Anti-HCMV IgM plus ELISA

ELISA for the detection of Anti-Cytomegalovirus-IgM-antibodies in human sera and plasma

Package size
REF 807 034 96 tests [IVD] complete test package

Intend of use
Infections with human cytomegalovirus (HCMV), a ß-herpes virus, are widespread throughout the world (rate of prevalence 40 - 100 %). While an infection with HCMV proceeds asymptomatically in the majority of immunocompetent persons, it can lead to serious complications in persons whose immune system has been weakened or is not yet fully developed (Hepatitis, Retinitis, Pneumonitis, etc.). HCMV is the most frequent pathogen of congenital infections. Approximately 10 % of children congenitally infected with HCMV show symptoms at birth (icterus, hepatosplenomegaly, petechial bleeding and chorioretinitis). Further groups of patients for whom an acute HCMV infection represents a serious threat are organ and bone-marrow transplant recipients and AIDS patients. Using serological tests the CMV-infection can be differentiated from an EBV- or Toxoplasma-infection. The detection of anti CMV IgM is a marker of acute infection [2, 3].

Principle of the method - classic ELISA

The anti-HCMV IgM substrate test is a highly sensitive solid-phase enzyme linked immunosorbent assay (ELISA) for in vitro diagnosis [IVD] which is based on a mixture of different natural HCMV antigens(1). If the sample being tested contains specific antibodies, these will bind to the antigen in the wells of the microplate. Non-specific antibodies are removed in a washing stage. To avoid false positive results which were mediated by rheumatoid factors (RF) and to reduce the competition with IgG the test should be run with RF-sorbtent. The resulting antibody-antigen complex is detected using a specific, enzyme-labelled monoclonal antibody directed against human IgM. The presence of bound antibodies is demonstrated using an enzyme reaction with TMB (3,3’,5’,5’-tetramethylbenzidine) as a substrate which results in a coloured product. The enzyme reaction is stopped using sulfuric acid. Measure the extinction at 450 nm using a spectral photometer (reference wavelength: 615-690 nm).

Kit contains

MTP 1
Microplate
12 single strips with 8 wells each, coated with different HCMV specific antigens

NC 1.0 ml
CMV IgM negative control (ready for use, human) preservative: 0.005 % gentamycin, 0.05 %, streptomycin 0.05 %, penicillin V.

PC 1.0 ml
CMV IgM positive control (ready for use, human) preservative: 0.005 % gentamycin, 0.05 % streptomycin, 0.05 %, penicillin V.

COS 1.0 ml
Cut-off Serum (ready for use, human) preservative: 0.005 % gentamycin, 0.05 % streptomycin, 0.05 %, penicillin V.

DIL 45 ml
Sample diluent (ready for use) preservative: 0.01 % neomycin-sulfat, 0.03 % chloramphenicol

CONJ 15 ml
Anti human IgM conjugat (ready for use) preservative: 0.025% penicillin V, 0.025% streptomycin-sulfat, 0.2% procoag-300

WB 6 ml
Wash buffer concentrate (500 x) preservative: 0.01 % 2-bromo-2-nitro-1,3-propanediol

SUB 13 ml
Substrate solution (ready for use) 3,3’,5’,5’-tetramethylbenzidine (TMB) solution < 0,05 % in H2O.

STOP 15 ml
Stop solution (ready for use), sulfuric acid < 1 N H2SO4

SB 1
Storage bag: polyethylene bag for storing remaining microplate strips.

FOL 1
Self-adhesive transparent foils: Self-adhesive transparent foils for sealing the microplate wells during incubation.

CR 1
Package insert

Preservatives: total concentration < 0,11%

Materials required but not supplied with the kit
micropipettes, spectrophotometer (450 nm, reference wavelength 615 – 690 nm), microplate washer, incubator for ELISA [MTP].

Washing and precautions
Do not incorporate reagents. Avoid contact with eyes and skin. All samples and materials used for the test must be treated as being potentially infectious and appropriately safety precautions taken. The controls are negative for 1/1, anti-HCV, HBsAg, anti-lues and elevated transaminases. Do not pipet with mouth. According to good laboratory practice wear gloves, laboratory coat and safety glasses. Liquids and non-combustible materials should be decontaminated with sodium hypochlorite (final concentration: 3 %, activity time at least 30 minutes). Liquid waste which contains acids must be neutralised before disposal. All materials that are to be re-used must be autoclaved for 1 hour at 121°C. The SUB is sensitive of light and has to be protected from light. The test must be performed by well-trained and authorised laboratory technicians. Testing is performed under aseptic and microbiologically controlled conditions.

