tris 20 mmol/L, sodium azide 0.95 g/L.

F. Blotting Paper.

Human sera used in the preparation of the positive and negative controls have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the controls should be handled cautiously as potentially infectious.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contamination is prevented during their use.

Indications of deterioration:

– Liquid components: Presence of particulate material, turbidity.
– Slides: rips in the sealing bag, macroscopic defects on the tissue section like scratches or tissue peelings off.

AUXILIARY REAGENTS

B. PBS (10x).
D. IgG FITC/Evans (M).
E. Mounting Medium.
C+ AICA Positive Control. Human serum containing anti-islet cells antibodies, sodium azide 0.95 g/L.
C–. Negative Control.

REAGENT PREPARATION

PBS: Dilute Reagent B 1/10 with distilled water. Stable for 1 week at 2-8ºC.

All other reagents are provided ready to use.

ADDITIONAL EQUIPMENT

– Moist chamber
– Wash tray
– Coverslips 24 x 60 mm
– Fluorescence microscope equipped with a 495 nm excitation filter and a 525 emission filter for FITC visualization.

SAMPLES

Serum or plasma collected by standard procedures. Stable for 1 week at 2-8ºC.

Dilute samples 1/4 in PBS (see Reagent Preparation) before assay.

For titration of positive samples, make two-fold serial dilutions starting from 1/4 in PBS.

PROCEDURE

1. Bring the reagents and samples to room temperature.
2. Place 1 drop (50 µL) of the diluted sample or Control on each slide well, making sure that it is completely covered (Note 1).
3. Incubate the slide for 30 minutes at room temperature (15-30ºC) into a moist chamber.
4. Drain sample drops off by gently tapping the inclinated slide. Avoid cross-contamination of the sera.
5. Rinse gently the slide with PBS (see Reagent Preparation) (Note 2).
6. Wash thoroughly the slide by immersing in a washing tray filled with PBS for 5 minutes. Change PBS and repeat wash.
7. Carefully dry off the slides by using the blotting paper provided. Keep the tissue section moist along the procedure.
8. Place 1 drop of Reagent D on each well. Incubate the slide for 30 minutes at room temperature (15-30ºC) into a moist chamber.
9. Wash (step 6) and dry (step 7).
10. Place several drops of Reagent E on the slide and cover with a coverslip avoiding the formation of air bubbles.

READING

Examine the slide using the fluorescence microscope (250-400x). For best results, the slides should be read immediately. Select reading fields in the inner part of the tissue section. Fluorescent intensity in the tissue edge is not representative of the slide preparation.

Sera showing intracellular staining with granular aspect of the islet cells at the recommended dilution should be considered positive.

Positive sera may be titered.

When none of the specific staining are observed, the result should be considered negative for these autoantibodies.

QUALITY CONTROL

Positive Control (C+) and Negative Control (C–) should be tested together with the patients samples, in order to verify the assay performance.

Positive Control (C+) should give the above described specific staining.

Negative Control (C–) should not give any specific staining.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

ASSAY CHARACTERISTICS

– IgG FITC/Evans (M) conjugate is valued against the WHO International Standard for FITC labeled sheep anti-human IgG for the demonstration of antibodies in human serum.
– The specificity of the AICA Positive Control has been verified against an internal reference serum
– Results obtained with BioSystems AICA kit in a comparative study with anti-GAD and anti-IA2 antibodies detection tests showed a good concordance. Details of this comparative study are available upon request.

DIAGNOSTIC CHARACTERISTICS

Indirect immmunofluorescence assay is the conventional method for the determination of anti-islet cell antibodies (AICA). Islet cells antibodies (AICA) are strongly associated with insulin-dependent diabetes mellitus[2,3].

BioSystems Islet Cells Antibodies (AICA) kit was used to test 128 sera, 78 from patients with type I diabetes mellitus and 50 from blood bank donors. The results showed a diagnostic sensitivity and specificity of 65% and 100%, respectively.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Avoid touching the tissue section fixed into the wells along the procedure.
2. Use a squeeze bottle or a pipette to wash the slides, avoiding cross-contamination among the adjacent samples.

BIBLIOGRAPHY

1. Melnicoff MJ. Immunofluorescence Methods for Microscopic Analysis. In: Howard GC ed. Methods in Nonradioactive Detection. Appleton & Lange, 1993.
2. Lipton R and LaPorte RE. Epidemiology of islet cell antibodies. Epidemiologic Reviews 1989; 11:182-203.
3. Hagopian WA and Lernmark A. Islet Cell Autoantibodies. In: James B. Peter and Yehuda Schoenfeld eds. Autoantibodies. Elsevier, 1996.

BioSystems S.A. Costa Brava 30, Barcelona (Spain)
Quality System certified according to
EN ISO 13485 and EN ISO 9001 standards