POINTE SCIENTIFIC
A MEDTEST COMPANY

CMV IgG

REF **T8005-96** 96-Test Set

IVD

in Vitro Diagnostic For Use Only

Intended Use: For the detection of human IgG antibodies to cytomegalovirus virus in human serum by enzyme immunoassay, as an aid in the determination of acute or reactivated infection with CMV. When used as a qualitative test, CMV IgG EIA aids in the assessment of the patient's immunological response to CMV. These reagents have not received FDA clearance for use in testing blood or plasma donors.

Summary of Test

1. **Prepare 1:51 dilutions of Calibrator(s), Controls and samples in the test set Diluent. Mix well.**
2. **Place 100 µl of the dilutions in the Coated Wells; reserve one well for the reagent blank.**
3. **Incubate at room temperature for 30 ± 5 minutes.**
4. **Drain wells thoroughly. Wash wells 4 times with Wash Solution and drain.**
5. **Place 2 drops (or 100 µl) of Conjugate in wells.**
6. **Incubate at room temperature for 30 ± 5 minutes.**
7. **Drain wells thoroughly. Wash wells 4 times with Wash Solution and drain.**
8. **Place 2 drops (or 100 µl) of Substrate in wells.**
9. **Incubate at room temperature for 30 ± 5 minutes.**
10. **Stop the enzyme reaction with 2 drops (or 100 µl) of Stop Reagent.**
11. **Read absorbance at 405 nm against reagent blank.**

Summary and Explanation of Test

Cytomegalovirus (CMV) is the causative agent of cytomegalic inclusion disease, a generalized infection of infants caused by intrauterine or early post natal infection. The disease may cause severe congenital abnormalities, such as microcephaly, motor disability and mental retardation in infants (1, 2, 3). Cytomegalovirus infection has also been associated with acquired hemolytic anemia, acute and chronic hepatitis, and an infectious mononucleosis-like syndrome. Subclinical infection may occur in adults (4). CMV infection can be transmitted to immunodeficient or immunosuppressed individuals, as a result of blood transfusion (5) or organ transplantation (6).

Serological tests, such as the CMV IgG EIA test, which detect the presence of CMV IgG antibodies, can aid in the diagnosis of diseases caused by cytomegalovirus. Test results are obtained after one and one-half hours incubation time. They are objective and normalized as index values, permitting uniformity of reporting.

Principle of the Test

Diluted samples are incubated in antigen-coated wells. CMV antibodies (if present) are immobilized in the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme labeled antibodies to human IgG) is added and incubated. If IgG antibodies to CMV are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

Reagents

Coated Wells	Coated with CMV antigen (strain AD 169). 12 eight-well strips.
Well Support	One.
Diluent*	25 mL (pink color). Phosphate-buffered saline with a protein stabilizer.
Calibrator 1*	0.3 mL. Human serum. Strongly reactive for CMV antibodies.
Calibrator 2*	0.3 mL. Human serum. Moderately reactive for CMV antibodies.
Positive Control*	0.3 mL. Human serum. Reactive for CMV antibodies.
Negative Control*	0.3 mL. Human serum. Non-reactive for CMV antibodies.
Conjugate	12 mL (green color). Goat anti-human IgG labeled with alkaline phosphatase (calf).
Substrate	12 mL. p-nitrophenyl phosphate.

Note: The substrate may develop a slight yellow color during storage. One hundred microliters of substrate should yield an absorbance value less than 0.35, when read in a microwell against air or water.

Wash Concentrate*	30 mL. Tris-buffered saline with Tween 20, pH 8.0. Prepare Wash Solution by adding the contents of the Wash Concentrate bottle to one liter of distilled or deionized water.
Stop Reagent	12 mL. Trisodium Phosphate 0.5 M.

* Contains 0.1% sodium azide.

Store these reagents according to the instructions on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

Other Materials Required

1. Microplate washer
2. Pipettors for dispensing 4, 100 and 200 µl
3. Timer
4. 1 or 2 liter container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm.

Precautions

1. For in-vitro diagnostic use.
2. Test samples, Calibrator(s), Controls and the materials that contact them, should be handled as potential biohazards. The calibrators and controls have been found to be negative for HIV, hepatitis B surface antigen and HCV antibodies by licensed tests. However, because no method can offer complete assurance that HIV, hepatitis B virus, HCV 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, or latest edition.
3. The concentrations of anti-CMV in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

4. Avoid contact with open skin.
5. Never pipet by mouth.
6. Certain of the test reagents contain sodium azide. Azides are reported to react with lead and copper in plumbing to form compounds that may detonate on percussion. When disposing of solutions containing sodium azide, flush drains with large volumes of water to minimize the build-up of metal-azide compounds.

