HSV 1 & 2 IgM
Enzyme Immunoassay

For Individual Laboratory to Complete:

Laboratory Name		
Adopted		
Reviewed		
Reviewed		
Revised		
Supercedes		

Method: **Diamedix Corp., Immunosimplicity®**
Manual or in conjunction with one of the Diamedix Automated EIA Systems such as the MAGO Plus, the DSX, or the DS2. For *In Vitro* Diagnostic Use.

Clinical Significance

Herpes simplex virus (HSV) is classified in the alphaherpesvirus subfamily of herpesviruses and is a linear DNA virus. Two antigenic types, HSV type 1 and HSV type 2, have been identified. The DNAs of both HSV types share approximately 50% of their base pairs. Due to this extensive sequence homology between the antigens from type 1 and type 2, subtyping analysis can only be performed if specific proteins or fragments of each of the subtypes are used in the assays (1,2,3,4).

HSV infections are extremely common and widespread and can involve mucocutaneous surfaces, internal organs and the central nervous system. HSV type 1 is usually acquired through contact with infectious salivary secretions whereas HSV type 2 infection is primarily transmitted by sexual contact. The initial infection caused by HSV type 1 or type 2 is followed by latent infection of neuronal cells in the dorsal root ganglia. Subsequent viral reactivation is accompanied by viral excretion from the original mucocutaneous sites of infection with or without concomitant appearance of clinical signs and symptoms (1,5). HSV transmission can result from direct contact with infected secretions from a symptomatic or an asymptomatic host. Although previous infection with HSV type 1 does not prevent infection upon exposure to HSV type 2, preexisting HSV type 1 immunity may modify the severity of HSV type 2 infection rendering it clinically mild or asymptomatic (1). The prevalence of HSV type 1 infections increases gradually from childhood reaching 70 to 80% in adult years. The prevalence of type 2 antibodies ranges from 15 to 50%,depending on a number of demographic variables (6,7).

The potential adverse outcome of HSV infection during pregnancy underscores the importance of determining the HSV immunological experience of the mother. Congenital and neonatal HSV can occur with primary or recurrent, symptomatic or aysmptomatic, maternal HSV infection. Infections usually result from exposure of neonates to virus being excreted by mothers at the time of vaginal delivery. If the neonate is exposed at delivery to a mother

with a recurrent infection the attack rate is probably less than 5%. However, if the mother is experiencing a primary infection at delivery the attack rate is probably greater than 50%. Neonates may present with infection localized to the skin, eyes and mucosa or the central nervous system, or with a disseminated infection (1,8).

Because of the high prevalence of past HSV infections in the general population, many patients who develop malignancy, an immunodeficiency such as AIDS or other diseases that require immunosuppressive therapy, may experience HSV infection. These infections, which may be primary or arise from reactivations, can be severe (1).

Both clinical and laboratory criteria are useful in establishing the diagnosis of HSV infection. Laboratory diagnosis is usually accomplished by isolating the virus in cell culture or by determining serologically the presence of HSV specific antigens or antibodies. In primary HSV infections, IgM antibodies usually appear between the third and seventh day after onset of symptoms. IgM antibody titers peak in four to six weeks and usually decline to undetectable levels after two months.IgM antibodies to HSV can sometimes be found in recurrent infections. However, production and detection of anti-HSV-IgM antibodies in patients with recurrent infections is less predictable and may be relatedto the severity of infection (!,5,7,8). IgG antibodies to HSV usually appear one to two weeks after the onset of infection and persist at various levels for life. Serological testing is most often performed using enzyme immunoassays (EIAs) which are easy to perform and more amenable to automation.

The Diamedix Immunosimplicity® Is-HSV 1 & 2 IgM Test Kit is an EIA procedure intended for the qualitative detection of IgM antibodies to HSV type 1 and/or type 2 antigens. The test can be performed either manually or in conjunction with one of the Diamedix Automated EIA Systems.

