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Diagnostic Automation Inc.

CMV IgM ANTIBODY

TEST SYSTEM

Catalog # : 371010-M

An Indirect Fluorescent Antibody (IFA) Assay for the detection of IgM Antibodies to Cytomegalovirus

INTENDED USE

The Diagnostic Automation, Inc. CMV IgM IFA test system is an indirect fluorescent antibody (IFA) assay designed for the 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 (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

Surveys show the incidence of congenital CMV infection to be from 0.5 to 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) (4,13,15). Clinical manifestations of CMV disease in immunocompromised patients ranges from CMV mononucleosis to pneumonia, hepatitis, pericarditis, and encephalitis (4).

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

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

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

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

PRINCIPLE OF THE ASSAY

The Diagnostic Automation, Inc. fluorescent CMV IgM test system is designed to detect IgM class antibodies to CMV antigen. The test system employs CMV infected substrate cells and fluorescein labeled anti-human IgM (μ chain specific). The test procedure involves three incubation steps:

1. Test sera are first treated to remove IgG and rheumatoid factor.
2. 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. 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.

KIT COMPONENTS

Reactive Reagents:

1. CMV Antigen Slides: Ten, 10-well substrate slides containing human fibroblasts infected with CMV (strain AD169). Approximately 10-15% of the cells are infected with CMV.
2. Anti-human IgM (μ chain specific), labeled with fluorescein. Contains 1.0% bovine albumin and Evans blue counterstain. Two, 1.5ml vials, lyophilized.
3. CMV Human Positive Control Serum: Two, 0.5ml vials, lyophilized.
4. CMV Human Negative Control Serum: Two, 0.5ml vials, lyophilized.

Non-reactive Components:

1. Phosphate-Buffered-Saline (PBS): Four packets. Sufficient to prepare 4 liters of PBS (0.01M phosphate, 0.15M NaCl, pH 7.2).
2. Mounting Fluid: One, 3.0ml vial of phosphate-buffered-glycerol, pH 7.6.

CAUTION: All reactive reagents, as well as buffered glycerol contain a preservative which may be toxic if ingested (thimerosal, mercury derivative 1:10,000).

PRECAUTIONS

- 1.The preservative may be toxic if ingested.
- 2.Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of the antibody to HIV-1 as well as for hepatitis B surface antigen and found to be negative (were not repeatedly reactive).

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus (HIV-1), hepatitis B virus, or other infectious agents are absent, this specimen/reagent, as well as patient samples 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”, 1993, and FDA LABELING GUIDELINES FOR *IN VITRO* DIAGNOSTIC REAGENT MANUFACTURERS, DEC., 1985.

- 3.Each working reagent has been optimized to identify CMV-IgM antibody.
- 4.Dilution or adulteration of these reagents may result in loss of sensitivity.
- 5.Reagents from other sources or manufacturers should not be used.
- 6.For *in vitro* diagnostic use.
- 7.Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
- 8.Avoid microbial contamination of reagents. Incorrect results may occur.
- 9.Do not allow the wells to dry once the assay has begun.
- 10.Incubation times or temperatures other than those specified may give erroneous results.
- 11.All reagents should be brought to room temperature (20-25°C) and mixed well before use.
- 12.Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
- 13.Evans blue dye is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
- 14.Although the slides have been inactivated, they should be handled as if capable of transmitting infection.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- 1.Small serological, Pasteur, capillary, or automatic pipettes.
- 2.Small test tubes, 13 x 100 mm or comparable.
- 3.Test tube racks.
- 4.Staining dish - A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
- 5.Cover slips: 24 x 60mm, thickness No. 1.
- 6.Distilled water.
- 7.One liter volumetric flask.
- 8.Clean wash bottle.
- 9.Moist 37°C incubation chamber.
- 10.IgG removal system (See Limitations, #2).
- 11.Properly equipped fluorescent microscope assembly.

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 200 W or 50 W			
Excitation filter	Barrier filter	Red Suppression filter	
KP490	K510 or K530	BG38	
BG12	K510 or K530	BG38	
FITC	K530	BG38	
Light Source: Tungsten - Halogen 100 W			
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

Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be employed in this assay (25,26). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. 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.

STORAGE CONDITIONS

- CMV Substrate Slides: -20°C or lower.
- Goat anti-human IgM labeled with FITC: 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
- Positive and negative human CMV IgM control sera: 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
- Phosphate-buffered-saline: Store at 2-25°C. Store reconstituted buffer at 2-8°C. Rehydrated PBS is stable for 30 days when stored at 2-8°C.
- Buffered glycerol (mounting media): Store at 2-8°C.

NOTE:

1. All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed.
2. Do not freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity.

PROCEDURE

Preparation of Reagents:

1. Phosphate-buffered-saline (PBS): Empty contents of one buffer packet into one liter of distilled water. Mix until all salts are thoroughly dissolved.
2. CMV human positive and negative control sera. Reconstitute with 0.5ml of distilled water.
3. Goat anti-human IgM FITC-labeled conjugate. Reconstitute with 1.5ml of distilled water. Alternately, aliquot in 0.5ml amounts and store at -20°C or lower in small tubes.

