Atlas Medical

CMV IgM ELISA Test Kit

An enzyme immunoassay (ELISA) for the qualitative detection of IgM antibodies to Cytomegalovirus (CMV) in human serum or plasma.

IVD **For *in vitro* diagnostic and professiona`l use only.**

2°C 8°C **Store at 2-8 °C**

Σ **96 Tests**

INTENDED USE

The CMV IgM assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro qualitative determination of IgG antibodies to cytomegalovirus (CMV) in Human serum and plasma.

SUMMARY

Cytomegalovirus (CMV) is a member of the Herpes virus family which includes Herpes Simplex virus (HSV) type 1 and 2, Varicella Zoster virus (VZV) and Epstein-Barr virus (EBV). It is a ubiquitous human pathogen transmitted through saliva, sexual contact, perinatally, organ transplantation or blood transfusion. In majority of the cases, the infection remains asymptomatic. However, CMV infection can cause serious illness in newborns and immunosuppressed individuals such as patients with AIDS, HIV, cancer or patients that received organ transplants.1 During immunosuppressive therapy, a reactivation of the latent virus or primary infection occurs frequently. For most newborns, CMV infections can be acquired before birth, during birth and later in life. The infection may cause severe congenital abnormalities such as microcephaly, motor disability, and mental retardation.2, 3, 4

Therefore, determining primary maternal infections and distinguishing primary from latent infection is of great importance. The presence of IgM antibodies indicates the presence of primary infection, while presence of IgG antibodies indicates immune status of patients.

The CMV IgM ELISA Test Kit is an immunoassay for the qualitative detection of the presence of IgM antibodies to CMV in serum or plasma specimen. The test utilizes recombinant CMV antigens to selectively detect IgM antibodies to CMV in serum or plasma.

PRINCIPLE

Capture method. Total duration of assay: **70 minutes**

This kit uses capture ELISA principle to detect CMV IgM. Polystyrene microwell strips are pre-coated with mouse anti-human IgM (anti-µ chain specific). During the first incubation step, all IgM antibodies binds to mouse anti-human IgM monoclonal antibody coated in microplate including the Anti-CMV IgM, the wells are washed to remove unbound serum proteins, and CMV antigens conjugated to the enzyme horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step, if there is CMV IgM antibody in sample, HRP-conjugated antigens will be bound to the anti-human CMV IgM complexes previously formed and the unbound HRP-conjugate is then removed by washing. Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively.

MATERIALS

Materials Provided

- **Coated Microplate**, 8 x 12 strips, 96 wells. Pre-coated.
- **Enzyme Conjugate**, (Red Cap) 1x11.0 mL/vial of HRP (horseradish peroxidase) labeled with CMV antigen. Contains 0.1% ProClin300 preservative.
- **Sample Diluent,** (White Cap) 1x11 mL/vial. ***Ready to use***.
- **Positive Control,** (Red Cap) 1x0.2 ml/vial.
- **Negative Control,** (Blue Cap) 1x0.2 mL/vial.
- **Wash Buffer,** (Transparent Cap) 1x25 mL/vial, (40X concentrated), PBS-Tween wash solution.
- **Substrate**, (Brown Cap) 1x11 mL/vial. Ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution,** (Yellow Cap) 1x6 mL/vial of 1 mol/L sulfuric acid.
- **Plate Lid: 2 Pieces.**
- **One package insert.**

Materials Required But Not Provided

- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

PRECAUTIONS

- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/ face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

STORAGE AND STABILITY

- Store at 2-8℃.
- Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

SPECIMEN COLLECTION AND PREPARATION

- Human serum or plasma is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Wash solution (40X dilution)

Add deionized water to the 40X concentrated Wash Solution Concentrate. Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

DIRECTIONS FOR USE

Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.

