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**User's Manual**

# Human CMV IgA ELISA Kit

REF **DEIA462**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

The test is intended for the diagnosis of CMV-caused or CMV-associated diseases such as infectious mononucleosis (IM), CMV syndrome, hepatitis in infants, intersticial pnemonia and active CMV infection in immunocompromised patients. The kit could be used for a complex characterisation of neurological or internal disorders and to test CMV seropositivity of blood and organ transplantation donors or acceptors. This examination can be supplemented with determination of IgM and IgG anti-CMV antibodies, eventually with IgG anti-CMV avidity test and with quantification of CMV-specific IgG in serum and cerobrospinal fluid.

## General Description

Human cytomegalovirus is a species of virus that belongs to the viral family known as Herpesviridae or herpesviruses. It is typically abbreviated as HCMV and is alternatively known as human herpesvirus-5 (HHV-5). Within Herpesviridae, HCMV belongs to the Beta herpesvirinae subfamily, which also includes cytomegaloviruses from other mammals. Although they may be found throughout the body, HCMV infections are frequently associated with the salivary glands. HCMV infection is typically unnoticed in healthy people, but can be lifethreatening for the immunocompromised, such as HIV infected persons, organ transplant recipients, or new born infants. After infection, HCMV has an ability to remain latent within the body over long periods. HCMV is found throughout all geographic locations and socioeconomic groups, and infects between 50% and 80% of adults in the United States (40% worldwide) as indicated by the presence of antibodies in much of the general population. Seroprevalence is age dependent: 58.9% of individuals aged 6 and older are infected with CMV while 90.8% of individuals aged 80 and older are positive for HCMV. HCMV is also the virus most frequently transmitted to a developing fetus. HCMV infection is more widespread in developing countries and in communities with lower socioeconomic status and represents the most significant viral cause of birth defects in industrialized countries. CMV "seems to have a large impact on immune parameters in later life and may contribute to increased morbidity and eventual mortality.

## Principles of Testing

The anti-CMV IgA is a solid-phase immunoanalytical test. The strips are coated by a mixture of specific antigens that bear immunodominant CMV epitopes. Anti-CMV antibodies, if present in the tested sera, bind to the immobilized antigens. The antibodies being in complexes with antigen are later on recognised by animal anti-human IgG antibodies labelled with horseradish peroxidase. The labelled antibodies are revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and the mild change in colour, if present, may be attributed to the reaction background.

## Reagents And Materials Provided

1. ELISA break-away strips coated with specific antigen (STRIPS Ag): 1 microplate
2. 1.3 mL Positive control serum r.t.u. *(CTRL+): 1 vial
3. 1.3 mL Negative control serum r.t.u. (CTRL-): 1 vial
4. 13 mL Anti-human IgA antibodies labelled with horseraddish peroxidase r.t.u. (Px-conjugate) CONJ: 1 vial

5. 55 mL Wash buffer 10x concentrated (WASH 10x): 1 vial
6. 60 mL Dilution buffer r.t.u. (DIL): 1 vial
7. 13 mL Chromogenic substrate (TMB substrate) r.t.u. (TMB): 1 vial
8. 13 mL Stop solution r.t.u. (STOP): 1 vial
9. Sealable pouch for unused strips
10. Instruction manual
11. Certificate of quality

* : ready to use

## Materials Required But Not Supplied

1. Distilled or deionised water for dilution of the Wash buffer concentrate.
2. Appropriate equipment for pipetting, liquid dispensing and washing.
3. Spectrophotometer/colorimeter (microplate reader - wavelenght 450 nm).

## Storage

Store the kit reagents at 2-8°C. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing. For more detailed information, please download the following document on our website.

## Reconstitution And Storage

1. Store the kit and the kit reagents at 2 - 10°C, in a dry place and protected from the light.
2. Store unused strips in the sealable pouch and keep the desiccant inside.
3. Store serum samples at 2 - 10°C up to one week. For longer period make aliquots and keep them at -20°C.
4. Avoid repeated thawing and freezing. Do not store diluted samples. Always prepare fresh.
5. Kits are shipped in cooling bags, the transport time of 72 hours have no influence on expiration.
6. If you find damage at any part of the kit, please inform the manufacturer immediately.
7. Expiration date is indicated at the ELISA kit label and at all reagent labels.

## Reagent Preparation

1. Allow all kit components to reach room temperature.
2. **Vortex samples and the Controls** in order to ensure homogeneity and mix all solution well prior use.
3. **Dilute serum samples 101x** in Dilution buffer and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). Do not dilute the Controls they are ready to use.
4. **Prepare Wash buffer** by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionized water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to 32 - 37°C in a water bath. Diluted Wash buffer is stable for one week if stored at 2 - 10°C.

