IVD

IGM ANTIBODY TO CYTOMEGALOVIRUS (ANTI-CMV) ELISA

Catalog No. E0901-2

96 tests

INTENDED USE

The Autobio anti-CMV IgM ELISA is intended for the qualitative determination of IgM antibodies to cytomegalovirus in human serum or plasma specimens (EDTA, heparin or sodium citrate).

INTRODUCTION

Human cytomegalovirus (CMV) is a member of the *Herpesviridae* family and is one of the seven human herpesviruses pathogenic for man. It is ubiquitous, species-specific and is spread by close human contact. The viral capsid, which has a DNA core, is icosahedral in shape and is formed of 162 capsomers. Surrounding the capsid, is one or more oval membranes containing lipids. CMV infection can be primary or secondary. Primary infection may be acquired through different transmission routes and in different periods of life (i.e., congenital and post-natal infections). Following primary infection, CMV enters a latency phase during which the virus can be found in B lymphocytes. Subsequent reactivation of viral replication (secondaryinfection) may take place concomitantly with changes in the relationship between host and virus, such as pregnancy, serious illness, immunosuppressive therapy or stress[1].

Congenital infection is transmitted transplacentally or at birth and can occur even if pregnant women already present antibodies to CMV (reinfection with exogenous virus), unlike rubella or toxoplasmosis. If seronegative women contract primary CMV infection during pregnancy, sequelae may be abortion, stillbirth or neonatal malformation. This is the case even if the birth of a normal, seronegative child is possible in almost 50% of maternal infections. The clinical picture of congenital CMV infection is always severe and includes psychomotor retardation, deafness, retinochoroiditis, microcephaly, hydrocephalus, cardiac disease, hepatitis, hepatosplenomegaly, thrombocytopoenia. The mortality rate is quite high. Most individuals (40-90%) acquire primary CMV infection during childhood or adulthood[2].

Post-natal infections are transmitted through close contact with infected biological fluids (urine, saliva, breast milk, semen, cervical secretions, faeces), infected blood products and, occasionally, organ transplants. In immunocompetent individuals, the clinical picture of post-natal CMV infection is usually mild or asymptomatic. The commonest signs include fever, malaise, and increased serum transaminase levels without jaundice.By contrast in immunocompromized patients (organ transplant recipients, patients with AIDS, lymphoproliferative diseases or cancer), symptoms may be severe because of disseminated and/or visceral infection, and include splenomegaly, pneumonia, haemolytic anemia, myocarditis and encephalitis. In these patients the disease may be fatal. The immune response to CMV involves synthesis of antibodies of the IgM class some weeks after infection by CMV and, one week later, of antibodies of the IgG class. Levels of IgM to CMV usually increase for some weeks and decrease slowly thereafter, in four to six months. Occasionally, IgM may circulate for years. Specific IgM assay is instrumental in diagnosing acute CMV infection, which remains difficult to identify on symptoms alone. However, it is not always possible to distinguish between primary and secondary infection, because reactivation may induce synthesis of IgM in immunocompromized patients. Specific IgG assay is useful in distinguishing subjects having acquired the disease from those who have not. This is particularly important in order to adopt suitable prophylaxis in susceptible individuals.

PRINCIPLE OF THE TEST

This assay is based on the capture ELISA method. Microtiter wells are pre-coated with anti-human IgM mono-

clonal antibodies (MAb). When anti-CMV IgMs are present in the sample, they react with anti-human IgM MAb and attach to the solid-phase. Non-reactive IgMs are removed with the wash fluid. Human IgMs bound to the solid phase then react with horseradish peroxidase (HRP) labeled CMV antigens. After incubation, the wells are washed with wash fluid to remove unbound enzyme conjugate. Substrate solution and chromogen solution are then added and incubated, resulting in the development of a blue color. The color development is terminated with stop solution, and the color turns to yellow and is measured spectrophotometrically at the wavelength of 450 nm. The concentration of anti-CMV IgM is directly proportional to the color intensity of the test sample.

MATERIALS PROVIDED

1. Anti-human IgM MAb Coated Microtiter Plate: microplate with anti-human IgM MAb coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: HRP labeled CMV antigen in stabilizing buffer (1 vial, 12.0 mL)
3. Negative Control: human serum/plasma non-reactive for anti-CMV diluted in buffer with preservatives (1 vial, 1.5 mL)
4. Positive Control: human serum/plasma reactive for anti-CMV IgM, diluted in buffer with preservatives (1 vial, 1.5 mL)
5. Wash Fluid Concentrate: PBS-Tween (1 bottle, 30.0 mL, 20×)
6. Substrate Solution: hydrogen peroxide (1 vial, 7.4 mL)
7. Chromogen Solution: tetramethylbenzidine (TMB) (1 vial, 7.4 mL)
8. Stop Solution: 1.0 M H_2SO_4 (1 vial, 7.4 mL)
9. Sample Diluent: buffer solution with preservatives (1 bottle, 12.0 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes and multichannel micropipettes of appropriate volume (the use of accurate pipettes with disposable plastic tips is recommended)
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Incubator
6. Disposable reagent troughs
7. Instrumentation
    1. Automated microplate strip washer
    2. Microplate reader

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 – 8℃ upon receipt. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Oened components will remain stable for at least 2 months, or until the expiration date, whichever is earlier, provided it is stored as prescribed above.

