LIGHT **DIAGNOSTICS**

CMV pp65 Antigenemia Immunofluorescence Assay

Detection of Cytomegalovirus pp65 Matrix Protein in Peripheral Blood Leukocytes

Cat. No. 3247i

FOR IN VITRO DIAGNOSTIC USE;
EXPORT ONLY

MILLIPORE™

28820 Single Oak Drive Temecula, CA 92590 • USA & Canada
Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Find the Millipore office closest to you:
www.millipore.com • custserv@millipore.com • techserv@millipore.com

This page left blank intentionally

The **Light Diagnostics™ Cytomegalovirus (CMV) pp65 Antigenemia Immunofluorescence Assay** (IFA) is intended for the qualitative detection and identification of lower matrix protein pp65 of CMV in isolated peripheral blood leukocytes.

For In Vitro Diagnostic Use; Export Only.

Summary And Explanation

CMV is a member of the family *Herpesviridae*. Synthesis of viral DNA and assembly of capsids occur in the nucleus where infective progeny are released by budding through the nuclear envelope. Also characteristic of herpesviruses, CMV undergoes periods of latency and reactivation.

CMV is species specific and has been isolated from many animal species (1). The first human CMV was isolated in 1956 from embryonic fibroblasts of adenoidal tissue (2). The terminal morphology of CMV infected permissive cells is that of a large cell with a prominent intranuclear inclusion (Cowdry Type A or "owl eye" cell). Such cells were identified in tissues of fatally infected infants, which gave rise to the name "cytomegalic inclusion disease (CID)" (3).

Humans are believed to be the only reservoir of human CMV and postnatal infections are acquired by close contact with individuals shedding virus. Transmission may be through saliva, urine, cervical and vaginal secretions, semen, breast milk, tears, feces, and blood (4,5,6). Infection rates vary with geographic location and socioeconomic conditions.

CMV infection has been detected in newborn infants and is the most commonly identified cause of congenital infection. Those infants that develop symptoms may exhibit severe disease with jaundice, hepatosplenomegaly, petechiae, and central nervous system abnormalities. The risk of infection is probably the same throughout pregnancy; CID occurs most often in fetuses infected during the first half of gestation. CMV may be transmitted to about 50% of the fetuses after primary maternal infection and about 10% of these will be clinically affected (7,8). Many congenitally infected infants appear normal at birth but subsequently develop neurologic sequelae. The route of transmission of CMV from mother to fetus has not been well elucidated. It is possible that the spread is hematogenous through cord blood or placental tissue and amniotic cells.

During the past decade an increasing population of immunosuppressed individuals has resulted in a resurgence of CMV as a major pathogen. Induced immunosuppression has occurred more frequently via chemotherapy and transplant regimens. CMV infection is common in patients receiving renal (9), bone marrow (10), heart (11), lung (12), and liver transplants (13). The spread of acquired immunodeficiency syndrome (AIDS) is also related to the CMV resurgence. CMV is the most common opportunistic viral infection in AIDS patients (14,15) and has been implicated as a cofactor in the pathogenesis of the HIV virus (16). CMV infection in AIDS has been implicated in pneumonitis (17), colitis (18,19), retinitis (20), and dementia (21).

Detection of CMV in blood leukocytes is closely associated with the clinical manifestations of CMV disease and is useful in the diagnosis of CMV infection (22). Rapid diagnosis of CMV disease may prevent delay in treatment using antiviral drugs such as ganciclovir and foscarnet. The **Light Diagnostics™ CMV pp65 Antigenemia Immunofluorescence Assay** is a rapid, sensitive method for detection of CMV in isolated leukocytes.