Storage
The reagents are stable up to the stated expiry dates on the individual labels when stored at 2-8°C. After opening reagents have to be used within 30 days. For repeatedly testing store the reagents immediately after usage at 2-8°C. The MTP sealed in an aluminium bag with a desiccant and must be at room temperature before opening. Return unused strips with the desiccant to the zip-lock bag and store in this way at 2-8°C. Do not touch the upper rim or the bottom of the wells with fingers.

Preparation of reagents
Dilute the wash buffer with demineralised or deionised water (e.g., 2ml diluted to 1 litre). The prepared buffer is stable for 1 week when stored at 2-8°C. All other test components are prepared ready for use. All reagents are lot specific and can not be used with kits of other lots. Do not use reagents of other manufacturers.

Sample preparation
Fresh serum or plasma samples, free from haemolysis should be used. Highly lipemic, icteric or microbially contaminated sera or plasma samples and concentrated immunoglobulin preparations can lead to unreliable test results. Avoid repeated freezing and thawing of the samples. If samples are to be transported, they must be packed in accordance with legal requirements for the transportation of infectious materials. The samples should not be inactivated, as unspecific reactions may otherwise occur.

Performance of test
The protocol (see pipetting procedure) has to be followed strictly. All controls and samples have to be diluted in the ratio 1:21 using sample diluent. Sera from patients were pretreated with RF-sorbtent. The qualitative determination of IgM antibodies can be performed using two alternative methods for manual or automated (ELISA processor) test runs. Sample dilution 1:21 with predilution in tubes

Dilute [PC], [NC] and [COS] samples 1:21 in a tube (e.g. 25 µl control or sample + 500 µl DIL). Mix well. The controls were not pretreated with RF sorbtent. Sera from patients were diluted in the ratio 1:21 too and pretreated with RF sorbtent (RFS). Samples will be diluted with RFS first and then the diluent is added (e.g., 25µl sample + 250 µl RF sorbtent + 250µl sample diluent, 1-2 dilution). The sample diluent is always added last. After pipetting, Mix well.

Washing procedure
The wash procedure is critical. Insufficient washing will result in poor precision and unspecific reactions.
W1: wash 5 times with wash buffer. For that remove the liquid in the well and dispense with 300µl washing buffer. Fill the well with at least 250µl washing buffer (total volume 550µl). This washing procedure is repeated 5 times. Tap out the plate briefly after washing. Do not allow the plate to dry out.

Pipetting procedure of IgM determination

The controls and the blank should be pipetted last. After pipetting the controls and samples immediately begin with incubation of the plate.

step 1 well [µl]

<table>
<thead>
<tr>
<th>A1/B1</th>
<th>C1/D1</th>
<th>E1/F1</th>
<th>G1/H1</th>
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<tbody>
<tr>
<td>Blank</td>
<td>200 [DIL]</td>
<td>[WC]</td>
<td>[PC]</td>
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Sample 1:21 (+ RF adsorption) from A2

Seal [MTP] using self-adhesive foils (not required in an ELISA processor*)

Incubation 60 ± 1 min., 37 ± 1°C

5x wash (s. W1) [WB]

step 2 well [µl]

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<tr>
<td>[WC]</td>
<td>100 [DIL]</td>
<td>100</td>
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Incubation 30 ± 1 min., 37 ± 1°C

5x wash (s. W1) [WB]

step 3 well [µl]

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<td>[WC]</td>
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Measure the extinction immediately or within 15 min after stop at 450 nm using a spectral photometer (reference wavelength: 615 - 690 nm).

* If an processors is used the operator has to validate the test under his own responsibility.

References
[1] The protocol
[2] The protocol
[3] The protocol

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Calculation of qualitative determination
After measuring the extinction values at 450 nm in all wells (reference filter: 615 - 690 nm), the mean value of the blanks is subtracted from the extinction values of the controls and samples. Mean extinction of blanks $\leq 0.100$ OD.
After subtraction of the blank, the control values must meet the following criteria of validity:

\[
\text{Mean OD-value of } \frac{\text{NC}}{\text{PC}} \leq 0.250 \quad \text{Mean OD-value of } \frac{\text{PC}}{\text{NC}} \geq 0.600
\]

Calculation of the Cut-off-value and grey area
The cut-off-value is dependent upon the mean OD value of the cut-off sample (CoS).