Principle of the Procedure

Diluted samples are incubated with HSV 1 & 2 antigens bound to the solid surface of a microtiter well. If IgM antibodies against HSV are present in the samples, they will bind to the antigen forming antigen-antibody complexes. Residual sample is eliminated by aspirating and washing. Conjugate (horseradish peroxidase-labeled anti-human IgM) is added and will bind to these complexes. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm (reference 600-630 nm) and is directly proportional to the concentration of IgM antibodies to HSV 1 & 2 antigens present in the sample.

Specimen Collection

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2 to 8°C). If assays are not completed within 48 hours, or the separated sample is to be

stored beyond 48 hours, samples are to be frozen at -20°C. Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen sera to room temperature slowly and mix gently, avoiding foam formation. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used. The NCCLS provides recommendations for collecting and storing blood specimens (10).

CAUTION: Serum samples must not be heat-inactivated prior to use.

Reagents

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded gold, coated with partially purified HSV-1 (MacIntyre strain) and HSV-2 (G-strain) antigens produced in E6 cells.
Cut-Off Calibrator	One vial with blue cap containing 0.25 ml of human serum preserved with 0.1% sodium azide, weakly reactive for HSV 1 and/or 2 IgM antibodies. The Cut-Off Calibrator is used to determine the cut-off of the assay.
Low Positive Control	One vial with white cap containing 0.25 ml of human serum reactive for HSV 1 and/or 2 IgM antibodies, preserved with 0.1% sodium azide. Assigned range printed on label. The positive control is used to control the low range of the assay.
Negative Control	One vial with black cap containing 0.25 ml of pre-diluted human serum preserved with 0.1% sodium azide, containing rheumatoid factor and specific anti-HSV IgG. Assigned range printed on label. The Negative Control is used to control the removal of IgG antibodies

Note: *Calibrator and controls are prepared from different serum lots.*

Sample D Diluent	One bottle with blue cap containing 60 ml Phosphate buffer with goat anti-human IgG and protein stabilizers. Contains 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Color-coded blue.
Wash S Concentrate (20X)	Two bottles with clear caps containing 50 ml of Phosphate buffered saline with Proclin™ 300, 15 ppm active ingredient. Color-coded light blue/green. Each bottle is sufficient to make 1 liter of wash solution.

Conjugate	One bottle with red cap containing 25 ml goat anti-human immunoglobulin M labeled with horseradish peroxidase. Also includes protein stabilizers and preservatives. Color-coded pink.
Substrate G	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethylbenzidine).
Stop M Solution	One bottle with white cap containing 30 ml of 1 N Phosphoric and 1N Hydrochloric acids. **CAUTION:** Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Precautions section.

Store these reagents at 2 to 8° C.

Other Materials Required

Manual Users:

1. Wash bottle or automated microplate washer
2. Pipettors capable of dispensing appropriate volumes
3. Timer
4. One liter graduated cylinder
5. One liter wash solution reservoir
6. Deionized or distilled water
7. Absorbent toweling
8. Tubes or microwell plate for sample dilution
9. Reader capable of reading absorbance at 450nm, reference at 600-630 nm.
10. Incubator capable of maintaining temperature of 37 ± 3°C

Diamedix Automated EIA System Users:

1. One liter graduated container
2. Deionized or distilled water
3. Dilution containers as appropriate to system
4. Sample and Reagent tips required by system
5. Reagent containers required by system

Warnings:

1. Handle samples, Calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the Calibrator and controls has been found negative for Hepatitis C, Hepatitis B surface antigen and HIV-1 and 2 antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1 and -2, Hepatitis C virus, Hepatitis B virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/ National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1993.

2. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.
4. Certain of the test reagents contain Proclin™ 300 as a preservative. When disposing of reagents containing Proclin™ 300, flush drains with copious amounts of water to dilute the active components below active levels.
5. Serum components contain sodium azide as preservative. Azides are reported to react with lead and copper in plumbing to form compounds that may become explosive. When disposing of solutions containing sodium azide, flush with copious amounts of water to minimize the build up of metal azide compounds.
6. Sodium azide inhibits conjugate activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.
7. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.
8. The substrate contains 3,3' 5,5' Tetramethylbenzidine (TMB) which has shown possible mutagenic effects in laboratory experiments.

Calibration

This test uses an in-house reference standard (or Calibrator). This Calibrator has been derived from weakly positive sera and is titrated to an absorbance value equivalent to the cut-off of the assay. Samples whose absorbances exceed this value are considered positive for HSV-1 and/or –2 antibodies and samples whose absorbances are less than this value are considered negative for HSV-1 and/or –2 antibodies. To account for the inherent variations in enzyme immunoassays an equivocal range of ± 10% has been included at the assay cut-off.

Quality Control

a) The Low Positive and Negative Controls must be included in each test run.

b) The absorbance of the Blank must be <0.100.

c) The absorbance of the Cut-Off Calibrator must be ≥0.150 when read against the reagent blank.

d) The Low Positive and Negative Controls must be within their assigned ranges.

If any of these criteria is not met, the run is invalid and must be repeated.

NOTE: Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. For guidance on appropriate quality control practices,

please refer to NCCLS document C24-A, Internal Quality Control Testing:Principles and Definitions.

Procedure

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 50 ml of Wash Concentrate (20X) to one liter of deionized or distilled H_2O.

Manual Users:

1. Prepare 1:101 dilutions of the Cut-Off Calibrator (in triplicate), controls and patient samples in Sample Diluent (e.g., by addition of 2 µl sample to 200 µl Sample Diluent or 5 µl sample to 500 µl Sample Diluent).
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of diluted Calibrator, controls and patient samples, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.

   NOTE: *Include one well which contains 100 µl of Sample Diluent as a reagent blank. This will ultimately be used to "zero" the photometer before reading test results.* ***DO NOT ADD CONJUGATE TO THE BLANK WELL.***
3. Allow the wells to incubate uncovered at 37 ± 3° C for 60 ± 5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling. Wash the wells by rinsing 3 times with at least 300 µl of Wash Solution. After adding the 3rd volume of Wash Solution, allow the wells to “soak” for at least one minute prior to final aspiration/emptying. When using an automated washer, follow the manufacturer’s instructions and set up the same wash procedure as described.
5. Place 100 µl of Conjugate into each well (*except the blank*), avoiding bubble formation.
6. Add 100 ul of Sample Diluent to the Blank well.
7. Allow the wells to incubate uncovered at 37 ± 3° C for 60 ± 5 minutes.
8. Wash the wells as described in Step 4 above.
9. Place 100 µl of Substrate into each well, avoiding bubble formation.
10. Allow the wells to incubate uncovered at 37 ± 3° C for 20 ± 2 minutes.
11. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
12. Read the absorbance of the wells at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 30 minutes of adding Stop Solution.

Diamedix Automated EIA System Users:

If using one of Diamedix's Automated EIA Systems, please refer to the corresponding Operating Manual for the test setup, procedure, and accessories/consumables needed.

Calculation of Results

Calculate the mean absorbance of the Cut-off Calibrator. **Note:** When calculating the mean absorbance value for the Cut-Off Calibrator exclude any absorbance value that deviates by more than 20% from the mean of the three absorbance values. Use the mean of the remaining two replicates in calculations. Exclusion of more than one of the three absorbance values invalidates the run.

Determine the Index Value for each patient sample or control using the following formula:

$$\frac{\textbf{Absorbance of Sample}}{\textbf{Mean Absorbance of Cut-off Calibrator}} = \textbf{Index Value}$$

The Diamedix Automated EIA Systems will calculate results using the above formula.

