**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 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 (13).

**LITERATURE REFERENCES**
5. Gupta RC, Dickson ER, M'Duff FC and Buggensato AH: Circulating IgG complexes in primary bilia.

**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 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 fluorescent evidence of the renal tubules indicates a positive result. Fluorescence of other cellular antigens such as nuclei, smooth muscle, connective tissue or a non-granular fluorescent 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

<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. Hemosiderosis</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 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
- FITC Conjugate with Evans Blue Counterstain: J501/J501-5
- This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.

CONJ IgG
- Mitochondrial antibody Positive Control no: C004N / C004N-0.5
- Universal Negative Control no: C000N/C000N-0.5

CONTROL

IF/IFA/PBS Buffer Pack no: R002
- 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
- Volumetric Flask (500 ml)
- Paper Towels – lint free
- Distilled Water
- Fluorescence Microscope
- Staining Dish and Slide Forceps
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REAGENT PREPARATION

KEY FOR OTHER SYMBOLS
- Manufacturer
- Contains sufficient for <x> 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 +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.

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.