MASTAZYME™ - CMV

A competitive enzyme immunoassay for the qualitative determination of antibodies to Cytomegalovirus in human serum or plasma.

Introduction

Cytomegalovirus (CMV) is a member of the Herpes virus family and is an important human pathogen. Most CMV infections are asymptomatic, especially in healthy adults, however the virus can cause severe illness and death in immunosuppressed patients such as transplant and cancer patients\textsuperscript{1, 2, 3}, Acquired Immunodeficiency Syndrome (AIDS) sufferers\textsuperscript{4}, and infants as a result of congenital or perinatal infection\textsuperscript{5}.

CMV is responsible for a broad spectrum of disorders including neurological diseases, myocarditis, pneumonia, hepatitis, CMV mononucleosis and gastrointestinal disorders\textsuperscript{6}. Disorders caused by CMV may occur by: \textsuperscript{6,7,8}
- a. primary exposure to CMV
- b. reactivation of a latent, pre-existing infection
- c. reinfection with the virus

CMV is the most common infectious cause of death in transplant recipients.\textsuperscript{1} CMV antibody positive recipients may be infected by reactivation of the virus while immunosuppressed, and CMV antibody negative patients may acquire primary CMV infections from virus-containing organs or blood products. Use of blood or organs from CMV antibody negative donors is effective in preventing CMV infection. CMV infected transplant recipients who show seroconversion or rising antibody levels to CMV survive longer and the possibility of death is reduced\textsuperscript{6,7,8}.

CMV is also the most common cause of intrauterine infection in humans, occurring in 1% of live births throughout the world\textsuperscript{5}. Congenital or perinatal acquired infections are normally asymptomatic at birth but later on in life symptoms such as deafness or mental deficiencies may become apparent. In severe cases, new-born infants may have multiple organ infections and the clinical prognosis may be bleak\textsuperscript{5}.

Serum antibodies are markers of past or present infection and hence serological tests for their detection are useful in clinical diagnosis of the disease.

Description

MASTAZYME™-CMV is a sensitive enzyme immunoassay for the detection of antibodies to cytomegalovirus in human serum and plasma and is ideal for screening serum and plasma samples.

Principle of the Test

MASTAZYME™-CMV is a competitive inhibition enzyme immunoassay for the detection of antibodies to cytomegalovirus in human serum or plasma.

Patient's serum or plasma, together with an aliquot of mouse monoclonal anti-CMV antibody conjugated to horseradish peroxidase is incubated in microtitre wells coated with cytomegalovirus antigen. Any specific antibodies in the patient's sample will compete with the conjugated cytomegalovirus antibodies for binding to the immobilised cytomegalovirus antigen on the microtitre wells. Thus, if there is a large amount of specific antibody in the patient's sample, little conjugate will bind. Conversely if there is little or no specific antibody in the patient's sample, most of the conjugate will bind.

Unbound material is then washed away and the bound conjugate remaining is detected by the addition of hydrogen peroxide and a chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). On incubation the enzyme reaction produces a blue colour. This reaction is terminated after a specified time with acid, converting any colour developed to an intense yellow, which can be read spectrophotometrically.

The intensity of the final colour reaction is an inverse measure of the amount of specific cytomegalovirus antibodies in the patient's serum or plasma.

Packaging and Ordering Details

MASTAZYME™-CMV is presented as kits for the performance of 480 tests.

Order code: EIA 802.
Contents

1. Microwell Strips
   Five packs of 12 x 8 microwell strips coated with cytomegalovirus antigen. Each set of strips is provided in a holder and sealed in a foil sachet containing silica gel as a desiccant.

2. Washing Buffer (Interchangeable between lots)
   Four bottles each with 100ml of phosphate buffered saline, containing Brij 35 and 0.01% Thimerosal as a preservative. Supplied as a 10x concentrate. Dilute in ultrapure water for use. If crystals are present, they should be dissolved at 37°C before dilution.

3. Positive Control
   One bottle with 1.0ml of human serum containing antibodies to CMV and diluted in 0.1M phosphate buffer. Contains 0.02% Bronidex and 0.05% phenol as preservatives. Supplied ready for use.

4. Negative Control
   One bottle with 1.0ml of serum negative for antibodies to CMV and diluted in 0.1M phosphate buffer. Contains 0.02% Bronidex and 0.05% phenol as preservatives. Supplied ready for use.

