Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

CMV IgG

Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-320 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare a 1:101 dilution of patient samples in Sample Diluent. Mix well.
   Note that Standards and Controls are ready-to-use.
2. Add 100 µl of Standards, Controls and diluted patient samples into the antigen wells.
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 cytomegalovirus (CMV) in human serum by indirect enzyme immunoassay to aid in the assessment of the patient's immunological response to CMV and to determine the immune status of individuals, including females of child-bearing age. The evaluation of acute and convalescent sera can aid in the diagnosis of primary infection, reactivated infection or reinfection with CMV. This product is not FDA cleared for use in screening blood and plasma donors.

SUMMARY AND EXPLANATION

Cytomegalovirus (CMV) is a herpes virus, which is responsible for a range of infections in humans of all ages. The vast majority of healthy children and adults who acquire CMV infection remain asymptomatic. Symptoms, if they are present, include fever, lethargy and atypical lymphocytosis that can mimic the symptoms caused by Epstein-Barr virus. However, the incidence and spectrum of disease in newborns, in organ transplant recipients and in AIDS and HIV-symptomatic individuals establish this virus as an important and significant human pathogen.[1] CMV infections can be acquired before birth (congenital), at birth (perinatal) or later in life (postnatal). Fewer than 5% of congenitally infected infants develop symptoms during the newborn period; possible manifestations range from severe disease with intrauterine growth retardation, jaundice, hepatosplenomegaly, petechiae, central nervous system abnormalities, and chorioretinitis, to more limited involvement. Symptomatic infants may die of complications within the first month of life; more commonly, they survive but are neurologically damaged. Newborns can also acquire infection at birth by contact with the virus in the birth canal. Such infants begin to excrete virus at 3 to 12 weeks of age but usually remain asymptomatic. Most postnatal infections are acquired by close contact with individuals who are shedding virus. Since CMV has been detected in several body fluids including saliva, urine, breast milk, tears, stool, vaginal or cervical secretions and semen, transmission can occur in a number of ways. CMV can also be transmitted by blood transfusion or organ transplantation.[1,2,3,4]

CMV infections are frequent and occasionally severe in immunosuppressed individuals such as patients with AIDS, HIV-virus, cancer patients and organ donor recipients. Such infections may represent reactivation of latent virus or primary infection introduced by blood transfusion or transplanted organ.[2,3,4,5]

Serologic procedures are useful in screening for evidence of past infection with CMV and in identifying individuals at risk for CMV. For the diagnosis of recent infection, paired sera should be obtained at least two weeks apart. CMV IgG antibodies generally appear 1 to 2 weeks after infection, reach peak levels in 6-10 weeks, and persist at various levels for life.[6]

The traditional methods for determining levels of CMV antibody such as the complement fixation (CF) or IHA (indirect hemagglutination) tests have been replaced by enzyme immunoassays (EIAs) which are more sensitive, easier to perform and more amenable to automation for screening large numbers of samples.

The Diamedix Immunosimplicity® *Is*-CMV IgG Test Kit is an EIA procedure intended for the qualitative and semi-quantitative detection of antibodies to CMV antigen. The results are objective and reported in EU/ml, which are traceable to in-house reference materials.

PRINCIPLE OF THE PROCEDURE

Diluted samples are incubated with CMV antigen bound to the solid surface of a microtiter well. If IgG antibodies against CMV 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 CMV antigen present in the sample.

REAGENTS

Each Is-CMV IgG Test Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded red, coated with partially purified CMV antigen (AD-169 strain produced in human fibroblasts).
0 EU/ml Standard	One vial with yellow cap containing 1.8 ml of pre-diluted human serum, non-reactive for CMV IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
10 EU/ml Standard	Two vials with green caps containing 1.8 ml of pre-diluted human serum, weakly reactive for CMV IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
160 EU/ml Standard	One vial with red cap containing 1.8 ml of pre-diluted human serum, highly reactive for CMV IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.

