Wampole Laboratories®

CYTOMEGALOVIRUS IgG ELISA II

REF. 425200CE

INTENDED USE

The Wampole Laboratories Cytomegalovirus (CMV) IgG ELISA test system is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG class antibodies to cytomegalovirus (CMV) in human serum. The test system is intended to be used to evaluate serologic evidence of previous or primary infection with CMV. 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

Of the newborn infants congenitally infected with CMV, 95% exhibit no clinically overt disease at birth (5). Of the remaining 5% of infected infants, clinical manifestations range from severe disease with jaundice, hepatosplenomegaly, thrombocytopenic purpura, cranial calcification and growth retardation to pneumonitis, hydrocephaly or microcephaly and ocular defects (6). Infants with severe manifestations of congenital CMV infection may expire early after birth due to secondary complications; however, most survive with consequent neurological damage (?)

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 immunocompromised 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).

The ELISA procedure was first described by Engvall and Perlman (19,20). Since then, ELISA test systems have been developed for detection of a wide variety of different antigens and antibodies, including antibodies to CMV (16, 17, 21). Besides ELISA, a number of other serologic procedures have been developed to detect antibodies to CMV. These include complement fixation (16,18), indirect immunofluorescence (18), indirect hemagglutination (18,22), and latex agglutination (22). When compared to other serologic tests for detection of antibodies to CMV, ELISA may be a very specific, sensitive and reliable method for detection of antibodies to CMV (16,17,18). The ELISA procedure allows an objective determination of antibody status to be made on a single dilution of the test specimen and is suitable for screening large numbers of patient samples.

PRINCIPLE OF THE ELISA ASSAY

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

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific 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 IgG (γ chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted 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. All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v)

PLATE	Plate. 96 wells configured in twelve 1x8-well strips coated with inactivated cytomegalovirus antigen (strain AD169.) The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (γ chain specific). Ready to use. One, 15 mL vial with a white cap.
CONTROL +	Positive Control (Human Serum). One, 0.35 mL vial with a red cap.
CAL	Calibrator (Human Serum). One, 0.5 mL vial with a blue cap.
CONTROL -	Negative Control (Human Serum). One, 0.35 mL vial with a green cap.
DILSPE	SAVe Diluent™ (Sample Diluent). One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. Note: Shake Well Before Use. (Product #: 4500CC). (NOTE: This reagent may be used with any Wampole ELISA test system utilizing Product #: 4500CC). NOTE: The SAVe Diluent™ will change color in the presence of serum.
SOLN TMB	TMB: One 15 mL amber bottle (amber cap) containing 3,3',5,5' -tetramethylbenzidine(TMB). Ready to use. Contains DMSO < 15% (w).
SOLN STOP	Stop solution: One 15 mL bottle (red cap) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF 10X	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 (26).

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 SAVe Diluent™, controls, and conjugate contain sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead or cooper 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. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used.
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. SAVe 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 (23, 24). No anticoaguiants 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 returned 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 SAVe Diluent™. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. The SAVe Diluent™ will undergo a color change confirming that the specimen has been combined with the diluent.
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 SAVe 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 antibody to CMV. A negative result indicates no current or previous infection with CMV. Such individuals are presumed to be susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgG antibody. If a primary infection is suspected, another specimen should be taken in 8-14 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 IgG antibody to CMV. A positive value indicates a current or previous infection with CMV. Such individuals are presumed to be at risk of transmitting CMV infection but are not necessarily currently contagious.
3. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested. Specimens that remain equivocal after repeat testing should be tested by an alternate serologic procedure such as the Wampole Laboratories indirect fluorescent antibody (IFA) test procedure.
4. To evaluate paired (acute and convalescent) sera, both samples must be tested in the same assay. If the acute specimen is negative and the convalescent specimen is positive, seroconversion has taken place and a primary CMV infection is indicated.

LIMITATIONS

1. The presence of IgG antibodies to CMV does not necessarily assure protection from future infection with CMV.
2. The antibody titer of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.
3. Test results for demonstration of seroconversion should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
4. Specimens containing antibodies to nuclear antigens (as are found in patients with systemic lupus erythematosus) may give false positive results in the Wampole CMV ELISA test.
5. Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, a second sample may be collected after 2-7 weeks and tested concurrently with the original specimen to look for seroconversion.
6. A positive CMV IgG test in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months (25). A negative test for IgG antibody in the neonate may help exclude congenital infection (15). The most definitive diagnosis of active CMV infection requires viral isolation.
7. The results of this test are qualitative and should be considered as either positive or negative for the presence of CMV IgG antibodies.

EXPECTED VALUES

The incidence of CMV infection varies with age, geographic location, and socioeconomic status (2). In the United States, 10 to 30% of children are seropositive for CMV by the age of 10 years (2). By age 35, about 50% of the population is seropositive. The seropositive rate among homosexual men has been reported to be greater than 90% (15,17).

PERFORMANCE CHARACTERISTICS

A. Comparative Study:

The Wampole CMV IgG ELISA test system was compared to a commercially available ELISA procedure. A total of 96 specimens from normal blood donors in the Northeastern United States were assayed by the two methods. These results are summarized below:

		Reference CMV IgG ELISA		
		Pos.	Neg.	Equivocal
Wampole CMV IgG ELISA	Pos.	54	2	2
	Neg.	2	31	2
	Equivocal	1	2	0

When compared to the other CMV IgG ELISA procedure, the Wampole CMV IgG ELISA showed a sensitivity of 96.4% (54/56), a specificity of 93.9% (31/33), and an overall agreement of 95.3% (85/89).

