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CYTOMEGALOVIRUS ANTIGEN SUBSTRATE SLIDE

NOTE: Changes highlighted

PRODUCT AVAILABILITY

The following Antigen Substrate Slides are available individually from Bion Enterprises, Ltd. (Bion):

Antigen Substrate Slides	Code No. REF
Cytomegalovirus	CM-2012
Σ Number of Tests	12-Wells

INTENDED USE

The Bion CYTOMEGALOVIRUS (CMV) ANTIGEN SUBSTRATE SLIDES may be used as the antigenic substrate in indirect fluorescent antibody assays for the qualitative and/or semi-quantitative determination of Cytomegalovirus IgG or IgM antibodies in human serum. Bion CYTOMEGALOVIRUS ANTIGEN SUBSTRATE SLIDES are intended for use as an aid in the diagnosis of primary infection, reinfection or reactivation of the latent virus and as a determination of immunological experience with CMV. This Test System is not intended for use by blood banks for blood donor screening.

SUMMARY AND EXPLANATION

CMV infections are widespread and usually asymptomatic; however, severe infections can be seen in newborns and immunocompromised individuals.[1] This virus may also persist as a latent or chronic infection.[2]

Neonatal infections can be congenital or prenatal. Ninety-five percent of congenital infections, or Cytomegalovirus Inclusion Disease (CID), are asymptomatic at birth but later may manifest neurologic abnormalities. The remaining 5% have classic CID with symptoms characterized by cerebral calcification, hepatomegaly, splenomegaly, jaundice, rash, microcephaly, pneumonia, and chorioretinitis.[3] The clinical symptoms of CID are very similar to those seen in congenital rubella, toxoplasmosis or congenital syphilis syndromes.[4,5,6,7] Perinatal infection is most often caused by exposure to the virus in the birth canal. Perinatal infected infants start excreting CMV three to twelve weeks after delivery and generally remain asymptomatic.[8]

The manifestations of symptomatic CMV infection in children and adults are similar to those of classic Epstein-Barr Virus (EBV) Infectious Mononucleosis, with fever, hepatitis, splenomegaly, lymphadenopathy, and viremia. In CMV mononucleosis syndrome, however, the heterophile is negative.[9]

CMV may be transmitted by blood transfusions and organ transplantation or reactivated by immunosuppression. There is also a high incidence of CMV in persons with acquired immune deficiency syndrome (AIDS).[10]

Individuals with primary infection of CMV or those who have had a previous experience with CMV and are experiencing a reinfection or reactivation, may be shedding infectious virus continuously or at intermittent periods and should be considered infectious to susceptible hosts.[11]

Methods for CMV antibody detection include Complement Fixation (CF), Neutralization (NT), Radioimmunoassay (RIA), Enzyme-Linked Immunosorbent Assay (ELISA), Indirect Hemagglutination (IHA), Immune-Adherence Hemagglutination (IAHA), and Immunofluorescence Assay (IFA). Antibodies detected by IHA and IFA appear within a few weeks after primary infection, whereas the CF and NT antibody responses follow 2 to 4 weeks later.[12] Of these, the CF test is least sensitive and cannot differentiate between IgG and IgM antibody classes. NT tests are technically complex and time-consuming and are usually reserved for seroepidemiologic studies. There is a lack of commercially available reagents for the IHA test. The solid phase immunoassays (IFA, EIA, and RIA) have the advantage of being sensitive, able to differentiate between the various antibody classes, and are commercially available.

PRINCIPLE OF THE IFA PROCEDURE

The Bion CYTOMEGALOVIRUS ANTIGEN SUBSTRATE SLIDES may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons[13] and further developed by Riggs, et al.[14] The procedure is carried out in two basic reaction steps:

Step 1 - Human serum is reacted with the antigen substrate. Antibodies, if present, will bind to the antigen forming stable antigen-antibody complexes. If no antibodies are present, the complexes will not be formed and serum components will be washed away.

