
















CMV - G ANTIBODY TEST SYSTEM

NOTE: Changes highlighted

SYMBOL DEFINITIONS

 = Consult Directions for Use	 = <i>In Vitro</i> Diagnostic Reagent
 = Store Away From Direct Light	 = Positive Control
 = Storage Temperature	 = Negative Control
 = Expiration Date	 = Endpoint Titer
 = Number of Tests	 = Code Number
 = Amount	 = Lot Number
 = Single Use Only	 = Contains biological material of animal origin
 = Contains human blood or plasma derivatives	

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.

Bion CMV-G KITS AND REAGENTS

<u>KITS and KIT COMPONENTS</u>	<u>CODE NO.</u>
CMV-G (CMV IgG Antibody) 120 Test Kit	CMG-120
CMV Substrate Slide, twelve wells	CM-2012
CMV IgG Positive Control Serum, 0.5 ml	CMG-2020
CMV Negative Control Serum, 0.5 ml	CMN-2010
Conjugate, IgG with Counterstain, 3.5 ml	CCG-9972
Mounting Medium, 3.5 ml	MM-9985
PBS Packet, One Liter	PBS-9990

INTENDED USE

The Bion CMV-G (Cytomegalovirus) ANTIBODY TEST SYSTEM is an indirect fluorescent antibody assay for the qualitative and/or semi-quantitative determination of CMV IgG antibodies in human serum. The Bion CMV-G ANTIBODY TEST SYSTEM is intended for use as an aid in the diagnosis of primary infection, reinfection or reactivation of the latent virus, and also as a determination of immunological experience with CMV. This Test System is not intended for use by blood banks for blood donor screening.

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 Enzyme-Linked Immunosorbent Assay (ELISA) and Immunofluorescence Assay (IFA). Antibodies detected by IFA appear within a few weeks after primary infection. The solid phase immunoassays (IFA and EIA) 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 CMV-G ANTIBODY TEST SYSTEM utilizes the indirect fluorescent antibody assay method first described by Weller and Coons¹² and further developed by Riggs, et al.¹³ 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 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

CMV ANTIGEN SUBSTRATE SLIDES

Ten 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. Stable in sealed foil pouch at 8°C, or lower, until labeled expiration date.

POSITIVE CONTROL SERUM

One vial containing 0.5 ml CMV positive IgG human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

When used undiluted as provided, specific fluorescent intensity of 3+ or greater should be seen. Optionally, the positive control can be titrated to endpoint. If titrated, the control should be serially diluted in PBS. When the control has been tested for the endpoint titer by Bion, an endpoint titer is printed on the positive control vial. Due to variations within each laboratory (fluorescent microscope, etc.), each laboratory should establish its own mean titer for each lot of positive control (generally \pm one dilution from stated endpoint).

NEGATIVE CONTROL SERUM

One vial containing 0.5 ml CMV negative human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

The control is intended to be used undiluted as provided. The staining should exhibit less than 1+ fluorescence.

MOUNTING MEDIUM

One dropper vial containing 3.5 ml phosphate buffered glycerol of pH 7.4 \pm 0.2. Stable at 2-8°C until labeled expiration date.

FLUORESCENT ANTIBODY CONJUGATE

Two ready to use dropper vials, each containing 3.5 ml fluorescein isothiocyanate labeled goat antihuman IgG (heavy chain specific) with 0.01% Evans Blue counterstain, protein stabilizer, less than 0.1% sodium azide and 0.001% thimerosal added. Stable at 2-8°C away from direct light until labeled expiration date.

PHOSPHATE BUFFERED SALINE (PBS)

Two one-liter packets of dry PBS. Stable in sealed packet at 25°C, or lower, until labeled expiration date.

BUFFER PREPARATION

Place contents of a one-liter PBS packet in a one-liter volumetric flask, add *distilled water to the one-liter mark, mix and leave several hours or overnight to dissolve. Reconstituted buffer should have a pH of 7.4 \pm 0.2. Adjust with 1N NaOH or 1N HCL if pH value is outside the stated range. Store in a clean screw capped bottle at 25°C or lower. Stable until labeled expiration date provided no gross contamination is seen. Do not use if pH changes, if the solution turns cloudy, or if a precipitate forms.

