Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

HSV 1&2 IgM
Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-350 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of Cut-Off Calibrator, controls and patient samples in Sample Diluent. Mix well.
2. Add 100 µl of diluted Cut-Off Calibrator, Controls and patient samples into the antigen wells. Reserve one well for reagent blank (100 µl of Sample Diluent).
3. Incubate at 37 ± 3°C for 60 ± 5 min.
4. Discard contents of the wells. Wash the wells 3 times with Wash Solution.
5. Add 100 µl of Conjugate to each well.
6. Incubate at 37 ± 3°C for 60 ± 5 min.
7. Wash the wells as in #4 above.
8. Add 100 µl Substrate Solution to each well.
9. Incubate at 37 ± 3°C for 20 ± 2 min.
10. Add 100 µl Stop Solution to each well.
11. Read the absorbances at 450/600-630 nm.

INTENDED USE

For the qualitative detection of IgM antibodies to herpes simplex virus (HSV) type 1 and/or type 2 in human serum by indirect enzyme immunoassay. This test can aid in the diagnosis of a primary or reactivated infection with HSV. The performance of this assay has not been established for use in neonates, infants, or on cord blood, and immunocompromised patients.

SUMMARY AND EXPLANATION

Herpes simplex virus (HSV) is classified in the alphaherpesvirus subfamily of herpes viruses 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 asymptomatic, 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 related to the severity of infection (1,5,7,8). 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.

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.

REAGENTS

Each Is-HSV 1 & 2 IgM Test Kit contains reagents for 96 tests.

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 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 negative range of the assay and to control the removal of IgG antibodies. *Note that the Cut-Off 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 20 (X)	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 1050 ml 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:

Wash bottle or automated microplate washer

Pipettors capable of dispensing appropriate volumes

Timer

One liter graduated cylinder

One liter wash solution reservoir

Deionized or distilled water

Absorbent toweling

Tubes or microwell plate for sample dilution

Reader capable of reading absorbance at 450 nm, reference at 600-630 nm

Incubator capable of maintaining temperature of 37 ± 3°C

Automated EIA Processor Users:

One liter graduated cylinder

Deionized or distilled water

Pre-dilution cups, strips or plates.

ProbeClean™ Concentrate, or tip washing detergent solution, if applicable.

WARNINGS AND PRECAUTIONS

For *in vitro* Diagnostic Use

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. Reagents containing Sodium Azide:

   (a) **CAUTION:** Some reagents in this kit contain Sodium Azide as preservative. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts", in the Manual Guide – Safety Management No. CDC-22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

   European Communities Hazardous Substance Risk Phrases (Regulation (EC) No 1272/2008)

   H300 –Fatal if swallowed.
   H310 – Fatal if contact with skin.
   EUH032 – Contact with acids liberates very toxic gas.
   H410 – Very toxic to aquatic life with long lasting effect.
   P264 – Wash all exposed external body areas thoroughly after handling.
   P302+P352 – IF ON SKIN: Wash with plenty of water and soap.
   P301+P310/P330 – IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.
   P270 – Do not eat, drink or smoke when using this product.
   P501 – Dispose of contents/container as hazardous waste.
   P391 – Collect spillage.
   P273 – Avoid release to the environment. Refer to special instructions/ Safety Data Sheet.

   (b) Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.
6. Avoid contamination of the TMB substrate solution with conjugate or other oxidants, which will cause the solution to change color prematurely.
7. Do not interchange reagents from different reagent lots except for Sample **D** Diluent, Wash **S** Concentrate, Substrate **G** and Stop **M** Solution.
8. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
9. Store unused reagents at 2 to 8°C.
10. Incubations above or below the recommended temperatures or times may give erroneous results.
11. 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.
12. Antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
13. (*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.
14. The concentrations 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.

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 (2-8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should 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 CLSI, formerly NCCLS, provides recommendations for collecting and storing blood specimens (10).

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

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 µl 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 each well at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 30 minutes of adding Stop Solution.

AUTOMATED EIA PROCESSOR USERS:

When using an Automated EIA Processor, refer to the Operator's Manual for the test setup and procedures.

NOTE: *Automated EIA Processor users must validate their equipment to demonstrate that the results obtained are equivalent to those obtained using manual assay.*

QUALITY CONTROL

1. The Low Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank must be < 0.100.
3. The absorbance of the Cut-Off Calibrator must be ≥ 0.150 when read against the reagent blank.
4. The Low Positive and Negative Controls must be within their assigned ranges.

