CMV IgM Test System

IVD

REF FA9001M

Rx Only

INTENDED USE

The ZEUS IFA Cytomegalovirus (CMV) IgM Test System is designed for qualitative detection of IgM 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 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.

Surveys show the incidence of congenital CMV infection to be from 0.5 - 2.5%, with 90% of the cases being asymptomatic. 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). 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). Although the majority of individuals contracting postnatal CMV infections remain asymptomatic, a small percentage of individuals will develop a negative heterophile-antibody infectious mononucleosis syndrome (2 - 4). 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, 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). 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), or protein G (24), ion exchange chromatography (22), precipitation of IgG with anti-human IgG serum (23), or the use of Zorba® IgG Removal Reagent (ZEUS Product #: FA003G).

PRINCIPLE OF THE ASSAY

The ZEUS IFA CMV IgM Test System is designed to detect IgM class antibodies to CMV antigen. The assay employs CMV infected substrate cells and fluorescein labeled anti-human IgM (µ chain specific). The assay procedure involves three incubation steps:

1. Step one; test sera are first treated to remove IgG and rheumatoid factor.
2. Step two; test sera are diluted in the phosphate-buffered-saline provided, added to the wells, and incubated. Antigen specific IgM antibody will bind to CMV antigen immobilized on the Slide. The Slides are washed to remove unbound antibody and other serum components.
3. Step three; Fluorescein labeled anti-human IgM Conjugate is added to the wells and the Slides are incubated. The Conjugate will react with the antigen specific IgM antibodies bound to the Slides in step 2. The Slides are washed to remove unbound Conjugate. The Slides are then mounted with a coverslip and read under a fluorescence microscope. A mixture of infected and uninfected cells on the Slide provide an internal control for nonspecific and autoantibody binding.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. SAVe Diluent® contains Sodium Azide (<0.1% w/v) as a preservative.**

• • •	1.	CMV IgM Antigen Substrate Slides: Ten, 10-well Slides containing human fibroblasts infected with CMV (strain AD169). Approximately 10 - 15% of the cells are infected with CMV. Also includes absorbent blotter and desiccant pouch.
CONJ	2.	Conjugate: Anti-human IgM (µ chain specific) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL +	3.	Positive Control (Human Serum): Will produce positive apple-green staining of the CMV infected cells. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL -	4.	Negative Control (Human Serum): Will produce no detectable staining of the CMV infected cells. One, 0.5mL, green-capped, vial. Ready to use.

DIL	SPE	5. SAVe Diluent®: One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
BUF	PBS	6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
MNTMED		7. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper tipped vials.

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical: SAVe Diluent® (Product #: FA005CC), Mounting Media (Product #: FA0009S), and PBS (Product #: 0008S).**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

PRECAUTIONS

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 Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
4. The Controls are **potentially bio-hazardous 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 Bio-safety 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 (20).
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 their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Conjugate, and Controls 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. This preservative may by toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. 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.
17. 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.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
22. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
6. Cover slips, 24 x 60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).
12. Incubator: 35 - 37°C.
13. IgG Removal System (see Limitations of the Assay).

The following filter systems, or their equivalent, have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

Transmitted Light		
Light Source: Mercury Vapor 200W or 50W		
Excitation Filter	Barrier Filter	Red Suppression Filter
KP490	K510 or K530	BG38
BG12	K510 or K530	BG38
FITC	K520	BG38
Light Source: Tungsten – Halogen 100W		
KP490	K510 or K530	BG38

Incident Light			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	Dichroic Mirror	Barrier Filter	Red Suppression Filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. 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.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (25, 26). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. 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 - 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 which 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 (31).

STORAGE CONDITIONS

2°C – 8°C	Unopened Test System.
	Mounting Media, Conjugate, SAVe Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
2°C – 25°C	Phosphate-buffered-saline (PBS) Packets.

