

AccuDiag™
Cytomegalovirus IgG (CMV IgG)
ELISA Kit

REF 1201-11



Test	CMV IgG ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	5µL Serum
Specificity	98%
Sensitivity	97%
Total Time	~75 min
Shelf Life	12 Months from the manufacturing date

INTENDED USE

The Diagnostic Automation, Inc. ELISA, CMV IgG is intended for use in evaluating a patient's serologic status to cytomegalovirus (CMV) infection.

SUMMARY AND EXPLANATION

Cytomegalovirus is a herpes virus and a leading biological factor causing congenital abnormalities and complications among those who receive massive blood transfusions and immunosuppressive therapy. About half of pregnant women who contract a primary infection spread the disease to their fetus. When acquired in-utero, the infection may cause mental retardation, blindness, and/or deafness.

Serological tests for detecting the presence of antibody to CMV can provide valuable information regarding the history of previous infection, diagnosis of active or recent infection, as well as in screening blood for transfusions in newborns and immuno-compromised recipients. CMV IgG ELISA is an accurate serologic method to detect CMV antibody for identification of CMV infection.

TEST PRINCIPLE

Purified CMV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the CMV IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The

intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

SPECIMEN COLLECTION AND PREPARATION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Microwell strips: purified CMV antigen coated wells (12 x 8 wells)
2. Enzyme conjugate: Red color solution 1 vial (12 ml)
3. Negative Calibrator: 0 IU/ml White cap 1 vial (150 µl)
4. Cut-off Calibrator: 1.2 IU/ml Yellow cap 1 vial (150 µl)
CMV G Index = 1.0
5. Positive Calibrator: 6 IU/ml Green Cap 1 vial (150 µl)
6. Positive Calibrator: 18 IU/ml Blue cap 1 vial (150 µl)
7. Negative Control: Range stated on label. Natural Cap 1 vial (150 µl)
8. Positive Control: Range stated on label. Brown Cap 1 vial (150 µl)
9. Washing concentrate 20x (H) 1 bottle (50 ml)
10. Sample diluent: Blue color solution 1 vial (22 ml)
11. TMB Chromogenic Substrate: Amber bottle 1 vial (12 ml)
12. Stop solution 1 vial (12 ml)

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer.
Prepare washing buffer by adding distilled or deionized water to 20x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5 µl of the test samples, negative control, positive control, and calibrators to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of Stop Solution to stop reaction.
Make sure there are no air bubbles in each well before reading
9. Read O.D. at 450 nm with a microwell reader.

RESULTS

1. Calculate the mean of duplicate calibrator value x_c .
2. Calculate the mean of duplicate positive control, negative control and patient samples.
3. Calculate the CMV G Index of each determination by dividing the mean values of each sample by calibrator mean value, x_c .

Example of typical results:

Cut-off Calibrator CMV G Index = 1.0

Calibrator O.D. = 0.685, 0.690 $x_c = 0.690$
 Patient sample O.D. = 1.373, 1.457 $x_s = 1.415$
 CMV G Index = $1.415 / 0.690 = 2.05$

INTERPRETATION

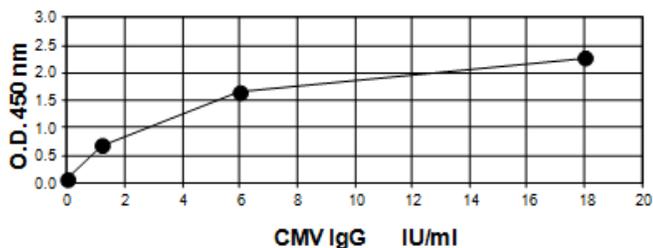
Negative: CMV G Index of 0.90 or less are seronegative for IgG antibody to CMV. (<1.1 IU/ml)

Equivocal: CMV G Index of 0.91-0.99 are equivocal. Sample should be retested.

Positive: CMV G Index of 1.00 or greater, or IU value greater than 1.2 are seropositive. It indicates prior exposure to the CMV virus. (> 1.2 IU/ml)

QUANTITATIVE ESTIMATION OF CMV IgG

For a quantitative estimate of anti-CMV IgG levels of positive specimens in IU/ml, OD of calibrator and positive control are plotted on Y-axis in graph versus their corresponding anti-CMV IgG concentration of 0, 1.2, 6 and 18 IU/ml on X-axis. The estimates of levels in patient sera are read off the graph using their individual OD values. For example:



PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity

A total of 85 patient samples were used to evaluate specificity and sensitivity of the test. The Diagnostic Automation, Inc. ELISA CMV IgG test results were compared to a commercial ELISA kit results.

		Reference ELISA			Total
		N	E	P	
DIAGNOSTIC	N	45(D)	0	1 (B)	46
	E	0	1	0	1
AUTOMATION	P	1(C)	0	38(A)	39
	Total	46	1	39	86

$$\text{Sensitivity} = A / (A+B) = 38 / 39 = 97\%$$

$$\text{Specificity} = D / (C+D) = 45 / 46 = 98\%$$

$$\text{Accuracy} = (A+D) / (A+B+C+D)$$

$$= (38+45) / (38+1+1+45) = 83 / 85 = 98\%$$

Precision

The precision of the assay was evaluated by testing three different sera of eight replicates over 3 days. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	8.9%	8.2%	7.2%
Inter-assay	9.7%	8.3%	7.8%

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The CMV G Index or IU/ml unit for Negative and Positive Control should be in the range stated on the labels.

LIMITATIONS OF PROCEDURE

1. Lipemic, hemolyzed, icteric or heat inactivated sera may cause erroneous results.
2. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

PRECAUTION

1. Potential biohazardous materials:
 The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

STORAGE

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

REFERENCES

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3. Starr, S.E. and H.M. Friedman. "Human CMV." Chapter 65. In Manual of Clin. Microbiol., 4th ed., Lennett, E.H. et al ed. Am. Soc. Microbiol. pp. 771-719, 1985.

<p>ISO 13485 ISO 9001</p> 	
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