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IVD | See external label | 2°C-8°C | Σ= 10 x 10 | REF 371010-M

CMV IgM IFA Kit

REF 371010-M

PRINCIPLE OF THE ASSAY

The DAI Immuno 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.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (25,26). 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.

EQUIPMENT AND MATERIALS

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 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

MATERIALS PROVIDED

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).

STORAGE CONDITIONS

1. CMV Substrate Slides: -20°C or lower.
2. 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.
3. 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.
4. Phosphate-buffered-saline: Store at 2-25ºC. Store reconstituted buffer at 2-8°C. Rehydrated PBS is stable for 30 days when stored at 2-8°C.
5. 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.

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.
6. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

PROCEDURE – STEPWISE

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 20µl 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 ± 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 ± 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 20µl conjugate to each well.
11. Incubate slides in a moist chamber at 37°C for 30 minutes ± 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.

CALCULATIONS/REPORTING RESULTS

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.

PROCEDURE NOTES

1. The preservative may be toxic if ingested.
2. 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 ().

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.

LIMITATIONS OF THE PROCEDURE

1. Substitution of other reagents or components of this kit are to be avoided. Since the components of this kit have been tested for maximum efficiency, DAI is not responsible for test performance if reagent substitution occurs.
2. 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.
3. It is now established that human CMV induces an FclgG receptor in the cytoplasm of infected human fibroblasts. This FclgG 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).
4. 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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Date Adopted	2016-05-11
REF 371010-M	DA-CMV IgM-IFA

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Revision Date: 2006-07-24