ASSAY PROCEDURE

1. Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
2. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted**.
   Diluting Patient Sera:
   a. It is recommended that test sera are pre-treated to remove IgG. Precipitation with anti-human IgG is recommended because this procedure is effective in removing all subclasses of human IgG and is less cumbersome to perform than other methods. After the pretreatment step, test sera should be at a 1:10 screening dilution (e.g.: 10µL of serum + 90µL of SAVe Diluent® or PBS).
   b. If patient samples are to be titrated to endpoint, one should pre-treat the serum to remove IgG and then make any subsequent dilutions with SAVe Diluent® or PBS**. NOTE: The SAVe Diluent® will undergo a color change confirming the combination of specimen with Diluent.**
   c. As an option, users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in SAVe Diluent® or PBS. When evaluated by ZEUS Scientific, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected end-point titer for each lot of Positive Control.
3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at 35 - 37°C for 60 ± 5 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash Slides for two, 5 minute intervals, changing PBS between washes.
7. Remove Slides from PBS one at a time. Invert Slide and key wells to holes in blotters provided. Blot Slide by wiping the reverse side with an absorbent wipe. CAUTION: Position the blotter and Slide on a hard, flat surface. Blotting on paper towels may destroy the Slide matrix. **Do not allow the Slides to dry during the test procedure**.
8. Add 20µL of Conjugate to each well and incubate at 35 - 37°C for 30 ± 5 minutes.
9. Repeat steps 5 through 7.
10. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.

NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.

QUALITY CONTROL

1. Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
2. It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described, results are invalid.
   a. Negative Control - characterized by the absence of intra-nuclear fluorescence and a red, or dull green, background staining of all cells due to Evans Blue. Use the reaction of the Negative Control serum as a guide for interpretation of patient results.
   b. Positive Control - characterized by apple-green fluorescent staining of inclusion bodies in the nucleus of infected cells which comprise 10 - 15% of the total cell sheet. The remainder of the cells should appear as red counter-stained cells with no fluorescence. Fluorescent staining of the nuclei of all the cells indicate the presence of antinuclear antibodies.
3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTE: The intensity of the observed fluorescence may vary with the microscope and filter system used.

INTERPRETATION OF RESULTS

A CMV reaction is positive when brightly fluorescent inclusion bodies are observed in the nucleus of infected cells. Uninfected cells appear a reddish-orange in color with no intra-nuclear inclusion staining. The endpoint titer is the highest dilution of patient sera showing 1+ to 2+ fluorescence. Absence of specific staining of CMV nuclear inclusions denotes a negative reaction. **Interpretation:**

Titer	Clinical Significance
<1:10	**Negative:** No detectable IgM antibody to CMV. This indicates no primary infection, reactivated infection, or re-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 IgM antibody. If a primary infection is suspected, another specimen should be taken with 7 days to look for the presence of CMV specific IgM. If the second specimen is positive, a primary, reactivated infection, or re-infection with CMV is indicated.
1:10 >	**Positive:** Detectable IgM antibody to CMV. This indicates a primary infection, reactivated infection, or re-infection with CMV. Such individuals are presumed to be at risk of transmitting CMV infection.

