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User's Manual

CMV IgM ELISA Kit

REF **DEIA-S21CM**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Cytomegalovirus Antibody (CMV) ELISA IgM Assay system is an enzyme-linked immunosorbent assay (ELISA) designed for the presumptive qualitative detection of IgM antibody to CMV in human serum and for the presumptive diagnosis of acute, recent, or reactive CMV infection. To adequately assess the patient's serological status, testing should be performed in conjunction with any of the other traditional CMV diagnostic technologies.

General Description

Cytomegalovirus (CMV) is a DNA virus herpes virus group. Widely distributed, other animals can be infected, causing the genitourinary system, central nervous system and liver disorders of various systems based infections, from mild asymptomatic infection until the serious defects or death. Cytomegalovirus also known as virus inclusion body cells, swelling of the infected cells and nuclear inclusion bodies with huge, hence the name.

CMV has the typical form of herpes virus, the HSV DNA structure and similar, but 5% larger than the HSV. The virus on the host or cultured cells with a high degree of species specificity, human cytomegalovirus (HCMV) can infect humans, and the proliferation of cells in the human fiber. The proliferation of the virus in cell culture slow replication cycle length, first isolated and cultured 30 to 40 days appear to be cenotaphic, characterized by rounded cell swelling, nuclear becomes larger, the nucleus around which a round there, "halo" of large addicted acid inclusions. CMV infection can affect the host organism in various organs and systems, but few opportunities for serious disease, target organ damage and with the children's age. Central nervous system damage (such as microcephaly, mental retardation, etc.) and congenital malformations were mainly seen in congenital infections; hepatitis, pneumonia can also be seen in infants and young children during infection. Therefore, infection after birth has been found in children with central nervous system damage and congenital malformations, be classified as caused by CMV infection is unscientific.

Of symptomatic CMV infection such as pneumonia, hepatitis diagnosis, rely solely on serology or urine, blood, positive results in the virological examination irregularities. The performance of these test results because the body can only exist with CMV or copied, but no orientation significance. Such as from lung disease, liver tissue in situ testing positive then, for the reliable, but the implementation difficulties. Thus, children with pneumonia should be taken to replace the lower respiratory tract secretions can cause the same disease or exclude other causes and pathogens, and strive to diagnose and reliable.

Principles of Testing

CMV ELISA test system is designed to detect IgM antibody to CMV in human sera. This product use polystyrene reaction lath solid phase adsorption monoclonal antibody(MAH IgM) , if the specimen has CMV IgM antibody, this antibody will combine with monoclonal antibody(MAH IgM) and Enzyme Labeling CMV antigen forms complex and showing on the plate, by chromogenic substrate. To detection CMV IgM antibody in human serum.

Reagents And Materials Provided

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label.

Coated Microtiter (PLATE)	96 wells configured in twelve well x 8 strips coated with CMV Composite. The plate is pouched with desiccant and sealed.
WASH BUFFER(40X) (20.0mL)	composition of PBST
SPECIMENS BUFFER (11ml)	calf serum
SUBSTRATE A (7.0mL)	composition of Hydrogen Peroxide
SUBSTRATE B (4.0mL)	composition of TMB
HRP Conjugate (CONJUGATE) (6.5ml)	Elisa labelled CMV antigen
STOP SOLUTION (6.0mL)	composition of sulfuric acid
NEGATIVE CONTROL (2.0mL)	Human serum. One vial containing IgM Negaitive Control with a blue cap, ready-to-use.
POSITIVE CONTROL (2.0mL)	Human serum. One vial containing IgM Positive Control with a red cap, ready-to-use.

Important Note: All kit components and serum samples should be allowed to equilibrate to room temperature before use.

Materials Required But Not Supplied

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 5µL to 200µL.
3. Wash bottle or microwell washing system.
4. Reagent grade water.
5. One liter graduated cylinder.
6. Laboratory timer to monitor incubation steps.
7. Disposal basin and disinfectant. (Example:10% household bleach, 0.5% sodium hypochlorite.)

