**Principle of the Test**

Purified β2GP1 antigens are coated on the surface of microwells. Diluted patient serum or plasma, and calibrators, are added to the wells. The Anti-β2GP1 specific antibodies, if present, bind to the antigens. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgA specific antibodies in the sample. The results are read by a microwell reader, and compared in a parallel manner with calibrators.

**Summary of Assay Procedure**

<table>
<thead>
<tr>
<th>Step</th>
<th>Room temperature (20-25°C)</th>
<th>Volume</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample dilution 1:101 = 5 µl / 500 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Washing buffer (3 times)</td>
<td>350 µl</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Diluted samples, controls &amp; calibrators</td>
<td>100 µl</td>
<td>30 minutes</td>
</tr>
<tr>
<td>4</td>
<td>Washing buffer (3 times)</td>
<td>350 µl</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Enzyme conjugate</td>
<td>100 µl</td>
<td>30 minutes</td>
</tr>
<tr>
<td>6</td>
<td>Washing buffer (3 times)</td>
<td>350 µl</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>TMB Chromogenic Substrate</td>
<td>100 µl</td>
<td>30 minutes</td>
</tr>
<tr>
<td>8</td>
<td>Stop solution</td>
<td>100 µl</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Reading OD 450 nm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Name and Intended Use**

The IMMUNOSPEC β2GP1 IgA Enzyme-linked Immunosorbent Assay (ELISA) is intended for the detection and semiquantitative determination of IgA antibodies to β2GP1 in human sera or plasma. The results of the assay are to be used as an aid in the diagnosis of certain autoimmune diseases thrombotic disorders, anti-phospholipid syndrome, SLE or lupus-like disorders.

**Summary and Explanation of the Test**

Cardiolipin autoantibodies (ACA) are described for various autoimmune diseases. The presence of anti-cardiolipin antibodies in systemic lupus erythematosus (SLE) can be related to the development of thrombocytopenia, in gynaecology they are supposed to cause intrauterine death or recurrent abortion.

**Materials Provided**

1. Microwell strips: β2GP1 antigen coated wells. 12 x 8 wells
2. Absorbent solution: Black Cap. 50 ml / bottle
3. Washing concentrate 10x. 100 ml / bottle
4. TMB Chromogenic Substrate: Amber bottle. 15 ml / bottle
5. Enzyme conjugate: Red color solution. 12 ml / bottle
7. Control set (1:101 prediluted): Negative and Positive controls. Ranges are indicated on each label. 1.0 ml / vial
8. Stop solution: 1.5 N acid solution. (HCl / H2SO4) 12 ml / bottle

**Warnings and Precautions**

1. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found nonreactive for Hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus, or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control / National Institutes of Health manual, “Biosafety in Microbiological and Biomedical Laboratories.” 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
5. To prevent injury and chemical burns, avoid contact with skin and eyes or inhalation and ingestion of the following reagents: Enzyme conjugate, TMB Chromogenic substrate and Stop solution.

**Preparation for Assay**

1. Prepare 1x washing buffer.
2. Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to make a final volume of 1 liter.
3. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

**ASSAY PROCEDURE**

1. Place the desired number of coated strips into the holder.
2. Prepare 1:101 dilution of test samples by adding 5 μl of the sample to 500 μl of sample diluent. Mix well. 
   
   **Do not dilute 1:101 prediluted Calibrators & Controls.**

3. Dispense 100 μl of diluted sera and prediluted calibrators & controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.

4. Remove liquid from all wells. Repeat washing three times with washing buffer.

5. Dispense 100 μl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.

6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.

7. Dispense 100 μl of TMB Chromogenic Substrate into each well and incubate for 30 minutes at room temperature.

8. Add 100 μl of Stop solution to stop reaction. 
   
   Make sure there are no air bubbles in each well before reading.

9. Read O.D. at 450 nm with a microwell reader.

**CALCULATION OF RESULTS**

1. Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of calibrator SAU values on the x-axis on a log-log graph paper or log-lin graph.

2. Using the O.D. value of each specimen, determine the concentration from the standard curve.

3. A typical example:

<table>
<thead>
<tr>
<th>Calibrator Set</th>
<th>β2GP1 IgA (SAU)</th>
<th>O.D. 450 nm</th>
<th>O.D. 450 nm Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 1</td>
<td>6.3</td>
<td>0.089</td>
<td>0.087</td>
<td>0.003</td>
<td>3.251</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>12.5</td>
<td>0.105</td>
<td>0.104</td>
<td>0.003</td>
<td>2.800</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>25</td>
<td>0.319</td>
<td>0.317</td>
<td>0.003</td>
<td>0.929</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>50</td>
<td>0.573</td>
<td>0.569</td>
<td>0.011</td>
<td>1.947</td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>100</td>
<td>1.130</td>
<td>1.154</td>
<td>0.021</td>
<td>1.853</td>
</tr>
<tr>
<td>Calibrator 6</td>
<td>200</td>
<td>2.175</td>
<td>2.218</td>
<td>0.061</td>
<td>2.742</td>
</tr>
</tbody>
</table>

**QUALITY CONTROL**

1. The negative control and positive control should be run with every batch of samples tested and the concentration must be within the range stated on its label.

