



Anti-Cytomegalovirus IgM (CMV IgM) Test System

Product Codes: 6625-300

1.0 INTRODUCTION

Intended Use: The Semi-Quantitative Determination of Anti-CMV Specific Antibodies of the IgM type by Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Congenital cytomegalovirus (CMV) infection is the most common congenital infection in developed countries with a prevalence rate of approximately 1% of all live births.¹ In fact, the prevalence of this infection is so high that roughly 40% of adults are infected by age 40.² Transmission of CMV to the fetus in women that are seropositive before pregnancy is extremely high.³ It is of high importance to screen for CMV infection due to it potentially causing deafness and slowed mental development in the fetus.⁴ Since CMV infection during gestation is associated with high risk to the fetus, antibodies against CMV should be screened at regular intervals throughout the course of a pregnancy to enable rapid medical intervention.

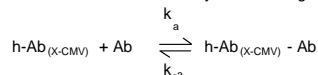
The Anti-CMV IgM Accubind® ELISA Test System is a semi-quantitative test designed to produce highly sensitive and specific results with a simple and brief protocol. The test utilizes recombinant chimeric CMV antigen (pp150+pp52) conjugated to horseradish peroxidase to detect native antibodies in the patient sample in a sequential sandwich type method.

3.0 PRINCIPLE

Sequential Sandwich ELISA Method (TYPE 10):

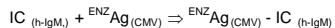
The reagents required for the sequential ELISA assay include immobilized antibody, circulating antibody to CMV, and enzyme-linked CMV antigen.

Upon adding a sample containing the anti-CMV antibody, reaction results between the anti-human IgM antibody that has been immobilized on the microwell and the antibody to form an immune-complex. The interaction is illustrated by the following equation:



Ab = Immobilized Antibody (Constant Quantity)
 h-Ab_(X-CMV) = Human Antibody (Variable Quantity)
 h-Ab_(X-CMV) - Ab = Immune Complex (Variable Quantity)
 k_a = Rate Constant of Association
 k_{-a} = Rate Constant of Disassociation

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked disease-specific antigen is then added to the microwells. This conjugate binds to the immune complex that formed.



IC_(h-IgM) = Immobilized Immune complex (Variable Quantity)

ENZAg_(CMV) = Enzyme-antigen Conjugate (Constant Quantity)

ENZAg_(CMV) - IC_(h-IgM) = Ag-Ab Complex (Variable)

The antigen enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing a serum reference equivalent to the positive-negative cut-off value, the absorbance value can be compared to the cut-off to determine a positive or negative result.

4.0 REAGENTS

Materials provided:

A. Anti-CMV IgM Controls – 2ml/vial - Icons PC, NC, CC

Three (3) vials of ready-to-use references for Anti-CMV IgM at positive, negative, and cut-off levels of IgM. Store at 2-8°C. A preservative has been added.

B. CMV IgM Enzyme Reagent – 13ml/vial - Icon

One (1) vial of chimeric recombinant CMV (pp150+pp52) horseradish peroxidase (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8°C.

C. Anti-hIgM Antibody Coated Plate – 96 wells - Icon

One 96-well microplate coated with anti-human IgM antibody and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Serum Diluent Concentrate – 20ml

One (1) vial of concentrated serum diluent containing buffer salts and a dye. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C

F. Substrate Reagent– 12ml/vial - Icon S^N

One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon

One (1) vial contains a strong acid (0.5 M H₂SO₄). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

- Fixed volume or variable volume pipette capable of delivering volumes ranging from 10 to 1000 µl with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.050 ml, 0.100 ml, and 0.350 ml volumes with a precision of better than 1.5%.
- Microplate washers or a squeeze bottle (optional).
- Microplate Reader with 450nm and 620nm wavelength absorbance capability.
- Absorbent Paper for blotting the microplate wells.
- Plastic wrap or microplate cover for incubation steps.
- Vacuum aspirator (optional) for wash steps.
- Timer.
- Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens used should be serum or plasma from blood. The usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA, heparin, or citrate (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of seven (7) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.200ml of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Serum Diluent

Dilute contents of Serum Diluent Concentrate to 200ml (1:10 Dilution) in a suitable container with distilled or deionized water. Store at 2-8°C.

2. Wash Buffer

Dilute contents of wash solution concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.