Test Principle

The **Light Diagnostics™ CMV pp65 Antigenemia Immunofluorescence Assay** utilizes an indirect immunofluorescence technique for identifying the lower matrix protein pp65 of human CMV in cytospin preparations of peripheral blood leukocytes. The blend of monoclonal antibodies provided will bind to CMV pp65 antigen present in formalin fixed leukocytes. Unbound monoclonal antibody is removed by washing with phosphate buffered saline (PBS). Fluorescein isothiocyanate (FITC) conjugated antibody will bind to the antigen-antibody complex. Unbound conjugate is removed by washing with PBS. FITC exhibits an apple green fluorescence when excited by ultraviolet light allowing visualization of the complex by fluorescence microscopy. Nuclear fluorescence indicates a positive specimen. Uninfected cells counterstain dull red due to the presence of Evans blue in the FITC conjugated antibody reagent.

Materials Provided

1. CMV pp65 Monoclonal Antibody - (Catalog No. 5097i) One dropper vial containing 5 mL of a monoclonal antibody blend against CMV pp65 antigen, protein stabilizer, 0.05% Tween 20, and 0.1% sodium azide.
2. Anti-Mouse IgG:FITC Conjugate - (Catalog No. 5024) One dropper vial containing 10 mL of FITC labeled anti-mouse IgG, 0.02% Evans blue, protein stabilizer and 0.1% sodium azide.
3. Separation Solution - (Catalog No. 5111US) One bottle containing 125 mL of PBS, Dextran and 0.1% sodium azide.
4. LyseStop - (Catalog No. 5099) One bottle containing 60 mL of PBS and 0.5% sodium azide.
5. Fixation Solution (5X) - (Catalog No. 5113) One bottle containing 220 mL of PBS, formalin, sucrose and 0.5% sodium azide.
6. Permeabilization Solution (5X) - (Catalog No. 5115) One bottle containing 220 mL of PBS, Nonidet P-40, sucrose, protein stabilizer and 0.5% sodium azide.
7. Phosphate Buffered Saline (PBS) - (Catalog No. 5087) Three packets of phosphate buffered saline salts.
8. Wash Supplement (100X) - (Catalog No. 5117) One bottle containing 30 mL of protein solution and 0.1% sodium azide.
9. Mounting Fluid - (Catalog No. 5013) One dropper bottle containing 10 mL of Tris buffered glycerin, a fluorescence enhancer and 0.1% sodium azide.

Materials Required But Not Provided

Equipment and Reagents:

- Cytocentrifuge (Shandon Lipshaw, Cytospin 3) cytocentrifuge slides, holders, funnels and filter cards.
- Laboratory centrifuge
- Fluorescence microscope with appropriate filter combination for FITC (excitation = 490 nm, emission = 515 nm) with 100x, 200x, and 400x magnification (dry objective)
- Hemocytometer or Coulter counter
- No. 1 microscope slide coverslips
- Sodium hypochlorite solution, 0.05% (1:100 dilution of household bleach)
- Humid Chamber
- Incubator (37^{o} C)
- Coplin staining jars
- Filter sterilized deionized or distilled water
- 0.2 M filtered deionized or distilled water

Stability and Storage

When stored at 2° to 8°C, the CMV kit is stable up to the expiration date printed on the kit label. Do not freeze or expose to elevated temperatures. Discard any remaining reagents after the kit expiration date.

Warnings and Precautions

- The sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides (23, 24). Upon disposal, flush with large volumes of water to prevent build-up in plumbing.
- Pooling or alteration of any reagent may cause erroneous results.
- Do not mix or substitute reagents from other manufacturers.
- Do not allow the slides to dry at any time during the staining procedure.
- Handle all specimens and materials coming in contact with them as potentially infectious and dispose of with proper precautions. Decontaminate with 0.05% sodium hypochlorite.
- Formaldehyde solution causes irritation of skin, eyes, nose, and throat. Avoid prolonged or repeated contact. Avoid prolonged breathing of vapor. Use adequate ventilation.
- Avoid contact with Evans blue (present in the Anti-mouse IgG:FITC conjugate) as it is a potential carcinogen. If skin contact occurs, flush with large volumes of water.
- Avoid contact with the Mounting Fluid, which contains a fluorescence enhancer that may be destructive to tissue of the mucous membranes. If contact occurs, flush with large volumes of water.
- Incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user.
- Performance of the fluorescence microscope is of critical importance in achieving satisfactory test results. Microscope objectives, bulb intensity and wattage and filters may affect results.
- Do not mouth pipette reagents.