- **Preparation:** Mark two wells as Negative control (e.g. B1, C1), two wells as Positive control (e.g. D1, E1) and one Blank (e.g. A1, neither samples nor HRPConjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
- **Adding Sample Diluent:** Add 100 µL of Sample Diluent into their respective wells except the Blank.
- **Adding Sample:** Add 10 µL of Positive control, Negative control, and Specimen into their respective wells except the Blank. Mix by tapping the plate gently. Use a separate disposal pipette tip for each specimen to avoid cross-contamination.
  Note: After adding Sample, the reagents in wells turns Blue color from Green
- **Incubating:** Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
- **Washing:** At the end of the incubation, remove and discard the plate

cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.

- **Adding Conjugate:** Add 100µl of HRP-Conjugate into each well except the Blank.
- **Incubating:** Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds.After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Coloring:** Add 100 µL of TMB Substrate into each well including the Blank. Incubate the plate at Room Temperature for 10 minutes avoiding light. The enzymatic reaction between the TMB substrate and the HRP-Conjugate produces blue color in Positive control and in CMV IgM positive sample wells.
- **Stopping Reaction:** Using a multichannel pipette or manually, add 50 µL of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and CMV IgM positive sample wells.
- **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbace at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results.
  Note: read the absorbance within 10 minutes after stopping the reaction).

QUALITY CONTROL AND CALCULATION

- Read the sample's optical density (OD) at 450nm with a micro plate reader.
- Mean negative control OD value ≤ 0.1 and positive control OD value ≥ 0.8, the test is valid, otherwise the test is invalid.
- Cut-Off value (C.O.) = Mean negative control OD value + 0.10 (Calculated by 0.05 when Mean negative control OD value is ≤ 0.05, calculated by actual value when Mean negative control OD value is > 0.05).

Positive Results: Sample O.D value ≥ C.O.

Specimens giving an absorbance equal to or greater than the Cut-Off value are considered initially reactive, which indicates that CMV IgM has probably been detected using CMV IgM ELISA. All initially reactive specimens should be retested in duplicates using CMV IgM ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for CMV IgM with CMV IgM ELISA.

Negative Results: Sample OD value < C.O

Specimens giving absorbance less than the Cut-Off value are negative for this assay, which indicates that no CMV IgM has been detected with CMV IgM ELISA, therefore the patient is probably not infected with CMV and the blood does not contain CMV IgM.

LIMITATIONS

- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

PERFORMANCE CHARACTERISTICS

Specificity

A study of 256 individuals was tested with ELISA kits and found that the specificity was 98.15%.

Sensitivity

Among 221 CMV IgM confirmed were positive when tested with this ELISA, and 219 samples of which were detected with positive, the sensitivity was 99.10%.

Precision

Intra-assay

CV ≤ 15%

Within-run precision has been determined by using 15 replicates of three specimens: a low positive, medium positive and a high positive.

Inter-assay

CV ≤ 20%

Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, medium positive and a high positive. Three different lots of the CMV IgM ELISA Test Kit have been tested using these specimens over a 5-day period.

Analytical specificity

Interferences are not observed up to concentrations of 1 mg/mL Acetaminophen, 0.2 mg/mL Gentistic Acid, 0.1 mg/mL Ascorbic Acid, 0.1 mg/ mL Acetosalisilyc Acid, 0.1 mg/mL Caffeine, 0.6 mg/mL Oxalic Acid, 2 mg/mL Bilirubin, 2 mg/mL Hemoglobin and 1 % Ethanol.

Rheumatoid factors do not interfere with test .

Cross-Reactivity are not observed in Syphilis, HBsAg, HCV , HCG positive specimens.

REFERENCES

1. "Viral Zone". ExPASy. Retrieved 15 June 2015..
2. ICTV. "Virus Taxonomy: 2014 Release". Retrieved 15 June 2015.
3. Ryan KJ, Ray CG, eds. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 556, 566–9. ISBN 0-8385-8529-9.

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REF	Catalogue Number		Temperature limit
IVD	In Vitro diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry