Wampole Laboratories®

CYTOMEGALOVIRUS IgM ELISA II

REF. 425250CE

INTENDED USE

The Wampole Laboratories CMV IgM ELISA test system is an enzyme-linked immunosorbent assay (ELISA) designed for the qualitative detection of IgM class antibodies to cytomegalovirus (CMV) in human serum. The test system is intended to be used to evaluate serologic evidence of primary or reactivated infection with CMV, and is for *in vitro* diagnostic use. This product is not FDA cleared (approved) for use in testing (*i.e.*, screening) blood or plasma donors.

SIGNIFICANCE AND BACKGROUND

Cytomegalovirus (CMV) infections are widespread and usually asymptomatic; however, the virus may persist as a latent or chronic infection (1). The relatively frequent incidence and often severe disease in newborns and immunosuppressed individuals clearly establishes this agent as an important human pathogen (2-4). CMV infections can be classified as follows:

Congenital	-	Acquired before birth.
Perinatal	-	Acquired at birth
Postnatal	-	Acquired after birth

The prognosis for congenitally infected infants who are asymptomatic at birth must be guarded. Ten to 25% may subsequently develop hearing loss (7). Five to 10% may exhibit various degrees of mental retardation and central nervous system motor disorders (5). Surveys show the incidence of congenital CMV infection to be from 0.5 to 2.5%. Consequently, a careful documentation of the long term effects of intrauterine infection is important (8).

Perinatally infected infants start excreting CMV 3 to 12 weeks after delivery and with rare exception, remain asymptomatic (9). Postnatal CMV infections are acquired through close contact with individuals who are shedding the virus (2). CMV has been isolated from saliva, urine, breast milk, cervical secretions, and semen. Consequently, the transmission of the virus may occur through a variety of mechanisms (6-8). Sexual transmission of the virus appears to contribute to the acquisition of the virus by young adults (10).

Although the age at which CMV infection is acquired varies with socioeconomic conditions, only about 10-15% of children in the United States are seropositive. By age 35 however, about 50% of the population is seropositive (2-4).

The majority of individuals contracting postnatal CMV infections remain asymptomatic (2-4). A small percentage of individuals will develop a negative heterophile-antibody infectious mononucleosis syndrome. CMV mononucleosis is characterized by fever, lethargy, and atypical lymphocytosis; whereas, in Epstein-Barr virus induced infectious mononucleosis, pharyngitis, lymphadenopathy, and splenomegaly are the chief clinical features (11-12).

In immunocompromised patients, CMV infections happen frequently, often from reactivation of latent infection, and may be life-threatening (2-4). These patients include allograft recipients, cancer patients, and patients with acquired immunodeficiency syndrome (AIDS) (4,13,15). Clinical manifestations of CMV disease in immunocompromised patients ranges from CMV mononucleosis to pneumonia, hepatitis, pericarditis, and encephalitis (4).

CMV infections may occur following blood transfusions, and the risk of infection increases with the number of donors and the volume of blood given (4). Primary infection in seronegative recipients may be contracted via blood from a seropositive donor. In seropositive recipients, a latent infection may become reactivated. Most transfusion acquired CMV infections are either subclinical or characterized by CMV mononucleosis (2-4). However, in specific groups of patients, considerable morbidity and mortality can result from a transfusion-acquired primary CMV infection. These patients are immune-compromised and include premature infants, pregnant women, cancer patients, and transplant recipients (4-14). In these patients, transfusion acquired CMV infections can be prevented by giving only blood from seronegative donors to seronegative recipients (4-14).

Serologic procedures which measure IgG antibodies to CMV can aid in the diagnosis of CMV infection when paired acute and convalescent sera are tested simultaneously and seroconversion or a significant rise in titer can be demonstrated (15). Also, serologic procedures may aid in the prevention of transfusion acquired CMV infections by assessing the serologic status of donors and recipients (4-14).

Antibody of the IgM class is produced during the first 2 to 3 weeks of infection with CMV and exists only transiently in most patients (16,17). Serologic procedures which measure the presence of IgM antibodies help discriminate between primary and recurrent infections since IgM antibodies are rarely found in recurrent infections (16).

High affinity IgG antibodies to CMV, if present in a sample, may interfere with the detection of IgM specific antibody (18,23). High affinity IgG antibody may preferentially bind to CMV antigen leading to false negative IgM results (18). Also, rheumatoid factor, if present along with antigen specific IgG, may bind to the IgG causing false positive IgM results (19). Both of the above problems can be eliminated by removing IgG from the sample before testing for IgM (20-23). Several different methods of separating IgG have been used. These include gel filtration (20), absorption with protein A (21), ion exchange chromatography (22), and precipitation of IgG with anti-human IgG serum (23).

