Mitochondrial Antibody ELISA

INTENDED USE
The Mitochondrial IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG class antibody to Mitochondrial in human serum or plasma. For research use only.

SUMMARY AND EXPLANATION
Mitochondrial Antibodies (MA) are directed against the E2 subunit of the pyruvate dehydrogenase enzyme complex located at the inner mitochondrial membrane (PDC-E2), the E2 subunit of the branched chain 2-oxo acid dehydrogenase complex (BCOADC-E2), the E2 subunit of the 2-oxo-glutarate dehydrogenase complex (OGDC-E2), protein X, and PDC-E1. MA are found in ~95% of patients with primary biliary cirrhosis (PBC). MA in low titers are common in chronic active hepatitis and their presence does not preclude response to corticosteroids. MA disappear in about one month after orthotopic liver transplantation (OLT) and decrease with cyclosporine treatment which might be useful in PBC. MA are found in ~1% of apparently healthy Caucasoid adults. Approximately 3% of patients with PBC have scleroderma, usually of the CREST syndrome variety. In addition, MA reactive with the PDC-E2 complex are found in some patients with CREST or diffuse scleroderma, sometimes in the absence of overt liver disease. Scleroderma typically precedes PBC in those patients with both diseases.

PRINCIPLE OF THE TEST

REFERENCES

WARNING
All GenWay kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of GenWay to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

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MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
<th>96 tests</th>
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<tbody>
<tr>
<td>1. Microwell coated with Mitochondrial antigen</td>
<td>12x8x1</td>
</tr>
<tr>
<td>2. Sample Diluent: 1 bottle (ready to use)</td>
<td>22 ml</td>
</tr>
<tr>
<td>3. Calibrator: 1 Vial (ready to use)</td>
<td>1.5 ml</td>
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<tr>
<td>4. Positive Control: 1 vial (ready to use)</td>
<td>1.5 ml</td>
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<tr>
<td>5. Negative Control: 1 vial (ready to use)</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>6. Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>7. TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>8. Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>9. Wash concentrate 20X: 1 bottle</td>
<td>25 ml</td>
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MATERIALS NOT PROVIDED
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY
1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light
ASSAY PROCEDURE
Bring all specimens and kit reagents to room temperature (18-26℃) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10μl of the sample to 200μl of sample diluent. Mix well.
3. Dispense 100μl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100μl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300μl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100μl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300μl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100μl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100μl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.4
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0