NOTE: Reconstitute reagents gently but thoroughly. If lyophilized reagents show evidence of rehydration prior to reconstituting, do not use. If reagents become cloudy, bacterial contamination should be suspected, and reagents should not be used.

TEST PROCEDURE

1. Remove substrate slides from freezer and allow them to reach room temperature (20-25°C). Tear open the protective envelope and remove slides containing the CMV infected cells. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE, THIS COULD DESTROY THE CELL SHEET ON THE SLIDE.**
2. Pretreat the test sera 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.
3. After the pretreatment step, test sera should be at a 1:10 screening dilution. The prediluted positive and negative serum controls, and a buffer control should be run each time the test is performed.
4. Identify each well with the appropriate patient sera and controls.
5. Spread 20 μ l of test and control sera over each appropriately labeled well being careful not to disturb the substrate cells with pipette tip.
6. Incubate slides in a moist chamber at 37°C for one hour \pm 5 minutes. **DO NOT ALLOW THE WELLS TO DRY OR NON-SPECIFIC STAINING WILL RESULT DUE TO DESTRUCTION OF CELL MORPHOLOGY.**
7. Take slides from the moist chamber and remove excess sera from the wells by gently rinsing slides with a stream of PBS. **DO NOT DIRECT THE STREAM OF PBS INTO THE TEST WELLS.**
8. Place slides in a staining dish and wash in PBS for two, 5 minute intervals \pm 2 minutes, with a change of PBS.
9. Remove slides from PBS solution. Dry mask area with blotters provided being careful not to disturb substrate in wells. **NOTE: DO NOT ALLOW SUBSTRATE WELLS TO DRY.**

10. Place slides in a moist chamber and add 20 μ l conjugate to each well.
11. Incubate slides in a moist chamber at 37°C for 30 minutes \pm 5 minutes. DO NOT ALLOW WELLS TO DRY.
12. Repeat steps 7, 8, and 9.
13. Add 3-4 drops of buffered glycerol to the mask area of each slide and coverslip. Avoid entrapment of air bubbles. Slides should be examined immediately at a total magnification of 200X.

QUALITY CONTROL

1. Positive, negative, and buffer controls should be run with each assay.
2. It is recommended that one read the positive and negative controls before evaluating test results. This will assist in establishing the positive and negative references required to interpret the test samples. If the controls do not appear as described, results are invalid.
3. The negative control is characterized by the absence of intra-nuclear fluorescence, and a red 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.
4. The positive control is 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, and these specimens may be difficult or impossible to interpret for anti-CMV IgM.
5. The intensity of the observed fluorescence may vary with the microscope and filter system used.

INTERPRETATION OF RESULTS

TITER CLINICAL SIGNIFICANCE

- | | |
|-------|---|
| <1:10 | NEGATIVE: No detectable IgM antibody to CMV. This indicates no primary infection, reactivated infection, or reinfection 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 reinfection with CMV is indicated. |
| >1:10 | POSITIVE: Detectable IgM antibody to CMV. This indicates a primary infection, reactivated infection, or reinfection with CMV. Such individuals are presumed to be at risk of transmitting CMV infection. |

LIMITATION OF THE ASSAY

1. Substitution of other reagents for components of this kit is to be avoided. Since the components of this kit have been tested for maximum efficiency, Diagnostic Automation Inc. is not responsible for test performance if reagent substitution occurs.
2. 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 (Diagnostic Automation Product).

3. 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).
4. 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.
5. 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).
6. CMV-specific IgM antibody may reappear during reactivation of CMV (15, 17, 27). Its presence is not limited to primary infection.
7. Results of the Diagnostic Automation Inc. CMV IgM IFA test are not by themselves diagnostic, and should be interpreted in light of the patient's clinical condition and the results of other diagnostic procedures.
8. Patients may continue to produce CMV-specific IgM antibody for 6-9 months or longer in the case of immunosuppressed patients following a primary infection (15, 26, 27, 28).
9. Heterotypic 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.
10. False positive nuclear or cytoplasmic staining of all cells may be observed due to nonspecific or auto antibody reactions such as antinuclear or mitochondria antibodies associated with systemic lupus erythematosus, or primary biliary cirrhosis, respectively.
11. 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.
12. 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.
13. 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).
14. 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).
15. 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).
16. 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 VALUES

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

Comparative Study

The Diagnostic Automation, Inc. CMV-IgM IFA 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 Diagnostic Automation Inc. CMV IgM IFA, and by the ELISA procedure. The results of this study are summarized below:

		Reference CMV IgM ELISA Procedure		
		Pos.	Neg.	Equivocal*
DAI CMV IgM IFA	Pos.	18	1	2
	Neg.	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.

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 Diagnostic Automation Inc. CMV IgM IFA 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.

Reproducibility:

Reproducibility studies reveal that this test kit performs within the reproducibility specifications of plus or minus one two-fold dilution when employed to determine endpoint titrations.

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