NOTE : The Diamedix CMV IgG 'Standards' serve as calibrators for the test system and are traceable to in-house reference materials and not to any recognized national or international standard preparation.

High Positive	One vial with white cap containing 1.8 ml of pre-diluted human serum, moderately reactive for CMV IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml range printed on label.
Low Positive Control	One vial with blue cap containing 1.8 ml of pre-diluted human serum, weakly reactive for CMV 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 CMV IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient.

Note: Standards 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' 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.

PRECAUTIONS

REAGENTS: For *in vitro* Diagnostic Use

1. Handle samples, standards, controls and the materials that contact them as potential biohazards. Each donor unit in the standards and controls has been found negative for Hepatitis B surface antigen and HIV-I 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-CMV 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 Process-ing of Blood Specimens, H18A3.)

For the diagnosis of recent CMV infection, paired sera should be obtained at least two weeks apart. However, antibody increases may not be detectable for up to 4 weeks after primary infection.

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

Note: *For qualitative assays*, the 10 EU/ml Standard only is required. This Standard should be assayed in triplicate. In addition, a Blank (100 µl Sample Diluent only, in the first well of the first strip) is required and will be used to "zero" the photometer before reading test results.

For semi-quantitative assays, all three Standards are required. No Blank is required; the 0 EU/ml Standard will function as the 'zero' and will be placed in the first well of the first strip. Standards can be run singly or in duplicate. High Positive, Low Positive and Negative Controls must be run for either assay option.

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 Standards, controls and diluted patient sample, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.
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. 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 High Positive, Low Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank or the 0 EU/ml Standard must be < 0.2.
3. The absorbance of the 10 EU/ml Standard must be higher than that of the Negative Control.
4. The absorbance of the 10 EU/ml Standard must be lower than that of the Low Positive Control.
5. The absorbance of the 160 EU/ml Standard must be greater than that of the High Positive Control.
6. The Low Positive Control must be within its assigned range.
7. The High Positive Control must be within its assigned range.
8. The Negative Control must be < 8 EU/ml.

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

Notes: The Negative and Positive Controls 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. Calculations

***Qualitative Assay*:** Qualitative results may be obtained using the 10 EU/ml Standard only in triplicate, following a single Blank well (100 µl Sample Diluent only). If performing the qualitative assay option, manually set the reader for absorbance mode or cut-off control test mode and calculate the mean absorbance for the three Standard wells.

Note: When calculating the mean absorbance exclude any absorbance value that deviates by more than 15% from the mean absorbance value. Calculate the mean absorbance value from the two remaining absorbances. Exclusion of more than one of the 3 absorbance values invalidates the run.

Example: *Absorbance values obtained for 10 EU/ml Standard: 0.256, 0.245, 0.258 (after subtraction of the Blank)*

Mean Absorbance of the 10 EU/ml Standard = 0.253

Sample Absorbance = 0.959

Index Values are then calculated as follows:

Sample Absorbance/Mean Absorbance of 10 EU/ml Standard= 3.79

When using an Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor), results are automatically calculated and expressed as Positive, Equivocal or Negative.

Semi-Quantitative Assay: Semi-quantitative results may be obtained from the point-to-point curve fit using all three Standards. For plate readers, the point-to-point option should be selected and Standard values entered accordingly. Index Values can be calculated by dividing the EU/ml values by 10 (the positive cut-off value).

An Automated Processor will calculate results using the point-to-point curve fit and will print results automatically.

Specimens which yield absorbances greater than that of the 160 EU/ml Standard may be reported as greater than 160 EU/ml or Index >16.0. Alternatively, such samples may be pre-diluted in Sample Diluent and retested. The resulting EU/ml or Index Value must be multiplied by the dilution factor for reporting.

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
< 8.0	< 0.80	Negative for anti-CMV IgG
8.0 – 9.9	0.8 – 0.99	Equivocal for anti-CMV IgG*
≥ 10.0	≥ 1.0	Positive for anti-CMV IgG

Note that when using the assay qualitatively the magnitude of the Index Value has no relevance and results should be reported as under 'Interpretation' above.