Step 2 - Fluorescein labeled antihuman IgG (or IgM) antibody is added to the reaction site which binds with the complexes formed in step one. This results in a positive reaction of bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope. If no complexes are formed in step one, the fluorescein labeled antibody will be washed away, exhibiting a negative result.

REAGENTS

Bion CYTOMEGALOVIRUS ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped twelve well slides with CMV (clinical specimen) infected human diploid fibroblast cells (foreskin) fixed onto each well. Each well contains an average of 5 or more nuclear inclusions per 200X field.

STORAGE AND STABILITY

The Bion CYTOMEGALOVIRUS ANTIGEN SUBSTRATE SLIDES are stable in sealed foil pouches at 8°C or lower until labeled expiration date.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of in vitro diagnsotics may perform the test. IVD
2. Substrate slides are for single use only and must not be used more than once.
3. Use with non-Bion reagents could result in erroneous results.
4. Care should be taken when handling substrate slides due to sharp edges.
5. The antigenic substrates have been fixed in acetone and contain no detectable live Cytomegalovirus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
6. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
7. Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
8. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
9. Refrigeration (2-8°C) of antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
10. Antigen substrate slides should not be used beyond stated expiration date.
11. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
12. Incubation times or temperatures other than those specified may give erroneous results.
13. Reusable glassware must be washed and thoroughly rinsed free of detergents.
14. Care should be taken to avoid splashing or generation of aerosols.
15. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera is freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
16. Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membranes.

SPECIMEN COLLECTION

Blood should be collected fasting or at least one hour after meals to avoid lipemic serum, as excess lipids may produce a "film" over the substrate. Aseptically collect 5-8 ml of blood by venipuncture. Allow the blood to clot at room temperature (20-25°C) before separating serum to avoid hemolysis which could interfere with test results. Specimens should be stored refrigerated at 2-8°C and tested within one week of collection. Long term storage should be at -20°C in aliquots to avoid repeated freezing and thawing. Do not store in self-defrosting freezer.

Avoid using contaminated sera as they may contain proteolytic enzymes which will digest the substrate. It is unnecessary to heat inactivate serum specimens prior to testing; however, sera that have been heat inactivated may be used.

When testing paired samples to look for evidence of recent infection, the acute specimen should be obtained as soon as possible after onset of illness and the convalescent specimen obtained 7-14 days later. Acute and convalescent specimens must be tested simultaneously, in the same assay, looking for a significant change in antibody titer between the paired sera. If the first specimen is obtained too late during the course of the infection, a significant rise in the antibody titer may not be detected.

PROCEDURE

Detailed descriptions of indirect immunofluorescence techniques may be found in the references listed in the bibliography.[15,16,17]

MATERIALS PROVIDED

Bion CYTOMEGALOVIRUS ANTIGEN SUBSTRATE SLIDES.
Lot Number provided on label. LOT

MATERIALS AVAILABLE FROM Bion

1. Fluorescent Antibody Conjugate with 0.01% Evans Blue counterstain
2. Cytomegalovirus Positive Human Control Serum
3. Cytomegalovirus Negative Human Control Serum
4. Phosphate Buffered Saline (PBS)
5. Mounting Medium

MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable test tubes (12 x 75 mm or comparable) and rack
2. Disposable serological pipettes
3. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl with disposable pipette tips
4. Pasteur pipettes and bulbs
5. Moist chambers
6. Plastic squeeze wash bottle
7. Coplin jars or staining dishes with slide racks
8. 24 x 60 mm #1 coverslips
9. Felt tip marking pen
10. Fluorescence microscope equipped with mercury or tungsten-halogen light source, a 390-490nm excitation filter and 515-520nm barrier filter, and optics to give a total magnification of 200X or 250X.The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

1. SPECIMEN PREPARATION

Screening:

Each laboratory should establish its own protocol for the preparation of serum screening dilutions. Most indirect fluorescent antibody staining procedures utilize a 1:10 dilution of each patient's serum which is prepared by adding 0.05 ml (50 µl) of the patient's serum to 0.45 ml of PBS.

