

Instructions for use

CMV IgM ELISA Kit

Qualitative assay for anti-CMV IgM antibodies

Product code GD85

96 tests

For *in vitro* research use

140108

1. Intended use

The CMV IgM kit is a rapid ELISA designed for the qualitative detection of IgM antibodies to cytomegalovirus (CMV) in human serum. The assay is intended to be used to evaluate serologic evidence of primary or reactivated infection with CMV, and is for *in vitro* research use only. Plasma samples may also be used.

2. Introduction

CMV infections can be classified as congenital (acquired before birth), perinatal (acquired at birth) or postnatal (acquired after birth). 95% of newborn infants congenitally infected with CMV exhibit no clinically overt disease at birth. Disease in the remaining 5% can be severe and may result in neurological damage in survivors or death early after birth.

The prognosis for congenitally infected infants who are asymptomatic at birth is variable. Many subsequently develop hearing loss and varying degrees of mental retardation and central nervous system disorders. Surveys show the incidence congenital CMV infection to be between 0.5 – 2.5%.

Perinatally infected infants start shedding the virus 3–12 weeks after delivery and generally remain asymptomatic. Postnatal CMV infections are usually asymptomatic and are acquired through close contact with individuals who are shedding the virus. A small percentage of individuals develop a negative heterophile-antibody infectious mononucleosis syndrome characterised by fever, lethargy and atypical lymphocytosis.

In immunocompromised patients e.g. allograft recipients, cancer patients and AIDS patients, CMV infections occur frequently, often from re-activation of latent infection, and may be life-threatening. CMV infections may occur following blood transfusions. Most transfusion acquired CMV infections are either sub-clinical or characterised by CMV mononucleosis. However, in immunocompromised patients, considerable morbidity and mortality can result from a transfusion-acquired CMV infection.

Serologic tests which measure IgG antibodies to CMV can aid in the diagnosis of CMV infection when paired acute and convalescent sera are tested simultaneously and seroconversion or a significant rise in titre can be demonstrated. Also serologic testing of blood transfusion donors and recipients can help prevent transfusion-acquired infection.

IgM antibodies are produced during the first 2 to 3 weeks of infection with CMV and exist only transiently in most individuals. Serologic procedures that measure the presence of IgM antibodies help to discriminate between primary and recurrent infections since IgM antibodies are rarely found in recurrent infection.

3. Principle of the test

Test sera are diluted (1:100) with the sample diluent provided. Anti-human IgG is added to the sample diluent sample to eliminate the possibility of interference by antigen-specific IgG and rheumatoid factor, if present. Diluted serum or plasma specimens are incubated for 20 minutes to allow specific antibodies to CMV to bind to the antigen-coated wells. After washing away unbound antibodies and other serum constituents, CMV specific IgM is detected using rabbit anti-human IgM conjugated to horseradish peroxidase. After 20 minutes incubation, unbound conjugate is removed by washing, and TMB enzyme substrate is added for 10 minutes. A blue colour develops if antibodies to CMV are present. Addition of stop solution gives a yellow colour and the optical densities of controls, 10 U/ml standard and samples are measured using a microplate reader.

4. Materials included in the Kit

- **Microplate** 96 wells in 12 x 8 break-apart strips, pre-coated with density gradient-purified CMV antigen.
- **Reagent 1:** Sample diluent 46 ml, (blue). Read the instructions before use.
- **IgG absorbent:** Anti-human IgG, 3 x 3.5ml. Read the instructions before use
- **Reagent 2:** Wash buffer concentrate (x 10), 100 ml.
- **Reagent 3:** Conjugate (peroxidase conjugated rabbit anti-human IgM), 12 ml, (green). Ready to use
- **Reagent 4:** TMB Substrate, 12 ml. Ready to use
- **Reagent 5:** Stop Solution, 12 ml. Ready to use
- **Positive control:** (red), 1ml liquid. Ready to use.
- **Standard:** 10 U/ml, (yellow), 1ml liquid. Ready to use.
- **Negative control:** (green), 1ml liquid. Ready to use.
- **Instructions for use**

5. Other equipment required

10mm X 60mm tubes for dilution, pipettes 5µl, 100µl, 1000µl; repeating dispenser 100µl, microplate reader with 450nm filter, microplate washing device. Distilled or de-ionised water, general laboratory apparatus.

Storage and precautions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for three months (or until its expiry date if less than three months). It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiry date.

The 10 U/ml standard and controls are manufactured from dilute non-infectious human serum. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

The stop solution contains 0.24M sulphuric acid and is non-corrosive.

6. Samples

Only freshly drawn and properly refrigerated sera or plasma should be used in this assay. Avoid haemolysed, lipaemic or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If delay in testing is anticipated, store test sera at -20°C. Avoid multiple freeze-thaw cycles.

7. Method

Ensure that all materials are at room temperature before beginning the procedure. We recommend that the 10 U/ml standard and the controls are always run in duplicate. Samples may be run singly or in duplicate.