* 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 primary or recent infection.

Note that the evaluation of paired sera can aid in the diagnosis of reactivated infection or reinfection but cannot distinguish between these conditions.

A negative result does not always exclude the possibility of CMV infection. The sample may have been collected before appearance of IgG antibody. If infection is suspected a second sample should be collected at least 10 days later and tested concurrently with the first sample to determine if seroconversion has occurred.

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*-CMV 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. In addition, paired sera should be evaluated within the linear range of the assay. The upper limit of the reportable range has been set at 160 EU/ml. In-house studies performed both manually and using the MAGO Plus have shown that a 3.1-fold or greater increase in the EU/ml ratio (convalescent serum EU/ml value / acute serum EU/ml value) corresponds to a four-fold increase in CMV IgG antibody level and a 1.9-fold increase in the EU/ml ratio corresponds to a two-fold increase in CMV IgG antibody level. Ratios in the range of 1.9 to 3.1 may be considered equivocal for significant increase status. In this case, paired samples can be retested or additional samples collected if necessary. If paired sera controls are desired, it is recommended that a four-fold dilution of the High Positive Control be made first in Sample Diluent. The undilute and

the 4-fold diluted material will provide a simulated serum pair. The Ratio of the undilute and 4-fold diluted material can then be compared against the established ratio.

CUT-OFF ESTABLISHMENT

The Diamedix *Is*-CMV IgG cut-off value was established to optimally differentiate those individuals with, from those without, immunological experience to CMV. The optimal cut-off value was determined by statistical analysis of the results of 177 sera shown to be negative by other test methods. The mean plus 3 SD (2.9 plus 3 x 1.7 = 8) was considered the equivocal decision point. The mean plus 4 SD (2.9 plus 4 x 1.7 = 9.7, rounded off to 10) 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 209 normal sera assayed manually by Diamedix in the *Is*-CMV IgG Test Kit and another commercially available test method. At the optimized cut-off level, the Diamedix *Is*-CMV IgG has a relative sensitivity of 100% and a relative specificity of 94% 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*-CMV 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. The test should be performed on serum. The use of whole blood or plasma has not been established.
4. The presence of IgG antibodies in a single serum sample is not sufficient to distinguish between active and past infection. A test for IgM antibodies may be performed for patients suspected of primary infection with CMV.
5. Performance of this assay has not been established on spectrophotometry utilizing a single wavelength.
6. The performance characteristics have not been established for prenatal populations or newborns.
7. The results on serum from immunosuppressed individuals must be interpreted with caution.
8. Studies demonstrating the effectiveness or monitoring of antiviral treatments have not been performed.
9. Definitive diagnosis of active CMV infection requires viral isolation. The presence of IgG antibody to CMV does not ensure protection from the disease.
10. The performance characteristics of the Diamedix *Is*-CMV IgG Test Kit with automated equipment other than the MAGO® Plus Automated EIA Processor have not been established.

EXPECTED VALUES

The prevalence of CMV antibodies in the normal population can vary depending on a number of factors such as age, geographical location, socio-economic status, race and type of test used. Prospective (fresh) sera from 214 individuals were evaluated in the CMV IgG Test Kit. Of these samples, 38 were obtained from patients in the southeastern US and 176 from patients in the western US. Eighty-two samples were obtained from males and one hundred and thirty-two from females. Of the female population, seventy-six (57.5%) were obtained from females of child-bearing age (18-45 years). Of these samples, 46 (61%) were positive, 29 (38%) were negative and one (1%) was equivocal.

Of the 214 samples tested, 137 (64%) were positive, 72 (34%) were negative and 5 (2%) were equivocal for anti-CMV IgG. The resulting prevalence of 64% for these populations is in general agreement with prevalence rates of 30% to 80% reported in US blood donors (2). Age distribution, geographic location and prevalence is provided in Table 1. Histograms demonstrating the distribution of EU/ml values in the negative and positive populations are shown in Figures 1 and 2.

