Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

CMV IgM Capture

Enzyme Immuno Assay Test Kit **For *In Vitro* Diagnostic Use**
Catalog No. 720-330 – 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 wells.
   Reserve the first well for reagent blank (100 µl of Sample Diluent).
3. Incubate at 37 ± 3°C for 60 ± 5 min.
4. Prepare the Enzyme Tracer by adding 2.9 ml of Tracer Diluent and 0.1 ml (100 µl) 30 X Tracer to each vial of lyophilized antigen. Note that 1 vial of lyophilized antigen is sufficient for at least 2 strips.
5. After incubation, discard the contents of the wells. Wash the wells 3 times with Wash Solution.
6. Add 100 µl of prepared Enzyme Tracer to each well.
7. Incubate at 37 ± 3°C for 60 ± 5 min.
8. Wash the wells as in #5 above.
9. Add 100 µl Substrate Solution to each well.
10. Incubate at 37 ± 3°C for 20 ± 2 min.
11. Add 100 µl Stop Solution to each well.
12. Read the absorbances at 450/600-630 nm.

INTENDED USE

For the qualitative detection of IgM antibodies to cytomegalovirus in human serum by capture enzyme immunoassay as an aid in the diagnosis of a recent or current CMV infection. This assay has not been cleared/approved by the FDA for use in testing (i.e. screening) blood or 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 pre sent, 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, hepatosple-nomegaly, petechiae, central nervous system abnormalities, and chorio-retinitis, to more limited involvement. Symptomatic infants may die of com-plications 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 for detecting CMV antibodies in patient sera. 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). IgM antibodies may appear as early as 5 days after the infection, rise sharply and fall to low levels or disappear within a few weeks or months.

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. Capture EIAs offer the additional advantage of avoiding interference due to rheumatoid factor and competing IgG antibodies.

The Diamedix Immunosimplicity® (*Is*)-CMV IgM Capture Test Kit is an EIA procedure intended for the qualitative detection of IgM antibodies to CMV antigen.

PRINCIPLE OF THE PROCEDURE

The *Is*-CMV IgM Capture Test Kit utilizes ELISA based on the antibody-capture technique. Diluted patient sera are incubated with mouse monoclonal antibody against human IgM bound to the solid surface of a microtiter well. Patient IgM is 'captured' by the surface bound antibody. Unbound serum components are washed away. The presence of patient anti-CMV IgM antibodies is 'detected' and bound by an immunocomplex, Enzyme Tracer, consisting of CMV antigen which is linked to a mouse monoclonal anti-CMV antibody conjugated to horseradish peroxidase. Enzyme 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 CMV antigen present in the sample.

REAGENTS

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

Anti-IgM Coated Wells	Twelve, 8-well microwell breakapart strips, color-coded pink, coated with mouse monoclonal anti-human IgM (heavy chain). Antibody designated IgM 1.1.
Cut-Off Calibrator	One vial with blue cap containing 0.25 ml of human serum preserved with 0.1% sodium azide, weakly reactive for CMV 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 preserved with 0.1% sodium azide. Assigned range printed on label. The Low 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, non-reactive for CMV IgM antibodies. The Negative Control is used to control the negative range of the assay. *Note: The Cut-Off Calibrator and Controls are prepared from different serum lots.*
Lyophilized Antigen	Six vials of lyophilized CMV antigen (partially purified with sucrose gradient centrifugation, Davis Strain, prepared from infected human fibroblast cells or AD-169 Strain, cultured in MRC-5, a diploid cell line of human lung origin).
30X Tracer	One vial with red cap containing 1.0 ml mouse monoclonal anti-CMV conjugated to horseradish peroxidase (30X concentrate) in stabilizer. The mouse monoclonal anti-CMV antibody (designated 2.5.5.6) recognizes an antigen present in the nucleus of cells one week post infection (an early antigen).
Tracer Diluent	One bottle with red cap containing 30 ml borate buffer. Also includes protein stabilizers, genta-mycin and Proclin® 300 as preservatives. Color-coded pink.
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.
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

