CMV-IgA-ELA Test PKS medac

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CMV-IgA-ELA Test PKS medac

Enzyme immunoassay with Pipetting Control System for the detection of IgA antibodies to cytomegalovirus (CMV)

Cat. no.: 112-PKS

FOR IN VITRO DIAGNOSTIC USE ONLY

INTRODUCTION

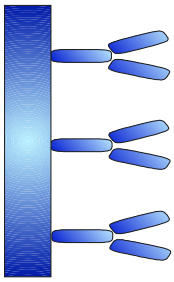
Cytomegalovirus (CMV) belongs to the family of human pathogenic *Herpesviridae* which is characterised by a double-stranded DNA genome. It is characteristic for these viruses to persist latently in the organism after primary infection. Reactivations of the virus can therefore occur under certain circumstances.

The course, CMV infections take in immunocompetent individuals, is normally without any symptoms. In individuals who suffer from immunosuppression (e.g. transplantation patients, HIV infected individuals, tumor patients, newborns) a variety of serious symptoms are observed.

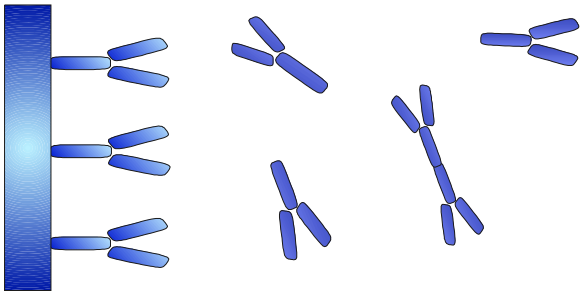
CMV is one of the most frequent pathogens in prenatal infection which can cause a variety of serious disorders, also including late damages in children born without symptoms.

Specific CMV IgA antibodies were detected in serum samples of transplantation patients and were correlated to specific CMV IgM antibodies. Therefore, CMV IgA antibodies represent a marker for acute infections. The detection of CMV-specific IgA in combination with CMV antigen and CMV-specific IgM leads to an enhanced specificity of the diagnosis of CMV disease in transplantation patients.

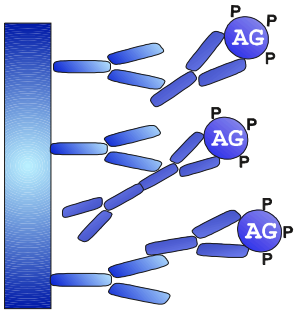
TEST PRINCIPLE



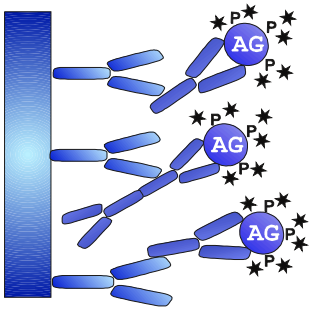
The plate is coated with anti-human IgA immunoglobulin.



IgA of the specimen is selectively bound to the wells.



The CMV-specific IgA antibodies bind to the peroxidase-labelled CMV antigen (AG = antigen, P = peroxidase).



Incubation with TMB-substrate (*). The reaction is stopped by the addition of sulfuric acid. The absorption is read photometrically.

Advantages of the test

- ☞ No unspecific reactions and no false positive results caused by rheumatoid factors.
- ☞ No blocking of IgA antibodies by high IgG titer.
- ☞ The Pipetting Control System allows to monitor visually each pipetting step through colour changes.
- ☞ The breakable microwell strips permit efficient use of the test.
- ☞ Suitable for automation on open ELISA devices.

KIT CONTENTS
Cat. no.: 112-PKS

1. **MTP**
   Microplate: 12 x 8 wells (with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, coated with goat anti-human IgA immunoglobulin, BSA and pH indicator, ready to use.

2. **CONTROL** | **-**
   Negative control: 1 vial with 1.5 ml, human serum, ready to use, contains BSA, phenol, ProClin™ 300 and gentamycine sulfate.

3. **CONTROL** | **+**
   Positive control: 1 vial with 1.5 ml, human serum, ready to use, contains BSA, phenol, ProClin™ 300 and gentamycine sulfate.

4. **WB**
   Wash buffer: 1 bottle with 100 ml, PBS/Tween (10x), pH 7.2 - 7.4, contains ProClin™ 300.

5. **VIR-DIL**
   Sample diluent: 1 bottle with 110 ml, PBS/Tween/BSA, pH 7.2 - 7.4, ready to use, contains ProClin™ 300.

