VIDAS® CMV IgM (CMVM)

IVD

The VIDAS® CMV IgM (CMVM) assay is intended for use on the instruments of the VIDAS family (Vitek® ImmunoDiagnostic Assay System) as an automated enzyme-linked fluorescent immunoassay (ELFA) for the qualitative detection of anti-CMV IgM antibodies in human serum. It is intended to be used as an aid in the diagnosis of cytomegalovirus infection. It is not intended for use in testing (screening) blood or plasma donors.

SUMMARY AND EXPLANATION OF THE TEST

Cytomegalovirus (CMV) is a herpesvirus which can cause primary and recurrent infections. CMV infections are common and usually asymptomatic. However, CMV can cause serious disease in infants and immunocompromised patients (1).

CMV infection in early life is either congenital or acquired. Congenital CMV infection is the most common of all congenital infections, and in 5% of infected neonates, serious neurological damage or even death can occur. Deafness is the most frequent problem associated with congenital CMV infection. Studies indicate that CMV could be responsible for approximately 12% of children with bilateral sensorineural hearing loss. Most congenital infections, however, are asymptomatic and the infant develops normally (1,2).

CMV infection can be acquired from individuals shedding the virus in saliva, urine, cervical secretions, breast milk, and other body fluids. CMV is also transmitted via blood transfusion or organ transplantation.

In immunocompromised (AIDS, cancer, or organ transplant) patients, CMV infections are frequent and can be severe. CMV is considered a significant cause of mortality for these patients (3).

Laboratory methods used to aid in the diagnosis of CMV infection include viral isolation from culture, direct staining of specimens, and serological techniques.

For viral isolation, human fibroblast cells best support the growth of CMV and are usually used. The time of appearance of CPE depends upon the amount of virus present in the specimen and ranges from 24 hours to 2 weeks or more (1).

Direct staining of infected cells will show characteristic large cells with prominent inclusions. Use of monoclonal antibodies can yield more rapid results.

Serological methods include Complement Fixation (CF), which has low sensitivity; Immune Adherance Hemagglutination assay (IHA), which is sensitive but difficult to perform; IFA, which is sensitive but requires tissue culture capability and is prone to nonspecific fluorescence, and EIA (4). EIA is easy to perform and is often the method used.

Detection of IgM antibodies to CMV can be useful in the diagnosis of current primary infection, particularly in pregnant women (5). IgM detection can be hampered by false positive results caused by rheumatoid factor and false negatives caused by concomitant IgG. These problems can be avoided by using techniques such as absorption (6).

PRINCIPLE OF THE PROCEDURE

The VIDAS CMV IgM (CMVM) assay is an enzyme-linked fluorescent immunoassay (ELFA) performed in an automated instrument. All assay steps and assay temperature are controlled by the instrument. A pipette tip-like disposable device, the Solid Phase Receptacle (SPR®), serves as the solid phase as well as the pipettor for the assay. The SPR is coated at the time of manufacture with CMV antigen (strain AD169). The VIDAS CMV IgM (CMVM) assay configuration prevents nonspecific reactions with the SPR. Reagents for the assay are in the sealed Reagent Strips.

After an IgG and rheumatoid factor absorption step, the sample is cycled in and out of the SPR for a specified length of time. Anti-CMV IgM antibodies present in the specimen will bind to the CMV antigen coating the interior of the SPR. Unbound sample components are washed away. Mouse monoclonal anti-human IgM antibodies conjugated with alkaline phosphatase are cycled in and out of the SPR and will attach to the human anti-CMV IgM 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. The intensity of the fluorescence is measured by the optical scanner in the instrument.

When the VIDAS CMV IgM (CMVM) assay is completed, the results are analyzed automatically by the instrument, a test value is generated, and a report is printed for each sample.

