Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

HSV 1&2 IgG

Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-340 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of patient samples in Sample Diluent. Mix well.
   Note that Calibrator and Controls are ready-to-use.
2. Add 100 µl of patient samples, Calibrator, and Controls 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 and semi-quantitative detection of IgG antibodies to herpes simplex virus (HSV) type 1 and/or type 2 in human serum by indirect enzyme immunoassay. This test is useful for indicating a past infection with HSV in a single specimen, including females of child-bearing age. The evaluation of acute and convalescent specimens, by demonstrating seroconversion or a significant increase in antibody level, can aid in the diagnosis of primary infection with HSV.

SUMMARY AND EXPLANATION

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 muco-cutaneous 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. IgG antibodies to HSV usually appear 1 to 2 weeks after the onset of infection and persist at various levels for life. Determination of HSV antibody status is diagnostically useful in several situations, including the demonstration of recent seroconversion after acute infection, the documentation of symptomatic past infections and the identification of allograft recipients or patients with cancer who are at risk for reactivation of latent HSV infection. Perhaps the greatest value of serologic tests lies in their ability to identify asymptomatic carriers.[4] Serological testing is most often performed using enzyme immunoassays (EIAs) which are easy to perform and more amenable to automation for screening large numbers of samples than other methodologies.

The Diamedix Immunosimplicity® *Is*-HSV 1 & 2 IgG Test Kit is an EIA procedure intended for the qualitative and semi-quantitative detection of antibodies to HSV type 1 and type 2 antigens. The results are objective and reported in EU/ml (ELISA Units per ml) which are traceable to in-house reference materials.

PRINCIPLE OF THE PROCEDURE

Diluted samples are incubated with HSV 1 & 2 antigens bound to the solid surface of a microtiter well. If IgG 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 IgG) 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 IgG antibodies to HSV 1 & 2 antigens present in the sample.

REAGENTS

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

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded light green, coated with sucrose density gradient purified HSV-1 (MacIntyre strain) and HSV-2 (G-strain) antigens produced in Vero cells.
Calibrator	Two vials with blue caps containing 1.8 ml of predi-luted human serum, highly reactive for HSV 1 & 2 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
Positive Control	One vial with white cap containing 1.8 ml of pre-diluted human serum, moderately reactive for HSV 1 & 2 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml range printed on label.
Negative Control	One vial with black cap containing 1.8 ml of pre-diluted human serum, non-reactive for HSV 1 & 2 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Note that the Calibrator and controls are prepared from different serum lots.
Sample **A** Diluent	One bottle with blue cap containing 60 ml Phosphate buffer with 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 1050 ml of wash solution.
Conjugate	One bottle with red cap containing 25 ml goat anti-human immunoglobulin G labeled with horseradish peroxidase. Also includes protein stabilizers and preservatives. Color-coded pink.
Substrate **HRP**	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethylbenzi-dine).
Stop **M** Solution	One bottle with white cap containing 30 ml of 1 N Phosphoric and 1 N 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.

PRECAUTIONS

REAGENTS: 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 B surface antigen and HIV-1 antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, 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.

ADDITIONAL PRECAUTIONS:

1. Do not interchange reagents from different reagent lots except for Sample **A** Diluent, Wash **S** Concentrate, Substrate **HRP** and Stop **M** Solution.
2. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
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 concentrations of anti-HSV IgG 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, (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18A3).

For the diagnosis of recent HSV infection, paired sera should be obtained at least 7-10 days apart.

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:

The Calibrator and Controls are provided ready to use: DO NOT DILUTE FURTHER.

1. Prepare 1:101 dilutions of the 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 Calibrator, controls and diluted 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.
3. Allow the wells to incubate uncovered at 37 ± 3º 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. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at 37 ± 3° C for 60 ± 5 minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.

9. Allow the wells to incubate uncovered at 37 ± 3° C for 20 ± 2 minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Read the absorbance of the wells at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 60 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 Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank must be < 0.2.
3. The Positive Control must be within its assigned range.
4. The Negative Control must be < 16.0 EU/ml.