If any one of these criteria are not met, the results are invalid and the test should be repeated.

NOTE: Additional controls may be tested according to guidelines or requirements of local, state or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to CLSI, formerly NCCLS, document C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions.

RESULTS

1. Calculation

Calculate the mean absorbance of the Cut-Off Calibrator. Note: When calculating the mean absorbance value of 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:

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

An Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor) will calculate results using the above formula.

Example: Absorbance values obtained for the Cut-Off 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

2. Interpretation of Results

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

3. 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".

CUT-OFF ESTABLISHMENT

The Diamedix *Is*-HSV 1 & 2 IgM cut-off value was established to optimally differentiate those individuals with, from those without IgM antibodies to HSV. The optimal cut-off value was determined by statistical analysis of one hundred and fifty-eight (158) normal sera shown to be negative by the *Is* method, as well by another test method. The mean and standard deviation of the absorbance values for these samples were 0.1177 and 0.0567 respectively. The cut-off was determined as being equal to the mean plus 3 standard deviations, 0.1177 + (3 X 0.0567) = 0.2878. The Cut-Off Calibrator has been titrated to equal this result. Therefore, the mean absorbance value of the Cut-Off Calibrator will be equal to the cut-off for the assay. To account for the inherent variation in EIA methods, an equivocal range of ± 10% has been included.

The appropriateness of the cut-off value was further verified by applying the principles from Receiver-Operating Characteristic (ROC) Curves to 258 sera assayed manually by Diamedix Corp. in the *Is*-HSV 1 & 2 IgM Test Kit and other commercially available test methods. At the optimized cut-off level, the Diamedix *Is*-HSV 1 & 2 IgM test has a relative sensitivity of 79% and a relative specificity of 90% based on comparison to the marketed tests.

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 wave-length.
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 antibodies 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 have already declined 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 amnestic 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 antibody level may also be observed in a primary or reactivated 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 the MAGO Plus Automated EIA Processor have not been established.

EXPECTED VALUES

The prevalence of HSV IgM antibodies can vary depending on a number of factors such as age, gender, geographical location, socio-economic status, race, sexual behavior, testing method used, specimen collection and handling procedures and clinical and epidemiological history of individual patients.

In the present study two hundred sera from South Florida blood donors were evaluated in the *Is*-HSV 1 & 2 IgM Test Kit. These sera were derived from 102 female donors and 98 male donors. Of these samples one hundred and fifty-eight (79%) were negative, thirty-two (16%) were positive and ten (5%) were equivocal. TABLE 1 shows the age and prevalence profile for this population. FIGURE 1 presents a histogram showing the distribution of Index values obtained. FIGURE 2 shows the distribution of values in the 59 positive samples tested by Diamedix. In addition to these samples, 77 sera from pregnant females were tested. For this group, 55 (71.4%) were negative, 13 (16.9%) were positive and 9 (11.7%) were equivocal for HSV IgM antibodies using the *Is*-HSV 1 & 2 IgM Test kit.

TABLE 1
Age Distribution and Prevalence of anti-HSV 1 & 2 IgM in a Normal S. Florida Population

	Number of Donors	% Seronegative	% Seropositive	% Equivocal
Total Number	200	79.0% (158)	16.0% (32)	5.0% (10)
Geographic Location: S. Fla	200			
Age: 10 – 19	18	77.8% (14)	22.2% (4)	0.0% (0)
20 – 29	47	70.2% (33)	23.4% (11)	6.4% (3)
30 – 39	74	81.1% (60)	13.5% (10)	5.4% (4)
40 - 49	40	80.0% (32)	12.5% (5)	7.5% (3)
50 - 59	11	100.0% (11)	0.0% (0)	0.0% (0)
60 - 69	9	77.8% (7)	22.2% (2)	0.0% (0)
>70	1	100.0% (1)	0.0% (0)	0.0% (0)
Gender				
Male	98	87.7% (86)	9.2% (9)	3.1% (3)
Female	102	70.6% (72)	22.5% (23)	6.9% (7)

