CMV IgG EIA ID: Pink

Enzyme Immunoassay (EIA) for the Detection of CMV IgG Antibodies in Human Serum.

For *In Vitro* Diagnostic Use Only 25177 • 96 Tests

CONTENTS

1 - INTENDED USE

For the detection of human IgG antibodies to cytomegalovirus 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.

2 - SUMMARY AND EXPLANATION OF THE 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.

3 - 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.

4 - REAGENTS

CMV IgG EIA Product Description
Catalog No. 25177 (96 Tests)

Component	Contents	Preparation
Coated Wells 12 eight-well strips	• Coated with CMV antigen (strain AD 169) • Pink wells	Use as supplied. Return unused strips to pouch and reseal. Do not remove desiccant.*
Well support 1 Frame	• Plate frame	Use as supplied.
Diluent** 1 bottle (25 mL)	• Phosphate-buffered saline with a protein stabilizer • Pink Color	Use as supplied.
Calibrator 1** 1 vial (0.5 mL)	• Human serum, strongly reactive for CMV antibodies • Index value shown on vial label	Dilute in Diluent as described.
Calibrator 2** 1 vial (0.5 mL)	• Human serum, moderately reactive for CMV antibodies • Index value shown on vial label	Dilute in Diluent as described.
Positive Control** 1 vial (0.5 mL)	• Human serum, reactive for CMV antibodies • Index value shown on vial label	Dilute in Diluent as described.
Negative Control** 1 vial (0.5 mL)	• Human serum, non-reactive for CMV antibodies	Dilute in Diluent as described.
Conjugate** 2 bottles (12 mL)	• Goat anti-human IgG labeled with alkaline phosphatase (calf) • Green color	Use as supplied.
Substrate*** 1 bottle (12 mL)	• p-Nitrophenyl phosphate	Use as supplied.
Wash Concentrate** 1 bottle (30 mL)	• Tris-buffered saline • Tween 20™ • pH 8.0	Dilute in 1 liter of distilled or deionized water.
Stop Reagent 1 bottle (12 mL)	• Trisodium phosphate 0.5 M	Use as supplied.

* The color of the desiccant does not affect the performance of the kit.
** Contains 0.1% sodium azide.
*** 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.

Store these reagents at 2-8°C up to the expiration date indicated on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water. Do not remove desiccant.

5 - 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.

6 - PRECAUTIONS FOR USERS

For *In Vitro* Diagnostic Use

1. Test samples, Calibrator(s), Controls, and the materials that contact them should be handled as potential biohazards. The calibrators and controls have been tested and found to be non-reactive for HIV, hepatitis B surface antigen, and HCV antibodies by licensed tests. However, no method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent. Handle reagents and patient samples as if capable of transmitting infectious disease following recommended *Universal Precautions* for bloodborne pathogens as defined by OSHA[14], Biosafety Level 2 guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[15], WHO *Laboratory Biosafety Manual*[16], and/or local, regional, and national regulations.
2. 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.
3. Avoid contact with open skin.
4. Never pipet by mouth.
5. Certain test reagents contain dilute **sodium azide**, which may be harmful if enough is ingested (more than supplied in kit). Azides are reported to react with lead and copper in plumbing to form compounds that may detonate on percussion. If disposing of solutions containing sodium azide down drains, flush with large volumes of water to minimize the build-up of metal-azide compounds. Dispose of contents and container in accordance with local, regional, national, and international regulations.
6. For more hazard information, refer to the product Safety Data Sheet (SDS), which is available at www.bio-rad.com and upon request.

7. Any lot number of the following reagents may be used with this assay, provided they have the correct catalog number and are not used beyond their labeled expiration date:
    - Diluent - Catalog # 25186
    - Substrate - Catalog # 25192
    - Wash Concentrate - Catalog # 25190
    - Stop Reagent - Catalog # 25191

    Do not mix any other reagents from different lots.
8. Do not use reagents beyond their stated expiration date.
9. Incubation times recommended in the Test Procedure section should be adhered to.
10. Unused Coated Wells should be kept in their resealable bag with desiccant and stored in the refrigerator.
11. This product should be used by qualified personnel.
12. There are no health hazards associated with the intact desiccant packet. Do not cut, split, or otherwise compromise it as dusts that may be generated could pose a health hazard. If the desiccant has been compromised, do not remove it from the plate pouch.

7 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE

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.

8 - TEST PROCEDURE

Materials Provided

See REAGENTS section on page 4.

EIA Procedure

1. Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use.
2. Prepare working wash solution by adding entire bottle of Wash Concentrate (30 mL) to 1 liter of water. Once diluted, the wash solution can be stored at room temperature for up

to two months, or at 4°C until the expiration date printed on the Wash Concentrate bottle.

3. 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 (Calibrator 2) may be used. For semi-quantitative assays, it is necessary to use Calibrator 1 and Calibrator 2 to prepare a calibration curve. The blank serves as the zero calibrator.