NOTE: If testing for IgM specific antibodies using an IgM specific fluorochrome conjugate, each patient serum specimen must be pre-treated to remove any IgG interference by separating the IgM from the IgG, and then running the screening test on the IgM eluate. Suggested methodologies are ion exchange chromatography,[18] or IgG immunoprecipitation.[19,20]

Semi-quantitation:

Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering protocol. The selection of either twofold or fourfold dilution procedures depends upon the experience level and training of the individual(s) reading the fluorescent antibody assay.

The following fourfold serial titration is suggested for IgG testing:

a. Prepare a 1:10 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 0.45 ml of PBS in tube #1.
b. Add 0.3 ml PBS to tubes #2, #3, #4, and #5.
c. Using a 100 µl pipette, transfer 0.1 ml (100 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.1 ml (100 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

The following twofold titration is suggested for IgM testing:

a. Prepare a 1:10 dilution of each patient's serum using one of the treatment methodologies mentioned in the "Screening NOTE" above. This will be designated as tube #1.
b. Add 0.2 ml PBS to tubes #2, #3, #4, and #5.
c. Using a 200 µl pipette, transfer 0.2 ml (200 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.2 ml (200 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

These titrations will have the following dilutions:

Fourfold	Twofold
Tube #1 = 1:10	Tube #1 = 1:10
Tube #2 = 1:40	Tube #2 = 1:20
Tube #3 = 1:160	Tube #3 = 1:40
Tube #4 = 1:640	Tube #4 = 1:80
Tube #5 = 1:2560	Tube #5 = 1:160

2. SLIDE PREPARATION

Remove reagents and as many slides as are required from the refrigerator or freezer and allow to equilibrate to room temperature (20-25°C) for at least five minutes. Remove slides from sealed foil pouches being careful not to touch the antigen surface. Identify each slide using a felt tip marking pen.

3. SPECIMEN APPLICATION

Using separate Pasteur pipettes, apply one drop (20-30 µl) of the positive control, one drop (20-30 µl) of the negative control and one drop (20-30 µl) of each patient serum dilution to individual wells of the slide. Do not touch the antigen surface with the pipette while dropping. Do not allow drops to mix, as cross contamination of samples between wells could cause erroneous results.

4. INCUBATION 1

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. THE ANTIGEN MUST NOT BE ALLOWED TO DRY DURING ANY OF THE FOLLOWING STEPS. Nonspecific binding may occur if the reagent is allowed to dry on the slide.

NOTE: For IgM testing, incubate the substrate slides in a moist chamber at 35-37°C for 90 minutes.

5. RINSE 1

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. Do not focus the PBS stream directly onto the wells. To prevent cross contamination tilt slide first toward wells 1-6 and, running a PBS stream along the midline of the slide, allow the PBS to run off the top edge of the slide. Then, tilt the slide toward wells 7-12 and repeat this procedure, allowing the PBS to run off the bottom edge of the slide.

6. WASH 1

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

7. CONJUGATE APPLICATION

Remove slides from the wash one at a time, shake off excess PBS, dry around outside edges if necessary and return each slide to the moist chamber. Apply one drop of an appropriate fluorescent antibody (IgG or IgM) conjugate with counterstain (diluted to its predetermined proper working dilution) to each well of each slide, making sure that each well is completely covered.

8. INCUBATION 2

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. Protect slides from excessive light.

NOTE: For IgM testing, incubate in a moist chamber at 35-37°C for 60 minutes.

9. RINSE 2

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. As suggested in step 5., do not focus PBS stream directly onto the wells.

10. WASH 2

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

11. COVERSLIP

Remove slides one at a time from the last PBS wash, shake off excess PBS and immediately add two to four drops of mounting medium across the slide. Tilt slide and rest the edge of the coverslip against the bottom of the slide allowing the mounting medium to form a continuous bead between the coverslip and slide. Gently lower the coverslip from the bottom of the slide to the top, being careful to avoid air bubbles. Drain excess mounting medium by holding the edge of the slide against absorbent paper. Wipe off back of slide.