KIT COMPOSITION (30 tests):

30 CMVM Reagent Strips	STR	Ready to use.
30 CMVM SPRs	SPR®	Ready to use. SPRs are coated with CMV antigen (strain AD169).
CMVM Standard 1 x 1 mL	S1	Ready to use. Anti-CMV IgM in human serum* diluent, with 0.1% sodium azide.
CMVM Positive Control 1 x 0.5 mL	C1	Ready to use. Anti-CMV IgM in human serum* diluent. Contains 0.1% sodium azide. MLE data indicate the Test Value (TV) range: Control C1 (+) Test Value Range.
Negative Control 1 x 1.9 mL	C2	Ready to use. Phosphate buffer + protein stabilizer of animal origin + preservatives.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar codes printed on the box label		
1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR®

The interior of the SPR is coated during production with purified CMV antigen. Each SPR is identified by the "CMVM" code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the VIDAS CMV IgM (CMVM) Reagent Strip

Well	Reagent
1	Sample Well
2	Sample Treatment: 300 µL of IgG and rheumatoid factor absorbant with 0.1% sodium azide.
3	Sample Treatment: 600 µL of IgG and rheumatoid factor absorbant with 0.1% sodium azide.
4	Pre-wash buffer: 600 µL of phosphate buffered saline (0.01 mol/L, pH 7.2) with protein and chemical stabilizers and 0.1% sodium azide.
5	Wash buffer: 600 µL of TRIS buffered saline (0.05 mol/L, pH 7.4) with 0.1% sodium azide.
6	Conjugate: 400 µL of mouse monoclonal anti-human IgM antibodies conjugated to alkaline phosphatase with 0.1% sodium azide.
7 - 8 - 9	Wash buffer: 600 µL of TRIS buffered saline (0.05 mol/L, pH 7.4) with 0.1% sodium azide.
10	Reading cuvette with Substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine DEA* (0.62 mol/L or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µL).

* Signal Word: **DANGER**

Hazard statement

H318: Causes serious eye damage.

Precautionary statement

P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Safety Data Sheet.

MATERIALS REQUIRED BUT NOT PROVIDED

- 100 µL pipettor or disposable transfer pipette which will dispense ≥ 100 µL
- Powderless disposable gloves
- For other specific materials, refer to the Instrument User Manual.
- Instrument of the VIDAS family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- This kit contains products of human 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 (see Laboratory biosafety manual - WHO - Geneva - latest edition).
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not 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 (do not ingest , do not inhale).
- Consider all patient specimens potentially infectious and observe routine biosafety precautions. 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, flush drains with large volumes of water to avoid build-up.
- The substrate (well 10) contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- 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. See the User Manual for cleaning spills on or in the instrument. Do not place solutions containing bleach in the autoclave.
- Routinely clean and decontaminate the instrument. See the User Manual for the appropriate procedures.
- Powderless gloves are recommended because powder has been reported as a cause of false results in some enzyme immunoassays.

STORAGE AND HANDLING

- Store the VIDAS® CMV IgM (CMVM) Kit at 2-8ºC. **Do not freeze reagents.** Return unused components to 2-8ºC.
- After opening the kit, check that the SPR® pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8ºC.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMEN COLLECTION AND PREPARATION

Whole blood should be collected and the serum separated by standard procedures. Do not heat the serum. Clarify samples containing particulate matter by centrifugation or filtration prior to testing.
If specimens cannot be tested on the day of collection, store them at 2-8ºC in stoppered tubes for up to five days. If longer storage is required, freeze sera at -25 ± 6°C for up to 2 months. Only one freeze-thaw cycle is recommended.
The use of plasma has not been established for this test.

INSTRUCTIONS FOR USE

For complete instructions, see the User Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.
If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.
Note: the master lot data need only be entered once for each lot.
It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User Manual).

Calibration

Calibration, using the standard provided in the kit, must be performed each time a new lot of reagents is opened, 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 standard, identified by S1, must be tested **in duplicate** (see User Manual). The standard value must be within the set RFV (Relative Fluorescence Value) range. If this is not the case, recalibrate.

Assay procedure

1. Remove necessary components from the kit and return all unused components to storage at 2-8ºC.
2. Allow components to reach room temperature (approximately 30 minutes).
3. 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.**
4. 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.
5. If needed, label the "CMVM" Reagent Label strips with the appropriate sample identification numbers.
6. Mix the standard, controls, and samples using a vortex- type mixer (for serum separated from the pellet).
7. **For this test, the standard, control, and sample test portion is 100 µL.**
8. 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.
9. Initiate the assay processing as directed in the User Manual. All the assay steps are performed automatically by the instrument.
10. Reclose the vials and return them to 2–8°C after pipetting.
11. The assay will be completed within approximately 60 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
12. Dispose of the used SPRs and strips into an appropriate receptacle.

