CYTOMEGALOVIRUS (CMV) IgG
ELISA Kit Protocol

(Cat. No.:EK-310-89)

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INTENDED USE

The CMV IgG ELISA is intended for use in evaluating a patient's serologic status to cytomegalovirus (CMV) infection. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

WARNINGS AND PRECAUTIONS FOR USERS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found non-reactive 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 from 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

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

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INTRODUCTION AND PROTOCOL OVERVIEW

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 the 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 diagnose active or recent infection and provide valuable information regarding the history of previous infection. These tests are also useful in screening blood for transfusions in newborns and immuno-compromised recipients. CMV IgG ELISA is an accurate serologic method to detect CMV IgG antibody for identification of CMV infection.

PRINCIPLE OF THE TEST

Purified CMV antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the CMV IgG specific-antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed

off and a solution of TMB Reagent 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 CMV IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrators and controls.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

STORAGE

1. Store the kit at 4°C. DO NOT FREEZE.
2. Keep microwells sealed in a dry bag with desiccants.
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.

LIST OF COMPONENTS

Materials Provided with the Kit:

- Microtiter Wells: purified CMV antigen-coated wells (12 x8 wells)
- Enzyme Conjugate Reagent (red color): Red cap. 1 vial (12 ml)
- Sample Diluent (green color): 1 bottle (22 ml)
- Negative Calibrator: 0 IU/ml. Natural cap. (150 µL/vial)
- Cut-off Calibrator. 1.2 IU/ml. Yellow cap. (150 µ L/vial), CMV IgG index= 1.0
- Positive Calibrator: 6 IU/ml. Red cap. (150 µL/vial)
- Positive Calibrator: 18 IU/ml. Green cap. (150 µL/vial)
- Negative Control: Range stated on label. Blue cap. (150 µL/vial)
- Positive Control: Range stated on label. Purple cap. (150 µL/vial)
- Wash Buffer Concentrate: 1 bottle (50 ml, 20x)
- TMB Reagent (One-Step), 1 vial (11 ml)
- Stop Solution: 1N HCl, Natural cap. 1 vial (11 ml)

SPECIMEN COLLECTION AND PREPARATION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 4°C. Mix well before use.

ASSAY PROCEDURE

Note: *Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-25°C).*

1. 1. Place the desired number of coated wells into the holder.
2. 2. Prepare 1:40 dilution of test samples, Negative Control, Positive Control, and Calibrator by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
3. 3. Dispense 100 µl of diluted sera, Calibrator, and Controls into the appropriate wells. For the reagent blank, dispense 100 µl of Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. 4. Incubate at 37°C for 30 minutes.
5. 5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
6. 6. Dispense 100 µl of Enzyme Conjugate into each well. Mix gently for 10 seconds.
7. 7. Incubate at 37°C for 30 minutes.
8. 8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
9. 9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. 10. Incubate at 37°C for 15 minutes.
11. 11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely**
    Note: Make sure there are no air bubbles in each well before reading.
13. Read O.D. at 450 nm **<u>within 15 minutes</u>** with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate cut-off calibrator value xc.
2. Calculate the mean of duplicate positive control (xp), negative control (xn) and patient samples (xs).
3. Calculate the CMV IgG Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, xc.

Example of typical results: Note: These O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.
Cut-off Calibrator CMV IgG Index = 1.0

1. Cut-off Calibrator (1.2 IU/ml) O.D. = 0.845, 0.850 xc = 0.848
2. Negative Control O.D. = 0.154, 0.169 xn = 0.162
   CMV IgG Index = xn / xc = 0.162/0.848 = 0.19
3. Positive Control O.D. = 1.284, 1.255 xp = 1.270
   CMV IgG Index = xp / xc = 1.270/0.848 = 1.50
4. Patient Sample O.D. = 2.392, 2.243 xs = 2.318
   CMV IgG Index = xs / xc = 2.318/0.848 = 2.73

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40
   5 µl / 200 µl
2. Three incubations at 37°C

Diluted Sample	Enzyme Conjugate	TMB Reagent (One-Step)
100 µl	100 µl	100 µl
30 min.	30 min.	15 min

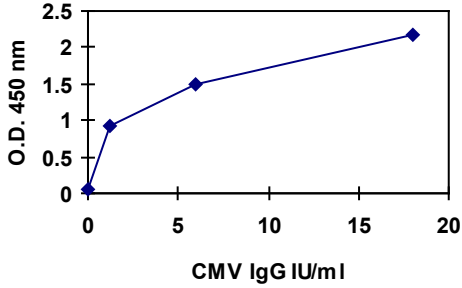
3. Stop with 100 µl of acid. Read O.D. at 450 nm

QUANTITATIVE DETERMINATION OF CMV IgG

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

CMV IgG (IU/ml)	A 450
0	0.056
1.2	0.930
6	1.496
18	2.167

Note: The Standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.



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 Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The CMV IgG or Index IU/ml unit for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

- Negative: CMV IgG Index less than 0.90 is seronegative for IgG antibody to CMV. (<1.2 IU/ml).
- Equivocal: CMV IgG Index between 0.91-0.99 is equivocal. Sample should be retested.
- Positive: CMV IgG Index of 1.00 or greater, or IU value greater than 1.2 is seropositive. It indicates prior exposure to the CMV virus. (>1.2 IU/ml)

PERFORMANCE CHARACTERISTICS

1. Specificity and Sensitivity:

A total of 199 patient samples were used to evaluate specificity and sensitivity of the test. The CMV IgG ELISA test results were compared to a commercial ELISA kit results:

Reference CMV IgG ELISA					
		N	E	P	Total
CMV IgG ELISA	N	82 (D)	0	0(B)	82
	E	2	0	0	2
	P	2 (C)	5	108 (A)	115
	Total	86	5	108	199

Sensitivity = A / (A+B) = 108 / 108 = 100.0%
Specificity = D / (C+D) = 82 / 84 = 97.6%
Accuracy = (A+D) / (A+B+C+D) = 190 / 192 = 99.0%

2. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Samples	1	2	3	4
# Reps.	24	24	24	24
Mean (IU/ml)	16.5	6.4	1.1	0.26
S.D. (IU/ml)	0.8	0.5	0	0.01
C.V. (%)	5.1	8.0	2.4	5.1

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of four different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Samples	1	2	3	4
# Reps.	20	20	20	20
Mean (IU/ml)	16.4	7.0	1.2	0.03
S.D. (IU/ml)	1.2	0.7	0.1	0.025
C.V. (%)	7.6	9.7	5.2	9.9

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. P. 771-719, 1985.

ASSAY DIAGRAM