PRINCIPLE OF THE ELISA ASSAY

The Wampole CMV IgM ELISA test system is designed to detect IgM class antibodies to CMV IgM in human sera. Wells of plastic microwell strips are sensitized by passive absorption with CMV IgM antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided. The Sample Diluent contains antihuman IgG that precipitates and removes IgG and rheumatoid factor from the sample leaving IgM free to react with the immobilized antigen. During sample incubation any antigen specific IgM antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgM (μ chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgM antibody immobilized on the solid phase in step 1. The wells are washed to remove unbound Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

MATERIALS PROVIDED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v).

PLATE	1. Plate. 96 wells configured in twelve 1x8-well strips coated with affinity purified 125kD capsid peptide purified from induced P3-HR1 cells. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgM (μ chain specific). Ready to use. One, 15 mL vial with a white cap.
CONTROL +	3. Positive Control (Human Serum). One, 0.35 mL vial with a red cap.
CAL	4. Calibrator (Human Serum). One, 0.5 mL vial with a blue cap.
CONTROL -	5. Negative Control (Human Serum). One, 0.35 mL vial with a green cap.
DILSPE	6. Sample Diluent. One 30 mL bottle (blue cap) containing Tween-20, bovine serum albumin, phosphate- buffered-saline, and goat anti-human IgG (γ-chain specific), (pH 7.2 ± 0.2). Purple solution, ready to use. Note: Shake Well Before Use. (Product #: 4500DM).
SOLN TMB	7. TMB: One 15 mL amber bottle (amber cap) containing 3,3',5,5' -tetramethylbenzidine(TMB). Ready to use. Contains DMSO < 15% (w).
SOLN STOP	8. Stop solution: One 15 mL bottle (red cap) containing 1M H_2SO_4. 0.7M HCl. Ready to use.
WASHBUF 10X	9. Wash buffer concentrate (10X): dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum controls are **POTENTIALLY BIOHAZARDOUS MATERIALS**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg. and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these

products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (36).

5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20-25°C) before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The sample diluent, controls, wash buffer, and conjugate contain sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

MATERIALS REQUIRED BUT NOT PROVIDED:

- ELISA microwell reader capable of reading at a wavelength of 450nm.
- Pipettes capable of accurately delivering 10 to 200µL.
- Multichannel pipette capable of accurately delivering (50-200µL)
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

STORAGE CONDITIONS

1. Store the unopened kit between 2° and 8°C.
2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
5. TMB: Store between 2° and 8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25° C) for up to 7 days or for 30 days between 2° and 8°C.
7. Sample Diluent: Store between 2° and 8°C.
8. Stop Solution: Store between 2° and 25°C.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (31, 32). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

   A. Manual Wash Procedure:

   a. Vigorously shake out the liquid from the wells.
   b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps a. and b. for a total of 5 washes.
   d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

   B. Automated Wash Procedure:

   If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

QUALITY CONTROL

1. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

	OD Range
Negative Control	≤ 0.250
Calibrator	≥ 0.300
Positive Control	≥ 0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.

4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

A. Calculations:

1. *Correction Factor*

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

2. *Cutoff OD Value*

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.

(CF x mean OD of Calibrator = cutoff OD value)

3. *Index Values or OD Ratios*

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example:

Mean OD of Calibrator	=	0.793
Correction Factor (CF)	=	0.25
Cut off OD	=	0.793 x 0.25 = 0.198
Unknown Specimen OD	=	0.432
Specimen Index Value or OD Ratio	=	0.432 / 0.198 = 2.18

B. Interpretations:

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	≤ 0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥ 1.10

1. An OD ratio ≤ 0.90 indicates no detectable IgM antibody to CMV. A negative result indicates no current infection with CMV. However, specimens taken too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, another specimen should be taken within 7 days and tested concurrently in the same assay with the original specimen to look for seroconversion.
2. An OD ratio ≥ 1.10 is positive for IgM antibody to CMV. A positive value indicates a primary or reactivated infection with CMV. Such individuals are presumed to be at risk of transmitting CMV infection.
3. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested and/or another specimen should be collected within 7 days and retested simultaneously with the original specimen. If the second specimen is positive, the patient is considered to have an active CMV infection.