A total of 156 samples from females of child-bearing age (18-45 years) were identified in the outside and in-house clinical studies. Of these samples 110 (70.5%) were positive, 43 (27.5%) were negative and 3 (2%) were equivocal in the *Is*-CMV IgG Test Kit.

TABLE 1

	Number of Donors	Prevalence
Total Number	214	64.0%
Geographic Locations:		
South Eastern US	38	65.8%
Western US	176	63.6%
Age		
0 – 10	55	56.4%
10 – 19	24	50.0%
20 – 29	43	62.8%
30 – 39	34	67.6%
40 – 49	22	63.6%
50 – 59	10	90.0%
60 – 90	26	80.8%

FIGURE 1
***Is*-CMV IgG Positive Population**

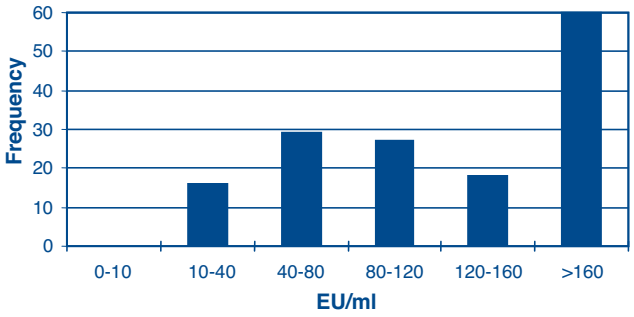
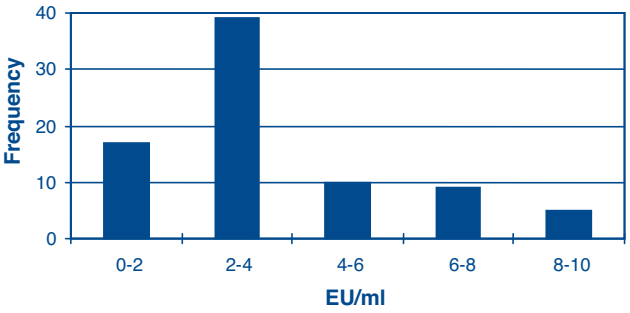


FIGURE 2
***Is*-CMV IgG Negative Population**



PERFORMANCE CHARACTERISTICS

A. Comparison Testing

A total of five hundred and eighty seven sera were tested for the presence of CMV IgG antibodies using the Diamedix *Is*-CMV 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 (26% fresh and 74% frozen). Samples were obtained from the S. Florida area. Forty-one (20.5%) of the samples were obtained from females of child-bearing age (18-45 years). Table 2 compares the results obtained for the *Is*-CMV IgG Test Kit and their currently used testing method.

Site #2 tested 178 samples (all fresh). Samples were obtained from the Midwest area. Seventy-one (40%) of the samples were obtained from females

of child-bearing age. Table 3 compares the results obtained for the *Is*-CMV IgG Test Kit and their currently used testing method.

TABLE 2
Is-CMV IgG - Site #1

		Positive	*Negative*	*Equivocal*
Other EIA	Positive	146 [28]	1	1 [1]
	Negative	1	47 [11]	3 [1]
	Equivocal	0	1	0

			*95% CI***
Relative Sensitivity	146/147	= 99.3%	96.3 – 100.0
Relative Specificity	47/48	= 97.9%	88.9 – 99.9
Overall Agreement*	193/195	= 99.0%	96.3 – 99.9

TABLE 3
Is-CMV IgG - Site #2

		Positive	*Negative*	*Equivocal*
Other EIA	Positive	113 [44]	4 [3]	2
	Negative	0	51 [20]	1 [1]
	Equivocal	0	6 [3]	1

			*95% CI***
Relative Sensitivity	113/117	= 96.6%	91.5 – 99.1
Relative Specificity	51/51	= 100.0%	93.0 – 100.0
Overall Agreement*	164/168	= 97.6%	94.0 – 99.3

* Equivocal results were excluded from calculations.