Xn HARMFUL
0.1% NaN_3
R 21/22: Harmful in contact with skin and if swallowed.
S24/25 - 36/37/39: Avoid contact with skin and eyes. Wear suitable protective clothing, gloves and eye/face protection.
For further information, refer to product MSDS.

Laboratory Quality Control

1. Do not interchange reagents from different reagent lots, except for Wash Concentrate, Substrate and Stop Reagent.
2. Do not use reagents beyond their stated expiration date.
3. Incubation times recommended in the Test Procedure section should be adhered to.
4. Unused Coated Wells should be kept in their resealable bag with dessicant, and stored in the refrigerator.

Specimen Collection

Sera should be separated from clotted blood and stored at 2 to 8° C short term, or frozen at –10° C or below, for longer periods. Multiple freeze-thaw cycles should be avoided. Samples containing visible particulate matter should be clarified by centrifugation; and grossly contaminated samples should not be used. Samples should not be heat-inactivated before testing.

Test Procedure

Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use. The test procedure follows:
1. Prepare 1:51 dilutions of test samples, Calibrator(s), Positive and Negative Controls, in the test set Diluent. For example: add 4 µl of sample to 200 µl of Diluent in a dilution well or tube, and mix well.
Note: For qualitative assays, a single Calibrator may be used; for semi-quantitative assays, it is necessary to use Calibrator 1 and Calibrator 2.
2. Place an appropriate number of Coated Wells in the Well Support.
Note: For combination testing (multiple assays per plate), the strips should be assembled on a white background with good lighting. Be sure to note the placement of each strip and the corresponding color.
3. Transfer 100 µl of each diluted Calibrator, Control and patient sample to the wells.
Note: Include one well which contains 100 µl of Diluent only. This will serve as the reagent blank and will be ultimately used to zero the photometer before reading the test results.
4. Incubate the wells at room temperature (20 to 25° C) for 30 ± 5 minutes.
5. Wash wells four times with at least 250 µL/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.
6. Place 2 drops (or 100 µl) of Conjugate into each well.
7. Incubate the wells at room temperature for 30 ± 5 minutes.
8. Wash wells four times with at least 250 µL/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.
9. Place 2 drops (or 100 µl) of Substrate into each well.
10. Incubate at room temperature for 30 ± 5 minutes.
11. Place 2 drops (or 100 µl) of Stop Reagent into each well.
12. Read and record the absorbance of the contents of each well at 405 nm against the reagent blank.
Note: Adjust the photometer to zero absorbance at 405 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.

Calculation of Results

Qualitative results may be calculated using a single calibrator. For semi-quantitative results, use a calibration curve consisting of two or more calibrators.

Single Calibrator (Calibrator 2)
Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Calibrator Index}}{} \times \text{Test Sample} = \text{Test Sample}$$

Calibrator	Absorbance	Index
Absorbance		

If the Calibrator is run in duplicate, use the average absorbance value to calculate results.

Calibration Curve

Alternatively, test results may be calculated from a three-point curve comprised of: Calibrator 1 (high-point), Calibrator 2 (mid-point) and the reagent blank (zero / origin), using a point-to-point curve fit.

The upper range of the curve may be expanded by adding additional points. For example: the concentration of Calibrator 1 may be increased 1.5-fold, and 2-fold, by adding 6 µl and 8 µl of Calibrator 1 to 200 µl of the test set Diluent, and transferring 100 µl of each dilution to coated wells. The Index, or IU/mL values, assigned to these points, should be 1.5 and 2 times respectively, the value shown on the Calibrator 1 label. *The extent to which the upper range of the standard curve may be expanded, will be limited by the absorbance range of the spectrophotometer being used.*

Test Validation Criteria

1. The Calibrator(s), Positive and Negative Controls must be included in each test run.
2. The absorbance values of Calibrator 1 and Calibrator 2, must be at least 0.8 and 0.4 respectively, when read against the reagent blank.
3. The absorbance value of the reagent blank should be less than 0.35.
4. The Negative Control must have an Index value less than 0.9.
5. The Positive Control must have an Index value within the range printed on the label. When performing qualitative tests, users may supply an alternative Positive Control if they wish.

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

Interpretation of Results

Index Value	Interpretation
< 0.9	Negative
≥ 0.9 < 1.1	Equivocal
≥ 1.1	Positive

The CMV IgG EIA cut-off values were based on statistical analyses, i.e. mean + 3 standard deviations, of serum specimens shown to be negative by other legally marketed devices. They were validated in tests of known positive and negative specimens (please see Performance Characteristics).