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, HCV 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 B virus or Hepatitis C 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. The concentrations of anti-CMV IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
3. Never pipette by mouth.
4. Avoid contact with open skin and mucous membranes.
5. 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.
6. 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.
7. Avoid contamination of the TMB substrate solution with conjugate other oxidants which will cause the solution to change color prematurely.
8. Do not interchange reagents from different reagent lots except for Sample **A** Diluent, Wash **S** Concentrate, Substrate **G** and Stop **M** Solution.
9. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
10. Store unused reagents at 2 to 8°C.
11. Incubations above or below the recommended temperatures or times may give erroneous results.
12. The capture ELISA 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.
13. Coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
14. *(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.*

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 Clinical and Laboratory Standards Institute (CLSI), formerly NCCLS, provides recommendations for collecting and storing blood specimens (8).

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.

Each vial of lyophilized antigen is sufficient for at least 2 strips. Reconstitute only the number of vials required. Discard any unused Enzyme Tracer after the day's testing is completed.

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 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 ENZYME TRACER TO THE BLANK WELL.***
3. Allow the wells to incubate uncovered at 37 ± 3°C for 60 ± 5 minutes.
4. As soon as the sample incubation has commenced, prepare the Enzyme Tracer by adding 2.9 ml of Tracer Diluent to each vial of lyophilized antigen. Mix until all the lyophilized material is reconstituted. Then add 100 µl of 30 X Tracer to each antigen vial and mix well. Allow the prepared Enzyme Tracer to sit at room temperature (18-30°C) for at least 30 minutes. Gently mix again immediately prior to use.
5. 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 each 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.

6. Place 100 µl of Enzyme Tracer into each well (*except the Blank*), avoiding bubble formation.
7. Add 100 µl Sample Diluent to the Blank well.
8. Allow the wells to incubate uncovered at 37 ± 3°C for 60 ± 5 minutes.
9. Wash the wells as described in Step 5 above.
10. Place 100 µl of Substrate into each well, avoiding bubble formation.
11. Allow the wells to incubate uncovered at 37 ± 3°C for 20 ± 2 minutes.
12. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
13. 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 Control must be within their assigned ranges.

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

NOTES: 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.

INTERPRETATION OF RESULTS

1. Calculation

Calculate the mean absorbance of the Cut-Off Calibrator. Note: When calculating the mean absorbance value for the Cut-off Calibrator exclude any absorbance value that deviates by more than 20% from the mean of the three absorbance values. Calculate the mean absorbance value from the two remaining absorbances. 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:

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

Example: Absorbance values obtained for the Cut-Off Calibrator: 0.356, 0.345, 0.368 (after subtraction of the Blank)

Mean Absorbance of the Cut-Off Calibrator = 0.356

Sample Absorbance = 0.959

Index Value = 2.69

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

2. Interpretation of Results

Index value	Interpretation
<0.90	Negative for anti-CMV IgM
0.90 - 1.09	*Equivocal for anti-CMV IgM
≥ 1.10	Positive for anti-CMV IgM

Note that the magnitude of the Index Value has no significance and results should be reported as under 'Interpretation' above.
In addition, note that a sample may have been collected too early during the course of the disease for IgM antibodies to have appeared and that an IgM antibody response may not occur or be below detectable levels during reactivation.

* When equivocal results are obtained, another specimen should be collected at least fourteen days later and tested in parallel with the initial specimen. If the second sample remains equivocal, antibody status cannot be determined. Other clinical and serological evidence should be sought in those cases. If the second sample is positive, seroconversion has occurred and may be indicative of a current or recent infection.

3. Reporting Results

When reporting results the following statement should be included: "The following results were obtained with the Diamedix Immunosimplicity (*Is*)-CMV IgM Capture EIA Test System. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present".