* Use deionized water with caution, as pH of this type of water may vary causing the pH of PBS to become unstable upon prolonged storage.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of *in vitro* diagnostics may perform the test.
2. Substrate slides are for single use only and must not be used more than once.
3. Care should be taken when handling substrate slides due to sharp edges.
4. The antigenic substrates have been fixed in acetone and contain no detectable live CMV. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
5. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
6. All reagents should be brought to room temperature (20-25°C) prior to use.
7. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
8. Refrigeration (2-8°C) of kit immediately upon arrival will insure stability until labeled expiration date.
9. Reagents should not be used beyond stated expiration date.
10. Substitution of components other than those provided may yield inconsistent results.
11. Do not expose conjugate to strong light during storage or use.
12. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
13. Incubation times or temperatures other than those specified may give erroneous results.
14. Reusable glassware must be washed and thoroughly rinsed free of detergents.
15. Care should be taken to avoid splashing and generation of aerosols.
16. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
17. 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.

WARNINGS AND PRECAUTIONS (continued)

16. Sera used to prepare positive and negative controls have been tested by an FDA approved method and found to be negative (were not repeatedly reactive) for the presence of Hepatitis B surface Antigen (HBsAg) and antibodies to Hepatitis C (HepCAb) and HIV 1 & 2. However, because no test method can offer complete assurance of the absence to these or other infectious agents, these reagents 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.
17. The preservatives used in conjugates and controls are toxic if ingested. Azides may react with copper or lead plumbing to form explosive metal azides. When disposing, flush drains with water to minimize build-up of azide and metal compounds.

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

MATERIALS PROVIDED

1. CMV Antigen Substrate Slides
2. Fluorescent Antibody Conjugate
3. Positive Control Serum
4. Negative Control Serum
5. Phosphate Buffered Saline (PBS)
6. Mounting Medium

MATERIALS REQUIRED BUT NOT PROVIDED

1. One liter volumetric flask or one liter graduated cylinder
2. Distilled water - CAP Type one or equivalent
3. One liter screw capped container
4. Disposable test tubes (12 x 75 mm or comparable) and rack
5. Disposable serological pipettes
6. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl, with disposable pipette tips
7. Pasteur pipettes and bulbs
8. Moist chambers
9. Plastic squeeze wash bottle
10. Coplin jars or staining dishes with slide racks
11. 24 x 60 mm #1 coverslips
12. Felt tip marking pen
13. Fluorescence microscope equipped with a 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 and/or 400X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

1. SPECIMEN PREPARATION

Screening:

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.

Semi-quantitation:

Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titrating protocol. The following fourfold serial titration is suggested:

- 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. This will give a fourfold titration with the following dilutions:

Tube #1 = 1:10
Tube #2 = 1:40
Tube #3 = 1:160
Tube #4 = 1:640
Tube #5 = 1:2560

TEST PROCEDURE (continued)

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.

NOTE: 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.

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 conjugate to each well of each slide, making sure 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.

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 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:¹⁴

- 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 the Bion CMV NEGATIVE CONTROL SERUM as provided with the Bion CMV-G ANTIBODY TEST SYSTEM, 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.¹⁶ This type of staining should be interpreted as negative for CMV antibodies.

Positive Control

Using the Bion CMV POSITIVE IgG CONTROL SERUM as provided with the Bion CMV-G ANTIBODY TEST SYSTEM, each field 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 titrated control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of each lot of Bion CMV POSITIVE IgG CONTROL SERUM must be determined.

There must not be more than a twofold difference (+/-) in titer from the stated endpoint. Each run should include the endpoint dilution, one fourfold dilution above and one 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 negative for CMV IgG 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 nuclear inclusions.