6. **ANTIGEN**
   CMV-IgA-ELA (Enzyme Labelled Antigen): 4 vials with 5.0 ml each, lyophilised, contains FCS, stained yellow, HRP-conjugated.

7. **TMB**
   TMB-substrate: 1 vial with 10 ml, ready to use.

8. **STOP**
   Stop solution: 2 vials with 11 ml each, 0.5 M sulfuric acid (H_2SO_4), ready to use.

1. STORAGE AND STABILITY

Material/Reagent	State	Storage	Stability
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with desiccant	6 weeks
Controls	opened	2...8 °C	6 weeks
Wash buffer	diluted	2...8 °C	6 weeks
Sample diluent	opened	2...8 °C	6 weeks
CMV-IgA-ELA	reconstituted	2...8 °C	5 days
TMB-substrate	opened	2...8 °C	6 weeks
Stop solution	opened	2...8 °C	until expiry date

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Water for injection (H_2O redist.). Use of deionised water can disturb the test procedure.

2.2. Adjustable micropipettes.

2.3. Clean glass or plastic containers for dilution of wash buffer and specimen.

2.4. Suitable device for microplate washing (e.g. multistepper or ELISA washer).

2.5. Incubator for 37°C.

2.6. Microplate reader with filters for 450 nm and 620 - 650 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature to prevent condensation.

Calculate the number of wells required.

3.1. Microplate
The aluminium bag has to be tightly resealed together with the desiccant after each removal of wells. Storage and stability of the wells are indicated under point 1.

Note: The microplate wells have a light green colour. Eventually occurring greenish brown stains inside the wells are due to the production process and do not influence the test performance.

3.2. Wash buffer
Mix one volume of wash buffer (10x) with nine volumes of water for injection (e.g. 50 ml wash buffer (10x) with 450 ml water). 10 ml of diluted wash buffer are needed for eight wells.

Crystals in the wash buffer (10x) have to be dissolved by warming (max. 37 °C) and/or stirring at RT.

3.3. CMV-IgA-ELA
Reconstitute the lyophilised CMV-IgA-ELA with 5.0 ml **sample diluent** each. Mix gently and take care that particles sticking to the closure are also dissolved.

After reconstitution, the CMV-IgA-ELA has a yellow colour and is ready to use.

Do not mix test specific reagents (microplate, controls, CMV-IgA-ELA) from different kit lots. In contrast to that, sample diluent, wash buffer, TMB-substrate and stop solution are generally exchangeable in all virological ELISA medac.

Reagents from other manufacturers must not be used in general.

Valid and reproducible results are only obtained if the test procedure is precisely followed.

4. SPECIMEN

4.1. The test is suitable for serum samples.

4.2. Pretreatment of sera, e.g. inactivation, is not necessary. However, they should neither be contaminated with microorganisms nor contain any red blood cells.

4.3. Sera have to be diluted 1 : 100 with sample diluent. They can be diluted further for titer determination.

5.A. TEST PROCEDURE

5.1. Cut the aluminium bag above the zip fastener and take out the required number of microplate wells (see 3.1.).

Microplate wells are ready to use and do not have to be pre-washed.

5.2. Leave well A1 empty as blank (see 6.A.). Add 50 µl each of the diluted sample as well as negative control and positive control in duplicates to the wells.

After pipetting the samples (pH neutral or basic fluid) the wells turn blue. A missing colour change in one well indicates that no sample or control has been added.

If necessary the microplate wells can be kept at RT in a humid chamber up to 30 min or at 2 - 8°C up to 60 min before proceeding.

5.3. Incubate the microplate wells for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.4. Reconstitute the CMV-IgA-ELA (see 3.3.).

5.5. After incubation wash the microplate wells three times with 200 µl wash buffer per well. Pay attention that all wells are filled. After washing, tap microplate wells on filter paper.

Do not allow the wells to dry out! Proceed immediately!

5.6. Add CMV-IgA-ELA (coloured yellow) to each well (except A1).

50 µl of ELA have to be pipetted into the wells if the test is done manually.

Please note:
When working with automated devices, 60 µl of ELA have to be pipetted into each well due to a higher evaporation in the incubation chambers of the devices.

The suitability of the test for automated devices was confirmed during the evaluation of the test. Nevertheless we recommend to verify the compatibility of the test with the devices used in the lab.

5.7. Incubate again for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.8. After incubation wash microplate wells again (see 5.5.).