CUT-OFF ESTABLISHMENT

The Diamedix *Is*-CMV IgM Capture Test Kit cut-off value has been established to optimally discriminate those individuals with, from those without, IgM antibodies to cytomegalovirus. The optimal cut-off value was determined by statistical analysis of one hundred and ninety-eight (198) normal sera shown to be negative for CMV IgM antibodies in this and other test methods. The mean and standard deviation of the absorbance values for these sera were 0.1190 and 0.0638 respectively. The cut-off was determined as being equal to the mean plus 3 standard deviations, 0.1190 + (3 X 0.0638) = 0.3104. The Cut-Off Calibrator has been titrated to equal this result. Therefore, the mean 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 cut-off value was further verified by applying the principles from Receiver-Operating Characteristic (ROC) Curves to two-hundred and fourteen (214) sera assayed manually by Diamedix in the *Is*-CMV IgM Capture Test Kit and another commercially available test method. At the optimized cut-off level, the Diamedix *Is*-CMV IgM Capture Test Kit has a relative sensitivity of 91% and a relative specificity of 99% based on comparison to the marketed test. Comparable values were obtained for MAGO Plus results.

LIMITATIONS

1. *Is*-CMV IgM Capture Test Kits are not intended to replace viral isolation and/or identification.
2. Assay performance characteristics have not been established for visual result determination.
3. Assay performance characteristics for the use of specimen matrices other than serum have not been established.
4. A negative result does not always exclude the possibility of active CMV infection. The sample may have been collected before the appearance of IgM antibody. If an infection is suspected, a second sample should be obtained at least 14 days later and tested in parallel with the first specimen to look for seroconversion or a significant rise in titer of IgM, which is indicative of a primary infection.
5. Specific IgM antibodies are usually detected in patients with recent or primary infections. In some cases, however, low levels of antibodies may persist for more than 12 months.
6. Assay performance characteristics have not been established with single wavelength spectrophotometers.
7. Re-infections or reactivation of latent infections may not be positive for specific IgM antibodies. Therefore, a negative result does not necessarily preclude a current re-infection or reactivation.
8. Isolation of the virus is the preferred method for diagnosing congenital CMV infection since such infections are often asymptomatic and serological evidence of CMV IgM antibody may be difficult to obtain.
9. Performance characteristics have not been established for newborns, for cord blood or for immunosuppressed individuals (including HIV-positive and pre-and post-transplant patients).
10. Performance characteristics of the Diamedix *Is*-CMV IgM Capture Test Kit with automated equipment other than the MAGO Plus Automated EIA Processor have not been established.

EXPECTED VALUES

The prevalence of CMV infection can vary depending on a number of factors such as age, gender, geographical location, socioeconomic status, race, type of test used, specimen collection and handling procedures, and clinical and epidemiological history of individual patients. In the present study two hundred sera from S. Florida blood donors were evaluated in the *Is*-CMV IgM Capture Test Kit. Of these samples one hundred and ninety-four (97%) were negative, two (1%) were positive and four (2%) were equivocal for CMV IgM antibodies. TABLE 1 shows the age and prevalence profile of this population. FIGURE 1 shows a histogram showing the distribution of Index values obtained. FIGURE 2 shows the distribution of values in a positive population tested by Diamedix Corporation.

TABLE 1:

Age Distribution and Prevalence of anti-CMV IgM in a Normal S. Florida Population

	Number	% Seronegative	% Seropositive	% Equivocal
Total Number	200	97.0% (194)	1.0% (2)	2.0% (4)
Geographic Location:	**South Florida**			
Age				
10-19	18	100.0% (18)	0.0% (0)	0.0% (0)
20-29	47	97.9% (44)	4.3% (2)	2.1% (1)
30-39	74	100.0% (73)	0.0% (0)	1.4% (1)
40-49	40	97.5% (39)	0.0% (0)	2.5% (1)
50-59	11	100.0% (10)	0.0% (0)	9.0% (1)
60-69	9	100.0% (9)	0.0% (0)	0.0% (0)
>70	1	100.0% (1)	0.0% (0)	0.0% (0)
Gender				
Male	98	97.0% (97)	1.0% (1)	2.0% (2)
Females	102	97.0% (97)	1.0% (1)	2.0% (2)