FIGURE 1
Distribution of *Is*-HSV 1 & 2 IgM Results in a Normal Population

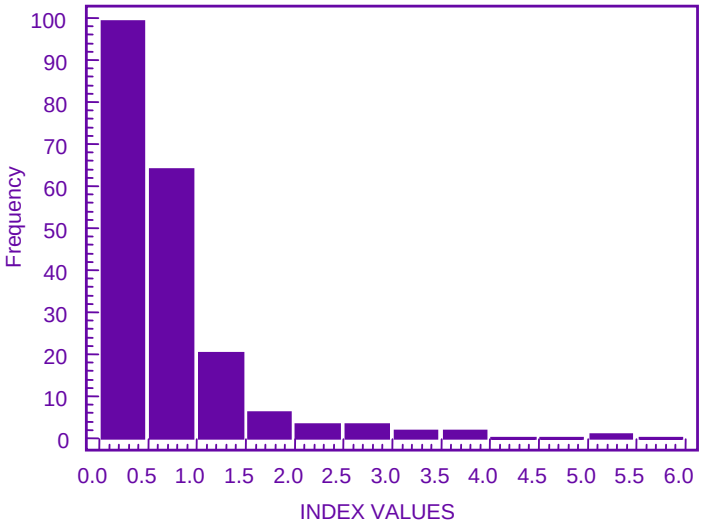
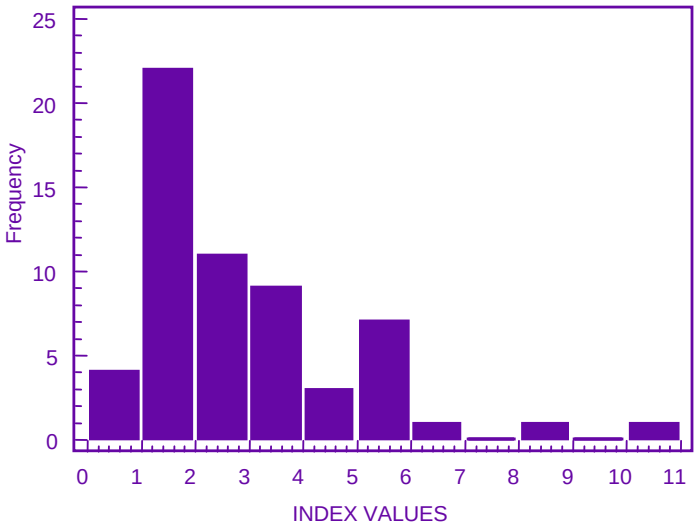


FIGURE 2
Distribution of *Is*-HSV 1 & 2 IgM Results in a Positive Population



PERFORMANCE CHARACTERISTICS

A. Comparison Testing

A total of five hundred and twenty-one sera were tested for the presence of HSV 1 & 2 IgM antibodies using the Diamedix *Is*-HSV1 & 2 IgM Test Kit and other marketed tests at two independent sites (site #1, California and site #2, New York) as well as at Diamedix Corp., Miami, FL (site #3). At site #3, testing was performed both manually and using the MAGO Plus Automated EIA Processor.

Site #1, a large commercial laboratory in California, not affiliated with the manufacturer, tested 132 samples. These samples consisted of 100 fresh samples submitted to the laboratory for HSV IgM testing and 32 frozen samples which had previously tested positive for HSV IgM antibodies using EIA and/or IFA methods. Samples came from a wide variety of geographic locations and from patients with ages ranging from 1 day to 81 years old. For the fresh samples, 60 were from females and 39 from males. Forty of the females were between the ages of 18 and 45 but were not specifically identified as prenatal. The remaining sample was not identified as regards gender. The current testing protocol for HSV IgM testing at site #1 involves screening samples on separate HSV 1 and HSV 2 IgM EIA test kits. Samples with negative results are reported as such. Samples with positive or equivocal results in either EIA test are then tested using HSV 1 and HSV 2 IFA methods for purposes of confirmation. TABLE 2 summarizes the initial testing using the Diamedix *Is*-HSV 1 & 2 IgM Test Kit and the other EIA test kits.

Site #2, a commercial reference laboratory in New York, not affiliated with the manufacturer, tested 130 samples. These samples consisted of 65 fresh samples and 65 frozen samples submitted to the laboratory for HSV IgM testing. Samples were obtained from various geographic regions and from patients with ages ranging from 4 to 88 years old. Fifty samples were from males and seventy-four from females. The remainder were not identified as regards gender. Fifty of the females were 18-45 years old. TABLE 3 compares the results obtained for the *Is*-HSV IgM test kit and the HSV IgM EIA kits currently used by the laboratory.

