**NAME AND INTENDED USE**

The HSV-2 IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to HSV-2 in human serum or plasma. For research use only.

**SUMMARY AND EXPLANATION OF THE TEST**

HSV-1 and 2 are viruses that are identical and can be identified by antibodies. Both types infect the same body sites, such as the mouth or genital area. They can cause disease in the nervous system, and both can cause encephalitis. HSV-1 is more common than HSV-2 and can recur and spread even when no symptoms are present. By the time they’re teenagers or young adults, about 50% of Americans have HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. 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 and increases in HSV IgG is an indicator of reactivation, current or recent infection. IgM antibody is present after primary HSV infection.

**PRINCIPLE OF THE TEST**

Diluted patient 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.

**MATERIALS PROVIDED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with HSV-2 antigen</td>
<td>12x8x1</td>
</tr>
<tr>
<td>Sample Diluent: 1 bottle (ready to use)</td>
<td>22 ml</td>
</tr>
<tr>
<td>Calibrator: yellow Cap. 1 Vial (ready to use)</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Positive Control: Red Cap. 1 vial (ready to use)</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Negative Control: Blue Cap. 1 vial (ready to use)</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Wash concentrate 20X: 1 bottle</td>
<td>25 ml</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

**REFERENCES**

3. 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.
5. Goodyear HM; McLeish P; Randall S; Buchan A; Skinner GR; Winther M; Rolland J; Morgan G; Harper JI. Immunological studies of herpes simplex virus infection in children with atopic eczema. Br J Dermatol 1996;134(1):85-93.
8. 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.
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. Potential biohazardous materials:
The calibrator and controls contain human source components which have been tested and found non-reactive 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. This kit is designed for research use only.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements in essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. 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 AND 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

Bring all specimens and kit reagents to room temperature (18-26°C) 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 10 μl of the sample to 200 μl of sample diluent. Mix well.
3. Dispense 100 μl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 μl 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 300 μl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 μl 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 300 μl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 μl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 μl 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.
4. Check Calibrator Factor (CF) value on the calibration chart.
5. 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 O.D. = 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-2 IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation
<0.9 No detectable antibody to HSV-2 IgG by ELISA.
0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1 Detectable antibody to HSV-2 IgG 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 for this kit are for research use only and are not intended to be used as a part of any official diagnosis.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

1. Precision and Specificity

<table>
<thead>
<tr>
<th>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>1.06</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>0.75</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>0.57</td>
<td>0.04</td>
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Example of typical results:

<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>1.08</td>
<td>0.09</td>
<td>8.3</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.63</td>
<td>0.06</td>
<td>9.5</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.24</td>
<td>0.03</td>
<td>12.5</td>
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</tbody>
</table>

2. Inter Assay Study

<table>
<thead>
<tr>
<th>Serum</th>
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<th>Mean</th>
<th>Standard Deviation</th>
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