4. Place 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.

5. 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 ultimately be used to zero the photometer before reading the test results.

6. Incubate the wells at room temperature (20 to 25°C) for 30 ± 5 minutes.
7. Wash wells four times, with at least 250 µL of wash solution per well. Do not allow the wells to soak between washes. Aspirate thoroughly after the last wash.
8. Place 2 drops (or 100 µL) of Conjugate into each well.
9. Incubate the wells at room temperature (20 to 25°C) for 30 ± 5 minutes.
10. Wash wells four times with at least 250 µL of wash solution per well. Do not allow the wells to soak between washes. Aspirate thoroughly after the last wash.
11. Place 2 drops (or 100 µL) of Substrate into each well.
12. Incubate at room temperature (20 to 25°C) for 30 ± 5 minutes.
13. Place 2 drops (or 100 µL) of Stop Reagent into each well. Tap the plate gently, or use other means to assure complete mixing.
14. 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.

9 - QUALITY CONTROL

Test Validation Criteria

1. The Calibrator(s), Positive and Negative Controls must be included in each test run.
2. The absorbance value of Calibrator 1 must be ≥ 0.800, when read against the reagent blank.
3. The absorbance value of Calibrator 2 must be ≥ 0.400, when read against the reagent blank.
4. The absorbance value of the reagent blank should be < 0.350.
5. The Negative Control must have an Index value < 0.9.
6. 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.
7. If any of these criteria are not met, the test is invalid and should be repeated. If the test is invalid, patient results can not be reported.

10-INTERPRETATION OF RESULTS

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:

$$\frac{\text{Calibrator 2 Index}}{\text{Calibrator 2 Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

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 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.*

Interpretation of Results

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

The CMV IgG EIA cutoff 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 the Bio-Rad CMV IgG EIA test. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgG level cannot be correlated to an endpoint titer.

11-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.

12-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.

13-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 Test #1	CMV IgG EIA		
	Positive	Equivocal	Negative
Positive	63	0	2
Negative	3	3	81

	95% C.I.**
Relative sensitivity*	89.3 to 99.6
Relative specificity*	89.9 to 99.3
Overall Agreement*	92.3 to 98.9

* Excluding equivocal results

** Calculated by the Normal 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 Test #2	CMV IgG EIA		
	Positive	Equivocal	Negative
Positive	97	0	4
Equivocal	1	0	0
Negative	1	2	58

	95% C.I.**
Relative sensitivity*	92.2 to 99.8
Relative specificity*	95.0 to 100
Overall Agreement*	94.1 to 99.5

* Excluding equivocal results

** 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 Test #3	CMV IgG EIA		
	Positive	Equivocal	Negative
Positive	101	0	0
Equivocal	0	0	0
Negative	0	0	42

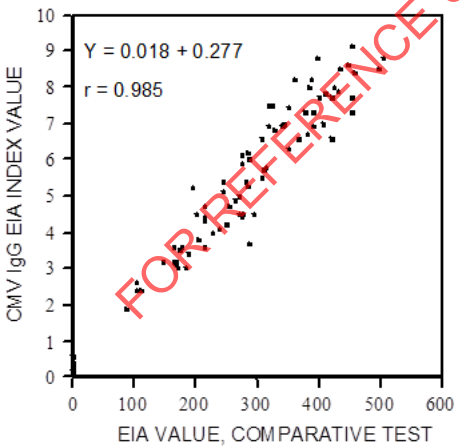
	95% C.I.**
Relative sensitivity*	96.4 to 100
Relative specificity*	91.6 to 100
Overall Agreement*	97.5 to 100

* Excluding equivocal results

** Calculated by the Normal Method[13]

The data obtained at Lab C and tabulated in Table 3 have 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.

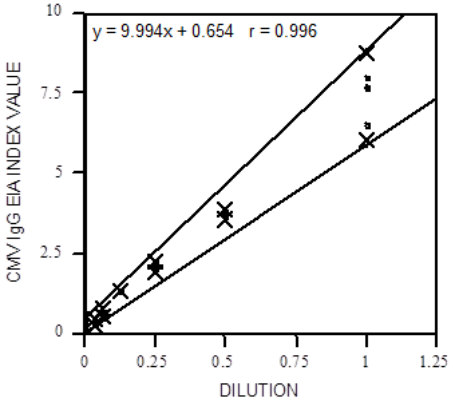
Figure 1: Results of tests of 121 serum specimens performed at Lab C, using the CMV IgG EIA test and another commercial test.



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 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 inter-assay precision tests performed at Lab A. Values were calculated from CMV IgG EIA Index values.

	INTRA-ASSAY			INTER-ASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	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 inter-assay precision tests performed at Lab B. Values were calculated from CMV IgG EIA Index values.

	INTRA-ASSAY			INTER-ASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	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 inter-assay precision tests performed at Lab C. Values were calculated from CMV IgG EIA Index values.

	INTRA-ASSAY			INTER-ASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	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	7.25
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 using 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.

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