QUALITY CONTROL

One positive control and one negative control are included in each VIDAS CMV IgM (CMVM) 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.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

RESULTS

Two instrument readings for fluorescence in the Reagent Strip's reading cuvette are taken for each specimen tested. The first reading is a background reading of the cuvette and substrate before the SPR is introduced into the substrate. The second reading is taken after the substrate has been exposed to the enzyme conjugate remaining on the interior of the SPR. The background reading is subtracted from the final reading to give a Relative Fluorescence Value (RFV) for the test result.

A test value is generated for each sample by forming a ratio from the RFV of the sample to that of the standard or a stored standard result. Test values from patient and control samples are compared to a set of thresholds stored in the computer. The thresholds and interpretations are given in the table below.

Thresholds and Interpretation of Results:

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

LIMITATIONS OF THE TEST

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

EXPECTED VALUES

CMV infections are quite common. Approximately 60-85% of the population is believed to be infected by age 18. For this reason, the incidence of CMV IgM positive samples is expected to be very low. At the VIDAS CMV IgM (CMVM) clinical trial site in the US, the positivity rate for CMV IgM in random samples submitted for CMV serology testing was 5.9%. At the trial site in central France, the positivity rate was 4.3%.

SPECIFIC PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

Two hundred three serum samples were tested at a single site in the US. Due to the low incidence of CMV IgM, 30 positive repository samples were added to the data set to help give an accurate estimate of assay sensitivity. Each sample was tested using the VIDAS CMV IgM (CMVM) assay on the VIDAS 30 instrument, a commercially available automated CMV IgM EIA, and a commercially available manual CMV IgM EIA. There were initially 8 (3.4%) VIDAS equivocal results. Upon retesting, all of them repeated as equivocal.

The following tables show the VIDAS CMV IgM (CMVM) assay compared to each of the EIAs tested, and to the final interpretation as described above.

Site A (Random Samples)
203 samples tested
6 equivocal results (not included in calculations)

		EIA 1*		EIA 2**		Final Interpretation	
		+	-	+	-	+	-
VIDAS	+	11	1	9	2	12	1
CMV IgM (CMVM)	-	1	183	1	183	0	184

Rel. Sens: Not calculated - insufficient # positives			
Rel. Spec.	99.5%	98.9%	99.5%
Spec. 95% CI	97-100	96.1-99.9	97-100
Rel. Agreemt	99.0%	98.5%	99.5%

* There was 1 equivocal in EIA 1.
** There were 2 equivocals in EIA 2.

Site A (Repository Samples)
30 samples tested
2 equivocal results (not included in calculations)
2 unresolved samples
(not included in Final Interpretation calculations)

		EIA 1*		EIA 2**		Final Interpretation	
		+	-	+	-	+	-
VIDAS	+	20	0	18	3	20	0
CMV IgM (CMVM)	-	3	3	2	4	1	5

Rel. Sens.	87.0%	90.0%	95.2%
Rel. Spec: Not calculated - insufficient # negatives			
Sens. 95% CI	66.4-97.2	68.3-99.8	76.2-99.9
Rel. Agreemt	88.5%	81.5%	92.6%

* There were 2 equivocals in EIA 1.
** There was 1 equivocal in EIA 2.

Site A (All Samples Combined)
233 samples tested
8 equivocal results (not included in calculations)
2 unresolved samples
(not included in Final Interpretation calculations)

		EIA 1*		EIA 2**		Final Interpretation	
		+	-	+	-	+	-
VIDAS	+	31	1	27	5	32	1
CMV IgM (CMVM)	-	4	186	3	187	1	189

Rel. Sens.	88.6%	90.0%	97.0%
Rel. Spec	99.5%	97.4%	99.5%
Sens. 95% CI	73-96.8	73.5-97.9	94.2-99.9
Spec. 95% CI	97.1-100	94.0-99.2	97.1-100
Rel. Agreemt	97.7%	96.4%	99.1%

* There were 3 equivocals in EIA 1.
** There were 3 equivocals in EIA 2.

Precision - VIDAS® 30
Intra-assay precision was evaluated at two sites using positive, low positive, and negative controls, each tested in a single work list. (NOTE: Different controls were used at each site). The results are given below.