Storage

2-8°C

Specimen Collection And Preparation

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A3: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. Avoid using hemolysed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera

may be stored between 2 and 8°C for no longer than 72 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

Assay Procedure

1. Remove the individual components from storage and allow them to equilibrate to room temperature for more than 15 minutes before use.
2. Prepare a 1:40 dilution of Wash Buffer with distilled water.
3. Add Specimens Buffer 100µL (2 drops) into the individual wells except the blank well, negative well and positive well (blank and positive wells set 1well, negative well set 2 wells).
4. Add 10µL specimen to the well, beating by pipettor repeatedly until liquid turn blue, dispense 50µL negative and positive control to the negative and positive well separately. Do not dispense liquid to blank control well.
5. Flick the microtiter wells for 30 seconds and mix well. Affix to sealing template, Incubate at 37°C for 20 minutes.
6. Take out, Add Wash Buffer to each well and absorb after 20 seconds. Repeat 5 times until each well is dry.
7. Dispense 1 drop (50µL) of HRP Conjugate to each well except the blank well. Gently vibration mixture, mix well, Affix to sealing template. Incubate at 37°C for 20 minutes.
8. Add Wash Buffer to each well and absorb after 20 seconds. Repeat 5 times until each well is dry.
9. Dispense 1 drop (50µL) of Substrate A and 1 drop (30µL) of Substrate B into individual well except blank well. Gently vibration mixture, mix well, Incubate at 37°C for 10 minutes.
10. Take out, adding 1 drop (50µL) of Stop Solution except the blank well ,mix well. Read result with a microwell reader.

Quality Control

1. Each time the assay is run a reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. Run validity is determined through the performance of the positive and negative controls as well as the blank.
3. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
4. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
5. Refer to NCCLS document C24-A3: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

Interpretation Of Results

1. Colorimetry: Read O.D at 450nm with a microwell reader.

Cut-off O.D=2.1×Negative Control O.D. (If the O.D value of the Negative Control is lower than 0.05, calculate as per 0.05; if the O.D. value is more than 0.05, calculate as the actual data).

Positive: Sample O.D≥ Cut-off O.D.

Negative: Sample O.D

2. Ratio method:

Positive: Sample OD/Cut off (S/Co)≥1

Negative: Sample OD/Cut off (S/Co)<1

Performance Characteristics

Clinical evaluation was conducted comparing the results obtained using Cytomegalovirus Antibody ELISA IgM Assay to a predicate device. The study included 163 serum samples from Russia, Ukraine, Togo, United States, and Northern Europe. 77 samples were identified as negative and 86 samples were identified as positive.

Out of the 77 negative samples, 57 samples were retrived from paitents who had previously been CMV inoculated.

PREDICATE DEVICE	ELISA Method	
	Positive	Negative
Positive	77	9
Negative	0	77

The results yielded a Specificity of 100% and a Sensitivity of 89.5%.

Precautions

1. For In Vitro Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly.
4. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Blood borne Pathogens.
5. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach a room temperature before starting the assay. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of

any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.

7. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of an accident or if you feel unwell, seek medical advice immediately.
8. The TMB Peroxidase Substrate is HARMFUL. Irritating to eyes, respiratory system and skin.
9. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
10. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
11. Reagents from other sources or manufacturers should not be used.
12. TMB Peroxidase Substrate Solution should be colorless, very pale yellow, very pale green or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used and dispense into a secondary container only what is needed to properly perform the assay.
13. Dilution or adulteration of these reagents may generate erroneous results.
14. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
15. Avoid microbial contamination of reagents. Incorrect results may occur.
16. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
17. Avoid splashing or generating aerosols.
18. Do not expose reagents to strong light during storage or incubation.
19. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
20. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
21. Do not allow the conjugates to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
22. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

Limitations

1. A diagnosis should not be made on the basis of anti-CMV results alone. Test results for anti-CMV should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. The use of hemolytic, lipemic, bacterially contaminated or heat inactivated specimens should be avoided. Erroneous results may occur.
3. The assay performance characteristics have not been established for matrices other than sera.
4. Assay performance characteristics have not been established for visual result determinations.
5. Caution should be used when evaluating samples obtained from immunosuppressed patients.