TABLE 2
***Is*-HSV 1&2 IgM – Site #1**

		Positive	Negative	Equivocal
Other EIAs (Combined)	Positive	57	21	0
	Negative	2	38	0
	Equivocal*	0	0	14

Overall Agreement 95/118 = 80.5%
**95% CI = 73.4 to 82.7

TABLE 3
***Is*-HSV 1&2 IgM – Site #2**

		Positive	Negative	Equivocal
Other EIAs (Combined)	Positive	27	2	3
	Negative	14	77	3
	Equivocal*	1	2	1

Overall Agreement 104/120 = 86.7%
**95% CI = 80.6 to 92.7

* Equivocal results excluded from calculations
**95% Confidence Intervals (CI) calculated by the Exact Method (9)

For Site #1, further testing of the discordant samples was performed by testing such samples using other methods. Of the 21 samples that were negative in the Is-HSV 1 & 2 IgM test but positive by the comparative EIA tests, all were negative in the confirmatory IFA test and 18 were negative in other EIA tests. Three were positive in the referee HSV 2 test. Of the 2 samples that were positive in the *Is*-HSV 1 & 2 IgM test and negative in the comparative tests, one was positive in the referee HSV 2 test.

For Site #2, further testing of the discordant samples was performed by testing such samples using other methods. Of the 2 samples that were negative in the *Is*-HSV 1 & 2 IgM test and positive in one of the two comparative tests, one was positive and one was negative in the referee (type 1 and 2) test. Of the 14 samples that were positive in the *Is*-HSV 1 & 2 IgM test and negative in the comparative tests, 12 were positive, one was equivocal and one was negative in the referee (type 1 and 2) test.

Site #3 (Diamedix Corp.) tested 259 samples (all frozen) by the manual method and 258 of these samples (one being QNS) by the MAGO Plus method. Two hundred of these samples were obtained from normal S. Florida blood donors and the remaining 59 sera from patients with positive serostatus. TABLES 4 and 5 compare the results obtained for the *Is*-HSV 1 & 2 IgM Test Kit and another marketed EIA test kit.

TABLE 4
***Is*-HSV 1&2 IgM – Site #3: Manual**

		Positive	Negative	Equivocal
Other EIAs (Combined)	Positive	74	17	7
	Negative	10	138	4
	Equivocal*	2	5	2

Overall Agreement 212/239 = 88.7%
**95% CI = 84.7 to 92.7

TABLE 5
***Is*-HSV 1&2 IgM – Site #3: MAGO Plus**

		Positive	Negative	Equivocal
Other EIAs (Combined)	Positive	74	12	12
	Negative	13	123	15
	Equivocal*	3	6	0

Overall Agreement 197/222 = 88.7%
**95% CI = 84.6 to 92.9

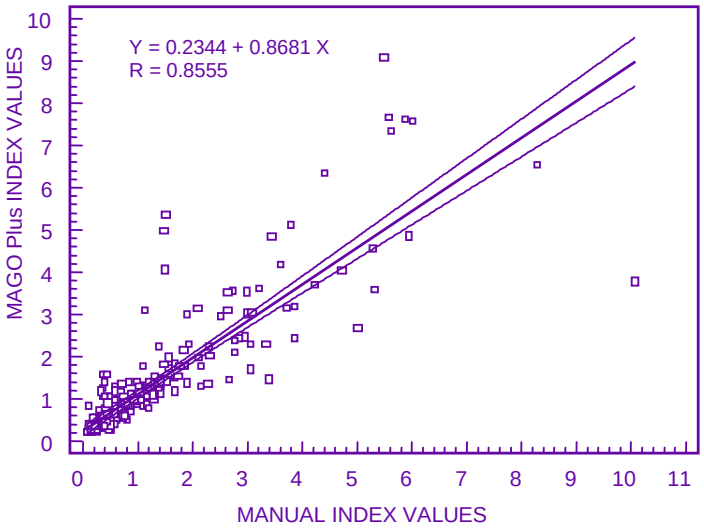
* Equivocal results excluded from calculations
**95% Confidence Intervals (CI) calculated by the Exact Method (9)

For site #3 (manual testing), further testing of the discordant samples revealed that of the 10 samples positive in the *Is*-HSV 1 & 2 IgM and negative in the comparative methods, 5 were positive, 3 were equivocal and 2 were negative in the referee method. Of the 17 samples negative in the *Is*-HSV 1 & 2 IgM and positive in the comparative methods, 11 were negative, 5 were positive and one was equivocal in the referee method. For the MAGO Plus testing, of the 13 samples that were positive in the *Is*-method and negative in the comparative tests, 7 were negative, 4 were positive and 2 were equivocal in the referee test. Of the 12 samples that were negative in the *Is*-method and positive in the comparative methods, 8 were negative, 3 were positive and one was equivocal in the referee method.

