Chemiluminescence ELISA

CMV IgM

Catalog No. CL10238 (96 tests)

SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:40 = 5 µL / 200 µL		
2	Diluted samples, controls & calibrators	100 µL	30 minutes
3	Washing buffer (3 times)	350 µL	
4	Enzyme conjugate	100 µL	30 minutes
5	Washing buffer (3 times)	350 µL	
6	Substrate A and Substrate B mixture	100 µL	5 minutes
7	Read with Luminometer in 5~30 minutes		

NAME AND INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

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 IgM Chemiluminescence 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 wells, and the CMV IgM 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 substrate A & substrate B mixture is added. The light generated (RLU) is proportional to the amount of IgM specific antibody in the sample. The results are read by a microwell luminometer compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1.Microwell Strips: purified CMV antigen coated wells (12 x 8 wells)

2.Absorbent Solution: Yellow Color Solution. 1 vial (22 ml)

3.Calibrator: Factor value (f) stated on label. Red Cap. 1 vial (150µL)

4.Negative Control: Range stated on label. Natural Cap. 1 vial (150 µL)

5. Positive Control: Range state on label. Brown Cap. 1 vial (150 uL)

6.Washing Concentrate 20X. 1 bottle (50 ml)

7.Enzyme Conjugate: Red color solution.1 vial (12 ml)

8.Substrate A: H_2O in buffer$_2$. Natural bottle.1 vial (6 ml)

9.Substrate B: Luminol in buffer. Amber bottle.1 vial (6 ml)

STORAGE AND STABILITY

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.

WARNINGS 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 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.

SPECIMEN COLLECTION AND HANDLING

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.

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.Prepare 1:40 dilutions by adding 5 µL of the samples, negative control, positive control, and calibrators to 200 µL of absorbent solution. Mix well.

2.Place the desired number of coated strips into the holder.

3.Dispense 100 µL of diluted sera, calibrators, and controls into the appropriate wells. 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 and 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.Mix equal volume of Substrate A & Substrate B, then dispense 100 µL of this mixture to each well.

8.Read RLU with a microwell luminometer within 5~30 minutes.

CALCULATION OF RESULTS

Determination of Index values

1. To obtain Cut off value: Multiply the RLU of Calibrator by Factor (f) printed on label of Calibrator.

2. Calculate the IgM Index of each determination by dividing the RLU values of each sample with obtained RLU value of Cut off.

NOTE: This factor (f) is a variable. It is specific for a lot manufactured and printed on label of Calibrator.

For example:

If Factor (f) value on label = 0.40
900110 x 0.40 = 360044

Sample	RLU	Mean RLU (A)	Calculated Cut off Value(B)	INDEX A/B	Interpretation
Calibrator f = 0.40	897142 903078	900110	360044		
Positive Control	1449370 1450960	1450165		4.02	Positive
Negative Control	6556 6978	6767		0.02	Negative
Patient Sample 1	1712547 1800691	1756619		4.88	Positive
Patient Sample 2	128620 118540	123580		0.34	Negative

QUALITY CONTROL

1. In order for the assay results to be considered valid the controls should be within the ranges indicated on the labels.

2. The RLU values vary with the different luminometer used.

3. Each laboratory should assay controls at levels in low, normal and elevated ranges for monitoring assay performance. Quality control trends should be maintained to monitor batch to batch consistency.

INTERPRETATION

CMV IgM Index	Interpretation
< 0.90	Negative for IgM to CMV
0.91 ~ 0.99	Equivocal, sample should be retested
1 ~ 2	Low positive
2 ~ 3	Moderate positive
> 3	High positive

PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity:
A total of 96 patient samples were used to evaluate specificity and sensitivity of the test. CMV IgM test results were compared to a commercial ELISA kit results:

		Reference ELISA			
		N	E	P	Total
CMV IgM	N	84 (D)	0	0 (B)	84
Chemiluminescence	E	0	0	0	0
ELISA	P	0(C)	0	12(A)	12
	Total	84	0	12	96

Sensitivity = A / (A+B) = 12 / 12 = 100%

Specificity = D / (C+D) = 84 / 84 = 100%

Accuracy = (A+D) / (A+B+C+D) = 96 / 96 = 100%

Expected Values:
49 random samples were determined with CMV IgM Chemiluminescence ELISA. The test results were computed as IgM Index using a chosen reference serum (cut off) as IgM index 1. None was found to be positive (0%), and 49 were found to be negative (100%). Others reported 1% positivity from 176 sera. A third set of 48 samples was found positivity 6.25%.

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	10.2%	7.9%	6.9%
Inter-assay	9.5%	8.2%	7.2%

LIMITATIONS OF THE TEST

1. To prevent false negative and false positive IgM test results caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit has been formulated to resolve these interferences. However, specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.

2. As with other serological tests, the results obtained with the CMV IgM ELISA serve only as an aid to diagnosis and should be interpreted in relation to other clinical and diagnostic findings.

3. IgM responses may vary in different individuals. It has been reported that 10-30 % of infants may fail to develop IgM antibody responses despite congenital CMV infection. Furthermore, up to 27 % of adults with primary CMV infection may not demonstrate an IgM response. Thus, the absence of CMV-specific IgM does not necessarily exclude the possibility of CMV infection.

4. The presence or absence of CMV IgG or IgM in pregnant women is of limited value in predicting congenital CMV infection. However, the presence of specific IgM in the circulation of the newborn is indicative of infection. Since serum samples obtained too early in infection may not contain detectable IgM antibody, a subsequent sample should be obtained 7 to 14 days later and test. In the case of cord blood, care should be taken to avoid contamination by maternal blood, and it is prudent to confirm positive IgM antibody results by testing a follow-up specimen from the newborn.

REFERENCES

1. Voler, A., J.E. Bidwell, et al. Manual of clinical immunology. Chapter 69. Rose, N. and Friedman, H. eds. Am. Soc. Microbiol. p.506, 1985.

2. Cremer, N.E. Antibodies in serodiagnosis of viral infection. p. 73. In Lennett E.H. ed. Laboratory diagnosis of viral infection. Mercel Dekker, Inc., New York, 1985.

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.

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