Specimen Collection

Five to 10 mL of whole blood, collected by venipuncture in heparin or EDTA tubes is sufficient for CMV pp65 detection. Patients with extreme neutropenia may require greater than 10 mL for testing. CMV pp65 analysis should be performed as soon as possible after collection of blood. Specimens should be transported and kept at room temperature (20 to 25^{o}C) until separation of leukocytes; samples may be stored for up to 24 hours with constant gentle mixing.

Specimen Processing

Reagent Preparation:

Prior to specimen processing, prepare the required volume of PBS, Fixation Solution, Permeabilization Solution and Wash Solution as follows:

PBS - Dissolve the contents of the PBS packet in 950 mL of filtered distilled or deionized water, mix thoroughly and adjust volume to 1 L with filtered distilled or deionized water.

Fixation Solution - Shake well before each use ensuring that the stock solution is completely mixed. Dilute 1/5 in filtered distilled or deionized water and mix thoroughly.

Permeabilization Solution - Shake well before each use ensuring that the stock solution is completely mixed. Dilute 1/5 in filtered distilled or deionized water and mix thoroughly.

Note: *The Permeabilization Solution will separate during storage and appear turbid when mixed.*

Wash Solution - Ensure that the Wash Supplement is completely mixed. Dilute 1/100 in PBS and mix thoroughly.

All other reagents are provided ready to use.

Processing:

1. Leukocyte separation

Add Separation Solution directly to the blood specimen at a ratio of 4:1 (blood:Separation Solution) and mix thoroughly. Incubate at 37^{o} C for 20 minutes. Transfer the leukocyte containing supernatant to a 15 mL conical centrifuge tube and centrifuge at ~300 x g for 10 minutes.

2. Erythrocyte lysing

Discard supernatant, resuspend the cell pellet in ~4.0 mL of filtered distilled or deionized water, vortex and incubate for 10 to 30 seconds. Add 1.0 mL of LyseStop, mix thoroughly and centrifuge at ~300 x g for 10 minutes. Discard supernatant.

Note: *Erythrocyte lysing may not be necessary in samples with few contaminating red blood cells. Erythrocyte lysing procedure should be repeated on samples with heavy contamination of red blood cells.*

3. Slide Preparation

Resuspend cell pellet in ~1 mL of PBS and determine cell concentration using a hemocytometer or Coulter Counter. Adjust the cell concentration to 1.0 x 10^{6} cells/mL in PBS. Using the cytocentrifuge, centrifuge 200 µL (2 x 10^{5} cells) of cell suspension onto a glass cytocentrifuge slide (prepare at least 3 slides per specimen) at 600-900 RPM for 3 to 4 minutes. Remove slides from the centrifuge, allow the slides to air dry, and fix immediately.

4. Fixation

Fix slides in Fixative Solution for 10 minutes at room temperature. Transfer slides to a Coplin jar containing Wash Solution. Repeat wash procedure using fresh Wash Solution.

5. Permeabilization

Permeabilize cells by immersing the fixed slides in Permeabilization Solution for 5 minutes at room temperature. Place slides in fresh Wash Solution then rinse in distilled or deionized water. Rapidly air dry slides. Stain immediately or store at 2° to 8°Cfor up to 18 hours. Slides may be stored desiccated at <-70°C for up to 12 months.

6. Control Slide Production

Control slides should be prepared from known positive and negative blood samples following the Slide Preparation, Fixation and Permeabilization procedures outlined in the Specimen Processing section. Control slides may be stored desiccated at < -70° C for up to 12 months. Positive samples, identified by antigenemia testing, should be used to produce positive control slides. Isolated leukocytes from positive samples should be diluted in known negative samples to product control slides. Dilutions should target 10 to 50 positive cells per cell spot. Known negative samples may be used to product negative control slides.

