INTENDED USE

The GenWay, Inc. Mouse HSV-1 IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) is used for the detection of IgG class antibodies to HSV-1 in mouse

SUMMARY AND EXPLANATION

HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. Several recent studies have shown the association of more than a dozen herpes viruses with cancer in man and various animals; for example with lymphoma and with squamous cell carcinoma of the lip and cancer of the cervix. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis; type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure. A significant increases in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV infection. The effect of virus dose and animal age on the appearance of acute and latent neurologic infection by HSV1 and HSV2 was studied in Balb/c and ICR mice inoculated in the footpad. At low viral doses, HSV2 was found to be 1,500 times more neurovirulent than HSV1. The Mp strain of herpes simplex virus type 1 (HSV1) induced a persistent infection in the mouse C 1300 neuronal cell line (clone N 115). C 1300 cultures infected at an MOI of 0.01 or 0.001 survived the initial infection and continued to produce infectious virus and viral antigens for 185 days and 31 days, respectively.

PRINCIPLES OF THE TEST

Diluted serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microwell coated with HSV-1 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>1.5ml</td>
</tr>
<tr>
<td>4. Positive Control: 1 vial (ready to use)</td>
<td>1.5ml</td>
</tr>
<tr>
<td>5. Negative Control: 1 vial (ready to use)</td>
<td>1.5ml</td>
</tr>
<tr>
<td>6. Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>7. TMB Substrate: 1 bottle (ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>8. Stop Solution: 1 bottle (ready to use)</td>
<td>12ml</td>
</tr>
<tr>
<td>9. Wash concentrate 20X: 1 bottle</td>
<td>25ml</td>
</tr>
</tbody>
</table>
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.

WARNINGS AND PRECAUTIONS

1. This kit is designed for research use only.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Control sera and sample diluent contain 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.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8° C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26 °C).

ASSAY PROCEDURE.

1. Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.
2. Place the desired number of coated strips into the holder.
3. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.

4. Dispense 100 µl 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.

5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.

6. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.

7. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.

8. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.

9. Add 100 µL of stop solution.

10. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter to 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= 0.400  
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

**QUALITY CONTROL**

The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should be greater than 1.2.

**INTERPRETATION**

The following is intended as a guide to interpretation of HSV-1 IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

1. **Antibody Index Interpretation**

   - **<0.9**  No detectable antibody to HSV-1 IgG by ELISA.
   - **0.9-1.1**  Borderline positive. Follow-up testing is recommend if clinically indicated.
   - **>1.1**  Detectable antibody to HSV-1 IgG by ELISA.

2. **Converting of Ab Index to IU/mL**

   As an option, Ab index may be converted to IU/ml by multiplying Ab index by 100. IU/ml values may then be interpreted as follows:
   - **<90 IU/ml**  No detectable IgG antibody to HSV-1 by ELISA
   - **90-110 IU/ml**  Borderline positive. Follow-up testing is recommended if clinically indicated.
   - **> 110 IU/ml**  Detectable IgG antibody to HSV-1 by ELISA

**LIMITATIONS OF THE TEST**

1. The test results obtained using this kit can not discriminate between HSV-1 and HSV-2 infection due to high cross reactivity between the two viruses. The results serve only as an aid to diagnosis and should be interpreted in relation to the patient’s history, physical findings and other diagnostic procedures.

2. Lipemic or hemolyzed samples may cause erroneous results.

**PERFORMANCE CHARACTERISTICS**

1. **Sensitivity and Specificity**

   336 patient sera were tested by this HSV-1 IgG ELISA and a reference ELISA method. 270 sera were positive and 56 were negative by both methods (97% agreement). The results are summarized below:

<table>
<thead>
<tr>
<th>Reference ELISA Kit</th>
<th>HSV-1 IgG ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>270</td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>276</td>
</tr>
</tbody>
</table>

2. **Precision**
Mouse-Rat HSV-1 IgG ELISA
Catalog No. 40-101-325094 (96 Tests)

Intra Assay Study

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1.23</td>
<td>0.06</td>
<td>4.87</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>0.66</td>
<td>0.04</td>
<td>6.10</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>0.33</td>
<td>0.02</td>
<td>6.06</td>
</tr>
</tbody>
</table>

Inter Assay Study

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1.77</td>
<td>0.15</td>
<td>8.47</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.93</td>
<td>0.09</td>
<td>9.47</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.21</td>
<td>0.02</td>
<td>14.2</td>
</tr>
</tbody>
</table>

REFERENCES

1. Markoulatos P; Fountoucidou P; Marinakis G; Krikelis V; Spyrou N; Vamvakopoulos N; Moncany ML. Clear detection and typing of herpes simplex virus types 1 and 2 by an indirect ELISA assay: comparison with three different combined methods--capture ELISA, restriction enzymes, and polymerase chain reaction. J Clin Lab Anal 1997; 11(3):146-53.


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