FIGURE 1
Distribution of *Is*-CMV IgM Results in a Normal Population

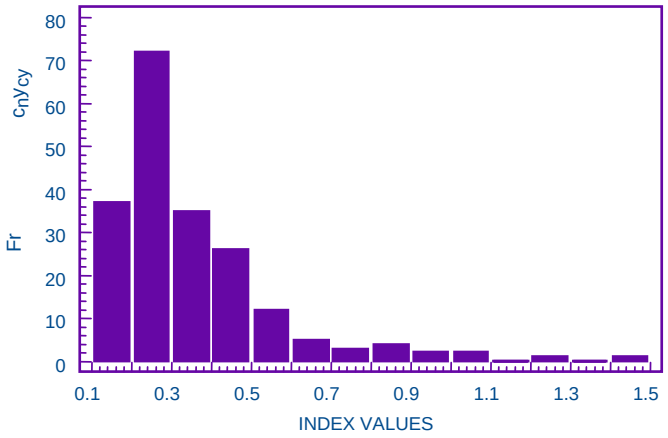
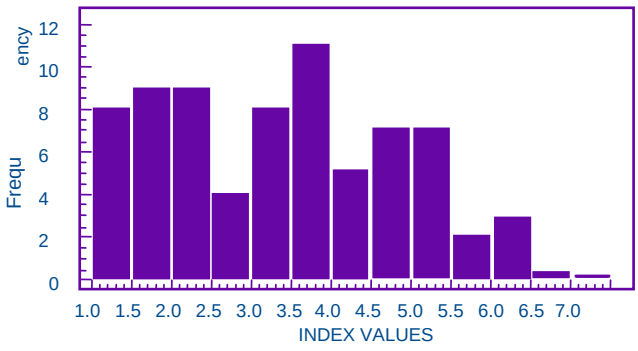


FIGURE 2
Distribution of *Is*-CMV IgM Results in a Positive Population



PERFORMANCE CHARACTERISTICS

A. Comparison Testing

A total of five hundred and eleven sera were tested for the presence of CMV IgM antibodies using the Diamedix *Is*-CMV IgM Capture Test Kit and other legally 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 167 samples. These samples consisted of 124 fresh samples submitted to the laboratory for CMV IgM testing and 43 frozen samples, which had previously tested positive for CMV IgM antibodies. Samples came from a wide variety of geographic locations and from patients with ages ranging from 4 days to 74 years old. For the fresh samples, 35 were from males and 87 from females. The remaining 2 samples were not identified as regards gender. TABLE 2 compares the results obtained for the *Is*-CMV IgM Capture Test Kit and their currently used IFA testing method.

Site #2, a commercial reference laboratory in New York, not affiliated with the manufacturer, tested 130 samples. These samples consisted of 50 fresh samples and 65 frozen samples submitted to the laboratory for CMV IgM testing as well as 15 frozen samples procured from a vendor based on their positive serostatus. Samples were obtained from various geographic regions. Sixty-six samples were from males and 64 from females. The remainder were not identified as regards gender. Ages of patients ranged from 1 day old to 77 years old. TABLE 3 compares the results obtained for the *Is*-CMV IgM Capture Test Kit and their currently used EIA testing method.

TABLE 2

Is-CMV IgM Capture - Site #1

		Positive	Negative	Equivocal
IFA	Positive	43	3	2
	Negative	4	110	5
	Equivocal *	0	0	0
				*95% CI***
Overall Agreement		153/160 = 95.6%		91.2 - 98.2

TABLE 3

Is-CMV IgM Capture- Site #2

		Positive	Negative	Equivocal
Other EIA	Positive	17	4	2
	Negative	1	106	0
	Equivocal *	0	0	0
				*95% CI***
Overall Agreement		123/128 = 96.1%		91.1 - 98.7

* Equivocal results were excluded from calculations.
** 95% Confidence Intervals (CI) calculated by the Exact Method (7).