2. The O.D. value of calibrators must be lower than 0.150 and the O.D. value of calibrator 200 SAU must be greater than 0.750. Additional controls may be prepared from human serum specimens and kept under -20°C.

**INTERPRETATION OF RESULTS**

Each laboratory is recommended to establish its own normal range based upon its own techniques, controls, equipments and patient population according to their own established procedures. The followings are a suggestive guideline.

Negative: < 20 SAU

Low positive: 20 – 40 SAU

Moderate positive: 40 – 70 SAU

High positive: > 70 SAU

A positive result suggests the possibility of certain autoimmune disease thrombotic disorders. A negative result indicates no β2 GP1 IgA antibody or levels below the detection limit of the assay.

**LIMITATIONS OF THE TEST**

1. Diagnosis cannot be made on the basis of anti β2 GP1 results alone. These results must be used in conjunction with information from clinical evaluation and other diagnostic procedures.

2. The clinical significance of β2 GP1 antibodies in diseases other than SLE is currently under investigation.

3. When negative anti β2 GP1 titers are found in the presence of clinical indications, a lupus anticoagulant, anti-cardiolipin or other additional testing is indicated.

4. It is to be expected that some samples can be anti-cardiolipin positive yet anti β2 GP1 negative. The anti β2 GP1 test is a more specific marker of thrombotic risk. The anticardiolipin test can produce false positive results due to cross-reactivity with dsDNA or certain infectious disease antibodies.

**PERFORMANCE CHARACTERISTICS**

**Sensitivity, specificity and accuracy:**

A total of 75 samples were assayed with the IMMUNOSPEC ELISA β2GP1 IgA (X values) and with a reference ELISA (1) (Y values). The correlation equation is

\[ Y = 0.9221 X + 0.5522 \]

\[ R^2 = 0.9375 \]

\( n = 75 \)

<table>
<thead>
<tr>
<th>Reference ELISA (1)</th>
<th>N</th>
<th>P</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference ELISA (2)</td>
<td>35</td>
<td>40</td>
<td>75</td>
</tr>
</tbody>
</table>

Sensitivity = \( A / (A+B) = 39 / (39 + 1) = 98 \% \)

Specificity = \( D / (C+D) = 32 / (3 + 32) = 91 \% \)

Accuracy = \( (A+D) / (A+B+C+D) = (39 + 32) / (39 + 1 + 3 + 32) = 71 / 75 = 95 \% \)

A second reference ELISA (2) was used to test 3 samples which reference ELISA (1) tested for negative and IMMUNOSPEC ELISA tested for positive. The results are all positive for the 3 samples. The samples tested for positive with reference ELISA (1) and negative with IMMUNOSPEC ELISA remains a positive result when it was tested with the reference ELISA (2).

**Precision:**

Statistic for CV, mean and SD were calculated for each of three samples from the results of 8 determinations in a single run for intra-assay. Inter assay precision was calculated from the result of 8 determinations of 8 different runs.

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>n</th>
<th>Mean SAU</th>
<th>SD</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A</td>
<td>8</td>
<td>14.9</td>
<td>0.35</td>
<td>2.38</td>
</tr>
<tr>
<td>Serum B</td>
<td>8</td>
<td>30.8</td>
<td>1.39</td>
<td>4.52</td>
</tr>
<tr>
<td>Serum C</td>
<td>8</td>
<td>58.9</td>
<td>0.99</td>
<td>1.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-assay</th>
<th>n</th>
<th>Mean SAU</th>
<th>SD</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A</td>
<td>8</td>
<td>15.6</td>
<td>0.38</td>
<td>2.4</td>
</tr>
<tr>
<td>Serum B</td>
<td>8</td>
<td>31.2</td>
<td>1.42</td>
<td>4.55</td>
</tr>
<tr>
<td>Serum C</td>
<td>8</td>
<td>59.3</td>
<td>1.05</td>
<td>1.77</td>
</tr>
</tbody>
</table>

**INTERFERENCE AND CROSS-REACTIVITY**

IMMUNOSPEC IgA test does not cross-react with the following positive samples tested: Rubella, Toxo, CMV, H. pylori, Measles, Mumps, VZV, RF and HSV.

**REFERENCES**


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