5. Calibration Control
   One bottle with 4.5ml of bovine serum in 0.1M phosphate buffer. Contains 0.02% Bronidex and 0.05% phenol as preservatives. Supplied ready for use.

6. Conjugate
   Three bottles each with 22ml of purified mouse monoclonal anti-cytomegalovirus antibody conjugated to horseradish peroxidase, in a phosphate buffered solution with stabilisers. Contains 0.02% Bronidex and 0.05% phenol as preservatives. Supplied ready for use.

7. TMB Substrate (Interchangeable between lots)
   One bottle with 60ml of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide in citrate buffer pH 3.8 with stabilisers. Supplied ready for use.

8. Stopping Solution (Red Cap) (Interchangeable between lots)
   Three bottles each with 22ml of 0.3M sulphuric acid. Supplied ready for use.

9. Plate Sealers
   Ten plastic self-adhesive plate sealers.

10. Instruction Leaflet
    Please read carefully before commencing test procedure.

Materials Required but not Provided

1. A precision microplate reader with 450nm filter with linearity up to at least 2.000.
2. 37°C incubator.
3. Vortex mixer.
4. Multichannel micropipettes (various).
5. Single channel micropipettes (various).
6. Disposable tips.
7. Manual or automated washing system.
8. Absorbent paper towels.
10. Fresh double distilled or good quality deionised water (ultrapure water).
11. Range of standard clean volumetric laboratory plastic and glassware.

Warnings and Precautions

1. The reagents in this kit are for in vitro diagnostic use only.
2. Read instructions carefully before conducting the assay. Do not modify the procedure.
3. Do not use the kit beyond the expiry date.
4. Wear appropriate protective clothing e.g. gloves while handling reagents and use appropriate facilities.
5. Avoid contamination of the wells with dust from disposable gloves.
6. Do not mouth pipette.
7. Use disposable plasticware where possible. Reusable glassware should be washed with 2M HCl and then rinsed thoroughly with deionised on high quality deionised water.
8. Do not re-use kit.
9. The kit contains material of human origin which have been tested and found to give a negative response by FDA-approved methods for the presence HbsAg and for anti-HIV-1, anti-HIV-2 and anti-HCV antibodies. As no diagnostic test can offer a complete guarantee regarding the absence of infective agents, all materials of human origin must be handled as potentially infectious. All precautions normally adopted in laboratory practice should be followed when handling material of human origin.
10. Do not mix reagents from different lots, as reagents have been standardised to give a correct reaction.
11. Do not cross-contaminate reagents or interchange caps on bottles. Use a separate pipette tip for each sample and reagent.
12. Do not allow wells to dry out during the assay procedure.
13. Protect all TMB solutions from exposure to direct light.
14. Do not expose plates to intense sunlight or similar adverse such effects as hypochlorite fumes while incubating.
15. The Stopping Solution contains acid, which is corrosive. Treat with appropriate care.
16. Dispose of contaminated plasticware and glassware according to local microbiological regulations. Spillages should be mopped with absorbent material and the area swabbed with 5.0% sodium hypochlorite solution.
17. Do not use microbially contaminated serum or plasma samples.
18. Preservatives are included in kit components as marked. Treat with appropriate caution.
19. Allow all reagents to equilibrate to room temperature before use.
20. Ensure that all sample material is thoroughly mixed before use e.g. using a vortex mixer. Poorly mixed samples may affect the test results.
21. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.
22. EIA tests can occasionally exhibit an “edge effect” which must be minimised by increasing the humidity during incubation steps. Plates must be covered with the plate sealers provided and incubated preferably in an incubator. Alternatively plates can be incubated in a waterbath with a rack or float to support the plates or in an approved analyser. CO₂ incubators must not be used.
23. For each supporting instrument used, read the manufacturer’s instructions carefully.

**Stability and Storage**

MASTAZYME™-CMV should be stored at 2-8°C and may be used until the expiry date given on the label.

Do not freeze reagents.

Coated microwell strips should be stored in the foil sachet provided. Once opened the sachet should be resealed with the closure provided. After opening the strips are stable for up to 4 weeks at 2-8°C. Diluted Washing Buffer may be stored for up to 5 days at room temperature or for up to 2 weeks at 2-8°C.