Staining Procedure

Stain three slides per specimen, two slides as duplicate tests and one slide as cell control.

1. Allow test slides and reagents to equilibrate to room temperature.
2. Circle the cell spot on the slides with an ImmunoPen or grease pencil (to contain the sample) and place the slides in a Coplin jar containing PBS for 3 to 5 minutes.

   Note: *Do not allow slides to dry at any time during the staining procedure.*
3. Shake excess buffer from slides and carefully dry the area surrounding the cell spot with a cotton swab. Overlay the cell spot of the duplicate test slides with sufficient CMV pp65 Monoclonal Antibody to cover with fluid (~40 µL; 1 drop). Overlay the cell spot of the cell control slide with PBS (~40 µL).
4. Incubate at 37°C for 30 minutes in a humid chamber.
5. Shake antibody from slides; wash slides in a Coplin jar containing PBS, repeat wash in fresh PBS.
6. Shake excess buffer from slides and carefully dry the area surrounding the cell spot with a cotton swab.
7. Add sufficient Anti-Mouse IgG:FITC Conjugate to cover each cell spot (~40 µL, 1 drop).
8. Repeat steps 4 and 5.
9. Rinse slides by dipping in a Coplin jar containing filtered distilled or deionized water.
10. Shake excess water from slides; carefully dry the area surrounding the cell spot with a cotton swab and mount coverslip using the Mounting Fluid supplied. Wipe excess fluid from the edges of the slides with a cotton swab.

    Note: *For best results, read slides immediately after staining. If slides are to be stored after staining, store at 2-8°C in a closed container in the dark.*

11. Examine, with a fluorescence microscope at 200 to 400x, for cells exhibiting the apple green fluorescence of FITC. Detailed examination may be carried out at 400x.

Interpretation Of Results

Control slides should be tested with each sample batch. The positive control cell spot should contain cells exhibiting bright, apple green nuclear fluorescence. Cell count performed on positive staining cells should fall within the targeted number of positive cells determined during control slide production. The negative control cell spot should contain cells exhibiting no specific fluorescence and staining a dull red due to the Evans blue counterstain. If the positive and negative controls cannot be clearly distinguished, the test should be considered invalid.

Examine the entire cell spot. The cell spot should appear as an even, continuous confluent smear of cells (~600 cells / 400x field). Percent confluency should be estimated. An inadequate sample is indicated by fewer than 50,000 cells present in the cell spot (< 25% confluent) and the test should be considered invalid.

The cells of the cell control slide should stain a dull red color due to the Evans blue counterstain. Fluorescent staining of cells or debris due to nonspecific binding of the conjugate should be ignored. It may be useful to examine the cell control slide prior to the test slides to distinguish specific nuclear staining.

A negative result is indicated by the absence of nuclear fluorescence. Negative cells stain a dull red color due to the Evans blue counterstain.

A positive reaction is indicated by the presence of cells exhibiting bright apple green fluorescence in the nucleus.