5.9. Add 50 µl of TMB-substrate to each well (also A1) and incubate for 30 min (± 2 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil in the dark. Positive samples turn blue.

5.10. Stop the reaction by adding 100 µl of stop solution to each well (also A1). Positive samples turn yellow.

Clean microplate wells from underneath before the photometric reading and take care that there are no air bubbles in the wells.

The reading should be done within 15 min after adding the Stop Solution.

5.B. TABLE FOR TEST PROCEDURE

	Blank (A1)	Negative control	Positive control	Sample
Negative control	-	50 µl	-	-
Positive control	-	-	50 µl	-
Sample	-	-	-	50 µl
Incubate for 60 min at 37 °C, wash 3 x with 200 µl wash buffer				
CMV-IgA-ELA	-	50/60 µl*)	50/60 µl*)	50/60 µl*)
Incubate for 60 min at 37 °C, wash 3 x with 200 µl wash buffer				
TMB-substrate	50 µl	50 µl	50 µl	50 µl
Incubate for 30 min at 37 °C in the dark				
Stop solution	100 µl	100 µl	100 µl	100 µl
Photometric reading at 450 nm (ref. 620 - 650 nm)				

*) manual/automatic procedure (see 5.6.)

6.A. CALCULATION OF RESULTS (VALIDITY)

* Read OD values at 450 nm (reference wavelength 620 - 650 nm).

* Subtract the OD value of the blank (well A1) from all other OD values.

* Validity Criteria
  - The mean OD value of the **negative control** has to be **< 0.150.**
  - The mean OD value of the **positive control** has to be **> 0.600.**

  Repeat the run if the results do not meet the specification.

* **Cut-off = mean OD value of the negative control + 0.140**

* **Grey zone = Cut-off ± 10 %**

6.B. INTERPRETATION OF RESULTS/LIMITATIONS OF THE METHOD

* Samples with OD values below the lower limit of the grey zone are reported as **NEGATIVE**.

* Samples with OD values within the grey zone are reported as **EQUIVOCAL**. These should be retested together with a fresh specimen taken 14 days later in order to determine a titer change.

* Samples with OD values exceeding the upper limit of the grey zone are reported as **POSITIVE**.

* The results should always be interpreted in connection with clinical data and additional diagnostic parameters.

* 59 potentially cross reactive serum specimen were tested. These samples were not reactive in the IgA test.

* Very high concentrations of lipids in negative samples can cause false positive results.
  High concentrations of Hemoglobin or Bilirubin do not influence the test results.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. SPECIFICITY

300 serum specimen from blood donors were tested with the CMV-IgA-ELA Test PKS medac. 296 sera were detected as negative. The other four sera - three of them were found to be equivocal and one weakly positive for IgA - were all detected as highly positive for CMV IgG and one also for CMV IgM.

The same 300 serum specimen were detected in another CMV-IgA-ELISA from a competitor. In this assay, 284 sera were found to be negative, two were equivocal and 14 were detected as positive.

7.B. SENSITIVITY

105 serum specimen were detected as CMV-IgA-positive with the above mentioned reference test. 101 sera were found to be positive, 2 were equivocal and 2 were detected as negative with the CMV-IgA-ELA test PCS medac. This results in a relative sensitivity of 96.2 %.

7.C. PRECISION

Sample	Intra-assay variation (n = 24)			Sample	Inter-assay variation (n = 11)		
	∅ OD	SD	CV (%)		∅ OD	SD	CV (%)
NC	0.047	0.007	15	**NC**	0.044	0.005	11
BC	0.341	0.014	4	**BC**	0.276	0.034	12
PC	1.601	0.047	3	**PC**	1.391	0.119	8
N° 1	0.047	0.006	13	**N° 1**	0.052	0.008	15
N° 2	0.223	0.010	4	**N° 2**	0.201	0.020	10
N° 3	2.473	0.052	2	**N° 3**	2.184	0.187	9

NC = Negative Control; BC = Weak Positive Control (not included in the kit); PC = Positive Control

GENERAL HANDLING ADVICES

* To avoid cross contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the shelf life.

* After use, all components of the testkit should be stored in the original package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFETY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of human origin have been tested and found to be negative for HBsAg, for antibodies to HIV-1/2 and to HCV. Nevertheless, it is strongly recommended that these materials as well as those of animal origin (see kit contents), should be handled as potentially infectious and used with all necessary precautions.

DISPOSAL CONSIDERATIONS

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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