When equivocal results are obtained, another specimen should be obtained two to three weeks later, and tested in parallel with the initial specimen. If the second specimen 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.

To determine a significant difference between acute/convalescent serum pairs, both specimens should be assayed concurrently. Dose response experiments performed at Laboratory C (Miami, FL), have shown that a 75 to 95 percent increase in the CMV IgG EIA Index value, corresponds to a two-fold increase in the CMV IgG antibody level; and a 150 to 190 percent increase in CMV IgG EIA Index value, corresponds to a four-fold increase in the CMV IgG antibody level.

Specimens which yield absorbance values above the range of the test set calibrator(s), or the microwell reader, may be pre-diluted in the test set Diluent and reassayed. The resulting Index value must be multiplied by the dilution factor. *Example: If the specimen has been pre-diluted 1:5 before testing, the resulting Index value should be multiplied by 5.*

The suggested method for reporting results is:

The following results were obtained with this CMV IgG EIA test. Values obtained with different manufacturer's assay methods may not be used interchangeably. The magnitude of the reported IgG level cannot be correlated to an endpoint titer.

Limitations

The results obtained with the CMV IgG EIA test serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.

To detect seroconversion, paired specimens should be collected during the acute and convalescent stages of infection, and tested concurrently.

Positive results with cord blood should be interpreted with caution. The presence of IgG antibodies to CMV in cord blood may be the result of passive transfer of maternal antibody to the fetus. A negative result however, may be helpful in ruling out infection.

Definitive diagnosis of active CMV infection requires viral isolation. The presence of IgG antibody to CMV does not assure protection from disease.

Titration experiments (please see Figure 2) have shown that the upper limit of linearity for CMV IgG EIA Index values is approximately 6.

The performance characteristics of the Rubella IgG test with automated analyzers have not been established.

Expected Values

The incidence of CMV antibodies is related to age, socio-economic condition, and geographic location of the test population (8, 9, 10). Up to 80 % of U.S. and European blood donors exhibit serological evidence of previous CMV infection (11, 12). Serum specimens obtained randomly from one hundred and forty-three healthy South Florida blood donors were assayed by the CMV IgG EIA test. One hundred and one specimens (71 %) were positive for antibodies to CMV. The Index values ranged from 1.9 to 10.7. Excluding the results for twenty-two strongly positive specimens which gave absorbance values above the range of the reader, the mean value of the positives was 5.5. The remaining forty-two specimens (29 %) were negative.

Performance Characteristics

Comparative Testing

CMV IgG EIA test results correlated very well with results of other serological tests. Sera from normal blood donors were assayed for the presence of CMV IgG antibodies, using the CMV IgG EIA test and three other commercial tests, at two independent laboratories (Lab A, Atlanta, GA, and Lab B, Gainesville, FL), and at Laboratory C (Miami, FL). These results are shown below in Tables 1, 2 and 3, respectively.

Table 1. Results of Tests of 152 Specimens (58% frozen and 42% fresh), from North and South Carolina, Alabama, Georgia and Florida, Performed at Laboratory A (Atlanta, GA), Using the CMV IgG EIA Test and Another Commercial Test.

Comparative	CMV IgG EIA				
Test #1	Positive	Equivocal	Negative		95%CI**
Positive	63	0	2	Relative sensitivity*	89.3 to 99.6
Negative	3	3	81	Relative specificity*	89.9 to 99.3
* Excluding equivocal results				Overall Agreement*	92.3 to 98.9

** Calculated by the Exact Method (13).

Table 2. Results of tests of 163 Specimens (66% frozen and 34% fresh), from North, Central and South Florida, Performed at Laboratory B (Gainesville, FL), Using the CMV IgG EIA Test and Another Commercial Test.

Comparative	CMV IgG EIA				
Test #2	Positive	Equivocal	Negative		95%CI**
Positive	97	0	4	Relative sensitivity*	92.2 to99.8
Equivocal	1	0	0		
Negative	1	2	58	Relative specificity*	95.0 to100
* Excluding equivocal results				Overall Agreement*	94.1 to 99.5

** Calculated by the Normal Method (13).

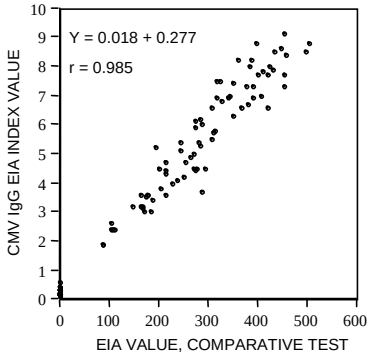
Table 3. Results of tests of 143 Specimens (100% frozen), from South Florida, Performed at Laboratory C (Miami, FL), Using the CMV IgG EIA Test and Another Commercial Test.