B. Correlation of Manual and MAGO Plus Results

The *Is*-HSV 1 & 2 IgM Test Kit has been developed for automated as well as manual use. To demonstrate the equivalence of the manual and MAGO Plus procedures, the results of 258 serum samples tested above were compared. A scattergram and regression line of the results obtained with 95% confidence intervals is shown in FIGURE 3.

FIGURE 3
Manual vs. MAGO Plus Correlation



C. Cross-Reactivity/Interference Studies

The specificity of the *Is*-HSV 1 & 2 IgM Test Kit was assessed by testing a number of sera containing relatively high levels of IgM antibody to other viruses, including other herpesviruses. A total of 29 known IgM-positive samples were tested. In addition, the effects of potential interference from rheumatoid factor (RF), anti-nuclear antibody (ANA), viral-specific IgG and heterophile antibodies were assessed by testing an additional 33 sera. Results are summarized in TABLE 6 and show some cross-reactivity with Epstein Barr Virus (EBV), Cytomegalovirus (CMV) and Toxoplasma. In addition, some interference was noted in one highly positive RF sample and in some ANA positive samples. Note that several of these samples were also positive in a commercially available HSV IgM test. TABLE 7 shows the lack of interference in samples containing relatively high levels of IgG antibodies and low levels of IgM antibodies before and after removal of the IgG-class antibodies.

TABLE 6

Specificity	# of Positive in *Is*-HSV 1 & 2 IgM
EBV IgM	2/8
Lyme IgM	0/3
VZV IgM	0/4
Rubella IgM	0/4
CMV IgM	1/5
Toxoplasma IgM	2/5
Heterophile Ab	0/4
RF	1/8
ANA	5/10
HSV IgG	0/11

TABLE 7

Sample #	Before IgG removal		After IgG removal	
	IgG EU/ml	IgM Index	IgG EU/ml	IgM Index
1	65.6	1.313	0.0	1.121
2	87.1	1.592	0.0	1.530
3	89.0	1.962	0.0	2.119
4	57.1	1.438	0.0	1.255
5	61.0	1.412	0.0	1.197
6	56.5	1.414	0.0	1.212
7	91.1	1.990	0.0	1.815

(Pos > 20 EU/ml)

D. Verification of IgM Specificity

To confirm that the *Is*-HSV 1 & 2 IgM Test Kit specifically detects IgM-class antibodies, 13 samples with moderate to high levels of antibodies were selected for testing. These samples were treated with Dithiothreitol (DTT) to destroy the IgM and were then retested in the *Is*-HSV 1 & 2 IgM Test Kit. The results in TABLE 8 show that these samples were rendered non-reactive following treatment with DTT confirming the specificity of the *Is*-HSV 1 & 2 IgM Test Kit for detecting IgM-class antibodies.

TABLE 8

Sample #	Untreated		Treated with DTT	
	Is-HSV 1 & 2 IgM		*Is*-HSV 1 & 2 Igm	
	Index	Interp	Index	Interp
1	3.279	POS	0.124	NEG
2	7.579	POS	0.482	NEG
3	4.804	POS	0.132	NEG
4	4.880	POS	0.381	NEG
5	3.252	POS	0.605	NEG
6	8.029	POS	1.54	NEG
7	3.986	POS	0.222	NEG
8	3.237	POS	0.173	NEG
9	4.421	POS	0.215	NEG
10	7.367	POS	0.511	NEG
11	7.182	POS	0.816	NEG
12	3.345	POS	0.633	NEG
13	5.975	POS	0.339	NEG

E. Precision

Six serum samples (two negative and four positive) as well as the kit Calibrator and controls were tested in triplicate in three separate runs for site #1 and #2 and in six separate runs for site #3. The precision studies were performed manually at the two independent testing sites (site #1 and site #2) and at site #3 (Diamedix Corp.) both manually and using the MAGO Plus Automated Processor. The results obtained are shown in Tables 9-12.