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

NOTE: The Negative and Positive Control are intended to monitor substantial reagent failure. The controls will not control all parts of the procedure such as technical dilution of patient specimens. The Positive Controls will not ensure precision at the assay cut-off. Users may wish to establish an in-house control having a quantitative value determined by replicate testing, at or near the cut-off to monitor the precision of the assay cut-off. 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 CLSI, formerly NCCLS, document C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions.

RESULTS

1. Calculation

Determine the EU/ml (ELISA) for each patient specimen or control using the following formula:

$$\frac{\text{EU/ml of Calibrator}}{\text{Absorbance of Calibrator}} \times \text{Absorbance of sample} = \text{EU/ml of sample}$$

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

Specimens which yield absorbances greater than that of the Calibrator may be reported as greater than 100 EU/ml or Index >5.0. Alternatively, such samples may be pre-diluted in Sample Diluent and retested. Several dilutions may be assayed simultaneously. Select the dilution that has an absorbance reading of about 50% of the absorbance reading of the Calibrator, calculate the EU/ml for this dilution and multiply by the dilution factor to obtain estimated values.

Example: If the specimen has been pre-diluted 1:5 before testing, the resulting EU/ml or Index Value should be multiplied by 5.

2. Interpretation of Results

EU/ml	Index Value	Interpretation
< 16.0	< 0.80	Negative for anti-HSV 1&2 IgG
16.0 – 19.9	0.8 – 0.99	Equivocal for anti-HSV 1&2 IgG*
≥ 20.0	≥ 1.0	Positive for anti-HSV 1&2 IgG

* 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 antibody status. If the second sample is positive, the patient can be considered to have a primary infection. The conversion of an individual patient's serum from negative to positive for antibodies to the infectious agent in question, is defined as seroconversion, and indicates active or recent infection.

A single positive result only indicates previous immunologic exposure. The level of antibody response or class of antibody may not be used to determine active infection or disease stage.

A negative result does not always exclude the possibility of active HSV infection. The sample may have been collected before the appearance of IgG antibody. If infection is suspected, a second sample should be collected at least 7 days later and tested concurrently with the first sample to determine if seroconversion has occurred. In addition, a negative result does not exclude the possibility of past infection, since, in some cases, antibody levels may fall to undetectable levels after primary HSV infection.

3. Reporting Results

When the EU/ml 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 IgG 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 IgG level cannot be correlated to an end-point titer."

When the assay is used semi-quantitatively, the following statement should be included when reporting results: "Timing of specimen collection for paired sera may be critical. In some patients, antibody titers may rise to significant levels and fall again to lower or undetectable levels within a month. Other patients may not develop significant antibody levels. Culture results, serology and antigen detection methods should all be appropriately used along with clinical findings for diagnosis."

4. Paired Sera

To determine a significant difference between acute/convalescent sera, both specimens should be run within the same assay. Paired sera should be evaluated within the reportable range of the assay. In addition, the EU/ml value for the acute serum should be less than 60. In-house studies performed both manually and using the MAGO Plus have shown that a 1.9-fold or greater increase in the EU/ml or Index ratio (convalescent serum EU/ml or Index Value / acute serum EU/ml or Index Value) corresponds to a four-fold increase in HSV IgG antibody level. The mean ratio obtained for two-fold dilutions was 1.4. Therefore, ratios in the range of 1.4 to 1.9 may be considered equivocal for significant increase status. If paired sera controls are desired, it is recommended that 1:2, 1:4 and 1:8 dilutions of the Calibrator be made first in Sample Diluent. The 1:2 and 1:8 dilutions will provide a simulated serum pair. The ratio of this simulated serum pair can then be compared against the established ratio.