12. READ

Examine stained slides as soon as possible using a properly equipped fluorescence microscope. It is recommended that slides be examined on the same day they are stained. If any delay is anticipated, store slides in the refrigerator (2-8°C) away from direct light and read the following day. Do not allow mounting medium to dry between slide and coverslip. If drying should occur, add additional mounting medium or recoverslip slide.

FLUORESCENT INTENSITY GRADING

Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia:[21]

4+ = Maximal fluorescence; brilliant yellow-green.
3+ = Less brilliant yellow-green fluorescence.
2+ = Definite but dull yellow-green fluorescence.
1+ = Very dim subdued fluorescence.

The degree of fluorescent intensity is not clinically relevant and has only limited value as an indicator of titer. Differences in fluorescence microscope optics, filters and light sources may result in differences of 1+ or more fluorescent intensity when observing the same slide using different microscopes.

QUALITY CONTROL

SPECIFICITY CONTROL

Both a positive and negative antibody control must be included with each run. These controls must be examined prior to reading test samples and should demonstrate the following results:

Negative Control:
Using a negative control serum on Bion CYTOMEGALOVIRUS ANTIGEN SUBSTRATE SLIDES, the infected cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

NOTE: CMV infection of an *in vitro* cell culture induces Fc-IgG receptors in the cytoplasm of infected cells. IgG antibody from the negative control attaches to these Fc receptor sites which then react with the antihuman IgG conjugate and appear as fluorescent perinuclear cytoplasmic inclusions in these cells.[22] This type of staining should be interpreted as negative for CMV antibodies.

Positive Control:
Using a positive control serum on Bion CYTOMEGALOVIRUS ANTIGEN SUBSTRATE SLIDES, the infected cells should exhibit well defined specific fluorescent staining of five or more nuclear inclusions per 200X field at an intensity of 3+ or greater. The remainder of the cells stain reddish-orange due to the counterstain.

Each control must demonstrate the expected reaction in order to validate the test. If the controls fail to appear as described above, the test results should not be reported and the test should be repeated. If upon repeat testing the controls still fail to show the proper reaction, do not report test results.

SENSITIVITY CONTROL

A titered control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of this control must be determined and there must not be more than a twofold difference (+/-) in titer from this determined endpoint. Each run should include the endpoint dilution, one twofold or fourfold dilution above and one twofold or fourfold dilution below the endpoint dilution. The more concentrated dilution should be positive and the less concentrated dilution negative. If the control does not behave as described, the test results are invalid and the tests should be repeated. If the control again fails to show the proper reaction upon repeat testing, do not report the test results.

READING OF TEST RESULTS

NEGATIVE

A serum dilution is considered to be negative for CMV antibodies if the cells exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain, or if the fluorescence observed is not the specific staining pattern of CMV.

A sample is considered negative for CMV antibodies if it exhibits less than 1+ fluorescence at a serum dilution of 1:10 and all greater dilutions, or if the fluorescence observed is not the specific staining pattern of CMV.

... Negative samples may exhibit fluorescent staining of the infected cells slightly greater than the negative control, but less than 1+.

... Nonspecific staining of all cells observed in some sera at low dilutions is most likely due to the presence of autoantibodies against cellular components in either the nucleus or cytoplasm.

... Staining of areas other than the viral infected cells should be interpreted as negative and attention should be directed to specific steps in the staining method (e.g., RINSE and WASH steps).

NOTE: CMV Infection of *in vitro* cell cultures can induce Fc-IgG receptors in the cytoplasm of infected cells which inexperienced personnel may interpret falsely as positive readings when doing IgG assays. IgG antibody from the patient attaches to these Fc receptor sites which then react with antihuman IgG conjugate and appear as fluorescent perinuclear cytoplasmic inclusions just outside the nuclear membrane of these cells. This can be differentiated from the specific nuclear inclusions of CMV fluorescent staining.[23] The perinuclear Fc-IgG receptor site staining should be interpreted as negative for CMV antibodies.