** 95% Confidence Intervals (CI) calculated by the Exact Method.[7]

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

For site#1, both discordant sera were negative when tested by a referee EIA. For site #2, the four discordant sera were negative when tested by a referee EIA.

Site #3 (Diamedix Corp.) tested 209 samples (all frozen) by the manual method and 206 of these samples (three being QNS) by the MAGO Plus method. Samples were obtained from S. Florida blood donors. Forty-four (21%) of the samples were from females of childbearing age. Tables 4 and 5 compare the results obtained for the *Is*-CMV IgG Test Kit and another marketed EIA method.

TABLE 4
Is-CMV IgG
Site #3: Manual

		Positive	*Negative*	*Equivocal*
Other EIA	Positive	152 [37]	0	0
	Negative	3	50 [6]	1
	Equivocal	2 [1]	1	0

			*95% CI***
Relative Sensitivity	152/152	= 100.0%	97.6 – 100.0
Relative Specificity	50/53	= 94.3%	84.3 – 98.8
Overall Agreement*	202/205	= 98.5%	95.8 – 99.7

TABLE 5
Is-CMV IgG
Site #3: MAGO Plus

		Positive	*Negative*	*Equivocal*
Other EIA	Positive	152	0	0
	Negative	6	43	2
	Equivocal	2	1	0

			*95% CI***
Relative Sensitivity	152/152	= 100.0%	97.6 – 100.0
Relative Specificity	43/49	= 87.7%	75.2 – 95.4
Overall Agreement*	195/201	= 97.0%	93.6 – 98.9

* Equivocal results were excluded from calculations.

** 95% Confidence Intervals (CI) calculated by the Exact Method.[7]

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

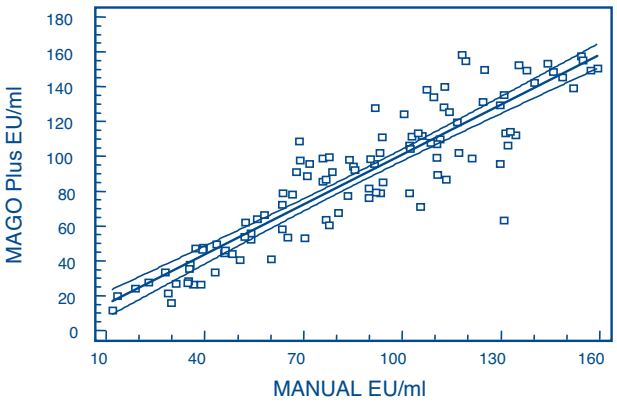
For site #3 (manual testing), two of the discordant sera were positive and the remaining sample was equivocal when tested by a referee EIA. For the six sera discordant when tested by the MAGO Plus, three were positive, two were negative and one was equivocal when tested by the referee EIA.

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*-CMV 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 108 serum samples tested in the comparison studies whose EU/ml results were within the linear range of the assay (10 to 160 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: 0.9563
Intercept: 5.2143
R: 0.9067

C. CDC Serum Panel Data

The following information was obtained with the Centers for Disease Control and Prevention (CDC) serum panel for CMV serology assays which was tested by the *Is*-CMV IgG Test Kit both manually and using the MAGO® Plus Automated 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 66% positive and 34% negative samples. The Diamedix *Is*-CMV IgG test demonstrated 99% (99 of 100) total agreement with the CDC results. Of the results obtained by Diamedix, there was 100% (66 of 66) agreement with the positive results using both the manual and automated

methods and 97% (33 of 34) agreement with the negative specimens using both the manual and automated methods.

D. Linearity

Several strongly positive serum specimens were diluted (2-fold) and separate dilutions were assayed, in duplicate, in the *Is*-CMV IgG Test Kit both manually and using the MAGO® Plus Automated 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 tested both manually and using the MAGO Plus. The results demonstrate a high degree of linearity throughout the reportable range of the assay when samples are tested either manually or by MAGO Plus.

FIGURE 4
Manual Linearity

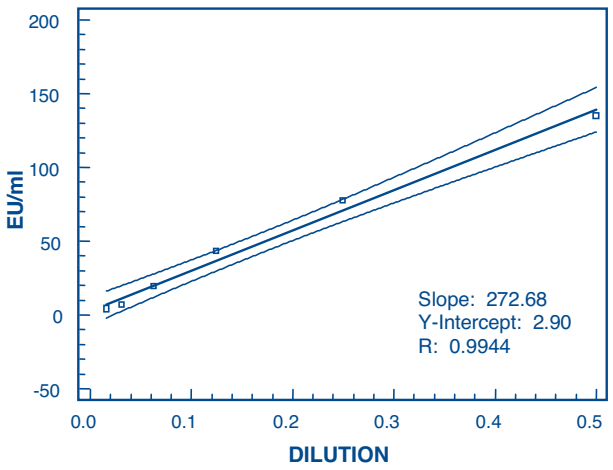
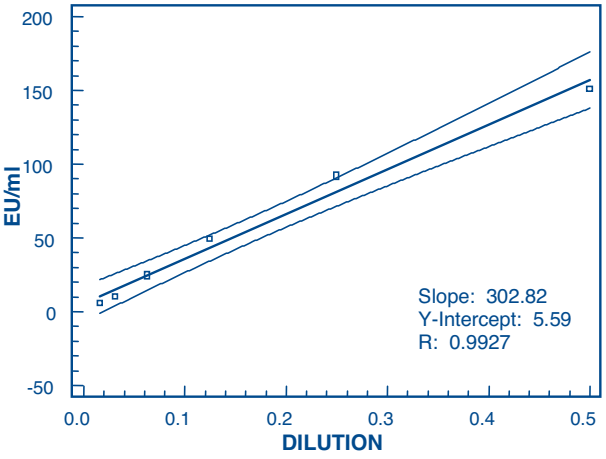


FIGURE 5
MAGO Plus Linearity



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 overall mean ratio obtained for four-fold dilutions was 3.80 (SD 0.66) and the overall mean ratio obtained for two-fold dilutions was 1.93 (SD 0.23). Overall, it was estimated a ratio of 3.1-fold or greater (mean ratio minus 1 SD) increase in *Is*-CMV IgG EU/ml values corresponded to a four-fold titer increase in CMV IgG antibody levels. A ratio in the range of 1.9 to 3.1 was considered equivocal for significant increase determination.

F. Cross Reactivity

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

TABLE 6

Analyte	CMV IgG	VZV IgG	HSV 1/2 IgG	Toxoplasma IgG	Rubella IgG	EBV IgG	Measles IgG
No. of Pos. Samples	0	45	35	6	38	45	43

G. Precision

Six serum samples (two negative and four positive) as well as the10 EU/ml kit Standard and kit controls were tested in triplicate in three separate runs. 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 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	5.0	3.76	75.20	4.1	2.11	51.46	4.1	2.12	51.71	4.4	2.44	55.45
B	4.7	0.44	9.36	4.9	0.31	6.33	4.8	0.26	5.42	4.8	0.31	6.46
C	28.5	7.09	24.88	21.6	2.02	9.35	21.9	2.20	10.05	24.0	5.12	21.33
D	39.4	9.88	25.08	38.2	3.81	9.97	38.9	3.97	10.21	36.8	5.68	14.64
E	55.0	0.31	0.56	61.1	1.40	2.29	62.6	1.38	2.20	59.6	3.59	6.02
F	139.3	7.61	5.46	138.6	6.41	4.62	141.9	6.43	4.53	139.9	6.11	4.37
10 STD	11.5	1.76	15.30	10.2	1.13	11.08	10.1	1.16	11.49	10.6	1.36	12.83
HPC	114.6	12.88	11.24	107.2	5.20	4.85	109.9	5.40	4.91	110.6	8.13	7.35
LPC	25.0	1.70	6.80	23.9	2.17	9.08	24.3	2.25	9.26	24.4	1.85	7.58
NC	3.1	0.23	7.42	2.6	0.25	9.62	2.7	0.31	11.48	2.8	0.33	11.79