TABLE 9
Site #1- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
H1	0.205	0.023	11.22	0.236	0.037	15.68	0.212	0.012	5.66	0.218	0.027	12.39
H2	0.270	0.018	6.67	0.306	0.012	3.92	0.244	0.026	10.66	0.273	0.032	11.72
H3	1.377	0.088	6.39	1.406	0.020	1.42	1.429	0.084	5.88	1.404	0.066	4.70
H4	3.250	0.241	7.42	3.067	0.267	8.71	3.392	0.256	7.55	3.236	0.262	8.10
H5	4.232	0.004	0.09	3.818	0.187	4.90	4.362	0.223	5.32	4.137	0.288	6.96
H6	7.858	0.339	4.31	6.401	0.130	2.03	7.552	0.294	3.89	7.271	0.705	9.70
POS	1.819	0.020	1.10	1.815	0.239	13.17	1.719	0.244	14.19	1.784	0.178	9.98
NEG	0.264	0.017	6.44	0.329	0.058	17.63	0.266	0.067	25.19	0.287	0.055	19.16

TABLE 10
Site #2- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
H1	0.224	0.072	32.14	0.191	0.090	47.12	0.233	0.026	12.88	0.216	0.063	29.17
H2	0.286	0.111	38.81	0.182	0.012	6.59	0.319	0.024	8.46	0.262	0.084	32.06
H3	1.087	0.044	4.05	1.367	0.514	37.60	1.946	0.117	6.94	1.466	0.464	31.65
H4	2.294	0.184	8.02	2.968	0.080	2.70	3.076	0.275	10.31	2.780	0.412	14.82
H5	3.538	0.633	17.89	4.337	0.411	9.48	5.553	0.046	0.96	4.476	0.957	21.38
H6	5.967	0.078	1.31	6.599	1.139	17.26	9.606	0.714	8.57	7.391	1.825	24.69
CAL	1.149	0.400	34.81	0.854	0.150	17.56	1.364	0.203	17.17	1.122	0.309	29.32
POS	2.109	0.613	29.07	1.689	0.197	11.66	2.404	0.168	8.06	2.067	0.458	22.16
NEG	0.445	0.168	37.75	0.235	0.024	10.21	0.374	0.080	24.62	0.351	0.134	38.18

TABLE 11
Site #3- Intra-Assay and Interassay Precision (Manual)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
H1	0.191	0.035	18.32	0.193	0.070	36.27	0.161	0.017	10.56	0.181	0.046	25.41
H2	0.316	0.049	15.51	0.268	0.018	6.72	0.235	0.019	8.09	0.273	0.045	16.48
H3	1.388	0.058	4.18	1.283	0.089	6.94	1.202	0.058	4.83	1.291	0.102	7.90
H4	3.424	0.085	2.48	2.646	0.280	10.58	2.398	0.107	4.46	2.823	0.481	17.04
H5	5.772	0.490	8.49	4.644	0.459	9.88	4.495	0.564	12.55	4.970	0.755	15.19
H6	8.538	0.825	9.66	7.668	0.469	6.12	7.339	0.750	10.22	7.848	0.837	10.67
c/o CAL	1.019	0.099	9.72	0.900	0.068	7.56	0.894	0.055	6.15	0.938	0.093	9.91
POS	1.474	0.144	9.77	1.233	0.182	14.76	1.334	0.162	12.14	1.347	0.184	13.66
NEG	0.351	0.054	15.38	0.291	0.054	18.56	0.346	0.105	30.35	0.329	0.076	23.10

TABLE 12
Site #3- Intra-Assay and Interassay Precision (MAGO Plus)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
H1	0.22	0.059	26.82	0.23	0.046	20.00	0.25	0.037	14.80	0.23	0.05	21.74
H2	0.35	0.053	15.14	0.36	0.025	6.94	0.38	0.053	13.95	0.36	0.05	13.89
H3	1.39	0.121	8.71	1.42	0.120	8.45	1.47	0.136	9.25	1.43	0.12	8.39
H4	2.69	0.478	17.77	2.54	0.263	10.35	2.54	0.293	11.54	2.59	0.34	13.13
H5	6.05	0.377	6.23	5.30	0.322	6.08	5.35	0.384	7.18	5.57	0.49	8.80
H6	8.33	0.509	6.11	7.62	0.681	8.94	7.86	0.537	6.83	7.94	0.62	7.81
c/o CAL	0.96	0.113	11.77	1.00	0.110	11.00	1.25	0.091	7.28	1.07	0.16	14.95
POS	1.42	0.289	20.35	1.22	0.283	23.20	1.32	0.096	7.27	1.32	0.24	18.18
NEG	0.37	0.143	38.65	0.40	0.060	15.00	0.44	0.089	20.23	0.40	0.10	25.00

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