Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ CMV IgM ELISA Kit

Cat# 1202-2

IVD See external Label 2°C 8°C Σ 96 Tests

Test	CMV IgM Elisa
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA : Antigen Coated Plate
Detection Range	Qualitative: Positive & Negative Control & Cut off
Sample	10µl Serum
Total Time	~ 60 min
Shelf Life	20 Months from the manufacturing date
Specificity	98.5%
Sensitivity	92.5%

INTENDED USE

The DAI Cytomegalovirus (CMV) IgM 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. This product is not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors. This test is intended for In Vitro diagnostic use only.

SUMMARY AND EXPLANATION

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). Scientists classify CMV infections as follows:

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

Health professionals must be guarded with the prognosis for congenitally infected infants who are asymptomatic at birth. Ten to 25% of infants may subsequently develop hearing loss (7). Five to 10% of infants 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 - 2.5%. Consequently, a careful documentation of the long-term effects of intrauterine infection is important (8).

Perinatally infected infants start excreting CMV three to 12 weeks after delivery, and with rare exception, remain asymptomatic (9). Acquisition of postnatal CMV infections can occur 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 acquisition age for CMV infection 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. Characteristics of CMV mononucleosis include 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, and 15). Clinical manifestations of CMV disease in immunocompromised patients range 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). Seronegative recipients may contract primary infection via blood from a seropositive donor. In seropositive recipients, reactivated latent infection may occur. 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 testing paired acute and convalescent sera simultaneously and seroconversion, or a significant rise in titer, can be demonstrated (15). In addition, serologic procedures may aid in the prevention of transfusion acquired CMV infections by assessing the serologic status of donors and recipients (4 - 14).

Production of the IgM class of antibodies begins during the first 2 to 3 weeks of infection with CMV and exists only transiently in most patients (16, 17). Serologic procedures that measure the presence of IgM antibodies help discriminate between primary and recurrent infections. Recurrent infections rarely create IgM antibodies (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). Remove IgG from the sample before testing for IgM to eliminate both of the above problems (20 - 23). Scientists have used several different methods of separating IgG. These include gel filtration (20), absorption with protein A (21), ion exchange chromatography (22), and precipitation of IgG with anti-human IgG serum (23).

TEST PRINCIPLE

The DAI 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 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.

SPECIMEN COLLECTION AND PREPARATION

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 could be used in this assay. 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. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (24).

MATERIALS AND COMPONENTS

Materials provided with the test kits

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): Controls, Calibrators and Sample Diluent.**

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.
2. **Conjugate:** Conjugated (horseradish peroxidase) goat anti-human IgM (µ chain specific). Ready to use. One, 15 mL vial with a white cap.
3. **Positive Control:** (Human Serum). One, 0.35 mL vial with a red cap.
4. **Calibrator:** (Human Serum). One, 0.5 mL vial with a blue cap.
5. **Negative Control:** (Human Serum). One, 0.35 mL vial with a green cap.
6. **Sample Diluent:** One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate- buffered-saline. Purple solution. Ready to use.
7. **TMB:** One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use.
8. **Stop solution:** One 15 mL bottle (red cap) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
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.**

NOTES:

The following components are not kit lot number dependent and may be used interchangeably with the DAI ELISA test system: TMB, Stop Solution, and Wash Buffer.
The kit also contains a Component Label containing lot specific information inside the Test System box.

Materials required but not provided

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

PRECAUTION

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. 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, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The 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, handle these products 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 (13).
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 Calibrator 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 upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the 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. Do not use reagents from other sources or manufacturers.

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. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a 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 Test System.

ASSAY 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.
3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) 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 5 times.

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.

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	

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. → *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. → *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. → *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

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 accordance

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 or previous 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 in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimen by using an alternate serologic procedure and/or re-evaluate by drawing another sample one to three weeks later.

PERFORMANCE CHARACTERISTICS

A. Comparative Study

A study was conducted to compare the Diagnostic Automation, Inc. ELISA CMV IgM Test System 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. Below is a summary of these results:

		Reference CMV IgM ELISA		
		Pos.	**Neg.**	**Equivocal**
Diagnostic Automation, Inc. CMV IgM ELISA	Pos.	25	1	2
	Neg.	2	66	2
	Equivocal	3	0	0

Test results of the two procedures, for three specimens, did not agree. A third commercial ELISA procedure for the detection of IgM antibodies retested the specimens and was in agreement with the DAI ELISA Test System.

B. Reproducibility

To assess the intra- and inter-assay variation of the test procedure, the 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:

Inra-assay (n=8)							Inter-assay(n=3)	
	Run # 1		**Run # 2**		**Run # 3**			
Serum #	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV
Serum # 1	6.48	3.7	7.68	3.1	5.82	4.7	6.66	11.6
Serum # 2	7.27	4.3	8.92	1.7	6.88	4.4	7.69	11.5
Serum # 3	2.73	6.0	3.19	7.7	2.69	4.0	2.87	7.9
Serum # 4	1.30	3.2	1.54	7.3	1.20	5.7	1.35	1.8
Serum # 5	0.55	7.2	0.66	12.2	0.55	8.3	0.59	9.1
Serum # 6	0.67	7.1	0.77	5.6	0.56	5.6	0.67	13.1

Cross Reactivity

Studies assessed the possible interference with the test procedure by sera containing rheumatoid factor or antinuclear antibodies. Ten rheumatoid factor positive sera specimens with latex agglutination titers from 1:80 to 1:640 by the Diagnostic Automation, Inc. ELISA CMV IgM procedure. After pretreatment with absorbent, all ten sera were negative in the Diagnostic Automation, Inc. ELISA CMV IgM procedure. Ten ANA positive sera with IFA titers of 1:80 to 1:1280 were tested by the Diagnostic Automation, Inc. ELISA CMV IgM procedure and nine of ten were negative. One serum with an ANA titer of 1:1280 was weakly positive but was also positive in another CMV IgM 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 ELISA CMV IgM Test System. None of five HSV IgM positive sera, and only one of nine VZ IgM positive sera were positive in the ELISA CMV IgM Test System.

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.

LIMITATIONS OF PROCEDURE

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.
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.
4. CMV specific IgM antibody may reappear during reactivation of CMV infection.
5. Results of the DAI 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.
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. 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.
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. However, CMV is the most common cause of congenital viral infection (16). In the United States, infection at birth affects approximately 1% of infants (16). CMV specific IgM usually develops after a patient has been clinically ill for at least a week or more. Most (83%) of patients produce IgM transiently within 16 weeks of seroconversion (16). However, some patients may continue to produce IgM for six to nine months after seroconversion (15 - 17).

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 .
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.

STORAGE

1. 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.

2. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
3. Unopened kit, Calibrator, Positive Control, Negative Control, TMB and Sample Diluent: Store between 2° and 8°C.
4. Wash Buffer (10X): Store between 2° and 25°C. 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.
5. Stop Solution: Store between 2° and 25°C.

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