Comparative	CMV IgG EIA				
Test #3	Positive	Equivocal	Negative		95%CI**
Positive	101	0	0	Relative sensitivity*	96.4 to 100
Equivocal	0	0	0		
Negative	0	0	42	Relative specificity*	91.6 to 100
* Excluding equivocal results				Overall Agreement*	97.5 to 100

** Calculated by the Exact Method (13).

The data obtained at Lab C and tabulated in Table 3, has been plotted below in Figure 1. Twenty-two specimens which were strongly positive in both assays, which gave results above the range of the reader, are not shown.

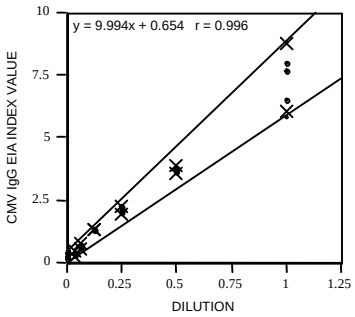
Figure 1. Results of Tests of 121 Serum Specimens Performed at Lab C, Using the CMV IgG EIA Test and Another Commercial Test.



Titration curve

Several strongly positive serum specimens were serially diluted (two-fold) in triplicate, and assayed by the CMV IgG EIA test. Typical results are shown in Figure 2.

Figure 2. Titration Curve for a Strongly Positive Specimen.



The triplicate data for each dilution are shown as points, the 95 % confidence limits for each set of triplicate data are indicated by (x's), and the 95 % confidence limits for the slopes and y-intercepts are represented by straight lines. The formula for the linear regression for the triplicate data is shown in Figure 2.

The results of the titration / dose response experiments were analyzed in order to relate changes in the CMV IgG EIA Index values to actual differences in antibody level. This analysis showed that a 75 to 95 percent increase in the CMV IgG EIA Index value, is equivalent to a two-fold increase in the antibody level; and a 150 to 190 percent increase in the Index value, indicates a four-fold change in the antibody level.

Specificity

The CMV IgG EIA test is specific for IgG antibodies directed against cytomegalovirus, and does not to cross-react with antibodies directed against other members of the herpes virus group. In tests of eleven sera which were negative for CMV antibody, all eleven were positive for varicella-zoster and Epstein-Barr antibodies, and six of eleven were positive for herpes simplex type 1 and type 2 antibodies.

Precision

Eight serum specimens (2 negative and 6 positive) and the CMV IgG EIA positive and negative controls, were assayed in triplicate, on three separate occasions. The precision experiments were performed manually at two independent laboratories (Lab A and Lab B), and at Laboratory C. These results are shown below in Tables 4, 5 and 6 respectively.

Table 4. Results Intra-assay and Interassay Precision Tests Performed at Lab A. Values were calculated from CMV IgG EIA Index values.

	INTRA ASSAY			INTERASSAY		
SAMPLE	MEAN	S.D	C.V. %	MEAN	S.D.	C.V. %
Pos. Control	3.8	0.265	7.0	3.7	0.244	6.6
Neg. Control	0.3	0.017	NA	0.3	0.022	NA
1	4.7	0.577	12.4	5.1	0.646	12.8
2	2.4	0.231	9.8	2.5	0.217	8.6
3	0.4	0.029	NA	0.5	0.059	NA
4	5.0	0.651	13.1	5.4	0.711	13.2
5	0.5	0.015	NA	0.5	0.059	NA
6	2.7	0.551	20.7	3.1	0.556	18.1
7	1.9	0.265	13.9	2.0	0.330	16.6
8	1.5	0.058	3.9	1.7	0.219	13.2

Table 5. Results Intra-assay and Interassay Precision Tests Performed at Lab B. Values were calculated from CMV IgG EIA Index values.

	INTRA ASSAY			INTERASSAY		
SAMPLE	MEAN	S.D	C.V. %	MEAN	S.D.	C.V. %
Pos. Control	2.7	0.115	4.2	2.9	0.169	5.9
Neg. Control	0.1	0.000	NA	0.3	0.172	NA
1	4.4	0.306	6.9	4.0	0.391	9.8
2	2.1	0.153	7.4	2.1	0.148	7.0
3	0.2	0.000	NA	0.2	0.000	NA
4	4.7	0.451	9.5	4.8	0.548	11.3
5	0.3	0.000	NA	0.3	0.033	NA
6	2.5	0.100	4.0	2.6	0.112	4.4
7	1.8	0.252	13.7	1.8	0.305	16.5
8	1.4	0.058	4.2	1.4	0.083	5.9

Table 6. Results Intra-assay and Interassay Precision Tests Performed at Lab C. Values were calculated from CMV IgG EIA Index values.

