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IVD	See external label	2°C-8°C	96 tests	REF 9063-11

CHEMILUMINESCENCE

CMV IgG

REF 9063-11

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, calibrators & controls	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 IgG Chemiluminescence ELISA is intended for use in evaluating a patient's serologic status to cytomegalovirus (CMV) infection. For investigational use only.

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 IgG Chemiluminescence ELISA is an accurate serologic method to detect CMV 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 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 substrate A & substrate B mixture is added. The light generated (RLU) is proportional to the amount of IgG 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. Sample diluent: Blue color solution. 1 vial (22 ml)
3. Washing concentrate 10x: 1 bottle (100 ml)
4. Enzyme conjugate: Red color solution. 1 vial (12 ml)
5. Substrate A: H_2O_2 in buffer. Natural bottle. 1 vial (6 ml)
6. Substrate B: Luminol in buffer. Amber bottle. 1 vial (6 ml)
7. Negative Calibrator: 0 IU/ml. Natural Cap. 1 vial (150 µl)
8. Cut-off Calibrator: 1.2 IU/ml. Yellow Cap. 1 vial (150 µl)
   CMV G Index = 1.0
9. Positive Calibrator: 6 IU/ml. Red Cap. 1 vial (150 µl)
10. Positive Calibrator: 18 IU/ml. Green Cap. 1 vial (150 µl)
11. Negative control: Range on label. Blue Cap. 1 vial (150 µl)
12. Positive control: Range on label. Brown Cap. 1 vial (150 µl)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 ^{o}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 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.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8 ^{O}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 10x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25 ^{O}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 sample diluent. 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. Calculate the mean of duplicate RLU values (B).
2. Calculate the CMV G Index of each determination by dividing the mean values of each sample (B) by Cut-off calibrator mean value (C).

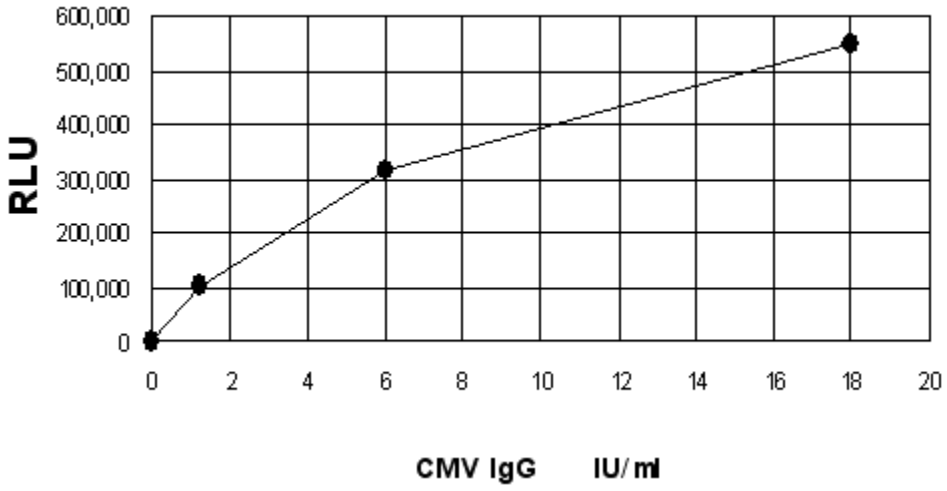
Example 1:

Sample	Well No	RLU (A)	Mean RLU (B)	INDEX B/C
Cut-off Calibrator	A1 B1	96221 102135	99178 (C)	1
Positive Calibrator	C1 D1	317570 320595	319083	3.2
Negative Control	E1 F1	29923 30895	30409	0.31
Positive Control	G1 H1	547029 560359	553694	5.58
Patient Sample	A2 B2	186167 195350	190758	1.9

Quantitative determination of CMV IgG IU/ml value

For a quantitative determination of anti-CMV IgG levels of specimens in IU/ml unit, RLU of calibrators are plotted on Y-axis in graph versus their corresponding anti-CMV IgG concentration 0, 1.2, 6, and 18 IU/ml on X-axis. The estimates of levels in patient sera are read off the point to point curve using their individual RLU values.

Example 2:



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

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)

PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity

A total of 86 patient samples were used to evaluate specificity and sensitivity of the test. CMV IgG test results were compared to a commercial ELISA kit results.

		Reference ELISA			
		N	**E**	**P**	**Total**
CMV IgG Chemiluminescence ELISA	N	45 (D)	0	1 (B)	46
	E	0	1	0	1
	P	1 (C)	0	38 (A)	39
	Total	46	1	39	86

Sensitivity = A / (A+B) = 38 / 39 = 97%

Specificity = D / (C+D) = 45 / 46 = 98%

Accuracy = (A+D) / (A+B+C+D)

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

Expected value and prevalence
48 random samples were determined with CMV IgG CLIA ELISA. 42 samples were found to be positive (88%) and 6 were found to be negative (12%). Another set of 49 random samples, the positivity were found to be 41%. Prevalence may vary depending on a variety of the factors such as geographical location, age, socioeconomic status, race, type of the test employed, specimen collection and handling procedures, clinical and epidemiological history.

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%

Cross-reactivity:

A study was performed to determine the cross-reactivity of CMV IgG CLIA test with positive IgG samples. The results indicated an absence of cross-reactivity of the test: H. pylori, Rubella, Toxo, HSV 1, HSV 2, Chlamydia trachomatis and ANA.

LIMITATIONS OF THE 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.

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.

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