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 nuclear, 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 1/10 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

INSTRUCTIONS FOR USE

Mitochondrial Antibody Test System

<table>
<thead>
<tr>
<th>Test</th>
<th>Rat Kidney</th>
<th>Mouse Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 Tests</td>
<td>K4806</td>
<td>K4807</td>
</tr>
<tr>
<td>96 Tests</td>
<td>K9606</td>
<td>K9607</td>
</tr>
<tr>
<td>50 Tests</td>
<td>K5006</td>
<td>K5007</td>
</tr>
<tr>
<td>100 Tests</td>
<td>K0006</td>
<td>K0007</td>
</tr>
</tbody>
</table>

Also for:
- 4 well for Rat kidney slides  S4102  S1102  S0102
- 8 well for Mouse kidney slides S4002  S8002  S5002  S0002

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.

SUMMARY 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).

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 1). 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 (9). 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 bound 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 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.
TABLE 1: INCIDENCE OF MITOCHONDRIAL ANTIBODIES IN VARIOUS DISORDERS

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mitochondrial Antibodies</th>
</tr>
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<tbody>
<tr>
<td>1. Primary Biliary Cirrhosis</td>
<td>greater than 90%</td>
</tr>
<tr>
<td>2. Chronic Active Hepatitis (HBsAg-Negative)</td>
<td>greater than 50%</td>
</tr>
<tr>
<td>3. Chronic Active Hepatitis (HBsAg-Positive)</td>
<td>greater than 60%</td>
</tr>
<tr>
<td>4. Cryptogenic Cirrhosis</td>
<td>30%</td>
</tr>
<tr>
<td>5. Alcoholic Cirrhosis</td>
<td>greater than 30%</td>
</tr>
<tr>
<td>6. Chronic Persistent Hepatitis</td>
<td>less than 20%</td>
</tr>
<tr>
<td>7. Hemochromatosis</td>
<td>greater than 50%</td>
</tr>
<tr>
<td>8. Cholangitis</td>
<td>23%</td>
</tr>
<tr>
<td>9. Hepatic Metastases</td>
<td>6%</td>
</tr>
<tr>
<td>10. Endocrine Disorders of Collagenoses</td>
<td>3-26%</td>
</tr>
<tr>
<td>11. Extra Hepatic Obstruction</td>
<td>less than 2%</td>
</tr>
</tbody>
</table>

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 controls 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: C000
- IFD/DFA: Buffer Pack no: R002
- PBS: 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


KEY FOR OTHER SYMBOLS

- Manufacturer
- Contained sufficient for < or > tests
- Ready for Use
- Temperature limitation
- IVD In vitro diagnostic medical device