LIMITATION OF THE ASSAY

1. A negative result does not rule out a primary or reactivated infection with CMV.
2. Since CMV specific IgM antibody usually does not develop until the patient has been clinically ill for a week or more, samples taken too early in the course of a primary infection may not have detectable levels of IgM (33).
3. In immunocompromised patients the ability to produce an IgM response may be impaired and CMV specific IgM may be falsely negative during an active infection (15, 34).
4. CMV specific IgM antibody may reappear during reactivation of CMV infection (15,17,33).
5. Results of the Wampole CMV IgM ELISA are not by themselves diagnostic and should be interpreted in light of the patient's clinical condition and the results of other diagnostic procedures.
6. Patients may continue to produce CMV specific IgM antibody for 6-9 months following a primary infection (15,27,33).
7. Isolation of CMV from urine or the presence of CMV IgM antibody during the first week of life usually provides a reliable diagnosis of congenital CMV infection (35). Specimens collected for viral isolation or for detection of CMV IgM beyond the first week after birth should not be used to distinguish congenital infection from infection acquired at or shortly after birth (35).
8. CMV specific IgG antibody may compete with IgM for binding sites and cause false negative results. Rheumatoid factor, if present along with CMV specific IgG, will cause false positive results. The absorbent incubation step will remove greater than 99% of IgG from the test specimens, and significantly reduce the incidence of false results.
9. Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr virus and give false positive results in the CMV-IgM ELISA.

EXPECTED VALUES

The incidence of CMV infection varies with age, geographic location, sexual behavior, and socioeconomic status (33). However, CMV is the most common cause of congenital viral infection (16,33). In the United States, approximately 1% of infants are infected at birth. (16,33)

CMV specific IgM usually develops after a patient has been clinically ill for at least a week or more (33). Most (83%) of patients produce IgM transiently within 16 weeks of seroconversion (16). However, some patients may continue to produce IgM for 6 to 9 months after seroconversion (15-17).

PERFORMANCE CHARACTERISTICS

Comparative Study:

The Wampole Laboratories CMV-IgM ELISA test system was compared to another commercially available ELISA test system for detection of IgM antibodies to CMV. A total of 101 serum samples, obtained from a reference laboratory, were assayed by the two methods. The results of this study are summarized below:

		REFERENCE ELISA		
WAMPOLE CMV IgM ELISA		POS.	NEG.	EQUIVOCAL*
	POS.	25	1	2
	NEG.	2	66	2
	EQUIVOCAL*	3	0	0
	Specificity = 98.5% (66/67) Sensitivity = 92.5% (25/27) Concordance = 96.8% (91/94) *Equivocal results were not included in the calculations for sensitivity, specificity, and concordance			

Test results of the two procedures for three specimens did not agree. These specimens were tested by a third commercial ELISA procedure for detection of IgM antibodies to CMV. Results of the third procedure for all three specimens were in agreement with the Wampole Laboratories test system.

Reproducibility:

To assess intra- and inter-assay variations of the test procedure, the Wampole CMV IgM ELISA was performed on six specimens with OD ratio values in the high positive, low positive, and negative ranges. Eight replicates of each sample were run on three consecutive days. The mean OD ratio and coefficient of variation (CV) were calculated for each sample. These data are shown below:

	Intra-Assay (n=8)						Inter-Assay (n=3)	
	Run #1		Run #2		Run #3			
	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV
Serum No. 1	6.48	3.7%	7.68	3.1%	5.92%	4.7%	6.66	11.6%
Serum No. 2	7.27	4.3%	8.92	1.7%	6.88%	4.4%	7.69	11.5%
Serum No. 3	2.73	6.0%	3.19	7.7%	2.69%	4.0%	2.87	7.9%
Serum No. 4	1.30	3.2%	1.54	7.3%	1.20%	5.7%	1.35	10.8%
Serum No. 5	0.55	7.2%	0.66	12.2%	0.55%	8.3%	0.59	9.1%
Serum No. 6	0.67	7.1%	0.77	5.6%	0.56%	5.6%	0.67	13.1%

Cross Reactivity:

Studies were done to assess the possible interference with the test procedure by sera containing rheumatoid factor or antinuclear antibodies. Ten rheumatoid factor positive sera with latex agglutination titers from 1:80 to 1:640 were tested by the Wampole Laboratories CMV IgM ELISA procedure. After pretreatment with absorbent, all ten sera were negative in the Wampole CMV IgM ELISA procedure. Ten ANA positive sera with IFA titers of 1:80 to 1:1280 were tested by the Wampole CMV IgM procedure and 9 of 10 were negative. One serum with an ANA titer of 1:1280 was weakly positive but was also positive in another ELISA procedure. These studies indicate that interference with the test procedure by rheumatoid factor and antinuclear antibodies is minimal.

Sera with IgM IFA titers to Herpes Simplex virus (1:8 - 1:640), and Varicella-Zoster virus (1:10 - 1:80) were tested for cross-reactivity with the CMV IgM ELISA test system. None of five HSV IgM positive sera, and only one of nine VZ IgM positive sera were positive in the CMV IgM ELISA test system.

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ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100 µL/well
3. ⟶ *Incubate 20 to 30 minutes*
4. Wash
5. Add Conjugate – 100 µL/well
6. ⟶ *Incubate 20 to 30 minutes*
7. Wash
8. Add TMB 100 µL/well
9. ⟶ *Incubate 10 to 15 minutes*
10. Add Stop Solution 50 µL/well - Mix
11. READ

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