CUT-OFF ESTABLISHMENT

The Diamedix *Is*-HSV 1 & 2 IgG cut-off value was established to optimally differentiate those individuals with, from those without, immunological experience to HSV. The optimal cut-off value was determined by statistical analysis of the results of 144 sera shown to be negative by the method as well as other test methods. The mean plus 3 SD (3.73 plus 3 x 3.86 = 15.3, rounded up to 16.0) was considered the equivocal decision point. The mean plus 4 SD (3.73 plus 4 x 3.86 = 19.2, rounded up to 20.0) was considered the positive cut-off point.

This cut-off value was further verified by applying the principles from Receiver-Operating Characteristic (ROC) Curves to 267 normal sera assayed manually by Diamedix Corp. in the *Is*-HSV 1 & 2 IgG Test Kit and another commercially available test method. At the optimized cut-off level, the Diamedix *Is*-HSV 1 & 2 IgG has a relative sensitivity of 100% and a relative specificity of 100% based on comparison to the marketed test.

The appropriateness of the cut-off was further confirmed using CDC Reference Sera (see Performance Characteristics).

LIMITATIONS

1. The results obtained with the *Is*-HSV 1 & 2 IgG 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 IgG EIAs are not intended to replace virus isolation and/or identification.
4. This test is not intended to be used 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 IgG does not imply protection from disease with one type-specific herpes (type 1 or 2) and does not provide protection from a second infection with the other type, however, it may lessen the severity of the second infection. In addition, individuals with past HSV infection may exhibit recurrent (reactivated) episodes of the past HSV infection.
9. Interpretation of serologic data for HSV must be completed with the understanding of cross-reactivity due to shared antigens and the chronic nature of HSV infections. 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. Therefore, definitive diagnosis of HSV typing should be made by viral isolation.
10. A significant increase in HSV antibody titer may not accompany recurrent herpes simplex disease. In addition, a significant increase in HSV antibody titer may not accompany first episode infection of the other type-specific HSV (e.g. HSV-1 infection followed by HSV-2 infection).
11. The performance characteristics have not been established for neonates, infants or on cord blood.
12. Results from immunosuppressed patients should be interpreted with caution.
13. The performance characteristics of the Diamedix *Is*-HSV 1 & 2 IgG Test Kit with automated equipment other than the MAGO® Plus Automated EIA Processor have not been established.

EXPECTED VALUES

The prevalence of HSV IgG antibodies in the normal population can vary depending on a number of factors such as age, geographical location, socioeconomic status, race, sexual behavior and testing method used. It is estimated that by the fourth decade 80% of the normal population has been exposed to HSV (7). The prevalence of anti-HSV antibodies also increases with the age of the population.

Sera from 100 randomly selected South Florida blood donors were evaluated in the *Is*-HSV 1 & 2 IgG Test Kit. These sera were derived from 52 female donors and 48 male donors. Eighty samples (80%) were positive for antibodies to HSV 1 & 2 IgG and twenty samples (20%) were negative for HSV 1 & 2 IgG antibodies. The age distribution and prevalence is shown in Table 1. Histograms showing the distribution of EU/ml values for the positive and negative populations are shown in Figures 1 and 2.

Thirty-seven of the female donors were of child-bearing age (18-45 years). Of the sera from these donors, 29 (78%) were positive and 8 (22%) were negative for HSV 1 & 2 IgG antibodies. In addition, a total of 210 samples from females of child-bearing age (18-45 years) were identified in the outside and in-house clinical studies. These included 45 samples from pregnant females tested by Diamedix Corp. Of these samples, 172 (82%) were positive and 38 (18%) were negative for anti-HSV 1 & 2 IgG.