Intra-assay Precision

	n =	Mean Test Value	Std Dev.	% CV
Site 1				
Positive	21	1.16	0.04	3.1
Low Positive	21	0.93	0.03	3.7
Negative	21	0.02	0.00	*
Site 2				
Positive	30	1.63	0.09	5.5
Low Positive	30	1.00	0.06	6.0
Negative	30	0.08	0.01	*

* CV was not calculated due to low test values

Inter-assay precision was evaluated at two sites using positive, low positive, and negative controls. At Site 1, each control was tested in triplicate in a single work list for 10 days and the mean Test Value for each day was used in the calculations. At Site 2, each control was tested in singlet in a single work list for 10 days. (NOTE: Different controls were used at each site). The results are given below.

Inter-assay Precision

	Mean Test Value	Std Dev.	% CV
Site 1			
Positive	1.23	0.08	6.5
Low Positive	0.98	0.06	5.9
Negative	0.01	0.00	*
Site 2			
Positive	1.51	0.06	4.0
Low Positive	0.96	0.05	5.2
Negative	0.14	0.06	*

* CV was not calculated due to low test values

mini VIDAS Precision

Intra-assay precision and inter-assay reproducibility were evaluated for the mini VIDAS by testing the VIDAS CMV IgM (CMVM) Positive and Negative controls and a low positive sample.

	n =	Mean Test Value	Std Dev.	% CV
Intra-assay:				
Positive	12	1.76	0.03	1.8
Low Positive	12	0.91	0.03	3.3
Negative	12	0.00	0.00	*
Inter-assay				
Positive	10	1.75	0.10	6.3
Low Positive	10	0.92	0.11	11.6
Negative	10	0.00	0.00	*

* CV was not calculated due to low test values

Cross-reactivity

Samples from patients positive for RF, ANA, EBV, VZV, or HSV that were negative for CMV IgM were tested in the VIDAS CMV IgM (CMVM) assay. The results are summarized in the table below.

Disease State	Number of samples	VIDAS Results
RF	20	18/20 Negative
ANA	16	15/16 Negative
EBV	17	15/17 Negative
VZV	5	5/5 Negative
HSV	4	3/4 Negative

Interference

Five RF positive, CMV IgM positive samples and five ANA positive, CMV IgM positive samples were also tested in the VIDAS CMV IgM (CMVM) assay. The results are shown in the table below.

Disease State	Number of samples	VIDAS Results
RF	5	5/5 Positive
ANA	5	5/5 Positive

ADDITIONAL DATA - EUROPEAN STUDY

Two hundred thirty-one serum samples were tested at a single site in France. Due to the low incidence of CMV IgM, 38 positive repository samples were added to the data set to help give an accurate estimate of assay sensitivity. Each sample was tested using the VIDAS CMV IgM (CMVM) assay on the VIDAS 30 instrument, a commercially available automated CMV IgM EIA, and a commercially available manual CMV IgM EIA. There were initially 5 (1.9%) VIDAS equivocal results. Upon retesting, all of them repeated as equivocal.

The following tables show the VIDAS CMV IgM (CMVM) assay compared to each of the EIAs tested, and to the final interpretation as described above.

Site B (All Samples Combined)
269 samples tested
5 equivocal results (not included in calculations)

		EIA 1*		EIA 2**		Final Interpretation	
		+	-	+	-	+	-
VIDAS® CMV IgM (CMVM)	+	34	1	31	5	36	0
	-	7	217	10	218	6	222

Rel. Sens. 82.9% 75.6% 85.7%
Rel. Spec 99.5% 97.8% 100.0%
Rel. Agreemt 96.9% 94.3% 97.7%
* There were 5 equivocals in EIA 1.

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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5. SCHAEFFER, L., et al. "Evaluation of Abbott CMV-M Enzyme Immunoassay for detection of cytomegalovirus immunoglobulin M antibody". Journal of Clinical Microbiology. 26:2041-2043. 1988.
6. CHOU, S., et al. "Immunoglobulin M to cytomegalovirus in primary and reactivation infections in renal transplant recipients". Journal of Clinical Microbiology. 25:52-55. 1987.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories:

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user
	Minor typographical, grammar, and formatting changes are not included in the revision history.

Release date	Part Number	Change Type	Change Summary
2015/01	13680C	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	KIT COMPOSITION (30 tests) WARNINGS AND PRECAUTIONS
2015/06	13680D	Technical	KIT COMPOSITION (30 tests) INSTRUCTIONS FOR USE
2016/12	13680E	Technical	KIT COMPOSITION (30 tests)

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