

Laboratory Procedure Manual

Analyte: **Cytomegalovirus (CMV) serology (IgG, IgM, and avidity)**

Matrix: Serum

Method: ELISA (ELFA)

As performed by: Dr. Sheila Dollard's Lab (Lab 116)

Contact: Dr. Sheila Dollard

Important Information for Users

Dr. Sheila Dollard's Lab (Lab 116) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
CMV_J	LBXIGG	Cytomegalovirus (IgG)
	LBXIGGA	Cytomegalovirus (IgG) avidity
	LBXIGM	Cytomegalovirus (IgM)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Cytomegalovirus (CMV) is a herpesvirus which can be a serious pathogen for infants and adults. As all herpesviruses, CMV persists in the human body and can cause recurrent infections. CMV infections are quite common with 60 to 85% of the population has been infected by age 18. 95% of all cases are asymptomatic.

Infection in pregnant women can lead to congenital infection. In most cases (95%), the neonate appears clinically normal. However, the other 5% have severe consequences such as jaundice, hepatosplenomegaly, intracerebral calcification, hydrocephalia, thrombocytopenia purpura, and ocular lesions. These infants may die soon after birth. CMV also causes severe infections in the immunocompromised (HIV positive, heart or kidney transplanted patients).

Biological diagnosis of CMV infection can be performed in three different ways:

- Direct staining of infected cells, using monoclonal antibodies conjugated with fluorescein,
- Culture and viral isolation from urine, semen, and bronchial wash specimens. Human fibroblast cells such as MRC5, WI-38, or IMR-90 are most often used for diagnostic purposes. Cytopathic effects can be seen within 1 to 3 weeks or more rapidly using a stain containing monoclonal antibodies conjugated with fluorescein.
- Serological methods are simple techniques for aiding in the diagnosis of CMV infection. Complement fixation is easy to perform but has low sensitivity. Indirect hemagglutination is more sensitive but less reproducible. Latex agglutination and ELISA are the two most commonly used methods.

The VIDAS is an automated instrument that performs an enzyme-linked fluorescent immunoassay (ELFA). The instrument controls all assay steps and assay temperature. Each CMV assay kit contains controls, standards, reagent strips, and SPR (Solid Phase Receptacle). After a sample dilution step, the sample is cycled in and out of the SPR for a specified length of time. Anti-CMV antibodies present in the specimen will bind to the purified CMV antigen coating the interior of the SPR. Unbound sample components are washed away.

A monoclonal anti-human IgG conjugated with alkaline phosphatase is cycled in and out of the SPR and will attach to any human IgG bound to the SPR wall. A final wash step removes unbound conjugate.

A fluorescent substrate, 4-methylumbelliferyl phosphate, is introduced into the SPR. Enzyme remaining on the wall of the SPR will catalyze the conversion of the substrate to the fluorescent product, 4-methylumbelliferone (450 nm). The intensity of the fluorescence is measured by the optical scanner in the instrument; it is proportional to the quantity of CMV IgG found in the sample.

When the VIDAS CMV Assay is completed, the results are analyzed automatically by the computer. The quantity of anti-CMV IgG present in the sample is calculated in reference to a calibration curve stored in the instrument. A report is printed for each sample.

2. SAFETY PRECAUTIONS

The 6.6% diethanolamine causes serious eye damage. If it gets in your eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to

do and continue rinsing. Recommended personal protective equipment (PPE) are as follows: wear protective glove, protective clothing, eye protection, and face protection.

CMV assay's are for in Vitro Diagnostic Use only or professional use only.