POSITIVE

A serum dilution is considered positive for CMV antibodies if fluorescent staining of well defined nuclear inclusions is observed in five or more of the cells per 200X field at an intensity of 1+ or greater, with the remainder of the cells staining reddish-orange due to the counterstain. The number of cells exhibiting a positive staining reaction and the type of fluorescent staining pattern should closely approximate that seen in the positive control.

A sample is considered positive for CMV antibodies if it exhibits the characteristic CMV staining pattern with a fluorescent intensity of 1+ or greater at a serum dilution of 1:10 or greater.

NOTE: Each field should contain cells that exhibit no apple-green fluorescence. Should most of the cells in the patient test wells fluoresce apple-green in the nucleus and/or cytoplasm, an autoimmune staining reaction due to the presence of autoantibodies should be considered.[24,25] It is recommended that such samples be diluted beyond the interference for better interpretation. It is possible that autoantibody staining may mask specific staining such that an interpretation cannot be made. Should this occur, test results should be reported as "Unable to interpret due to the presence of interfering antibodies."

TITRATION

If a semi-quantitative titration is performed, the result should be reported as the reciprocal of the last dilution in which 1+ apple-green fluorescent intensity of the specific staining pattern is detected. When reading fourfold serial dilutions, endpoints can be extrapolated where necessary.

EXAMPLE OF ENDPOINT EXTRAPOLATION:
1:10 = 4+
1:40 = 3+
1:160 = 2+
1:640 = +/-

The extrapolated endpoint is reported as 320.

TROUBLESHOOTING

Possible solutions to problems that may occur in immunofluorescent assays are discussed in an accompanying brochure entitled "TROUBLESHOOTING IN IMMUNOFLUORESCENCE".

INTERPRETATION OF RESULTS

Detection of the presence of Cytomegalovirus antibodies indicates a current or previous infection with CMV. A significant (fourfold or greater) increase in titer between acute and convalescent serum samples and/or a positive test for IgM specific antibodies usually indicates recent or active infection be it a primary infection, reinfection or reactivation of latent virus.

NOTE:
1. Antibodies to CMV do not confirm immune status but only indicate previous exposure.
2. Individuals who have had a previous experience with CMV or are experiencing reinfection, reactivation or primary infection, may be shedding infectious virus continuously or at intermittent periods and should be considered infectious to susceptible hosts.[11]