TABLE 1

	Number of Donors	Prevalence
Total Number	100	80%
Geographic location: South Eastern US	100	80%
Age		
10 – 19	13	61.5%
20 – 29	23	69.6%
30 – 39	40	82.5%
40 – 49	13	92.3%
50 – 59	5	100.0%
60 - 69	6	100.0%

FIGURE 1
***Is*-HSV 1 & 2 IgG Positive Population**

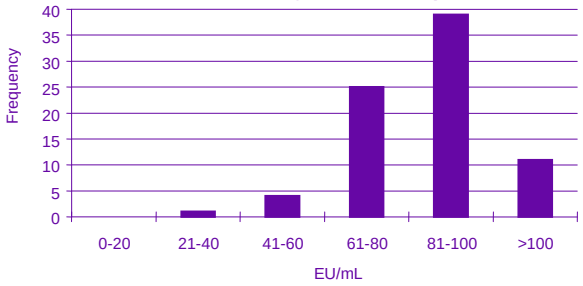
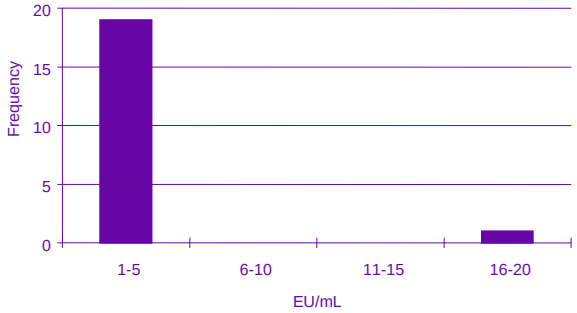


FIGURE 2
***Is*-HSV 1 & 2 IgG Negative Population**



PERFORMANCE CHARACTERISTICS

A. Comparison Testing

A total of six hundred and forty-five sera were tested for the presence of HSV 1 & 2 IgG antibodies using the Diamedix *Is*-HSV 1 & 2 IgG Test Kit and two other marketed tests at two independent sites (site #1, Miami, FL and site #2, Salt Lake City, Utah) 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 tested 200 samples (19% fresh and 81% frozen). Samples were obtained from the S. Florida area. Forty-seven (24%) of the samples were obtained from females of childbearing age (18-45 years). Table 2 compares the results obtained for the *Is*-HSV 1& 2 IgG Test Kit and their currently used testing method.

Site #2 tested 178 samples (all fresh). Samples were obtained from the West region. Seventy-two (40%) were obtained from females of childbearing age (18-45 years). Table 3 compares the results obtained for the *Is*-HSV 1 & 2 IgG Test Kit and their currently used testing method.

TABLE 2
***Is*-HSV 1 & 2 IgG - Site #1**

		Positive	Negative	Equivocal
Other EIAs	Positive	156 [42]	0	1
	Negative	3	38 [5]	2

		*95% CI**
Relative Sensitivity	156/156 = 100.0%	97.7-100.0
Relative Specificity	38/41 = 92.7%	80.1-98.5
Overall Agreement**	194/197 = 98.5%	95.6-99.7

TABLE 3
HSV 1 & 2 IgG - Site #2

		Positive	Negative	Equivocal
Other EIAs	Positive	119 [50]	1	1
	Negative	3 [1]	54 [21]	0

		*95% CI**
Relative Sensitivity	119/120 = 99.2%	95.4-100.0
Relative Specificity	54/57 = 94.7%	85.4-98.9
Overall Agreement**	173/177 = 97.7%	94.3-99.4

[ ] denotes samples from females of childbearing age (18-45 years)

* 95% Confidence Intervals (CI) calculated by the Exact Method (9)

** Equivocal results were excluded from calculations

For site #1, the three discordant sera were negative when tested by a referee EIA. For site #2, the three sera positive by the *Is*-method and negative by the other EIA were positive by a referee method. The sample that was negative by the *Is*-method and positive by the other EIA was negative when tested by a referee method.

Site #3 (Diamedix Corp.) tested 267 samples (all frozen) by the manual method and 259 of these samples (eight being QNS) by the MAGO Plus method. Two hundred and four sera were obtained from normal S. Florida blood donors, 45 sera were derived from pregnant females (15 from each trimester) and 18 sera were specifically obtained for their seronegative status. Of the total number of samples tested, 89 (33%) were derived from females of childbearing age (18-45 years). Tables 4 and 5 compare the results obtained, by manual and MAGO Plus testing, for the *Is*-HSV 1 & 2 IgG Test Kit and another marketed EIA method.