	INTRA ASSAY			INTERASSAY		
SAMPLE	MEAN	S.D	C.V. %	MEAN	S.D.	C.V. %

Pos. Control	2.9	0.200	6.9	3.0	0.179	5.9
Neg. Control	0	0.000	NA	0	0.133	NA
1	4.4	0.000	0.0	4.4	0.273	6.2
2	2.0	0.208	10.6	2.1	0.217	10.4
3	0.0	0.058	NA	0.1	0.044	NA
4	5.3	0.493	9.2	5.3	0.363	6.8
5	0	0.000	NA	0.1	0.097	NA
6	2.7	0.115	4.3	2.7	0.179	6.6
7	1.6	0.100	6.3	1.7	0.120	7.2
8	1.4	0.100	7.1	1.4	0.101	7.0

CDC Panel Results

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 CMV IgG EIA test. The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement by the CDC.

The panel consists of 66 % positive and 34 % negative samples. The CMV IgG EIA test demonstrated 99 % (99 of 100) total agreement with the CDC results. Of the results obtained by Laboratory C, there was 100 % (66 of 66) agreement with the positive results and 97 % (33 of 34) agreement with the negative specimens.

References

1. Hanshaw, J.B., Scheiner, A.P. Moxley, A.W. Gaev, L., Abel, V. and Scheiner, B.: School Failure and Deafness After “Silent” Congenital Cytomegalovirus Infection. N. Eng. J. Med. 295: 468-470, 1976.
2. Reynolds, D.W., Stagno, S., Stubbs,K.G., Dable,A.J., Livingston, M.M., Saxon, S.S., Alford, C.A.: Inapparent Congenital Cytomegalovirus. N. Eng. J. Med. 790: 291-296, 1974.
3. Stern, H. Cytomegalovirus Vaccine: Justification and Problems. In: Waterson, A.P. (ed.), Recent Advances in Clinical Virology, Edinburgh: Churchill Livingstone. 117-134, 1977.
4. Krech, U., Jung, J. Cytomegalovirus Infections in Man. S. Karger, Basel, Switzerland. 1971.
5. Foster, K.M. Jack, I. A Prospective Study of the Role of Cytomegalovirus In Post-transfusion Mononucleosis. N. Eng. J. Med. 280: 1311-1316, 1969.
6. Fiala, M., Payne, J.E., Berne, T.V., Moore, T.C., Montgomerie, J.Z., Chatterjee, S.N., and Guze, L.B. Epidemiology of Cytomegalovirus Infections After Transplantation and Immunosuppression. J. Infect. Dis. 132: 421-433, 1975.
7. Duncan, R.J.S., The Use of ELISA for Rapid Viral Dianosis: Antibody Detection. In *ELISA and Other Solid Phase Immunoassays*, D.M. Kemeny and S.J. Challacombe, eds. John Wiley & Sons, Ltd., Chichester.
8. Wentworth, B.B., and Alexander, E.R., Seroepidemiology of Infections due to Members of the Herpesvirus Group, Am. J. Epidemiol. 94: 496-507, 1971.
9. Stagno, S. et al.,Congenital Cytomegalovirus Infection: The Relative Importance of Primary and Recurrent Maternal Infection. N. Engl. J. Med. 306: 945-949, 1982.
10. Alford, C.A., et al., Epidemiology of Cytomegalovirus. In The Human Herpesviruses, 159-171, Nahmias, Dowdle and Schinazi, eds. Elsevier: New York, 1981.
11. Klemola, E., et al, Cytomegalovirus Mononucleosis in Previously Healthy Individuals. Ann. Intern. Med., 71:11-19, 1969.
12. Prince, A.M., et al, A Serologic Study of Cytomegalovirus Infections Associated with Blood Transfusions. N. Engl. J. Med., 284: 1125-1131, 1971.
13. Gardner, M.J. and Altman, D.G., Confidence Intervals Rather Than Hypothesis Testing. Brit. Med. J., 292: 746-750,1986.

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Lot No.

Expiration Date

96 Tests

Catalog No.

In vitro diagnostic use only

Temperature Limitations

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