

INTENDED USE

The ZEUS IFA Cytomegalovirus (CMV) IgG Test System is designed for the qualitative and semi-quantitative detection of anti-CMV IgG antibody in human serum by the indirect fluorescent antibody (IFA) technique, 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

Following the isolation of Cytomegalovirus, it has become quite clear that these viruses are important human pathogens. 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 or immunodeficient individuals following CMV infections clearly establishes this agent as an important human pathogen (2, 3). CMV infections can be classified as follows:

- Congenital - Acquired prior to birth.
- Perinatal - Acquired at birth.
- Postnatal - Acquired during adult life.

In congenital CMV inclusion disease (CID) 95% of the offspring infected exhibit no clinically overt disease at birth (4). Of the remaining 5% of CID infected infants, clinical manifestations of the disease range from severe disease with jaundice, hepatosplenomegaly, thrombocytopenic purpura, cranial calcification, and growth retardation to pneumonitis, hydrocephaly or microcephaly, and ocular defects (1). Infants with CID may expire early after birth due to secondary complications; however, most survive with concurrent neurological damage (2).

The prognosis for CID infected infants with no overt clinical disease must be guarded. Ten to 25% may develop hearing loss (5). Five to 10% may exhibit various degrees of mental retardation and central nervous system motor disorders (4). Recent surveys show the incidence of congenital CMV to be from 0.5 - 2.5%. Consequently, a careful documentation of the long-term effects of intrauterine infection is important (6). Perinatal infected infants start excreting CMV 3 to 12 weeks after delivery and remain asymptomatic except for rare exceptions (7). Postnatal CMV infections increase with age presumably due to close contact with individuals who are shedding the virus. CMV has been isolated from saliva, urine, breast milk, cervical secretions, and semen. Consequently, the transmission of the virus can occur through a variety of mechanisms (1, 6). The majority of individuals acquiring postnatal CMV infections remain asymptomatic. Some recognized clinical syndromes of postnatal CMV infections include heterophile antibody, negative mononucleosis syndrome, and pulmonary CID associated with hepatitis. CMV mononucleosis in young adults 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 (8, 9).

Pulmonary CID associated with hepatitis is seen in patients on immunosuppressive therapy or with immunodeficiency diseases. Such patients (transplant recipients, leukemia, and immunosuppressed patients) appear to be highly susceptible to CMV infection (9 - 12). The ZEUS IFA CMV IgG Test System is helpful in determining:

1. The history of a previous infection with CMV.
2. Whether or not previous infections have occurred and therefore help determine the patient's relative susceptibility to a primary infection.
3. Undiagnosed congenital diseases and etiology of prolonged fever in immunosuppressed or deficient patients.
4. CMV etiology in young adults with heterophile negative infectious mononucleosis.
5. Recent infection with CMV virus by demonstrating a four-fold rise or fall in antibody titer, or by demonstrating the presence of IgM CMV antibody.

PRINCIPLE OF THE ASSAY

The ZEUS IFA CMV IgG Test System is designed to detect circulating CMV IgG antibodies in human sera. The assay employs CMV infected substrate cells and FITC-labeled goat anti-human IgG (γ chain specific) adjusted for optimum use dilution and free of nonspecific background staining. The reaction occurs in two steps:

1. Step one is the interaction of CMV antibodies in patient sera with CMV infected substrate cells.
2. Step two is the interaction of FITC-labeled anti-human IgG (γ chain) with the CMV-IgG antibody attached to the CMV localized in the nucleus of the infected cells.

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 IgG 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: Goat anti-human IgG (γ 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 $\pm$ 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, dropper 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.
 - b. A CD containing all ZEUS IFA Product Inserts, providing instructions for use.

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 be 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).

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

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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	

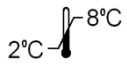
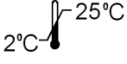
Incident Light			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	Dichroic Mirror	Barrier Filter	Red Suppression Filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

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.

- Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (23, 25). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
- 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 (26).

STORAGE CONDITIONS

	Unopened Test System.
	Mounting Media, Conjugate, SAvE Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
	Phosphate-buffered-saline (PBS) Packets.

ASSAY PROCEDURE

- 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.**
- Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted.** Prepare a 1:16 dilution (e.g.: 10µL of serum + 150µL of SAvE Diluent® or PBS) of each patient serum. **The SAvE Diluent® will undergo a color change confirming that the specimen has been combined with the Diluent.**
Dilution Options:
 - As an option, users may prepare initial sample dilutions using PBS, or Zorba-NS (Zorba-NS is available separately. Order Product Number FA025 – 2, 30mL bottles).
 - 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 endpoint titer for each lot of Positive Control.
 - When titrating patient specimens, initial dilutions should be prepared in SAvE Diluent®, PBS, or Zorba-NS (Zorba-NS is available separately. Order Product Number FA025 – 2, 30mL bottles). or Zorba-NS and all subsequent dilutions should be prepared in SAvE Diluent® or PBS only. Titrations should not be prepared in Zorba-NS.
- With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
- Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
- Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
- Wash slides for two, 5 minute intervals, changing PBS between washes.
- 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.**
- Add 20µL of Conjugate to each well.
- Repeat steps 4 through 7.
- 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

- Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
 - 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.
 - 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.
 - 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 off all the cells indicate the presence of antinuclear antibodies.
 - 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
<1:16 (Negative)	Non-Diagnostic
1:16 or Greater (Positive)	Considered positive for CMV antibody. Since a high percentage (80%) of the population over 35 years of age is seropositive, the interpretation of a single bleeding may be difficult. A four-fold rise or fall in titer however is evidence of a recent infection. (1, 19, 20, and 22). Alternatively, if a recent primary infection is suspected, specimens should be evaluated for anti-CMV IgM antibody (13, 14, and 21).

LIMITATIONS OF THE ASSAY

- A single serological antibody titer to CMV should not be used as the only criteria for diagnosis. The patients clinical data and laboratory test results should be carefully reviewed by a medical authority.
- It is now established that human CMV induces an FcIgG receptor in the cytoplasm of infected human fibroblasts. This FcIgG receptor may result in a false positive reading because the anti-human IgG conjugate attaches to patient IgG attached to infected cell membrane Fc receptor sites. To circumvent this problem, restrict positive CMV reactions to intra-nuclear inclusion staining only (16). Receptors for IgM or IgA have not been observed by this technique (17).

3. After CMV infections many patients develop Rheumatoid factor. The Rheumatoid factor (IgM) reacts with CMV specific IgG antibodies forming a complex (18). The IgG in this complex may attach to the CMV infected substrate cells.

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