SPECIMEN COLLECTION, PREPARATION, TRANSPORT AND STORAGE

1. Plasma specimens may be used with this test but serum is the recommended specimen type for this assay.
2. When plasma specimen is used, it is recommended to use 1.5 g/L EDTA, 10.9 mmol/L sodium citrate or 20 – 30 U/mL herapin as the anticoagulant.
3. Collect all blood samples observing universal precautions for venipuncture.
4. Any turbidity and particulate matters might interfere with the test, hence must be removed by centrifugation before testing.

5. Allow samples to clot before centrifugation.
6. Specimens could be stored at room temperature for up to 8 hours. For specimens which are not to be assayed within 8 hours of collection, they must be stored at 2 – 8℃ for no more than 48 hours. Specimens to be transported or stored for a longer period should be stored frozen at - 20℃ or a lower temperature. Avoid multiple freeze-thaw cycles. After thawing, ensure specimens are thoroughly mixed and brought to room temperature before being assayed.
7. Avoid grossly hemolytic and lipemic specimens.
8. Do not add sodium azide into the specimen as a preservative.

PRECAUTIONS AND WARNINGS

1. for *in vitro* diagnostic use only
2. This package insert must be fully understood prior to operation. The operation must be stringently in accordance with the instruction for use.
3. Micropipette tips are not interchangeable to eliminate cross contamination.
4. Specimens added must be mixed thoroughly. The presence of bubbles must be eliminated.
5. The microtiter plate must be washed completely. Each well must be fully injected with Wash Fluid. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, liquids in each well must be dried. The microtiter plate should be stroked onto absorbent paper to remove residual water droplets. It is recommended to wash the microtiter plate with an automated microplate stripwasher.
6. Wear disposable gloves when dealing with specimens and reagents. Wash hands after operations. All specimens must be regarded as potentially infectious. Waste material must be disposed of safely according to relevant local and national requirements.
7. Avoid any skin contact with all reagents. Stop Solution contains H_2SO_4, in case of contact, wash thoroughly with water.
8. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. Obtain the assays from the fridges. Place at room temperature (18 – 25℃) and equilibrate for at 30 minutes.
2. Mix the reagents by gently inverting or swirling.
3. Carry out a 1:20 dilution of the Wash Fluid Concentrate with distilled water.
4. Calibrate the temperature of the incubator at 37℃. Only use after the temperature is stabilized.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. Do no reuse the plate covers.
4. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
5. Replace caps on reagents immediately. Do not switch caps.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Leave 1 well for the blank, add 100 L of Negative Control to the next 3 wells, then 100 L of Positive Control to the following 2 wells. Add 100 L of Sample Diluent into each of the rest of the wells, and then add 10 L of specimen into each well with added Sample Diluent.
3. Mix thoroughly by shaking on a vortex mixer for 10 seconds.
4. Apply the plate seal, Incubate at 37℃ for 45 minutes.
5. Wash 6 times (an automated microplate strip washer is recommended), strike the microtiter plate onto absorbent paper at the end of the last wash cycle.
6. Add 100 L of Enzyme Conjugate Reagent into each well except for the blank well.
7. Repeat steps 3, 4 and 5.

8. Dispense 50 μL of Substrate Solution, then 50 μL of Chromogen Solution into each well. Gently mix and incubate at 37℃ for 10 minutes without exposure to sunlight.
9. Terminate the reaction by adding 100 μL of Stop Solution to each well. Mix thoroughly on a vortex mixer.
10. Immediately after mixing, read the absorbance of each well at 450 nm using 620 – 630 nm as the reference wavelength. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

CALCULATION OF RESULTS

1. Test is valid only if absorbance of Positive Control ≥0.7, and absorbance of Negative Control ≤ 0.1.
2. Calculation of the cut-off value

Cut-off value = 0.1 + mean absorbance of Negative Control replicates (in case the mean absorbance of Negative Control replicates ＜0.05, use 0.05 instead of the actual mean）

INTERPRETATION OF RESULTS

The specimen is positive when the absorbance ≥ the cut-off value, otherwise, the specimen is negative.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

70 positive results were obtained by testing 70 specimens positive for anti-CMV IgM. The sensitivity is 100%.

2. Specificity

466 negative results were obtained by testing 470 specimens negative for anti-CMV IgM. The specificity is 99.1%.

3. Precision

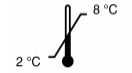
The coefficient of variance (CV) calculated from 10 replicate runs is not greater than 15%.

LIMITATIONS

1. This assay is only intended as an aid in clinical diagnosis. Assay values must be considered in combination with clinical examination, patient history and other assay values.
2. This assay is only suitable for aiding in the diagnosis of patients at the early stage of CMV infection. It is not to be used for screening blood donors.
3. It is recommended to re-test specimens with assay values close to the cut-off value.
4. A negative result could not exclude CMV infection. Because there is a delay in the generation of anti-CMV IgMs and this delay differs from person to person. The negative result may be due to insufficient amount of IgMs present in the body at the time of test.
5. False positive results might be obtained. Suspected results could be re-tested with this assay or other confirmative tests.
6. Anti-CMV IgMs may be present for more than half a year in some patients's body, consequently, a positive result might not definitely indicate a recent infection.

SYMBOLS

LOT	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**

	**TEMPERATURE LIMITATION**
	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES

1. Anet K., IDA O., et. al. *J. Infect. Dis.* **1985,** 151, 772.
2. Revello, M. G. and Gerna, G. *Clin. Microbiol. Rev.* **2002,** 15, 680.

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