A sample is considered negative for CMV IgG 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.¹⁵ The perinuclear Fc-IgG receptor site staining should be interpreted as negative for CMV antibodies.

POSITIVE

A serum dilution is considered positive for CMV IgG antibodies if, at an intensity of 1+ or greater, there is fluorescent staining of well defined nuclear inclusions in five or more of the cells per 200X field 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 IgG 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.^{16,17} 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

RESULT	INTERPRETATION OF SINGLE SAMPLE RESULTS
Less than 10	Negative - Indicates no previous infection with CMV and susceptibility to this agent. NOTE: This may represent a primary infection with the humoral immune response not yet developed to detectable levels. If infection with CMV is still suspected, a second specimen should be obtained 7-14 days later, and the paired specimens tested simultaneously, looking for a seroconversion.
10 or Greater	Positive for CMV antibodies. This may represent: <ol style="list-style-type: none"> 1. a primary infection, reinfection, or reactivation of latent virus; 2. a previous experience with CMV; 3. a passively acquired antibody from recent blood transfusions, organ transplantation, or transplacental transfer.

- NOTES:**
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 a reinfection, reactivation or primary infection, may be shedding infectious virus continuously or at intermittent periods and should be considered infectious to susceptible hosts.¹⁸
 3. The titer of a single specimen should not be the only criteria used to aid in the diagnosis of CMV infections (primary infection, reinfection, or reactivation of latent virus). Paired samples (acute and convalescent) must be collected and tested simultaneously in the same assay to look for a seroconversion or significant rise in titer.
 4. Testing for IgM specific CMV antibodies may help to confirm a diagnosis of active CMV infection when only a single specimen is available or in prenatal cases.¹⁹

ACUTE RESULT	CONVALESCENT RESULT	INTERPRETATION OF PAIRED SAMPLE RESULTS
Less than 10	Less than 10	Not likely to be an acute CMV infection. NOTE: This may represent a primary infection if time of obtaining the second specimen is too soon after the first. If this condition is suspected, obtain a third specimen 7-14 days after the second specimen and run the three simultaneously, looking for a seroconversion.
Less than 10	10 or Greater	Most likely a primary infection with CMV unless the individual has recently acquired passive antibody.
10 or Greater	10 or Greater but with less than a fourfold difference in titer from the acute specimen	Not significant evidence of current infection. Most likely a previous experience with CMV. This may represent: <ol style="list-style-type: none"> 1. a primary infection, reinfection, or reactivation of latent virus, but the interval between the first and second specimens may not have been long enough for development of a fourfold rise in antibody titer. If this condition is suspected, obtain a third specimen 7-14 days after the second specimen and run the three simultaneously looking for a significant rise in antibody titer; 2. a primary infection, reinfection, or reactivation of latent virus if first specimen is obtained late after onset and antibodies have already reached a plateau; 3. a passively acquired antibody from transplacental transfer, blood transfusion, etc. NOTE: Testing for IgM specific CMV antibodies may help to confirm a diagnosis of active CMV infection when there is less than a fourfold difference in titer between the acute and convalescent specimens.
10 or Greater	10 or Greater with a fourfold or more difference in titer from the acute specimen	Usually indicates an active or recently active CMV infection, be it a primary infection, reinfection, or a reactivation of a latent virus.

LIMITATIONS OF THE PROCEDURE

1. CMV IgG 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. 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. The method of choice to diagnose CMV infection is viral isolation.¹⁹
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.¹⁶
9. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochochondrial antibodies (AMA) often seen in primary biliary cirrhosis.¹⁷ 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. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, as well as the experience level of personnel performing the assay.
11. 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.
12. 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.

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 performing IgG assays. Antihuman 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.¹⁵ 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.²⁰

Approximately 45% of women of middle-class and 18% of women of lower socioeconomic background are susceptible to CMV.²⁰ 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.

