CYTOMEGALOVIRUS (CMV) IgM ENZYME IMMUNOASSAY TEST KIT
Catalog Number: BC-1091

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ENZYME IMMUNOASSAY FOR THE DETECTION OF IgM ANTIBODIES TO CYTOMEGALOVIRUS (CMV) IN HUMAN SERUM

FOR INVESTIGATIONAL USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

CMV IgM Enzyme Immunoassay

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40

5 µl / 200 µl

2. Three incubations at 37°C

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

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

INTENDED USE

The CMV IgM ELISA is intended for use in the detection of IgM antibodies to Cytomegalovirus (CMV) infection in human serum.

INTRODUCTION

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. The CMV IgM ELISA is an accurate serologic method to detect CMV IgM 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 IgM 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 IgM specific-antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

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 (blue color): 1 bottle (22 ml)
- Negative Control: Range stated on label. Natural cap. (150 µL/vial).
- Cut-off Calibrator: Yellow cap. *CMV IgM* Index = 1 (150 µL/vial)
- Positive Control: Range stated on label. Red cap. (150 µL/vial)
- Wash Buffer Concentrate (20x): 1 bottle (50 ml)
- TMB Reagent (One-Step): 1 vial (11 ml)
- Stop Solution (1N HCl): Natural cap. 1 vial (11 ml)

STORAGE OF TEST KITS AND INSTRUMENTATION

1. Store the kit at 2-8°C.
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.

WARNING AND PRECAUTIONS

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.

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 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Place the desired number of coated wells into the holder.
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. 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.
4. Incubate at 37°C for 30 minutes.
5. At the end of the incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
6. Dispense 100 µl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
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.

11. Read O.D. at 450 nm ***within 15 minutes*** with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate calibrator value x_c.
2. Calculate the mean of duplicate positive control (x_p), negative control (x_n) and patient samples (x_s).
3. Calculate the CMV IgM Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, x_c.

Example of typical results: Note: The 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 IgM Index = 1.0

1. Cut-off Calibrator O.D. = 0.856, 0.830 $x_c = 0.843$
2. Negative Control O.D. = 0.072,0.071 $x_n = 0.072$
   CMV IgM Index = $x_n / x_c = 0.072 / 0.843 = 0.09$
3. Positive Control O.D. = 1.592, 1.641 $x_p = 1.617$
   CMV IgM Index = $x_p / x_c = 1.617 / 0.843 = 1.92$
4. Patient sample O.D. = 1.465, 1.411 $x_s = 1.438$
   CMV IgM Index = $x_s / x_c = 1.438 / 0.843 = 1.71$

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 IgM Index for Negative and Positive Control should be in the range stated on the Certificate of Analysis.

INTERPRETATION

Negative:	CMV IgM Index less than 0.90 is negative for IgM antibody to CMV.
Equivocal:	CMV IgM Index between 0.91-0.99 is equivocal. Sample should be retested.
Positive:	CMV IgM Index of 1.0 or greater is positive for IgM antibody to CMV.

PERFORMANCE CHARACTERISTICS

I. Specificity and Sensitivity:

A total of 329 patient samples were used to evaluate specificity and sensitivity of the test. The CMV IgM ELISA test results were compared to a commercial ELISA kit results:

		Reference CMV IgM ELISA			
		N	E	P	Total
CMV IgM ELISA	N	159(D)	10	13(B)	182
	E	6	1	7	14
	P	11(C)	2	120(A)	138
	Total	133	13	140	329

Sensitivity = A / (A+B) = 120 / 133 = 90.2%

Specificity = D / (C+D) = 159 / 170 = 93.5%

Accuracy = (A+D) / (A+B+C+D) = 279 / 303 = 92.1%

II. 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:

Sample	1	2	3	4
# Reps.	24	24	24	24
Mean A_{450}	1.998	1.045	0.550	0.157
S.D. (A_{450})	0.053	0.044	0.024	0.010
C.V. (%)	2.7	4.2	4.4	6.4

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:

Sample	1	2	3	4
# Reps.	20	20	20	20
Mean A_{450}	2.098	1.107	0.570	0.185
S.D. (A_{450})	0.083	0.080	0.043	0.025
C.V. (%)	3.9	7.2	7.6	13.5

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.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES

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