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

		Positive	Negative	Equivocal
Other EIA	Positive	212 [76]	0	0
	Negative	0	54 [13]	0
	Equivocal	0	1	0

		*95% CI**
Relative Sensitivity	212/212 = 100.0%	98.3-100.0
Relative Specificity	54/54 = 100.0%	85.4-98.9
Overall Agreement**	266/266 = 100.0%	98.6-100.0

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

		Positive	Negative	Equivocal
Other EIA	Positive	211	1	0
	Negative	1	45	2
	Equivocal	0	0	0

		*95% CI**
Relative Sensitivity	211/212 = 99.5%	97.4-100.0
Relative Specificity	45/46 = 97.8%	88.5-99.9
Overall Agreement**	256/258 = 99.2%	97.2-99.9

[ ] denotes samples from females of childbearing age (18-45 years)

* 95% Confidence Intervals (CI) calculated by the Exact Method (9)

** Equivocal results were excluded from calculations

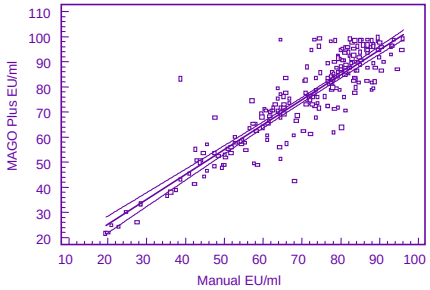
For site #3 (manual testing), there were no discordant samples. For the MAGO Plus testing, the sample that was negative by the *Is*-method and positive by the other EIA was positive when tested by the referee method. The sample that was positive in the *Is*-method and negative in the other EIA was negative in the referee method.

NOTE: Please be advised that 'relative' refers to the comparison of the assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgment can be made on the comparison assay's accuracy to predict disease.

B. Correlation of Manual and MAGO Plus Results

The *Is*-HSV 1 & 2 IgG 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 229 serum samples whose results were within the assay's critical range of 20-100 EU/ml were plotted. 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



Slope: 0.990
Intercept: 5.470

R: 0.8994
(95%CI: 0.871-0.922)

C. CDC Serum Panel Data

The following information was obtained with the Centers for Disease Control and Prevention (CDC) serum panel for HSV serology assays which was tested by the *Is*-HSV 1 & 2 IgG Test Kit both manually and using the MAGO® Plus Automated EIA Processor. The results are presented as a means to convey further information on the performance of this assay with a masked characterized serum panel. Results were submitted to the CDC for their interpretation and evaluation. This does not imply an endorsement of the assay by the CDC.

The panel consists of 100 samples representing individuals with antibodies to HSV type 1 only, HSV type 2 only, HSV type 1 and type 2 as well as samples with no HSV antibodies. The panel consists of 72% positive and 28% negative samples. The Diamedix *Is*-HSV 1 & 2 IgG Test Kit demonstrated 97% (97 of 100) total agreement with the CDC results using the manual method and 98% total agreement using the automated method. Of the results obtained by Diamedix, there was 96% (69 of 72) agreement with the positive results using the manual method and 97% (70/72) agreement using the MAGO Plus method. For negative samples there was 100% agreement (28/28) using either the manual or automated method.

D. Titration of Strongly Positive Samples

Several strongly positive serum specimens were diluted (two-fold) and separate dilutions were assayed, in duplicate, in the *Is*-HSV 1 & 2 IgG Test Kit both manually and using the MAGO® Plus Automated EIA Processor. Representative linear regression graphs and scattergrams of the mean results with 95% confidence intervals are presented in Figures 4 and 5 for one patient sample. EU/ml values were linear with the log of the serum dilution as determined by linear regression. The results were comparable for samples tested either manually or by MAGO Plus.