- This kit contains products of human and animal origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions.
- Consider all patient specimens potentially infectious and observe routine biosafety precautions. Though the CMV virus coating the inside of the SPR has been inactivated, handle the SPRs as if they were infectious. Dispose of all used components and other contaminated materials by acceptable procedures for potentially biohazardous human blood products.
- Do not mix reagents or disposables from different lots.
- Kit reagents contain 0.1% sodium azide which could react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- Powderless gloves are recommended as powder has been reported as a cause of false results in some enzyme immunoassays.
- The reading cuvette with substrate contains an irritant agent (6.6% diethanolamine).
- Spills should be wiped thoroughly after treatment with liquid detergent and a solution of household bleach containing at least 0.5% sodium hypochlorite to inactivate infectious agents.
- The instrument should be routinely cleaned and decontaminated.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Not applicable

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

Whole blood should be collected and the serum separated by standard procedures. Samples containing particulate matter should be clarified by centrifugation or filtration prior to testing. Serum should not be heated. If specimens cannot be tested on the day of collection, they should be stored at 2-8° C in stoppered tubes for up to five days. If longer storage is required, the sera should be frozen at $-25 \pm 6^{\circ}\text{C}$. Avoid repeated cycles of freezing and thawing. Plasma has not been established for this test. Specimens with obvious microbial contamination should not be tested. Paired serum specimens should be tested concurrently.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

Biomerieux VIDAS, Biomerieux VIDAS CMVG Assay kit (30204-01), Biomerieux VIDAS CMVM Assay kit (30205-01), 1000µL pipettor, 100µL pipettor, Pipette tips, Powderless disposable gloves, Safety goggles, Lab coat

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Calibration, using the calibrator provided in the kit, must be performed upon receipt of a new lot of reagents after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit. The calibrator, identified by S1, must be tested in duplicate. The calibrator value must be within the set Relative Fluorescence Value range. If this is not the case, recalibrate.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

For CMVG:

- A. Remove necessary components from the kit and return all unused components to storage at 2 - 8°C.
- B. Allow components to reach room temperature (approximately 30 minutes).
- C. Use one "CMVG" strip and one "CMVG" SPR for each sample, control or calibrator to be tested. Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.
- D. The test is identified by the code "CMVG" on the instrument (to do so, refer to the Instrument User Manual). The calibrator must be identified by "S1", and tested in duplicate. If the positive control is to be tested, it should be identified by C1. If the negative control is to be tested, it should be identified by C2.
- E. If needed, label the "CMVG" Reagent Strips with the appropriate sample identification numbers.
- F. Mix the calibrator, control, and sera using a vortex-type mixer (for serum separated from the pellet).
- G. For this test, the calibrator, control, and sample test portion is 100 µL.
- H. Insert the "CMVG" Reagent Strips and SPRs into the appropriate position on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
- I. Initiate the assay as directed in the User Manual. All the assay steps are performed automatically by the instrument.
- J. Reclose the vials and return them to 2–8°C after pipetting.
- K. The assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
- L. Dispose of the used SPRs and strips into an appropriate recipient.

For CMVM:

- A. Remove necessary components from the kit and return all unused components to storage at 2-8°C.
- B. Allow components to reach room temperature (approximately 30 minutes).

- C. Use one "CMVM" strip and one "CMVM" SPR for each sample, control or standard to be tested. Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.
- D. The test is identified by the "CMVM" code on the instrument. The standard must be identified by "S1", and tested in duplicate. If the positive control is to be tested, it should be identified by C1. If the negative control is to be tested, it should be identified by C2.
- E. If needed, label the "CMVM" Reagent Label strips with the appropriate sample identification numbers.
- F. Mix the standard, controls, and samples using a vortextype mixer (for serum separated from the pellet).
- G. For this test, the standard, control, and sample test portion is 100 µL.
- H. Insert the "CMVM" Reagent Strips and SPRs into the appropriate position on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
- I. Initiate the assay processing as directed in the User Manual. All the assay steps are performed automatically by the instrument.
- J. Reclose the vials and return them to 2–8°C after pipetting.
- K. The assay will be completed within approximately 60 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
- L. Dispose of the used SPRs and strips into an appropriate receptacle.

9. REPORTABLE RANGE OF RESULTS

For CMVG:

Test Value Thresholds AU/mL *	Interpretation
< 4 AU/mL	Negative
≥ 4 to < 6 AU/mL	Equivocal
≥ 6 AU/mL	Positive

NOTE: AU = Arbitrary Unit.