References

1. Weller, T. H. 1971. The Cytomegalovirus: Ubiquitous agents with protean clinical manifestations. N. Eng. J. Med. 285:203-214, 267-274.
2. Smith, M. G. 1956. Propagation in tissue cultures of cytopathogenic virus from human salivary gland disease, Proc. Soc. Exp. Biol. (NY) 92:424-430.
3. Hanshaw, J. B. 1968. Cytomegalovirus. *In* S. Gard, C. Hallue, K. F Meyer (ed.) Virology Monographs. New York: Springer-Verlag. p. 2-23.
4. Lang, D. J. and J. F. Krummer. 1975. Cytomegalovirus. *In* Semen: observations in selected populations. J. Infec. Dis. 132:472-473.
5. Reynolds, D. W., et al. 1973. Maternal cytomegalovirus excretion and prenatal infection. N. Eng. J. Med. 289:1-5.
6. Stagno, S., et al. 1980. Breast milk and the risk of cytomegalovirus infection. N. Eng. J. Med. 302:1073-1076.
7. Freij, B. J. and J. L. Sever. 1988. Herpesvirus infections in pregnancy: risk to embryo, fetus and neonate. Clin. Perinatol. 15:203-231.
8. Alford, C. A. and W. A. Britt. 1990. Cytomegalovirus. *In* B. N. Fields and D. M. Knipe. (ed.) Virology, 2nd Ed. New York: Raven Press. p. 1981-2010.
9. Marker, S. C., et al. 1981. Cytomegalovirus infection: A quantitative prospective study of three hundred twenty consecutive renal transplants. Surgery, 89:660-671.
10. Guytat, D., et al. 1987. Incidence and prognosis of cytomegalovirus infections following allogenic bone marrow transplantation. J. Med. Virol. 23:393-399.
11. Weingerg, A., et al. 1990. Cytomegalovirus and other herpesvirus infections in heart and bone marrow transplant recipients. Rev. Inst. Med Trop. Sao Paulo. 32:338-345.
12. Smyth, R. L., et al. 1990. Experience of cytomegalovirus infection in heart-lung transplant recipients. Transplan. Proc. 22:1822-1823.
13. King, S. M., et al. 1990. Cytomegalovirus infections in pediatric liver transplantation. Am. J. Dis. Child. 144:1307-1310.
14. Baskar, J. F., et al. 1983. Cytomegalovirus infection of murine testicular leydig cells. Infect. Immunol. 40:726.
15. Biggar, R .J., et al. 1983. Seminal fluid excretion of cytomegalovirus related to immunosuppression in homosexual men. Brit. Med. J. 286:2010.
16. Campbell, A. E. 1990. Interrelationships between human immuno-deficiency virus and cytomegalovirus. Arch. Immunol. Et Therapiae Experimen. 38:165-170.
17. Laurence, J. 1986. CMV infections in AIDS patients. Infect. Surg. 5:603-610.
18. Dieterich, D. T. and M. Rahmin. 1991. Cytomegalovirus colitis in AIDS: Presentation in 44 patients and a review of the literature. J. AIDS. 4 (Suppl. 1): S36-S41.
19. Kitler, D. P. 1991. Cytomegalovirus colitis and wasting. J. AIDS. 4 (Suppl. 1): S36-S41.
20. Hardy, W. D. 1991. Combined ganciclovir and recombinant human granulocyte-macrophage colony stimulating factor in the treatment of cytomegalovirus retinitis in AIDS patients. J. AIDS. 4 (Suppl. 1): S22-S28.
21. Petito, C. K., et al. 1986. Neuropathology of Acquired Immunode-ficiency Syndrome (AIDS): An autopsy review. J. Neuropathol. and Exp. Med. 45:635-646.
22. Meyers, J. D., P. Ljungman, and L. D. Fisher. 1990. Cytomegalovirus excretion as a predictor of cytomegalovirus diseases after marrow transplantation: Importance of cytomegalovirus viremia. J. Infect. Dis.. 1162:373-380.

23. “Procedures for decontamination of plumbing systems containing copper and/or lead azides”, Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health, Rockville, MD. 1976
24. Center for Disease Control Manual Guide: Safety Management, No. CDC-22, Atlanta, GA. April 30, 1976, “Decontamination of Laboratory Sink Drains to Remove Azide Salts.”

Warranty

These products are warranted to perform as described in their labeling and in **Millipore** literature when used in accordance with their instructions. THERE ARE NO WARRANTIES, WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND **Millipore** DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. **Millipore** sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of **Millipore** to repair or replace the products. In no event shall **Millipore** be liable for any proximate, incidental or consequential damages in connection with the products

Cat. No. 3247i

February 2009
Revision I: 40129i