**Specimen Collection**

No special preparation of the patient is necessary. Serum or plasma samples can be used. The use of anticoagulants such as citrate, heparin, or EDTA does not interfere with the test. Highly lipaemic, icteric or contaminated samples should be avoided. If a new sample cannot be obtained, such samples should be clarified using a 0.45μm filter or by centrifugation at 3000rpm for 10 minutes.

Serum or plasma samples may be stored at 2-8°C for up to 7 days or at ~20°C for longer term storage. Freezing or thawing of the samples or heat inactivation for 30 minutes at 56°C does not affect the results.

**Test Procedure**

1. Prepare a 1 in 10 dilution of the concentrated Washing Buffer in ultrapure water as required. Allow 18ml of ultrapure water and 2ml of Washing Buffer per strip.
2. Ensure all reagents and samples have reached room temperature (15-30°C) before testing.
3. Ensure that the foil sachet containing the coated microwell strips has reached room temperature to prevent condensation forming on the strips when the sachet is opened.
4. Open the sachet carefully, remove from the holder the strips that are not needed and replace them in the sachet, leaving only those required for the assay in the holder.
5. Reseal the sachet with the closure provided and replace the pack in refrigerated storage at 2-8°C.
6. Using a micropipette, dispense 30μl ± 10% of the Calibrator into the first two wells of the first strip of each run and dispense 30μl ± 10% of each sample and Positive and Negative Controls into the other wells according to a pre-planned template. Use a separate pipette tip for each sample. Do not allow the pipette tip to scrape the surface of the well. Note:- the extinction value of the calibrator may differ from batch to batch.
7. Immediately after adding the samples to the wells dispense 100μl ± 10% of conjugate to all wells and mix thoroughly by tapping the side of the frame gently.

Note: - To prevent contamination of the conjugate it is advisable to transfer the required amount of cytomegalovirus into a second container and dispense into the wells from there.

8. Seal the strips with the plate sealer provided and incubate the strips at 36-40°C for 60 ± 5 minutes.

9. After incubation remove the seal from the wells and wash them 4 times with the diluted Washing Buffer either manually using a wash bottle or with a positive pressure automatic plate washer, allowing a soaking time of 20-30 seconds between each wash.

a. manual procedure:- aspirate the contents from each well using an 8 channel handwashing accessory attached to a vacuum line fitted with a collection trap or shake the contents of the wells into a suitable container containing disinfectant. Fill all wells with diluted Washing Buffer from a wash bottle and aspirate or shake out the contents as before. Repeat the filling and emptying process for a total of 4 times. Finally, invert the plate and bang firmly onto absorbent paper towels to remove any residual fluid from the wells. Check that no fluid remains in the wells and blot dry the surface of the plate using a fresh absorbent paper towel.

b. automatic procedure:- using an automatic plate washer or processor, aspirate the contents of the wells and wash the wells a total of 4 times in accordance with the manufacturers instructions. It is important to check that the washing jets are clean, and unblocked, and are filling the wells completely each time (approx. 0.4ml).

10. Dispense 100μl ± 10% of TMB substrate into each well. A multichannel pipette is recommended for this step.

Note:- To prevent contamination of the TMB substrate it is advisable to transfer the required amount of substrate into a second unused disposable plastic container and dispense into the wells from there. Do not return unused substrate to the original container. Occasionally some blue colour is observed in the TMB substrate as a result of a reversible photochemical reaction.

If this is observed the substrate should be stored overnight in the dark. Contamination of the substrate usually produces a deep blue colour which is not reversible and hence the substrate should be discarded.

11. Incubate the plate at room temperature (15-30°C) for 15 ± 2 minutes preferably in a darkened place.

12. After incubation stop the reaction by adding 100μl ± 10% of Stopping Solution to all wells in the same sequence and at the same time interval as the TMB substrate.

13. Gently tap the frame to ensure uniform mixing of reagents.

14. Within 30 minutes of adding the Stopping Solution read the absorbance of each well at 450 ± 5 nm on a suitable microplate reader. Blank the plate against air.