For CMVM:

Test Value Thresholds	Interpretation
< 0.70	Negative
≥ 0.70 to < 0.90	Equivocal
≥ 0.90	Positive

For Avidity:

Test Value Thresholds	Interpretation
< 0.70	Low Avidity
0.70 to 0.80	Intermediate
> 0.80	High Avidity

10. QUALITY CONTROL (QC) PROCEDURE

One positive control and one negative control are included in each VIDAS CMV kit. These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. The instrument will only be able to check the control values if they are

identified by C1 and C2. Results cannot be validated if the control values deviate from the expected values.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Repeat test

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

For CMVG:

1. Sera collected very early in the acute stage of disease may have IgG levels < 4 AU/mL.
2. The VIDAS CMV IgG Assay demonstrates a linear dilution response to concentration.
3. Positive test results from cord blood should be interpreted with caution. The presence of total or IgG CMV antibodies in cord blood is usually the result of passive transfer from the mother to the fetus. A negative test, however, may be useful in excluding current infection, but the most definitive diagnosis of active CMV infection requires viral culture.
4. The titer of a single specimen should not be used to aid in the diagnosis of recent infection. Paired (acute and convalescent) samples should be collected and tested concurrently to look for seroconversion which may be indicative of primary or recent infection.
5. Positive test results may not be valid in persons who have received blood transfusions or other blood products within the past several months.
6. Increases in antibody level or seroconversion may indicate recent antigenic stimulation but per se are not confirmatory either of recent primary infection or of reactivation of a pre-existing latent process with active viral excretion.
7. Lack of a significant increase in antibody level does not exclude the possibility of CMV infection.
8. No cross-reactivity has been observed with the VIDAS CMV IgG Assay. However, rare heterotypic responses of cytomegalovirus antibodies have been reported in conjunction with herpes simplex virus, influenza A virus, and Mycoplasma pneumoniae. The virus causing the infection may not always demonstrate the greater rise in antibody level. Frequently, a differential diagnosis can be made on the basis of the fact that antibody to the infecting virus type is absent or at a very low titer in the acute-phase specimen, whereas antibody to the viral heterotype is already present.

For CMVM:

1. Positive test results may not be valid in persons who have received blood transfusions or other blood products within the past several months.
2. IgM responses can vary from patient to patient. A negative result in the VIDAS CMV IgM assay does not preclude the possibility of recent primary CMV infection.
3. Increases in antibody level or seroconversion may indicate recent antigenic stimulation but per se are not confirmatory either of recent primary infection or of reactivation of a pre-existing latent process with active viral excretion. Results from the VIDAS CMV IgM assay must be used in conjunction with clinical symptoms and patient history.
4. Serum samples with total IgG concentrations of ≥ 20 mg/mL may cause interference in the VIDAS CMV IgM assay due to incomplete absorption of the IgG. Samples with IgG concentrations of ≥ 20 mg/mL should not be tested in the VIDAS CMV IgM assay.

5. Use of the VIDAS CMV IgM assay in cord blood or neonatal serum samples has not been validated.

13. REFERENCE RANGES (NORMAL VALUES)

For CMVG:

Test Value Thresholds AU/mL *	Interpretation
< 4 AU/mL	Negative
≥ 4 to < 6 AU/mL	Equivocal
≥ 6 AU/mL	Positive

NOTE: AU = Arbitrary Unit.

For CMVM:

Test Value Thresholds	Interpretation
< 0.70	Negative
≥ 0.70 to < 0.90	Equivocal
≥ 0.90	Positive

For Avidity:

Test Value Thresholds	Interpretation
< 0.70	Low Avidity
0.70 to 0.80	Intermediate
> 0.80	High Avidity

14. CRITICAL CALL RESULTS ("PANIC VALUES")

None

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Stored at 2-8° C in stoppered tubes.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

None

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Not applicable

19. SUMMARY STATISTICS AND QC GRAPHS

Not applicable

REFERENCES

Biomerieux, Inc. VIDAS CMG IgG (CMVG) [REF 30204-01]. France: Biomerieux; 2016/12.
Biomerieux, Inc. VIDAS CMG IgM (CMVM) [REF 30205-01]. France: Biomerieux; 2019/02.