1. Assemble the number of strips required for the assay.
2. Prepare only sufficient IgG-absorbent-containing sample diluent for the number of samples to be tested. Add one part **IgG absorbent** to 4 parts of **Reagent 1 Sample Diluent** as shown in the examples below and mix thoroughly. Discard any unused IgG-absorbent-containing diluent.

Approx Nr of samples	Volume of sample diluent (ml)	Volume of IgG Absorbent (ml)
24	10	2.5
48	20	5.0
72	30	7.5
96	40	10.0

3. Dilute patient samples 1:100 (e.g. 5µl serum plus 0.5 ml diluent). It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

4. Dispense 100 µl of the negative control, the 10 U/ml standard, the positive control and the diluted patient sample into the wells.
5. Incubate for **20** minutes at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.
6. Dilute the Wash Buffer (**Reagent 2**) 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.
7. After 20 minutes, decant or aspirate the well contents and wash the wells 3 times using an automatic plate washer or the manual wash procedure (see below). Careful washing is the key to good results. Blot the wells on absorbent paper before proceeding. **Do not allow the wells to dry out.**

Manual Wash Procedure:

Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process two more times.

8. Dispense 100µl of Conjugate (**Reagent 3**) into each well. This reagent is colour coded green. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent! Incubate the wells for **20** minutes at room temperature.
9. After 20 minutes, discard the well contents and carefully wash the wells four times with wash buffer. Ensure that the wells are completely washed. Blot the microplate on absorbent paper to remove final drops of wash fluid. **Do not allow the wells to dry out.**
10. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate (**Reagent 4**) into each well. Incubate the plate for **10** minutes.
11. Add 100µl of Stop Solution (**Reagent 5**) to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.
12. Read the optical density in a microplate reader within 10 minutes.

8. Quality control

The expected optical density values for the negative and positive controls and the 10 U/ml standard are given on the certificate included in the kit.

9. Interpretation

Negative samples: OD < 10 U/ml standard OD
Positive samples: OD >= 10 U/ml standard OD

A negative result indicates no current or previous infection with CMV. Such individuals are presumed to be susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, another specimen should be taken within 7 days and tested concurrently in the same assay with the original specimen to look for seroconversion.

A positive result indicates a primary or a reactivated infection with CMV. Such individuals are presumed to be at risk of transmitting CMV infection.

10. Limitations

1. Results of the Genesis Diagnostics CMV IgM assay are not by themselves diagnostic and should be interpreted in

conjunction with the patient's clinical condition and the results of other diagnostic tests.

2. A negative result does not rule out a primary or reactivated infection with CMV.
3. CMV-specific IgM antibody usually does not develop until the patient has been clinically ill for a week or more. Therefore, samples taken too early in the course of a primary infection may not have detectable levels of IgM.
4. Patients may continue to produce CMV specific IgM antibody for 6-9 months following a primary infection.
5. Heterotypic IgM antibody responses may occur in patients with Epstein-Barr virus and give false positive results.

11. Expected values

The incidence of CMV infection varies with age, geographical location, sexual behaviour and socio-economic class. CMV specific IgM usually develops after a patient has been clinically ill for at least a week or more. Most patients produce IgM transiently within 16 weeks of seroconversion. However, some patients continue to produce IgM for 6-9 months after seroconversion.

12. Performance characteristics

Comparative study:

The Genesis Diagnostics CMV IgM kit was compared with another commercially available ELISA procedure for the detection of IgM antibodies to CMV. The Genesis kit showed 100% agreement with the other ELISA. The results are summarised below.

Comparative Study (n=70)	Reference CMV IgM ELISA kit	
	+	-
Genesis	10	0
Diagnostics	0	60
CMV IgM kit	-	-

13. Assay characteristics

Within assay coefficient of variation < 12%
Between assay coefficient of variation < 12%

Method Summary

- Mix IgG absorbent and Sample Diluent 1:4 and dilute all samples 1:100
- Dispense 100µl of 10 U/ml standard, each control and diluted sample into the microplate wells
- Incubate for **20** minutes at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate (**Reagent 3**) into each well
- Incubate at room temperature for **20** minutes
- *Wash the wells four times*
- Add 100µl of TMB Substrate (**Reagent 4**) to each well
- Incubate at room temperature for **10** minutes
- Add 100µl Stop Solution (**Reagent 5**) to each well
- Read the optical density at 450nm

Further Reading

Drew WL: Diagnosis of cytomegalovirus infection. Rev Infect Dis 10:5468-5475, 1988
Booth JC *et al*: Comparison of enzyme-linked immunosorbent assay, radioimmunoassay, complement fixation, anticomplement immunofluorescence and passive agglutination techniques for detecting cytomegalovirus IgG antibody. J Clin Pathol 35: 1345-1348, 1982
Dylewski JS *et al*: Large scale serological screening for cytomegalovirus antibodies in homosexual males by enzyme-linked immunosorbent assay. J Clin Micro 19: 200-203, 1984
Stagno S *et al*: Immuno-globulin M antibodies detected by enzyme-linked immunosorbent assay in the diagnosis of cytomegalovirus infections in pregnant women and new born infants. J Clin Micro 21: 930-935, 1985