Anti-Mitochondrial Antibody Test System

RESULTS

Primary Biliary Cirrhosis (PBC) is a chronic intrahepatic cholestasis found more frequently in women than in men with an incidence that is highest in the 30-60 age group. The diagnosis of PBC is based upon clinical observations, histological findings on liver biopsy, increased alkaline phosphatase activity, elevated IgM levels, and presence of mitochondrial antibodies.

A positive result is observed as granular fluorescence in the cytoplasm of the renal tubules. The fluorescence is limited to the cytoplasm of the proximal and distal tubular epithelium. Fluorescence of other cellular antigens such as nuclei, smooth muscle, or non-granular fluorescence limited to the central (lumen) portion of the proximal tubules should not be reported as positive MA.

Titre Interpretation:

The titre is the highest dilution of patient's serum showing weak (1+) fluorescence of the renal tubular epithelium:

Less than 1/20	Normal, negative
1/20 - 1/80	Positive. May be suggestive of liver disease. Repeat with a fresh specimen in 2 weeks.
1/160 or greater	Presumptive primary biliary cirrhosis.

The titre range in PBC is from 1:10 to 1:6,000. About 50% of PBC patients have titres between 1/2,000 to 1/6,000. MA titres do not appear to change with time or therapy so cannot serve as monitors of response to therapy.

TEST LIMITATIONS

1. No diagnosis should be based upon a single serological test result, since various host factors must be taken into consideration.
2. Clinical manifestations, histological finds on liver biopsies, elevation of IgM and in-creased alkaline phosphatase values should all be considered in the final diagnosis of PBC.
3. Liver and kidney microsomal antibody stains proximal tubules preferentially whereas MA reacts with distal tubules more strongly than with proximal tubules.
4. A normal serum IgM is strong evidence against the diagnosis of PBC as increased concentration of this immunoglobulin is the dominant abnormality in this disease (12,13).
5. Anti-smooth muscle antibody can be detected in 30-50% and antinuclear antibody in 25-46% of patients with PBC (13).

LITERATURE REFERENCES

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INSTRUCTIONS FOR USE

Mitochondrial Antibody Test System

	48 Tests	96 Tests	50 Tests	100 Tests
Rat kidney system	**K4806**	**K9606**	**K5006**	**K0006**
Mouse kidney system	**K4807**	**K9607**	**K5007**	**K0007**
Also for:	4 well	8 well	5 well	10 well
Mouse kidney slides	**S4002**	**S8002**	**S5002**	**S0002**
Rat kidney slides	**S4102**	**S8102**	**S5102**	**S0102**

Mitochondrial antibody Positive control **C004N**

INTENDED USE

The Bio-Diagnostics Mitochondrial Antibody Test System is an immunofluorescent antibody (IFA) test to detect the presence of mitochondrial antibodies in human serum.

SUMARY AND EXPLANATION

Mitochondrial Antibody (MA) as a circulating autoantibody in chronic liver disease is of great clinical importance in the differential diagnosis of chronic active hepatitis (CAH) from chronic persistent hepatitis (CPH), and in the diagnosis of primary biliary cirrhosis (PBC) (1). Tests for the detection of MA are recommended as an alternative to surgical exploration, as the presence of high titre MA can provide confirmatory evidence in the diagnosis of PBC (2,3). Both CAH and PBC have many overlapping immunologic features and may represent a continuum of a single disease entity (4). MA titres in PBC do not appear to have any correlation with clinical activity, since they do not vary with the severity or progression of the disease, and cannot serve as a monitor of response to therapy or provide prognostic information (5).

MA are present in sera of patients with a variety of liver disorders but are only present in high titre in the majority of patients with PBC. Studies have demonstrated that MA titres greater than 1/40 are found only in patients with PBC. The detection of MA by the indirect immunofluorescent technique is most useful in the differential diagnosis of extrahepatic obstruction in which only less than 2% of these patients possess this antibody and only at low titre (Table I). Rat or mouse kidney is utilised for MA detection in this test system.

PRINCIPLE OF THE TEST

The MA reaction involves circulating antibodies that bind to the inner lipoprotein membrane and cristae of mitochondria (7). These antibodies are not organ or tissue specific and may be found in many different tissues which are abundant in mitochondria (8). Mitochondrial rich cells line the proximal and distal tubules of the rat or mouse kidney which is used as the test substrate in indirect immunofluorescent procedures. MA are primarily of the IgG class but may also include IgA and IgM (9).

The primary test reaction involves circulating mitochondrial antibodies present in the patient's serum, which attach to their homologous mitochondrial 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.

Bright granular cytoplasmic fluorescence of the renal tubules indicates a positive result. Fluorescence of other cellular antigens such as nuclei, smooth muscle, connective tissue or a non-granular fluorescence limited to the central portion of the proximal tubules should not be reported as positive MA.

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TABLE 1 : INCIDENCE OF MITOCHONDRIAL ANTIBODIES IN VARIOUS DISORDERS

	Disease	Mitochondrial Antibodies
1.	Primary Billiary Cirrhosis	greater than 90
2.	Chronic Active Hepatitis (HbSAg-Negative)	greater than 50%
3.	Chronic Active Hepatitis (HbSAg-Positive)	greater than 60%
4.	Cryptogenic Cirrhosis	30 %
5.	Alcoholic Cirrhosis	greater than 30%
6.	Chronic Persistent Hepatitis	less than 20%
7.	Hemochromatosis	greater than 50%
8.	Cholangitis	23%
9.	Hepatic Metastases	6%
10.	Endocrine Disorders of Collangenoses	3-26%
11.	Extra Hepatic Obstruction	less than 2%

WARNINGS AND PRECAUTIONS

1. All human components have been tested by radioimmunoassay for (HBsAg) and HTLVIII/LAV by an FDA approved method and found to be negative (not repeatedly reactive). However, this does not assure the absence of (HBsAg) or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The reagents included in the kit contain either 0.1% sodium azide or 0.01% thiomersal as a preservative. Although this is at a low concentration, 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	Rat or mouse kidney substrate antigen slides
CONJ **IgG**	FITC Conjugate with Evans Blue Counterstain: J501/J501-5.This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
CONTROL +	Mitochondrial antibody Positive Control no: C004N / C004N-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.

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

Anti-Mitochondrial Antibody Test System

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. 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 1/20 (1 part patient sample to 19 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 1/20, (i.e. 1/20, 1/40, 1/80, 1/160, 1/320, 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. 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 the wells to blotter holes. Place slide on top of the blotter. Wipe the back of the slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. **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.

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.