Example: Absorbance values obtained for the Calibrator: 0.276, 0.288, 0.258 (after subtraction of Blank)

Mean Absorbance of Cut-off Calibrator = 0.274

Sample Absorbance = 1.150

Index Value 1.150/ 0.274 = 4.2

Reference Ranges

Index < 0.90	Negative for anti-HSV 1 and/or 2 IgM.
Index ≥ 1.1	Positive for anti-HSV 1 and/or 2 IgM.
Index 0.90-1.09	Equivocal for anti-HSV 1 and/or 2 IgM*.

* When equivocal results are obtained, another specimen should be collected ten to fourteen days later and tested in parallel with the initial specimen. If the second sample is also equivocal, the patient is negative for primary or recent infection, and equivocal for IgM antibody status.

A negative result does not always exclude the possibility of active HSV infection. The sample may have been collected before the appearance of IgM antibody. If infection is suspected, a second sample should be collected at least 7 days later and tested concurrently with the first sample.

Reporting Results

When the Index value is reported for a single specimen the following statement should be included: "The following results were obtained with the Diamedix Immunosimplicity Is-HSV 1 & 2 IgM EIA Test System. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present. The magnitude of the reported IgM level cannot be correlated to an end-point titer."

Procedure Notes

1. Do not interchange reagents from different reagent lots except for Sample **D** Diluent, Wash **S** Concentrate, Substrate **G** and Stop **M** Solution.
2. Do not use reagents beyond their expiration date.
3. Store unused reagents at 2 to 8° C.
4. Incubations above or below the recommended temperatures or times may give erroneous results.
5. The EIA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
6. Antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
7. *(Manual Procedure Only)* The washing procedure is very important and requires special attention. (Please refer to the Procedure section)

   NOTE: *Improperly washed* wells *may* give *erroneous results.*
8. The concentration of anti-HSV 1 & 2 IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

Limitations

1. The results obtained with the Is-HSV 1 & 2 IgM Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
2. Assay performance characteristics have not been established for visual result determination.
3. HSV 1 & 2 IgM EIAs are not intended to replace virus isolation and/or identification.
4. This test is not intended to be used as the sole criterion for the diagnosis of current herpes simplex infection in pregnant women. The presence of HSV should be demonstrated by isolation of live virus.

5. The test should be performed on serum. The use of whole blood or plasma has not been established.
6. Performance of this assay has not been established on spectrophotometry utilizing a single wavelength.
7. The continued presence or the level of antibody cannot be used to determine the success or failure of therapy.
8. The presence of HSV 1 and/or 2 IgM may indicate a primary or reactivated infection but cannot distinguish between these conditions.
9. A negative result does not necessarily rule out a primary or reactivated infection since samples may have been collected too early in the course of disease or too late in the course of disease when IgM levels are below detectable levels.
10. Due to commonly shared antigens, infections with one type of HSV in the presence of antibody to the heterologous type, may produce an anamnestic response with the pre-existing antibody to become more elevated than the antibody titer of the infective agent of the current infection. Definitive diagnosis of HSV typing should be made by viral isolation.
11. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr virus and give false positive results in HSV 1 & 2 IgM EIA tests. A heterotypic rise in anti-HSV IgM antibody level may also be observed in a primary or re-activated VZV infection.
12. Since rheumatoid factor (RF) binds to IgG in immunocomplexes, false positive results may arise in sera with RF and specific IgG. False negatives may arise due to specific IgG competing with specific IgM The goat anti-human IgG in Sample **D** Diluent diminishes RF interference and minimizes competing specific IgG in test samples. Sample **D** Diluent removes >95% of the IgG at levels up to 1400 mg/dl. Samples with IgG levels >1400 mg/dl should be interpreted with caution.
13. The prevalence of the analyte will affect the assay’s predictive value.
14. The performance characteristics have not been established for neonates, infants or on cord blood.
15. Results from immunosuppressed patients should be interpreted with caution.
16. The performance characteristics of the Diamedix Is-HSV 1 & 2 IgM Test Kit with automated equipment other than Diamedix Automated EIA Systems have not been established.

References

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