*Herpes-simplex* virus-1 and/or -2 IgM ELISA Kit

Qualitative assays for anti-*Herpes simplex* IgM antibodies

For Research Use Only. Not for use in diagnostic procedures.

Changes from previous version 04/18/02 – ALPCO:
Changes are not critical to the assay procedure.

Catalog Number: 27-GD88
Size: 96 Wells
Version: 060405 - ALPCO 07/20/05

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**Intended use**

The *Herpes simplex* virus-1 and/or -2 IgM ELISA is designed for the qualitative determination of IgM antibodies to *Herpes Simplex* Virus (HSV) in human serum. Plasma samples may also be used. The assay is intended to be used to evaluate serologic evidence of primary or reactivated infection with HSV, and is for research use. Not for use in diagnostic procedures.

**Introduction**

*Herpes simplex* infections are caused by two antigenically distinct strains of the common virus *Herpes simplex*. HSV-1 is usually associated with infections in the oropharyngeal area and eyes while HSV-2 causes most genital and neonatal infections.

Following infection, a latent infection is established in sensory neurons, and recurrent infection results from reactivation of latent infection. HSV infections are usually localized to the initial site of infection. However, serious localized or disseminated disease may occur in immunocompromised individuals including newborn infants, cancer patients and transplant recipients.

HSV infections are transmitted by virus-containing secretions through close personal contact. Infection is classified as either primary or recurrent. Both forms are often subclinical and asymptomatic. Primary symptomatic HSV-1 infections are characterised by gingivostomatitis associated with fever, malaise and tender swollen cervical lymph nodes. The most common form of recurrent HSV-1 is herpes labialis in which vesicles appear on the lips, nostrils or skin around the mouth. Genital HSV infections manifest as multiple ulcerative lesions accompanied by pain, fever, dysuria and lymphadenopathy.

The most severe complication of genital HSV infection is neonatal disease. HSV is transmitted from the mother to the neonate during birth. Of mothers with an active infection, the risk of transmission to infants is as high as 40%. About 60-80% of infants who develop neonatal herpes are born to women who are asymptomatic of genital herpes at the time of birth. Infants infected with HSV appear normal at birth but generally develop symptoms during the newborn period. Of the infants with neonatal HSV, about half will die if not treated, and about half of the surviving infants will develop severe neurological or ocular sequelae.

**Principle of the test**

Diluted serum or plasma specimens (1:100) are incubated for 20 minutes to allow specific antibodies to HSV to bind to the antigen-coated wells. Anti-human IgG is added to the sample diluent sample to eliminate the possibility of interference by antigen-specific IgG and rheumatoid factor, if present. After washing away unbound antibodies and other serum constituents, HSV specific IgM is detected using rabbit anti-human IgM conjugated to horseradish peroxidase. After 20 minutes incubation, unbound conjugate is removed by washing, and TMB-enzyme substrate is added for 10 minutes. A blue color develops if antibodies to HSV are present. Addition of stop solution gives a yellow colour and the optical densities of controls, 10 U/ml standard and samples are measured using a microplate reader.

**Materials included in the Kit**

- **Microplate**: 96 wells in 12 X 8 break-apart strips, pre-coated with a mixture of inactivated HSV-1 and HSV-2 strain antigens.
- **Reagent 1**: Sample diluent 46 ml, (blue). Read the instructions before use.
- **IgG absorbent**: Anti-human IgG, 3 x 3.5ml. Read the instructions before use.
- **Reagent 2**: Wash buffer concentrate, 75 ml. Dilute before use.
- **Reagent 3**: Conjugate (peroxidase conjugated rabbit anti-human IgM), 12 ml, (green). Ready to use
- **Reagent 4**: TMB Substrate, 12 ml. Ready to use
- **Reagent 5**: Stop solution, 12 ml. Ready to use
- **Positive control**: (red), 1ml liquid. Ready to use.
- **Standard**: 10 U/ml (yellow), 1ml liquid. Ready to use.
- **Negative control**: (green), 1ml liquid. Ready to use.
- **Instructions for use**

**Other equipment required**

- 10mm X 60mm tubes for dilution, pipettes 10µl, 100µl, 1000µl; repeating dispenser 100µl, microplate reader with 450nm filter, microplate washing device. Distilled or de-ionized water, general laboratory apparatus.

**Storage and precautions**

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for three months (or until its expiry date if less than three months). It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiry date.

The 10 U/ml standard and controls are manufactured from dilute non-infectious human serum. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

The Stop Solution contains 0.25M sulphuric acid.

**Samples**

Only freshly drawn and properly refrigerated sera or plasma should be used in this assay. Avoid hemolysed, lipemic or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If delay in testing is anticipated, store test sera at – 20°C. Avoid multiple freeze-thaw cycles.

**Method**

Ensure that all materials are at room temperature before beginning the procedure. We recommend that the 10 U/ml standard and the controls are always run in duplicate. Samples may be run singly or in duplicate.