LIMITATIONS OF THE PROCEDURE

1. CMV antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.
2. A single serological IgG antibody titer to CMV should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific CMV antibodies may provide more meaningful data.
3. A negative test result does not necessarily rule out current or recent infection. The specimen may have been collected too early in the disease before demonstrable antibody is present.
4. Lack of significant rise in titer does not exclude the possibility of recent infection but may indicate an acute phase specimen was obtained too late.
5. In some instances, high IgG or IgM antibody levels in the first of paired specimens may prevent the detection of increases in total antibody, resulting in apparently stationary total antibody titer.
6. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
7. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
8. Antinuclear antibodies (ANA) present in serum may interfere with the CMV IFA test. They can be differentiated from CMV staining in that ANAs stain the nuclei in all cells; whereas, CMV antibodies exhibit nuclear inclusion staining in an average of only five to fifteen cells per 200X field.[24]
9. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.[25] They can be differentiated from the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, CMV antibodies exhibit staining in only an average of five to fifteen cells per 200X field.
10. Positive test results from cord blood or neonates should be interpreted with caution. The presence of CMV IgG antibodies in cord blood is usually the result of passive transfer from mother to the fetus. A negative test, however, may be useful in excluding possible infection. 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, preferably within the first five days of life.[26] The method of choice to diagnose CMV infection is viral isolation.[27]
11. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, IgG/IgM separation methods, as well as the experience level of personnel performing the assay.
12. If both the positive and negative control substrate cells are not visible when viewed using the fluorescence microscope, it may be necessary to replace or realign the light source and check the specific filters.
13. Cell culture substrate slides may exhibit nonspecific fluorescence due to contamination of antibodies or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.
14. If testing for IgM specific antibodies, the presence of Rheumatoid Factor (RF) in serum may cause a false positive reaction if pathogen specific IgG is also present. Routine RF tests may not be sensitive enough to detect small amounts of RF which exist within the normal range, but are sufficient to cause a false positive reaction in the more sensitive IFA technique.[30] Therefore, all sera should be treated by ion exchange chromatography[18] or IgG immunoprecipitation[19,20] before testing to eliminate possible RF interference.
15. If testing for IgM specific antibodies, high titers of specific IgG when present in the patient serum may compete with the pathogen specific IgM for the antigen sites resulting in a false negative IgM reaction.[30] Therefore, all sera should be treated by ion exchange chromatography[18] or IgG immunoprecipitation[19,20] before testing to avoid this possible problem.
16. Two methods such as immunoprecipitation and ion exchange chromatography have been commonly used for neutralizing or removing possibly interfering IgG antibodies prior to testing for specific IgM antibodies in IFA tests. Immunoprecipitation neutralizes all classes of IgG while not affecting the IgM levels; however, high levels of IgG may need to be treated with proportionally increased amounts of the precipitating reagent. Ion exchange chromatography will only eliminate IgG subclasses 1, 2 and 3 with subclass 4 (usually less than 5% of the total IgG) remaining in the fraction with the IgM. Also, only a portion of the IgM antibodies can be recovered.[29]

SPECIFIC LIMITATIONS OF THE CMV ASSAY

1. Presence or absence of active infection with CMV can only be inferred from serologic data and should be confirmed by viral isolation whenever possible.
2. CMV antibody tests should not be used by themselves for the diagnosis of current CMV infection in pregnant women. The presence of Cytomegalovirus should be demonstrated by direct viral isolation methods.
3. The presence of IgG or total antibody does not imply protection from disease.
4. CMV induces Fc-IgG receptors in the cytoplasm of infected cells which inexperienced personnel may interpret falsely as a positive reading when doing IgG assays. Anti-human IgG conjugate reacts with the patient IgG which is attached to the Fc-IgG receptor sites. This can be differentiated from the specific CMV fluorescent staining in that "receptor site" staining is outside the nuclear membrane of the cells; whereas, CMV staining is in the form of nuclear inclusions.[22] This type of staining should be interpreted as negative.

EXPECTED VALUES

The prevalence of antibody to CMV in any given population has been shown to be highly dependent on age and socioeconomic status. In the United States, at least 70% to 85% of adults from the low socioeconomic sector have CMV antibodies. CMV infection early in life is common among this group, with the peak period being the first year of life. At least 50% to 60% of adults from middle-class background have antibodies to CMV. After early childhood, the rate of acquisition of CMV is approximately 1% per year in the middle-class population of the United States.[28]

Approximately 45% of women of middle-class and 18% of women of lower socioeconomic background are susceptible to CMV.[28] If exposure is suspected, a specimen should be taken as soon after exposure as possible. Absence of detectable antibody indicates susceptibility. A convalescent specimen must be run simultaneously with the first specimen to determine whether or not infection, apparent or inapparent, has resulted from exposure.

SPECIFIC PERFORMANCE CHARACTERISTICS

Bion CMV ANTIGEN SUBSTRATE SLIDES have been evaluated for the presence of specific CMV antigen using commercial monoclonal antisera. In each case, positive reactions were identified with the CMV infected cell cultures when stained with its corresponding antisera. Also, there was no cross-reactivity with other specific viral or chlamydial monoclonal antisera and the CMV antigen.[29] Each laboratory should determine its own performance characteristics using all reagents assembled to perform the IFA test.