For Site #1, further resolution of the discordant samples was performed by testing such samples using referee EIA methods. The three samples negative by the *Is*-CMV IgM Capture Test Kit and positive by IFA were also negative in a referee capture EIA method. Of the four samples that were positive in the *Is*-CMV IgM Capture Test Kit and negative by IFA, only three were available for additional testing. These were negative using a referee capture EIA method.

For Site #2, further resolution of the discordant samples was performed in a similar manner. The four samples that were negative in the *Is*-CMV IgM Capture Test Kit and positive by the other EIA were also negative in the referee capture EIA method. The sample that was positive in the Is-CMV IgM Capture Test Kit and negative in the other EIA was also positive in the referee capture EIA method.

Site #3 (Diamedix Corp.) tested 214 samples (all frozen) by both the manual and the automated method. Of these samples, 111 were from normal blood donors. Thirty-eight samples were obtained from pregnant, transplants or AIDS patients and, where possible, were either classified as having a primary infection or a reactivation. Twenty-six samples were multiple bleeds that had positive IFA titers. The remaining samples were obtained from serum vendors based on their serostatus. TABLES 4 and 5 compare the results obtained for the *Is*-CMV IgM Capture Test Kit and another marketed capture EIA method.

TABLE 4

Is-CMV IgM Capture - Site #3 : Manual

		Positive	Negative	Equivocal
Other EIA	Positive	72	7	2
	Negative	1	120	1
	Equivocal *	1	5	1

95% CI**

Overall Agreement 196/204 = 96.1% 92.4 - 98.3

TABLE 5

Is-CMV IgM Capture - Site #3 : MAGO Plus

		Positive	Negative	Equivocal
Other EIA	Positive	73	8	4
	Negative	0	118	4
	Equivocal *	3	4	0

95% CI**

Overall Agreement 191/199 = 96.0% 92.2 - 98.2

* Equivocal results were excluded from calculations.
** 95% Confidence Intervals (CI) calculated by the Exact Method (7).

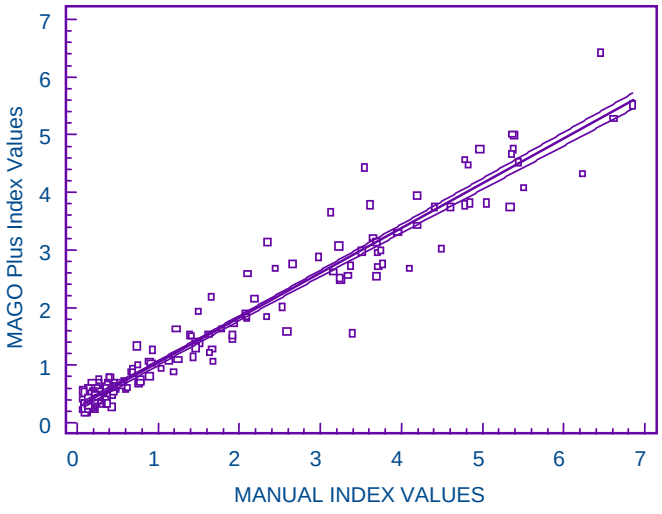
For Site #3 (manual testing), further resolution of the discordant sera revealed that of the 7 sera negative in the *Is*-CMV IgM Capture Test Kit but positive in the other EIA, 4 were negative and 3 were positive by a referee capture EIA method. The serum that was positive in the *Is*-CMV IgM Capture Test Kit and negative in the other EIA was negative by the referee method. For MAGO Plus testing, of the 8 sera that were negative in the *Is*-CMV IgM Capture Test Kit but positive in the other EIA, 5 were negative and 3 were low positive by the referee capture EIA method.