1. **Assemble the number of strips required for the assay.**
2. **Prepare only sufficient IgG-absorbent-containing sample diluent for the number of samples to be tested. Add one part IgG absorbent to 4 parts of Reagent 1 Sample Diluent as shown in the examples below and mix thoroughly. Discard any unused IgG-absorbent-containing diluent.**

<table>
<thead>
<tr>
<th>Approx Nr of samples</th>
<th>Volume of sample diluent (ml)</th>
<th>Volume of IgG Absorbent (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>48</td>
<td>20</td>
<td>5.0</td>
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<tr>
<td>72</td>
<td>30</td>
<td>7.5</td>
</tr>
<tr>
<td>96</td>
<td>40</td>
<td>10.0</td>
</tr>
</tbody>
</table>

This protocol is for reference purposes only. DO NOT use this copy to run your assay; use the protocol included with the kit ONLY.
3. Dilute patient samples 1:100 in IgG-absorbent-containing sample diluent (5 µl serum plus 0.5 ml IgG-absorbent containing sample diluent). It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

4. The controls and the 10 U/ml standard are ready to use. Dispense 100 µl of the negative control, the 10 U/ml standard, the positive control and the diluted patient sample into the wells.

5. Incubate for 20 minutes at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.

6. Dilute the wash buffer (Reagent 2), 1:14 in distilled/de-ionized water to make sufficient buffer for the assay run. The diluted wash buffer is stable for two months at 2 - 8°C.

7. After 20 minutes, decant or aspirate the well contents and wash the wells 3 times using an automatic plate washer or the manual wash procedure (see below). Careful washing is the key to good results. Blot the wells on absorbent paper before proceeding. Do not allow the wells to dry out.

Manual Wash Procedure:
Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process two more times.

8. Dispense 100 µl of Conjugate (Reagent 3) into each well. This reagent is color coded green. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent! Incubate the wells for 20 minutes at room temperature.

9. After 20 minutes, discard the well contents and carefully wash the wells 4 times with wash buffer. Ensure that the wells are completely washed. Blot the microplate on absorbent paper to remove final drops of wash fluid. Do not allow the wells to dry out.

10. Using a repeating dispenser, rapidly dispense 100 µl of TMB Substrate (Reagent 4) into each well. Incubate the plate for 10 minutes.

11. Add 100 µl of Stop Solution (Reagent 5) to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.

12. Read the optical density in a microplate reader within 10 minutes.

Quality control
The expected optical density (OD) values for the negative and positive controls and the 10 U/ml standard are given on the Quality Control Certificate included in the kit.

Interpretation
Negative samples: OD < 10 U/ml standard OD
Positive samples: OD >/= 10 U/ml standard OD
1. A negative result indicates no current or reactivated infection with HSV-1 or HSV-2
2. A positive result indicates a primary or reactivated infection with HSV-1 and/or HSV-2.
3. Specimens obtained too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, another specimen should be taken in 7-14 days and tested concurrently in the same assay with the original specimen to determine seroconversion.

Limitations
1. A negative result does not rule out a primary or reactivated infection with HSV-1 or HSV-2 because samples may have been obtained too early in the course of infection, or IgM titres may have declined below detectable levels.
2. HSV-1 or HSV-2 antibody test results will not indicate the site of infection. The test is not intended to replace viral isolation.
3. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Bar virus and give false positive results.
4. Results of the Herpes simplex IgM ELISA are not by themselves diagnostic and should be interpreted in light of the patient's clinical condition and the results of other diagnostic procedures.
5. In immunocompromised patients, the ability to produce an IgM response may be impaired and HSV-specific IgM may be falsely negative during an active infection.

Expected Values
The incidence of HSV infection varies with age, geographical location, sexual behaviour and socioeconomic status. IgM antibody to HSV may persist for up to 9 months following a primary infection in some patients.

Performance characteristics
Comparative study:
The HSV-1 and/or HSV-2 IgM kit was compared with other commercially available ELISA assay for the detection of IgM antibodies to HSV-1 and/or HSV-2. The results are summarized below.

<table>
<thead>
<tr>
<th>Comparative Study</th>
<th>Reference HSV-1/2 IgM ELISA kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n= 100)</td>
<td>+</td>
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<tr>
<td>Genesis Diagnostics</td>
<td>35 1</td>
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<tr>
<td>HSV-1/2 IgM kit</td>
<td>-</td>
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<td></td>
<td>1 63</td>
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**Assay characteristics**

Within Assay Imprecision < 12%

Between Assay Imprecision < 12%

**Method Summary**

- Mix IgG absorbent and Sample Diluent 1:4 and dilute all samples 1:100
- Dispense 100µl of each control, the 10 U/ml standard and diluted sample into the microplate wells
- Incubate for 20 minutes at room temperature.
- **Wash the wells three times**
- Dispense 100µl of Conjugate (Reagent 3) into each well
- Incubate at room temperature for 20 minutes
- **Wash the wells four times**
- Add 100µl of TMB Substrate (Reagent 4) to each well
- Incubate at room temperature for 10 minutes
- Add 100µl Stop Solution (Reagent 5) to each well
- Read the optical density at 450nm

**References**


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