A study of 43 normal sera from the Mid-Atlantic United States were tested using the Bion CMV-G ANTIBODY TEST SYSTEM. Antibodies to CMV were found in 27 (62.8%) sera and no antibodies were found in 16 (37.2%) sera.^{21,22}

SPECIFIC PERFORMANCE CHARACTERISTICS

To investigate the relative specificity and sensitivity of the Bion CMV-G ANTIBODY TEST SYSTEM, one hundred eighteen serum specimens were compared qualitatively and fifteen serum specimens were compared semi-quantitatively with another commercially available indirect fluorescent CMV IgG antibody test system.

As summarized in TABLE 1, there was 95% overall agreement between the two systems. Four specimens were considered to be greater than 1:10 (positive) but less than 1:20 on the BION Test System; whereas, on the other test system, three of these specimens were considered greater than 1:16 (positive) by one reader and all four specimens were considered less than 1:16 (negative) by another reader.

In addition, two specimens were considered less than 1:10 (negative) on the BION Test System and greater than 1:16 (positive) on the other system.^{21,22}

TABLE 1 - SUMMARY OF RELATIVE COMPARISON TESTING

		BION KIT		Relative Sensitivity	Relative Specificity
		Positive	Negative		
OTHER KIT	Positive	73	2	73/75	
	Negative	4(3+/-)	39		39/43

SPECIFIC PERFORMANCE CHARACTERISTICS (continued)

In addition, fifteen serum specimens were titrated for IgG antibodies to CMV on both the Bion CMV-G ANTIBODY TEST SYSTEM and another commercially available CMV IgG Antibody Test System to investigate the sensitivity of the Bion CMV-G TEST SYSTEM. As summarized in TABLE 2, all fifteen specimens ranging in titer from less than 1:10 to 1:10,240 agreed with no more than one twofold difference (+/-) in titer with both systems. The tables represent the results from two independent readers. If the readers differed, both results are given.²¹

TABLE 2 - SUMMARY OF RELATIVE SENSITIVITY TESTING

Spec.#	BION	Other	Spec.#	BION	Other
1	<10	<10	9	80/160	40/80
2	<10	<10	10	640	640
3	<10	<10	11	640	320
4	10	10	12	320/640	160/320
5	20	10/20	13	2560	1280
6	80/160	80/160	14	640/1280	640
7	40	20/40	15	10,240	5120
8	160	80			

Interlot and intralot precision of the Bion CMV-G ANTIBODY TEST SYSTEM was evaluated by testing ten serum specimens (2 negative and 8 positive over a range of titers) using four different lot numbers of slides, as well as, testing them three times on slides from the same lot. There was no more than a twofold difference (+/-) in titer between any of the comparison testings, which is within the confidence limits of this methodology. None of the tests vacillated between a positive or a negative result. Summaries of this data are presented in TABLE 3 and TABLE 4.²¹

TABLE 3 - SUMMARY OF INTERLOT PRECISION

Spec.#	Lot 1	Lot 2	Lot 3	Lot 4
1	<10	<10	<10	<10
2	<10	<10	<10	<10
3	20	10	10	10/20
4	20/40	10/20	10/20	20
5	160	160	160/320	160
6	160	160	80	160
7	320	320/640	320	320
8	320/640	320/640	320/640	640
9	1280/2560	1280	1280	1280/2560
10	5120/10240	5120/10240	5120/10240	5120/10240

TABLE 4 - SUMMARY OF INTRALOT PRECISION

Spec.#	1st Test	2nd Test	3rd Test
1	<10	<10	<10
2	<10	<10	<10
3	10/20	10/20	10/20
4	20	20/40	20
5	80/160	160	80/160
6	160/320	160	160
7	320	320	320/640
8	320/640	320/640	320/640
9	1280	1280	1280
10	5120/10240	5120/10240	5120/10240

As the Cytomegalovirus is a member of the Herpes Virus 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-G ANTIBODY TEST SYSTEM 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-G ANTIBODY TEST SYSTEM. All eight specimens were negative on the Bion CMV-G 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 5. Results reflect test results at screening dilutions of 1:10.²¹

TABLE 5 - SUMMARY OF HERPES GROUP 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+

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