B. Correlation of Manual and MAGO Plus Results

The *Is*-CMV IgM Capture 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 the 214 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



C. Cross Reactivity/Interference Studies

The specificity of the *Is*-CMV IgM Capture Test Kit was verified by testing a number of sera containing relatively high levels of IgM antibody to other viruses as determined using commercially available test kits. These included other herpesviruses. A total of 40 known IgM-positive sera 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 30 sera. These data are shown in TABLE 6. TABLE 7 shows the lack of interference in samples containing 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 Samples	# Positive in Is-CMV IgM Capture
EBV IgM	8	0
Lyme IgM	3	0
HSV IgM	15	0
VZV IgM	4	0
Rubella IgM	5	0
Toxoplasma IgM	5	0
Heterophile	4	0
Antibody RF	5	0
ANA	10	0
CMV IgG	11	0

TABLE 7

Sample #	Before IgG Removal		After IgG Removal	
	IgG Index	IgM Index	IgG Index	IgM Index
1	11.55	3.99	0.72	3.95
2	21.64	2.68	0.80	2.55
3	7.21	1.57	0.32	1.42
4	9.21	1.82	0.00	1.41
5	14.04	2.03	0.00	1.85
6	4.28	1.66	0.10	1.67
7	12.52	2.02	0.00	1.40
8	8.71	2.06	0.53	2.42
9	7.05	1.93	0.04	1.75
10	6.47	1.97	0.64	1.71
	IgG Pos > 1.0		IgM Pos ≥ 1.10	

D. Verification of IgM Specificity

To confirm that the *Is*-CMV IgM Capture Test Kit specifically detects IgM-class antibodies, 15 samples with moderate to high levels of CMV IgM antibodies were selected for testing. These samples were treated with dithiothreitol (DTT) to destroy the IgM and were then retested in the *Is*-CMV IgM Capture Test Kit. The results in TABLE 8 show that these samples were rendered negative following treatment with DTT confirming the specificity of the *Is*-CMV IgM Test Kit for detecting IgM-class antibodies.

TABLE 8

Sample #	Untreated		Treated with DTT	
	Is-CMV IgM Capture		Is-CMV IgM Capture	
	Index	Interp	Index	Interp
1	2.068	POS	0.213	NEG
2	3.377	POS	0.214	NEG
3	4.860	POS	0.476	NEG
4	4.064	POS	0.574	NEG
5	1.371	POS	0.242	NEG
6	3.086	POS	0.182	NEG
7	1.499	POS	0.240	NEG
8	2.296	POS	0.385	NEG
9	3.073	POS	0.356	NEG
10	2.738	POS	0.501	NEG
11	1.902	POS	0.277	NEG
12	2.755	POS	0.304	NEG
13	1.775	POS	0.240	NEG
14	1.302	POS	0.209	NEG
15	3.229	POS	0.229	NEG

E. Precision

Six serum samples, as well as the kit controls, were tested to assess the precision of the *Is*-CMV IgM Capture Test Kit. Sites #1 and #2 tested samples in triplicate in three separate runs on three different days. Site #3 (Diamedix Corp.) tested samples in triplicate in two separate runs on three different days both manually and using the MAGO Plus Automated EIA 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 (n=9)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
C1	0.198	0.006	3.03	0.247	0.023	9.31	0.271	0.018	6.64	0.239	0.036	15.06
C2	0.213	0.003	1.41	0.270	0.007	2.59	0.290	0.021	7.24	0.258	0.036	13.95
C3	1.433	0.005	0.35	1.548	0.021	1.36	1.692	0.031	1.83	1.558	0.114	7.32
C4	1.839	0.032	1.74	1.979	0.031	1.57	2.269	0.043	1.90	2.029	0.192	9.46
C5	3.148	0.044	1.40	3.275	0.218	6.66	3.519	0.027	0.77	3.314	0.198	5.97
C6	3.772	0.032	0.85	4.065	0.109	2.68	4.305	0.064	1.49	4.047	0.240	5.93
LPC	1.363	0.031	2.27	1.545	0.011	0.71	1.612	0.041	2.54	1.507	0.115	7.63
NC	0.181	0.010	5.52	0.222	0.022	9.91	0.224	0.035	15.63	0.209	0.030	14.35