LIMITATIONS OF THE ASSAY

1. IgG antibodies, if present in the sample, may interfere with determination of IgM titers to the organism. High affinity IgG antibodies may preferentially bind to antigenic determinants leading to false negative IgM titers (18). Also, IgM rheumatoid factor may bond to the antigen specific IgG leading to false positive IgM titers. Both of these problems can be eliminated by removing IgG from the samples before testing for IgM. Several different methods of separating IgG have been used. These include gel filtration (20), absorption with protein A (21), ion exchange chromatography (22), precipitation of IgG with anti-human IgG serum (23), or the use of Zorba®-IgG Removal Reagent.
2. A negative result does not rule out a current or recent infection. IgM responses may be variable in different persons. Absence of CMV specific IgM does not exclude the possibility of CMV infection. It has been reported that 10 to 30% of infants may fail to develop IgM antibody responses despite congenital infection with cytomegalovirus (16, 17). Additionally, up to 27% of adults with primary CMV infection may demonstrate no CMV IgM antibody response (16).
3. 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 (27). If CMV infection is suspected, a second sample should be obtained 7 to 14 days later and tested for the presence of CMV specific IgM antibody.
4. 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 (25, 26).
5. CMV-specific IgM antibody may reappear during reactivation of CMV (15, 17, and 27). Its presence is not limited to primary infection.
6. Results of the ZEUS IFA CMV IgM Test System are not be themselves diagnostic, and should be interpreted in light of the patient's clinical condition and the results of other diagnostic procedures.
7. Patients may continue to product CMV-specific IgM antibody for 6 - 9 months or longer in the case of immunosuppressed patients following a primary infection (15, 26, 27, and 28).
8. Hetertypic IgM antibody responses may occur in patients infected with Epstein-Barr or Varicella-Zoster viruses, and give false positive results in the CMV-IgM IFA (29). Heterotypic IgM antibody responses to CMV have been reported in as many as 30% of patients with infectious mononucleosis (30). Polyclonal stimulation of B lymphocytes by EBV seems the most likely mechanism. However, reactivation of latent CMV is also a possibility.
9. False positive nuclear or cytoplasmic staining of all cells may be observed due to nonspecific or autoantibody reactions such as antinuclear or mitochondrial antibodies associated with systemic lupus erythematosus, or primary biliary cirrhosis, respectively.
10. Nonspecific staining of all cells may be observed in some sera at low dilutions and may be difficult to interpret. These results should not be used.
11. The endpoint reactions may vary due to the type of microscope employed, the light source, age of bulb, filter assembly, and filter thickness. Each laboratory should establish its own criteria for reading of endpoints using appropriate controls.
12. Because of the possibility of contamination of cord blood with maternal IgM, it is prudent to confirm positive viral IgM antibody results on cord blood samples by testing a follow-up specimen from the infant (28).
13. The presence or absence of CMV IgM antibodies in pregnant women experiencing primary CMV infections is unrelated to the transmission of infection *in utero* (16).
14. Because of all the complications of serological diagnosis of congenital infection, virus isolation from urine in the first week of life remains the best way to diagnose intrauterine involvement (16, 17).
15. Infants with perinatally acquired CMV infections do not manifest serum CMV IgM antibodies until two to three weeks after birth. Newborns with congenital (prenatally acquired) CMV infections usually show CMV IgM antibody and/or virus. Therefore, detection of CMV IgM antibody, and isolation of virus from urine in the first week of life, provides a definitive diagnosis of congenital CMV infection. Detection of CMV IgM and/or virus after those times does not distinguish congenital from perinatal infection.

EXPECTED RESULTS

CMV specific IgM usually develops after a patient has been clinically ill for at least a week or more (27). Most 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). In immuno-suppressed transplant patients however, CMV IgM antibody characteristically persists for long periods of time (> 2 years) (26).

PERFORMANCE CHARACTERISTICS

1. Comparative Study:

The ZEUS IFA CMV IgM Test System was compared to a commercially available ELISA test system for detection of IgM antibodies to CMV. Serum samples from patients suspected of having active CMV infection were obtained from a reference laboratory in the Northeastern United States. Sera from normal blood donors were obtained from a blood bank. After pretreatment to remove IgG, 86 serum samples were tested by the ZEUS IFA CMV IgM Test System, and by the ELISA procedure. The results of this study are summarized below:

Reference CMV IgM ELISA Procedure

		Positive	Negative	Equivocal*
ZEUS IFA CMV IgM Test System	Positive	18	1	2
	Negative	2	65	2

Specificity - 90.5% (65/66) Sensitivity - 90.0% (18/20) Concordance - 96.5% (83/86)

* Equivocal results were not included in the calculations for sensitivity, specificity, and concordance.

Three samples gave discrepant results between the two test systems. A second commercially available CMV-IgM ELISA was used to resolve discrepancies. Of the two samples that were negative by IFA and positive by ELISA, one was positive by the second ELISA, and one was negative. A third sample which was IFA positive/ELISA negative was also positive by the second ELISA.

2. Cross Reactivity:

To assess the cross-reactivity by IgM antibodies to other herpes viruses, sera with IgM antibodies (IFA 1:8 - 1:640) to Herpes-Simplex Virus type 2, Epstein-Barr virus, and Varicella-Zoster were tested by the ZEUS IFA CMV IgM Test System. None of the HSV-2 (n=5) or VZ (n=9) sera were positive, and only 4 of 21 EBV sera were positive.

3. Reproducibility:

Reproducibility studies reveal that the ZEUS IFA CMV IgM Test System performs within the reproducibility specifications of plus or minus one two-fold dilution when employed to determine endpoint titrations.

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