As the Cytomegalovirus is a member of the Herpesvirus Group, a study was performed to insure there were no cross-reactions between IgG antibodies to the other members of this group and the Cytomegalovirus antigen on the BION CMV substrate slide. Eight serum specimens with IgG antibodies to Epstein-Barr Virus (EBV), Herpes Simplex Type 1 (HSV 1), Herpes Simplex Type 2 (HSV 2) and/or Varicella Zoster Virus (VZV) were tested using the BION CMV substrate slides. All eight specimens were negative on the BION CMV substrate. Therefore, false positive reactions for CMV antibody will most likely not be obtained when exposed to the other Herpes Group Viruses. The data summary is presented in TABLE 1. Results reflect test results at screening dilutions of 1:10.[29]

TABLE 1 – Summary of Herpes Group IgG Specificity Study

Spec. #	CMV	EBV	HSV 1	HSV 2	VZV
1	<10	3-4+	+/-	<10	2-3+
2	<10	3-4+	4+	3-4+	3+
3	<10	3-4+	4+	3-4+	3-4+
4	<10	3-4+	<10	<10	3-4+
5	<10	3-4+	<10	<10	2+
6	<10	3+	<10	<10	3-4+
7	<10	3+	<10	<10	3-4+
8	<10	3-4+	3-4+	3-4+	1-2+

In addition, a cross-reactivity study of IgM tests done by IFA was conducted to insure that IgM antibodies to one virus would exhibit no specificity for other antigen substrates. A total of 40 serum specimens were treated to separate IgG and IgM and then tested for IgM. Ten specimens had IgM antibodies to EBV; ten specimens had IgM antibodies to HSV; ten specimens had IgM antibodies to CMV, and ten specimens had IgM antibodies to VZV. These 40 specimens were then tested against all five antigen substrates.

As shown in TABLE 2, there did not appear to be any specific pattern of cross-reactions between antibodies of any one virus against the other four antigens except between HSV types 1 and 2. There was some other multiple positive results. Some with comparatively low titers (*) may be of little or questionable significance while others had higher titers (**) which may be of importance.[29]

TABLE 2 – Summary of Herpes Group IgM Specificity Study

# of Spec.	EBV	HSV 1	HSV 2	CMV	VZV
9	160-1280	<10	<10	<10	<10
1	1280	<10	<10	10*	<10
10	<10	80-320	20-160	<10	<10
9	<10	<10	<10	10-160	<10
1	80**	<10	<10	640	<10
7	<10	<10	<10	<10	40-320
1	>80**	20*	20*	<10	640
1	<10	20*	20*	<10	80
1	20*	<10	<10	<10	80

Several limitations must be kept in mind when interpreting IgM serology tests. This is true even after separation treatment of IgG and IgM eliminates the problems of Rheumatoid Factors and IgG competitive binding. IgM specific antibody responses can occur not only with primary infection but can be attributed to reactivation or reinfection particularly in CMV, HSV, EBV and VZV infections.[27,30] It is also well known that dual infections can occur and have been reported between several members of the Herpesvirus Group.[10,31,32,33] In addition, with some viruses such as CMV and EBV, IgM specific antibodies may continue for many months. Thus, a person may have residual IgM from one infection and become infected with a different agent.[27,30] It is not uncommon for one childhood infection to follow closely after another. Also, an infection with a new agent may cause an anamnestic response in IgM from the recent previous infection.[34]

Sometimes requesting a convalescent specimen looking for a change in antibody levels between paired specimens may help to clarify the actual current infection from the previous past infection.

IgM serology performed by IFA is very attractive since it combines specificity with sensitivity and in most cases only a single serum sample is required. However, the need for careful interpretation of the significance of positive IgM tests in relationship to patients' clinical situations must be emphasized. It is essential to have an awareness of understanding of the many problems associated with IgM testing to avoid the many pitfalls that can trap the most experienced of workers. IgM results must always be interpreted with caution.[29]

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