Sample-OD $\geq$ CoS-OD \hspace{1cm} Sample Anti-HCMV positive
Sample-OD $< \text{grey zone}$ \hspace{1cm} Sample Anti-HCMV negative
CoS-OD $>$ Sample-OD $\times 0.9$ \hspace{1cm} grey zone (questionable)

Interpretation of the result
Samples with an extinction value below the grey zone are considered to be negative. If a sample has an extinction value equal to or greater than the grey zone, it is considered to be HCMV-specific IgM antibodies. If the OD-value of the tested sample is within the grey area (questionable result) we recommend to request for a follow up sample.

Performance
200 sera from healthy blood donors and 110 patients out of different clinical stages of acute HCMV infections (transplant and immunocompromised patients of different clinical stages of infection/confirmation by antigenemia assay: PCR, virus culture or IgG seroconversion, three commercial available seroconversion panels) were tested in comparison with an reference ELISA.
The IgM Plus ELISA mediated a sensitivity of 98% and a specificity of 97.6%. 20 serum-EDTA heparin-/-citrat-samples were tested, in each of one individual were analysed (20 individuals),10 out of 20 samples were spiked with sera from patients with acute HCMV infection. The results which were obtained were in agreement for all samples.

The intra assay variability of the anti-HCMV IgM Plus ELISA was investigated by pipetting reactive samples in 8 wells of one plate. Using this procedure the following coefficient of variation (CV) were obtained: high reactive sample (3.17%) and low reactive sample 1.59%. The inter assay variability was evaluated by testing a sample in 8 subsequent runs. A CV of 7.6% with a) exceeding or plate was not incubated directly after finishing of pipetting.

b) Wavelength fault: Measurement without reference filter will increase OD values approximately + 0.120 OD

3) Yellow coloration in all wells: (see 2a, 2b)
a) WE contamination; Prepare new washing buffer
b) DI or CONJ contamination; Repeat test with reagents from unopened vials. Use reagents under less microbial conditions.

c) Washing fault: Too intensive washing or mechanic contact of samples

b) Temperature too low or fall below incubation time.

d) Incubation fault: Temperature too high, incubation time was exceeded or plate was not incubated directly after finishing of pipetting.

e) OD values approximately + 0.120 OD

4) Mean value of $\frac{\text{PC}}{\text{NC}}$ below $\leq 0.600$ OD
a) Exceed of expire date.
b) Temperature too low or fall below incubation time.
c) Washing fault: Too intensive washing or mechanic contact of manifold and solid phase of the well.
d) Contamination of PC or 3b.

5) Mean value of $\frac{\text{PC}}{\text{NC}}$ higher than $\geq 0.250$ OD: (see 1 and 2 a-d)
a) NC was not pipetted subsequent to pipetting of samples; Pipet all samples prior to pipetting of blanks and controls.
b) Contamination with the lid of the PC.

to take into consideration EBV specific serology assays and the detection of anti-glycoprotein B (gB) specific antibodies (Anti-HCMV rec. gB IgG ELISA, article number: (087 035)

Limitations of the method
The detection of antibodies is an indirect method of detecting a pathogen. Negative results obtained with the Anti-HCMV IgM Plus ELISA do not exclude the involvement of HCMV with disease. On the other hand a positive result is not sufficient to define illness. The specific knowledge of further clinical information and the patient’s case history are important prerequisites for correct interpretation. The following direct methods supplement the serological diagnosis of HCMV: antigenemia assay, PCR and virus cultivation. Sera from patients with an primary EBV-Infection, which are positive for heterophilic antibodies, can cause false positive results using the Anti-HCMV IgM Plus ELISA. For identification we recommend to request for a follow up sample.

Trouble Shooting Guide

1) Unexpected high rate of reactive results: Samples and controls were pipetted prior to pipetting of DIL or mixing was insufficient.

2) Mean blank value higher than criteria of validity, $\geq 0.100$ OD:
a) SUB turned blue due to oxidation or contamination.
b) Washing fault: Perform 5x wash cycles/washing step, use Biot-Rad STAB as contained in the kit.
c) Incubation fault: Temperature too high, incubation time was exceeded or plate was not incubated directly after finishing of pipetting.
d) Wavelength fault: Measurement without reference filter will increase OD values approximately + 0.120 OD

3) Yellow coloration in all wells: (see 2a, 2b)
a) WE contamination; Prepare new washing buffer
b) DIL or CONJ contamination; Repeat test with reagents from unopened vials. Use reagents under less microbial conditions.

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5) Mean value of $\frac{\text{PC}}{\text{NC}}$ higher than $\geq 0.250$ OD: (see 1 and 2 a-d)
a) NC was not pipetted subsequent to pipetting of samples; Pipet all samples prior to pipetting of blanks and controls.
b) Contamination with the lid of the PC.