FIGURE 4
Manual

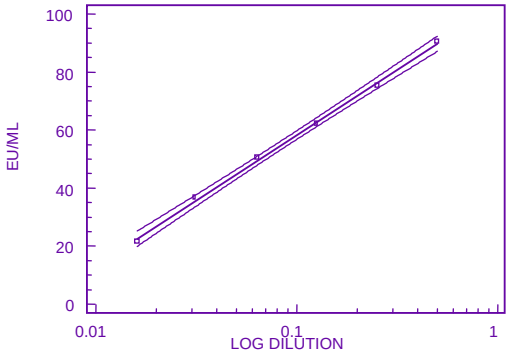
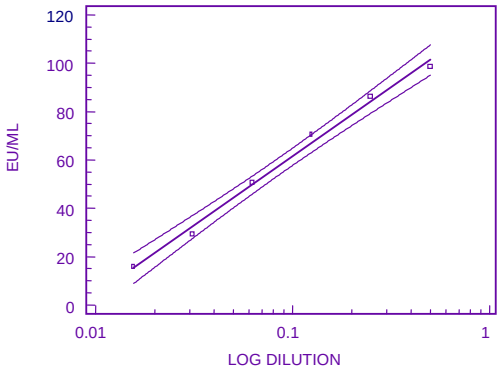


FIGURE 5
MAGO Plus



E. Semi-Quantitative Data

Serum pairs were obtained by preparing multiple two-fold dilutions of several strongly positive sera. Ratios for dilutions representing a four-fold and two-fold difference in antibody level were evaluated as a serum pair both manually and using the MAGO Plus. The mean ratio obtained for the four-fold dilutions was 1.9 and the mean obtained for the two-fold dilutions was 1.4. Thus it was estimated that a ratio of 1.9-fold or greater corresponded to a four-fold increase in HSV 1 & 2 antibody levels. A ratio in the range of 1.4 to 1.9 was considered equivocal. For MAGO Plus testing ratios of 2.16 and 1.46 were obtained for the four- and two-fold dilutions respectively.

F. Cross Reactivity

Sera containing IgG antibodies to viruses potentially cross-reactive to HSV have been tested in the *Is*-HSV 1 & 2 IgG Test Kit. Forty-nine sera negative for antibodies to HSV in the *Is*-HSV 1 & 2 IgG Test Kit as well as in another marketed test were positive for antibodies to one or more viruses. The data in the following table indicate that no cross-reactivity should be expected with the *Is*-HSV 1 & 2 IgG Test Kit from these analytes.

TABLE 6

Analyte	HSV 1/2 IgG	VZV IgG	CMV IgG	Toxoplasma IgG	Rubella IgG	EBV IgG	Measles IgG
# of Pos. Samples	0	48	21	2	46	47	45

G. 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 EIA Processor. The results obtained are shown in Tables 7-10.

TABLE 7
Site #1- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY		
	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%
A	2.3	0.53	4.42	2.2	0.21	9.55	4.8	0.15	3.13	3.1	1.32	42.58
B	2.0	0.06	3.00	1.8	0.06	3.33	3.4	0.59	17.35	2.4	0.80	33.33
C	34.7	5.00	14.41	32.5	2.82	8.68	34.5	3.15	9.13	33.9	3.43	10.12
D	48.6	1.08	2.22	42.0	1.10	2.62	48.9	1.40	2.86	46.5	3.54	7.61
E	89.7	3.45	3.85	80.3	2.84	3.54	82.7	1.90	2.30	84.2	4.85	5.76
F	100.7	7.77	7.72	90.5	9.94	10.98	94.7	6.76	7.14	95.3	8.43	8.85
CAL	107.8	5.96	5.53	98.7	6.45	6.53	98.1	4.16	4.24	101.6	6.77	6.66
POS	47.4	1.99	4.20	43.7	1.08	2.47	45.1	1.35	2.99	45.4	2.09	4.60
NEG	0.7	0.15	21.43	0.7	0.06	8.57	0.8	0.12	15.00	0.7	0.10	14.29