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

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
C1	0.192	0.052	27.08	0.226	0.018	7.96	0.330	0.030	9.09	0.249	0.070	28.11
C2	0.171	0.058	33.92	0.213	0.006	2.82	0.345	0.058	16.81	0.243	0.088	36.21
C3	1.339	0.032	2.39	1.513	0.088	5.82	1.536	0.029	1.89	1.463	0.105	7.18
C4	1.881	0.092	4.89	2.117	0.046	2.17	1.971	0.104	5.28	1.989	0.127	6.39
C5	3.168	0.070	2.21	3.239	0.111	3.43	3.115	0.068	2.18	3.174	0.091	2.87
C6	3.589	0.201	5.60	3.731	0.060	1.61	3.543	0.118	3.33	3.621	0.147	4.06
CAL	0.988	0.073	7.39	0.928	0.043	4.63	0.975	0.041	4.21	0.964	0.055	5.71
LPC	1.570	0.037	2.36	1.512	0.034	2.25	1.462	0.060	4.1	1.515	0.061	4.03
NC	0.243	0.059	24.28	0.172	0.019	11.05	0.351	0.070	19.94	0.255	0.091	35.69

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

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=18)		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
A	0.254	0.019	7.48	0.296	0.088	29.73	0.317	0.059	18.61	0.289	0.065	22.49
B	0.300	0.089	29.67	0.258	0.031	12.02	0.258	0.091	35.27	0.272	0.074	27.21
C	1.640	0.028	1.71	1.589	0.076	4.78	1.625	0.074	4.55	1.618	0.063	3.89
D	2.456	0.144	5.86	2.008	0.214		1.854	0.429		2.191	0.291	13.28
E	4.056	0.167	4.12	3.480	0.479	13.76	2.344	0.539	22.99	3.671	0.437	11.9
F	5.110	0.336	6.58	4.309	0.637	14.78	3.479	0.356	10.23	4.613	0.618	13.4
c/o CAL	1.122	0.078	6.95	0.938	0.147	15.67	0.955	0.150	15.71	1.005	0.148	14.73
LPC	1.866	0.121	6.48	1.514	0.180	11.89	1.636	0.264	16.14	1.672	0.238	14.23
NC	0.283	0.051	18.02	0.221	0.027	12.22	0.286	0.126	44.06	0.263	0.081	30.8

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%
A	0.40	0.104	26.00	0.29	0.071	24.48	0.36	0.078	21.67	0.35	0.094	27.09
B	0.60	0.172	28.67	0.45	0.069	15.33	0.46	0.142	30.87	0.50	0.145	28.83
C	1.80	0.125	6.94	1.53	0.096	6.27	1.42	0.104	7.32	1.58	0.193	12.19
D	2.70	0.266	9.85	2.43	0.430	17.70	2.36	0.413	17.50	2.50	0.386	15.46
E	4.50	0.241	5.36	3.46	0.197	5.69	3.33	0.232	6.97	3.76	0.577	15.33
F	5.53	0.477	8.63	4.46	0.636	14.26	4.33	0.575	13.28	4.77	0.766	16.05
c/o CAL	1.17	0.161	13.76	1.04	0.141	13.56	1.13	0.148	13.10	1.14	0.155	13.58
LPC	1.34	0.217	16.19	1.77	0.334	18.87	1.67	0.335	20.06	1.84	0.395	21.43
NC	0.48	0.203	42.29	0.40	0.117	29.25	0.39	0.081	9.00	0.42	0.141	33.49

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