Introduction:

Mitochondrial Antibody (MA) are circulating autoantibodies in chronic liver disease and are of clinical importance in the differentiation of chronic active hepatitis (CAH) from chronic persistent hepatitis (CPH), and is particularly useful in the diagnosis of primary biliary cirrhosis (PBC). Tests for the detection of mitochondrial antibodies (MA) are recommended as an alternative to surgical exploration, as the presence of high titer MA can provide confirmatory evidence in the diagnosis of PBC. Both CAH and PBC have many overlapping immunologic features and may represent a continuum of a single disease entity. MA titers 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.

MA are present in sera of patients with a variety of liver disorders but are only present in high titer in the majority of patients with PBC. Recent studies have demonstrated that MA titers 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 titer (Table I). Rat or mouse kidney is utilized for MA detection in this test system.

TABLE I

INCIDENCE OF MITOCHONDRIAL ANTIBODIES IN VARIOUS DISORDERS

	Disease	Mitochondrial Antibodies
1.	Primary Biliary 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 Collagenoses	3-26%
11.	Extra Hepatic Obstruction	less than 2%

Principles:
The MA reaction involves circulating antibodies that bind to the inner lipoprotein membrane and cristae of mitochondria. These antibodies are not organ or tissue specific and may be found in many different tissues which are abundant in mitochondria. 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.

Since MA will react with kidney tubules, thyroid epithelial cells and stomach parietal cells, SCIMEDX offers two and three tissue sections per well to help differentiate organ specific antibodies. A rat kidney and monkey thyroid slide #2504 facilitates this type of immediate differentiation in one well.

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 while the serum covers the antigen surface. A secondary reaction then follows a rinsing period which 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.

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1501L (3.0 ml)/15015L (5.0 ml) with Evans Blue Counterstain is to be stored at 2-8 C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label. This reagent contains antibodies which will react with the human IgG, IgM and IgA Immunoglobulin classes.

2. The antigen slides of rat or mouse kidney sections must be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.

3. MA positive control No. 2202L (1.0 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

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 1:20 in PBS. **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 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:

Primary Biliary Cirrhosis (PBC) is a chronic intrahepatic cholestasis found more frequently in women than in men, with an incidence which is highest in the 30-60 age group. The diagnosis of PBC is based upon clinical observations, histologic 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.

Titer Interpretation:

MA The titer 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. Suggestive of liver disease. Repeat with a fresh specimen in two weeks.

1:160 or greater
Presumptive primary biliary cirrhosis.

The titer range in PBC is from 1:10 to 1:6,000 with about 50% of PBC patients having titers between 1:2,000 to 1:6,000. MA titers do not appear to change with time or therapy and cannot serve as monitors of response to therapy.

Limitations of Procedure:

1. No diagnosis should be based upon a single serologic test result, since various host factors must be taken into consideration.
2. Clinical manifestations, histologic finds on liver biopsies, elevation of IgM and increased 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.
5. Anti-smooth muscle antibody can be detected in 30-50% and antinuclear antibody in 25-46% of patients with PBC.

Precautions:

1. All human components have been tested by radio-immunoassay 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. 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 artifacts.

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MITOCHONDRIAL ANTIBODY TEST SYSTEM

For In - Vitro Diagnostic Use

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Fax: 973.625.8796
www.scimedx.com

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