TABLE 8
Site #2- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY		
	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%
A	5.2	0.23	4.42	3.6	0.66	18.33	1.8	0.42	23.33	3.6	1.53	42.50
B	2.9	0.06	2.07	2.0	0.98	49.00	1.3	0.21	16.15	2.1	0.84	40.00
C	30.0	1.17	3.90	28.3	1.21	4.28	26.3	0.59	2.24	28.2	1.84	6.52
D	49.9	1.21	2.42	37.6	1.55	4.12	36.9	0.93	2.52	41.5	6.41	15.45
E	84.2	3.61	4.29	76.9	1.02	1.33	75.4	2.46	3.26	78.8	4.64	5.89
F	99.8	3.36	3.37	91.4	11.07	12.11	94.3	1.86	1.97	95.2	6.93	7.28
CAL	94.6	7.08	7.48	95.3	1.63	1.71	100.3	3.67	3.66	96.7	4.87	5.04
POS	52.5	1.46	2.78	45.0	3.59	7.98	46.3	1.63	6.52	47.9	4.07	8.50
NEG	0.8	0.06	7.50	0.4	0.06	15.00	0.4	0.35	87.5	0.6	0.27	45.00

TABLE 9
Site #3-Intra-Assay and Interassay Precision (Manual)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY		
	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%
A	1.3	0.06	4.62	1.3	0.15	11.54	1.9	0.11	5.79	1.5	0.31	20.67
B	1.3	0.17	13.08	1.3	0.13	10.00	1.5	0.23	15.33	1.4	0.20	14.29
C	26.7	1.36	5.09	28.5	0.59	2.07	27.9	1.22	4.37	27.7	1.30	4.69
D	37.9	2.31	6.09	40.4	2.01	4.98	39.6	2.13	5.38	39.3	2.29	5.83
E	69.8	2.08	2.98	74.7	2.28	3.05	73.5	3.17	4.31	72.7	3.24	4.46
F	86.9	2.62	3.01	90.6	1.69	1.87	92.0	2.56	2.78	89.8	3.12	3.47
CAL	88.9	3.74	4.21	94.3	2.01	2.13	95.1	3.02	3.18	92.8	3.98	4.29
POS	45.6	1.42	3.11	50.7	1.29	2.54	48.7	2.08	4.27	48.3	2.67	5.53
NEG	0.8	0.06	7.50	0.9	0.39	43.33	0.7	0.10	14.29	0.8	0.24	30.00

TABLE 10
Site #3- Intra-assay and Interassay Precision (MAGO Plus)

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY		
	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%	MEAN EU/ml	SD	CV%
A	2.2	0.50	22.73	1.7	0.81	47.65	2.5	0.66	26.40	2.1	0.71	33.81
B	1.0	0.28	28.00	1.7	1.39	81.76	1.3	0.33	25.38	1.3	0.84	64.62
C	27.9	1.94	6.95	28.3	1.99	7.03	31.5	2.13	6.76	29.2	2.53	8.66
D	42.5	1.90	4.47	37.9	4.30	11.35	42.5	2.58	6.07	41.0	3.68	8.98
E	85.0	6.18	7.27	78.1	4.15	5.31	84.7	2.72	3.21	82.6	5.40	6.54
F	82.4	4.70	5.70	76.7	4.16	5.42	84.2	2.82	3.35	81.1	4.98	6.14
CAL	107.3	3.80	3.54	85.5	15.99	18.70	93.0	10.90	11.72	95.2	14.19	14.19
POS	53.5	4.56	8.52	51.5	3.82	7.42	55.5	3.14	5.66	53.5	4.02	7.51
NEG	1.2	0.28	23.33	0.9	0.78	86.67	1.5	0.54	36.00	1.2	0.58	48.33

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