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Cytomegalovirus IgM CLIA

REF

Catalog No.C30-901

INTENDED USE

Immunospec Cytomegalovirus IgM CLIA kit provides material for the detection of IgM-class antibodies to Cytomegalovirus in human serum or plasma using chemiluminescence detection technology.This assay is intended for *in vitro* professional use only.

SUMMARY AND EXPLANATION

Cytomegalovirus (CMV) belongs to Herpes virus family and is transmitted through saliva, sexual contact, perinatally, or through blood transfusions or organ transplantation.Infection with CMV appears to be worldwide and common despite the relative rarity of clinical disease.CMV causes most of the congenital virus infections in humans, with an incidence ranging from 0.2 to 2.2% of live births in different populations. Intrauterine transmission of the virus can occur at any time during gestation, but most infants are probably infected during birth or after birth from ingesting CMV-infected maternal milk. Disease of newborn with CMV infection is an often severe, fatal illness, usually affecting the salivary glands, brain, kidneys, liver and lungs.

After the primary infection, CMV can persist in a dormant state as a latent infection.During immunosuppressive treatment of patients (e.g. recipients of organ transplants), latent infection can be activated and appear as a secondary infection.

CMV is one of the most serious and frequent pathogens in AIDS patients. CMV pneumonia, a life threatening infection may occur in about 20% cases BMT (Bone Marrow Transplant) patients. The ability to distinguish primary from latent infection is of great importance in as much as primary maternal infections have greater pathological potential for fetus.

Diagnosis is made mainly by serological findings of antibodies (IgG and IgM classes) to CMV. However, it is necessary to test the specimen for specific IgM; presence of specific IgM antibodies indicates the primary infection, whereas presence of specific IgG antibodies indicates the immune status of patients. .

PRINCIPLE OF THE TEST

Immunospec test for the assay of CMV IgM is based on the principle of the capture of these immunoglobulins and subsequent identification of those, which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The u-capture is performed using monoclonal antibodies bound to the solid phase (microtitration strips). The antigen is composed of purified and inactivated Cytomegalovirus antigen.

REAGENTS

Immunospec Cytomegalovirus IgM CLIA kit contains sufficient reagents for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED	QUANTITY
Anti-human IgM Antibody-Coated Microtitration Strip	One Plate
Wash Concentrate	One Bottle
Sample Diluent	One Bottle
Substrate A	One Bottle
Substrate B	One Bottle
Negative Control	One Vial
CutOff Control	One Vial
Positive Control	One Vial
CMV-HRP Conjugate	One Bottle

MATERIAL NOT PROVIDED

- Microtitration plate luminometer
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl, and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent materials for blotting the strips

Anti-human-IgM -antibody Coated Microtitration Strips:

One stripholder containing 12x8 (96) microtitration wells coated with anti-human-IgM-antibodies. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate:

One bottle, 100 ml, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent:

One bottle, 100 ml, containing a protein solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

Cytomegalovirus IgM Controls:

Three vials, each 2 ml of human serum in a 0.01 M phosphate buffer with 0.09% sodium azide as a preservative. The value for Calibrator 2 represents the Cut-Off control units values are reported on the labels of the vials. Store at 2-8°C until expiration date.

CMV-HRP Conjugate:

One bottle, 12 ml, containing anti-human IgM monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

Substrate A:

One bottle, 6 mL containing substrate A. Store at 2-8°C until expiration date.

Substrate B :

One bottle, 6 mL, containing A. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product.Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete

washing will adversely affected the outcome and assay precision.. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination with Substrate Solution and Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and Substrate can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS:

Wash Solution:

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 ml of the Wash Concentrate into a clean container and dilute by adding 900 ml of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips:

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Substrate : Atleast 30 minutes prior to use mix 1 part of Substrate in 1 part of Substrate B (Example : 1ml of Substrate A and 1 ml Substrate B).The two components should be mixed thoroughly by inversion and store the substrate mixture at RT, avoid exposure to light.

Assay Procedure:

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate. .

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Assay Buffer.
3. Pipette 100 µL of each diluted serum sample and controls to the appropriate wells. Leave one well for the blank, performed using 100 µL of the substrate mixture.
4. Incubate for 15 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
6. Add 100 µL of CMV-HRP-Conjugate into each well.
7. Incubate for 15 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µL of substrate mixture to each well using a dispenser.
10. Shake the wells for 10 sec.

Read the wells within 3 minutes using a microplate luminometer

RESULTS

Calculate the mean RLU for each control and unknown.

Qualitative results:

If the RLU of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgM.

Calculate the ratio between the average RLU value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the late phase of infection, when only IgG antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

Subtract the value of the blank from all the other readings. The RLU values of cut off control must be at least >10. Positive control must have a RLU at least 1.5 times that of cut off.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

125 human sera were analyzed by this Cytomegalovirus IgM CLIA and reference Elisa method. Out of 125 samples, 11 were positive for the presence of IgM antibodies to CMV by Immunospec CLIA, and reference method showed 12 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
Immunospec	11	114	0	0
Test A ElA	12	113	0	1

REFERENCES

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