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	3.8	0.40	10.53	3.0	0.74	24.67	2.5	0.44	17.60	3.1	0.75	24.19
B	4.7	0.06	1.28	3.3	0.81	24.55	3.8	0.87	22.89	4.0	0.86	21.50
C	14.1	1.75	12.41	14.2	3.41	24.01	17.2	0.31	1.80	15.2	2.44	16.05
D	30.0	3.44	11.47	27.8	1.10	3.96	23.5	3.05	12.98	27.1	3.70	13.65
E	60.5	8.38	13.85	45.2	2.00	4.42	43.7	2.26	5.17	49.8	9.23	18.53
F	96.3	12.92	13.42	97.8	6.27	6.41	92.3	0.95	1.03	95.5	7.60	7.96
10 STD	9.4	1.21	12.87	9.7	1.04	10.72	8.3	1.74	20.95	9.1	1.35	14.84
HPC	85.7	2.47	2.88	89.0	3.06	3.43	84.9	2.09	2.46	86.6	2.95	3.41
LPC	22.5	1.58	7.02	22.5	1.60	7.11	20.4	2.20	10.78	21.8	1.91	8.76
NC	3.0	0.42	14.00	3.1	0.10	3.23	2.8	0.12	4.12	3.0	0.25	8.33

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	2.2	1.64	74.54	1.9	0.56	29.47	1.8	0.60	33.33	2.0	1.01	50.50
B	3.9	1.85	47.44	2.8	0.94	33.57	3.5	0.49	14.00	3.4	1.25	36.76
C	10.0	0.75	7.50	9.5	0.59	6.21	10.5	0.94	8.95	10.0	0.86	8.60
D	26.4	1.69	6.40	25.8	2.59	10.04	28.6	1.00	3.50	26.9	2.14	7.96
E	45.9	7.47	16.27	43.0	4.21	9.79	46.1	6.41	13.90	45.0	6.00	13.33
F	99.1	4.61	4.65	92.7	7.61	8.21	100.5	3.88	3.86	97.4	6.32	6.49
10 STD	9.6	1.05	10.94	9.2	0.67	7.28	9.9	0.59	5.96	9.6	0.81	8.44
HPC	96.1	12.08	12.57	94.7	9.67	10.21	96.6	11.66	12.07	95.8	10.54	11.00
LPC	24.9	2.53	10.16	24.0	0.88	3.67	25.6	1.34	5.23	24.8	1.76	7.10
NC	3.5	0.64	18.29	3.6	0.44	12.22	3.6	0.29	8.06	3.6	0.45	12.50

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	2.8	0.74	26.42	2.8	0.96	34.28	3.3	0.43	13.03	3.0	0.74	24.67
B	4.1	0.45	10.98	4.3	0.56	13.02	3.8	0.56	14.74	4.1	0.54	13.17
C	14.0	3.11	22.21	15.5	1.13	7.29	14.1	0.76	5.39	14.5	1.97	13.59
D	31.2	4.59	14.71	34.1	4.00	11.73	35.8	6.47	18.07	33.7	5.21	15.46
E	50.5	4.65	9.21	56.5	4.37	7.73	55.1	6.01	10.91	54.0	5.44	10.07
F	102.8	4.88	4.75	115.1	9.30	8.07	121.5	7.64	6.29	113.1	10.67	9.43
10 STD	10.3	1.36	13.20	10.3	0.77	7.48	11.9	0.68	5.71	10.8	1.19	11.02
HPC	108.6	8.67	7.98	109.5	3.69	3.37	111.9	9.38	8.38	110.0	7.35	6.68
LPC	30.2	3.46	11.46	32.1	2.70	8.41	30.1	1.52	5.05	30.8	2.70	8.77
NC	3.2	0.95	29.69	3.2	0.55	17.19	3.1	0.70	22.58	3.2	0.71	22.19

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