Test Procedure Summary

2. Select the required number of microwell strips.
3. Dispense 30μl ± 10% of Calibrator, Controls and serum or plasma samples into predetermined wells of the strip.
4. Add 100μl ± 10% of conjugate to all wells in use.
5. Seal the wells, mix contents and incubate at 36-40°C for 60 ± 5 minutes.
6. Wash all wells 4 times with working strength Washing Buffer.
7. Dispense 100μl ± 10% of TMB substrate to all wells.
8. Incubate at room temperature (15-30°C) for 15 ± 2 minutes.
9. Stop the reaction by adding 100μl ± 10% of Stopping Solution to all wells and mix the contents.
10. Read the absorbance at 450 ± 5 nm within 30 minutes of stopping, and interpret the results.
Results and Interpretation

a. Calculation of Results

1. Calculate the average absorbance value of the Calibrator.

2. Calculate the cut-off value as follows:
   \[ \text{Cut-off} = \frac{\text{average absorbance at 450nm of the Calibrator}}{\text{Calibrator Factor (as stated on the Calibrator bottle label)}} \]
   Example:
   \[ \text{average absorbance of Calibrator} = 1.100 \]
   \[ \text{Calibrator Factor} = 0.60 \]
   \[ \text{Cut-off} = 1.100 \times 0.60 = 0.66 \]

b. Interpretation by Absorbance Values

Specimens giving Absorbance values \( A_{450} \) within 10% of the cut-off value should be considered equivocal and should be retested. If samples appear in the equivocal range on retesting, a further specimen should be obtained from the patient for further testing.

Specimens giving \( A_{450} \) values outside the equivocal range and below the cut-off value of the Calibrator should be considered positive for CMV antibodies.

Specimens giving \( A_{450} \) values outside the equivocal range and above the cut-off value of the Calibrator should be considered negative for CMV antibodies.

c. Interpretation by Antibody Index

Use of the Antibody Index permits a comparison of results from different assay runs.

\[ \text{Antibody Index} = \frac{\text{absorbance of test serum or plasma}}{\text{cut-off value}} \]

Antibody Index values of between 0.9 - 1.1 should be counted as an equivocal range for the assay. Specimens giving results within this range should be retested. If samples appear in the equivocal range on retesting, a further specimen should be obtained from the patient for further testing.

Specimens with an Antibody Index equal to or less than 0.9 should be considered positive for CMV antibodies.

Specimens with an Antibody Index equal to or greater than 1.1 should be considered negative for CMV antibodies.

Validation of Test

The test is valid if:

1. The average absorbance of the Calibrator Control should be greater or equal to 0.600.
2. The average absorbance of the Positive Control at 450nm divided by the average absorbance of the Calibrator Control at 450nm should be less than or equal to 0.500.

Interpretation

If the absorbance value for the sample tested is lower than the cut-off value, the sample is considered positive for the presence of anti-cytomegalovirus antibodies.

If the absorbance value for the sample tested is greater than the cut-off value, the sample is considered negative for the presence of anti-cytomegalovirus antibodies.

Limitations of the Test

1. The test is not able to discriminate between the presence of IgG and IgM antibodies.
2. The test should not be used on its own for clinical diagnostic purposes but should be considered along with all serological tests, clinical history and other aspects of patient management to be considered diagnostically significant.
3. A negative result does not exclude the possibility of disease.

Performance Characteristics

a. Reproducibility

Reproducibility was studied by testing 27 negative and 27 positive samples. The CV% were found to be 6.4 and 5.8% respectively. Variations between runs were studied using a panel of serum samples which were positive, negative and borderline for the presence of anti-CMV antibodies. The samples were tested in 10 different runs. The CV% values ranged from 1.4% to 4.4%.

b. Analytical specificity

Samples containing antibodies against Epstein Barr Virus (n=40), Herpes Simplex Virus (n=33), and anti-nuclear (n=44), along with samples containing high levels of bilirubin (n=40), proteins (n=20), lipids (n=24), haemoglobin (n=20) and rheumatoid factor (n=40) were tested. No disagreement was found when the results were compared with those from another commercial test.
c. Diagnostic Sensitivity and Specificity

704 human sera from healthy blood donors were analysed. Samples were tested in parallel on the MASTAZYME™-CMV kit and on another commercial specific anti-CMV IgG EIA kit. 418 negative samples and 265 positive samples were found to be in agreement between the 2 methods. The 20 samples not in agreement were subjected to further study using 2 other commercial kits based on different analytical methods. In conclusion the data showed the MASTAZYME™-CMV to have a sensitivity of 99.3% and a specificity of 99.8%.

References