3. Patient Sample Dilution (1/100)

For example, dispense 0.010ml (10µl) of each patient specimen into 0.990 ml (990 µl) of serum diluent or 0.0101 ml (10.1 µl) into 1 ml (1000 µl). Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note : Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

****Test Procedure should be performed by a skilled individual or trained professional****

- Format the microplates' wells for each control sample and patient specimen to be assayed in duplicate. Dilute the patient or any external control samples 1/100 (see Reagent Preparation Section 8.0) **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
- Pipette 0.100 ml (100µl) of the appropriate control or diluted patient specimen into the assigned well for IgM determination. **DO NOT SHAKE THE PLATE AFTER SAMPLE ADDITION**
- Cover and incubate 30 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
- Add 350µl of wash buffer (see Reagent Preparation Section 8.0), decant (blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to**

dispense the wash. Decant the wash and repeat four (4) additional times.

- Add 0.100 ml (100µl) of CMV IgM Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION

- Cover and incubate for thirty (30) minutes at room temperature.
- Wash the wells five (5) times with 350 µl wash buffer by repeating steps (4 & 5) as explained above.
- Add 0.100 ml (100µl) of Substrate Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells. Do not use the Substrate Reagent if it looks blue.**
- DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**
- Incubate at room temperature for twenty (20) minutes.
- Add 0.050ml (50µl) of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix. **Always add reagents in the same order to minimize reaction time differences between wells.**
- Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

10.0 INTERPRETATION OF RESULTS

A Cut-Off Control (CC) and kit specific Cut-Off Factor is used to ascertain the positivity or negativity of samples. Follow the following procedure to interpret the sample results.

- Record the absorbance of all samples obtained from the printout of the microplate reader as outlined in Example 1.
- Multiply the average absorbance of the Cut-Off Control by the Cut-Off Factor to obtain the Cut-Off Value.
- Divide the average absorbance of each sample by the Cut-Off Value and multiply by 10 to obtain the relative value unit (RV).
- If RV <9, the sample is negative for Anti-CMV IgM and if RV >10, the sample is positive for Anti-CMV IgM.

Samples with RV that fall within the range of 9-10 are considered borderline and should be retested with a new blood draw for reevaluation.

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

Interpretation of Samples

IgM < 9 RV	Negative
IgM 9-10 RV	Borderline
IgM > 10 RV	Positive

EXAMPLE 1 (Cut Off Factor = 1.00)

COV = MeanCC x COF
 COV = Cut-Off Value
 MeanCC = Mean Absorbance of Cut-Off Control
 COF = Cut-Off Factor (See Certificate of Analysis)
COV = 0.745 x 1.00 = 0.745

Sample I.D.	Abs	Mean Abs	RV	Pos/Neg
Negative	0.054	0.057	±0.745 x 10 = 0.77	Negative
	0.061			
Cut-Off	0.736	0.745	±0.745 x 10 = 7.40	Cut-Off
	0.754			
Positive	3.046	3.059	±0.745 x 10 = 41.9	Positive
	3.071			
Patient 1	0.671	0.697	±0.745 x 10 = 9.36	Borderline
	0.724			
Patient 2	1.090	1.088	±0.745 x 10 = 14.6	Positive
	1.086			
Patient 3	0.098	0.105	±0.745 x 10 = 1.41	Negative
	0.112			

*The data presented in Example 1 is for illustration only and **should not** be used in lieu of a Cut-Off Control run and Cut-Off Factor with each assay. **In this example, since the Cut-Off Factor = 1.00, the average absorbance of the Cut-Off Value = 1.00 x Cut-Off Control.**

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. Maximum Absorbance (Positive control) > 1.5
2. Positive control RV > 15
3. Negative control RV < 6

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the Cut-Off control.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-CMV in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. The Anti-CMV IgM AccuBind® ELISA Test System is a semi-quantitative assay and gives quantities of IgM only in relative units to a cut-off that is traceable to an international standard.
11. Samples, which are contaminated microbiologically, should not be used.
12. Any patient samples used in manufacturing have been heat inactivated prior to handling. However, treat all samples, including the control samples, as potentially hazardous or infectious.
13. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
14. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
15. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
16. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. **Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, **Monobind shall have no liability.**
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

6. The clinical significance of the result should be used in evaluating the possible presence of CMV infection. However, **clinical inferences should not be solely based on this test** but rather as an adjunct to the clinical manifestations of the patient and other relevant tests such as Histology, nasopharyngeal swab, etc. A positive result does not indicate and does not distinguish between infection or contagiousness of CMV. Similarly, a negative result does not eliminate the absence of a CMV infection but rather a very low titer of antibody that may be related to the early stages of disease.