B. Reproducibility

To assess intra-and inter-assay variation of the test procedure, the Wampole CMV IgG ELISA was performed on four specimens with OD ratio values in the high positive, mid 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 #2			
	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV
Serum #1	4.62	8.9%	3.6	6.8%	6.3	6.3%	4.07	10.0%
Serum #2	2.53	4.8%	2.46	7.0%	11.8	11.8%	2.37	7.4%
Serum #3	1.13	10.8%	1.33	14.7%	6.6	6.6%	1.21	7.1%
Serum #4	0.23	10.1%	0.35	16.1%	14.1	14.1%	0.27	18.9%

CROSS REACTIVITY

Ten serum samples that were negative in the Wampole CMV IgG ELISA were tested by the indirect fluorescent antibody assay for the presence of IgG antibodies specific for Varicella-Zoster (VZ), Epstein-Barr Virus Viral Capsid Antigen (EBV-VCA) and Herpes Simplex Virus type 1 (HSV-1). All ten of the samples tested positive for EBV-VCA and HSV-1 IgG and 2 of the 10 samples were positive for VZ IgG. These results indicate that the Wampole CMV ELISA test system does not cross-react with antibodies to other herpes viruses.

REFERENCES

1. Jordon MC:Latent infection and the elusive cytomegalovirus. Rev. Infect. Dis. 5:205-215, 1983.
2. Starr SE:Cytomegalovirus. Ped. Clin. N. Am. 26:282-293, 1979.
3. Weller TH:Clinical spectrum of cytomegalovirus infection. In: Nahmias AJ, Dowdle WR, and Schinazi RF, eds. The Human Herpes Viruses, an interdisciplinary perspective. Elsevier/North Holland Publishing Co., New York, pp. 20-30, 1980.
4. Adler SP:Transfusion-associated cytomegalovirus infections. Rev. Infect. Dis. 5:977-993, 1983.
5. Melish ME and Hanshaw JB:Congenital cytomegalovirus infection: Development progress of infants detected by routine screening. Am. J. Dis. Child. 126:190-194, 1973.
6. Reynolds DW, Stagno S, and Alford CA:Laboratory diagnosis of cytomegalovirus infections. In: Lennette EH and Schmidt NJ, eds. Diagnostic Procedures for Viral, rickettsial, and Chlamydial Infections, 5th ed., American Public Health Association, Washington, DC, 1979.
7. Nankervis G:Long term follow-up of cytomegalic inclusion disease of infancy, Pediatrics 46:403-410, 1970.
8. Stagno S, Reynolds DW, Huang ES, Thames SD, Smith RJ, and Alford CA: Congenital cytomegalovirus infections: Occurrence in an immune population. N. Engl. J. Med. 296:1254-1258, 1978.
9. Stern H:Isolation of cytomegalovirus and clinical manifestations of infection at different ages. Br. Med. J. 1:665-669, 1968.
10. Handsfield HH, Chandler SH, Caine VA, Meyers JD, Corey L, Medeiros E, and McDougall JK:Cytomegalovirus infection in sex partners: Evidence for sexual transmission. J. Infect. Dis. 151:344-348, 1985.
11. Jordon MC, Rousseau WE, Noble GR, Stewart JA, Noble CR, and Chin TDY: Spontaneous cytomegalovirus mononucleosis. Ann. Intern. Med. 79:153-160, 1973.
12. Umetsu M, Chiba Y, Horino K, Chiba S, and Kakao T:Cytomegalovirus-mononucleosis in a newborn infant. Arch. Dis. Child. 50:396-398, 1975.
13. Simmons RL, Matas AJ, Rattazzi LC, Balfour HH, Howard RJ, and Najarian JS: Clinical characterization of the lethal cytomegalovirus infection following renal transplantation surgery. Surgery 82:537-546, 1977.
14. Yeager AS, Grumet FC, Hafleigh EB, Arvin AM, Bradley JS, and Prober CG: Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. J. Pediatrics 98:281-287, 1981.
15. Drew WL:Diagnosis of cytomegalovirus infection. Rev. Infect. Dis. 10:5468-5475, 1988.
16. Booth JC, Hannington G, Bakir TMF. Stern H, Kangro H, Griffiths PD, and Heath RB:Comparison of enzyme-linked immunosorbent assay, radioimmunoassay, complement fixation, anticomplement immunofluorescence and passive hemagglutination techniques for detecting cytomegalovirus IgG antibody. J. Clin. Pathol. 35:1345-1348, 1982.
17. Dylewski JS, Rasmussen L, Mills J, and Merigan TC:Large scale serological screening for cytomegalovirus antibodies in homosexual males by enzyme-linked immunosorbent assay. J. Clin. Micro. 19:200-203, 1984.
18. Phipps PH, Gregoire L, Rossier E, and Perry E:Comparison of five methods of cytomegalovirus antibody screening of blood donors. J. Clin. Micro. 18:1296-1300, 1983.
19. Engvall E, and Perlman P: Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochem. 8:871-874, 1971.
20. Engvall E and Perlman P:Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen coated tubes. J. Immunol. 109:129-135, 1972.
21. Voller A, Bartlett A, and Bidwell DE:Enzyme immunoassays with special reference to ELISA techniques. J. Clin. Pathol. 31:507-520, 1978.
22. Adler SP, McVoy M, Biro VG, Britt WJ, Hider P, and Marshall D:Detection of cytomegalovirus antibody with latex agglutination. J. Clin. Micro. 22:68-70, 1985.
23. Procedures for the collection of diagnostic blood specimens by venipuncture - Second Edition; Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
24. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
25. Starr SE, and Friedman HM:Human cytomegalovirus. Manual of Clin. Microbio. 4th edition, ch. 65, pp. 711-719, 1985.
26. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.

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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Press 2 for Technical Service
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