13.0 EXPECTED RANGES OF VALUES

Known negative and positive CMV IgM samples were used to determine expected values for the Anti-CMV AccuBind® ELISA Test System. Based on the international standards, the following cut-off point was established.

Presence of CMV antibodies Confirmed

IgM > 10 RV

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The precision of the Anti-CMV IgM AccuBind® ELISA Test System was determined on six different patient sera of varying levels. The data summary is collected in the tables below.

Table 1: Total Precision

Sample	Mean Value (RV)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Patient 1	10.52	0.56	5.32	1.04	9.90
Patient 2	16.07	0.96	5.96	1.75	10.89
Patient 3	5.12	0.25	4.86	0.68	13.18
Patient 4	21.93	1.45	6.63	2.80	12.76
Patient 5	3.70	0.21	5.79	0.63	16.99
Patient 6	1.69	0.19	11.44	0.37	21.68

The above data was collected using three different kits in forty assays in duplicate over 20 days.

Table 2: Reproducibility of Interpretation

Sample	Number Negative	Number Borderline	Number Positive
Patient 1	2/80	20/80	58/80
Patient 2	0/80	0/80	80/80
Patient 3	80/80	0/80	0/80
Patient 4	0/80	0/80	80/80
Patient 5	80/80	0/80	0/80
Patient 6	80/80	0/80	0/80

14.2 Sensitivity and Specificity

The sensitivity and specificity of the CMV IgM AccuBind® ELISA Test system was determined by measuring 126 different samples from a random population on the Monobind kit and another commercially available ELISA test. The results are tabulated below.

Commercial Interpretation	Monobind Interpretation		Total
	Positive	Negative	
Positive	11	2	13
Negative	18	95	113
Total	29	97	126

Monobind Interpretation	Proportion	Wilson 95% Confidence Interval
True Positives (Sensitivity)	84.6%	54.6-98.1%
True Negatives (Specificity)	84.1%	76.0-90.3%
False Positives	15.9%	9.7-24.0%
False Negatives	15.4%	1.9-45.4%

14.3 Linearity

The linearity of the Anti-CMV IgM AccuBind® ELISA test system was tested by diluting human serum samples containing high levels

of IgM against CMV (15 to 30 RV) with the serum diluent solution. The system produces excellent linearity up to 29.7 RV and as low as 2.0 RV.

16.0 REFERENCES


1. Lazzarotto T., Spezzacatena P., et al. Anticytomegalovirus Immunoglobulin G Avidity in Identification of Pregnant Women at Risk of Transmitting Congenital CMV Infection. *Clin Diagn Lab Immunol* 1999. **6**(1): 127-129.
2. Mana H., Yassine H., et al. The Current State of Cytomegalovirus (CMV) Prevalence in the MENA Region: A Systematic Review. *Pathogens* 2019. **8**(213).
3. Prince H., Leber A. Validation of an In-House Assay for Cytomegalovirus Immunoglobulin G (CMV IgG) Avidity and Relationship of Avidity to CMV IgM Levels. *Clin Diagn Lab Immuno* 2002. **9**(4): 824-827.
4. Tomtishen J. Human cytomegalovirus tegument proteins. *Virology* 2012. **9**(22).
5. Schroeder H. Jr., Cavacini L., Structure and function of immunoglobulins. *J Allergy Clin Immunol* 2010. **125**(2 Suppl 2):S41-S52.

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MP6625

DCO: 1646
Product Code: 6625-300

Size	96(A)	192(B)
	Reagent (fill)	
A)	2ml set	2ml set
B)	1 (13ml)	2 (13ml)
C)	1 plate	2 plates
D)	1 (20ml)	2 (20ml)
E)	1 (20ml)	1 (20ml)
F)	1 (12ml)	2 (12ml)
G)	1 (8ml)	2 (8ml)

For Orders and Inquires, please contact










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Please visit our website to learn more about our products and services.

Glossary of Symbols (EN 980/ISO 15223)

 In Vitro - Diagnostic Medical Device	 Temperature Limitation Storage Condition (2-8° C)	 Consult Instructions for Use
 Catalogue Number	 Contains Sufficient Test for Z	 Batch Code
 Used By (Expiration Day)	 Date of Manufacturer	 Manufacturer