5. **Do not dilute** Px-conjugate, TMB substrate and Stop solution, they are ready to use.

## Assay Procedure

1. Allow the vacuum-closed aluminium bag with strips to reach a room temperature. Withdraw the adequate number of strips and put unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
2. Pipette 100 µL of Dilution buffer and Control sera and serum samples to the wells according to the pipetting scheme in Figure 1 : fill first well with Dilution buffer DIL to determine reaction background. Fill the next two wells with Positive control serum CTRL+. The next well fill with Negative control serum CTRL-. The remaining wells fill with diluted tested sera (S1...). It is satisfactory to apply one serum into one well (S1, S2, S3, ...). However, if you want to minimize a laboratory error, apply CTRL+ in triplet and remaining sera in doublets.
3. Incubate 60 minutes (5 min) at room temperature.
4. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µl/well of Wash buffer. Avoid cross-contamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
5. Add 100 µL of Px-conjugate CONJ into each well.
6. Incubate 60 minutes (5 min) at room temperature.
7. Aspirate and wash four times with 250 µl/well of Wash buffer.
8. Dispense 100 µl of TMB substrate into each well.
9. Incubate for 10 minutes (+/-30 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Cover the strips and keep them in the dark during the incubation with TMB substrate.
10. Stop the reaction by adding 100 µL of Stop solution STOP. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.
11. Read the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use reference reading at 620-690 nm.

**Fig.1 Pippetting scheme**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **a** | DIL | S5 | | | | | | | | | | |
| **b** | CTRL+ | … | | | | | | | | | | |
| **c** | CTRL+ | | | | | | | | | | | |
| **d** | CTRL- | | | | | | | | | | | |
| **e** | S1 | … | | | | | | | | | | |
| **f** | S2 | | | | | | | | | | | |
| **g** | S3 | | | | | | | | | | | |
| **h** | S4 | | | | | | | | | | | |

## Calculation

Determine the Positivity Index for each serum sample as follows:

1. Compute the cut-off value
2. Compute the Positivity Index according to the following formula:
   **Sample Positivity Index = Sample absorbance / cut-off value**
3. Express a serum reactivity according to Table 1 (Semiquantitative interpretation of results)

Table 1: Semiquantitative interpretation of the results

| Index value | Evaluation |
|---|---|
| < 0.9 | Negative |
| 0.9 - 1.10 | +/- |
| > 1.11 | Positive |

**Note:** An equivocal sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later.

Example of calculation:

OD of CTRL+ = 1.430; 1.490; 1.448

Mean OD CTRL+ absorbance = 1.456

Sample absorbance = 0.800

Correction factor = 0.16

Cut-off value = 1.456*0.16 = 0.233

Sample Positivity Index = 0.800/0.233 = 3.43

## Interpretation Of Results

Presence of IgA anti-CMV in serum can be related to acute or recent active CMV infection (either the primary infection and reactivation, including the asymptomatic one). After recovery, IgA antibodies may persist in the patient´s serum for several months. IgA anti-CMV antibodies may be present in as much as 10 per cent of healthy seropositive individuals. Serological findings must be interpreted in the context with the clinical symptoms and with the other laboratory results only.

## Precision

The intraassay variability (within the test) and the interassay variability (between tests) were performed with samples of variable absorbance values.

## Sensitivity

Assessment of diagnostic effectivity was performed using comparative testing of 90 CMV IgA negative and 58 CMV IgA-positive serum samples on ELISA and two alternative commercial IVD kits. Diagnostic sensitivity of the test was 100% and the specificity 96.29%.

## Recovery

Measured values of recovery test for every Lot are between 80-120% of expected values.

## Interferences

Haemolytic and lipemic samples have no influence on test results to minimal concentration 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL triglycerides.

## Precautions

1. Manufacturer guarantees performance of the entire ELISA kit.
2. Follow the assay procedure indicated in the Instruction manual.
3. Wash solution, TMB substrate, Stop solution and Dilution buffer are compatible and interchangeable between CD kits unless otherwise stated in the instruction manual.
4. Controls, TMB substrate, Dilution buffer and Px-conjugate contain preservative ProClin 300®.
5. Avoid microbial contamination of serum samples and kit reagents.
6. Avoid cross-contamination of reagents.
7. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
8. Variations in the test results are usually due to:
   * Insufficient mixing of reagents and samples
   * Inaccurate pipetting and inadequate incubation times
   * Poor washing technique or spilling the rim of well with sample or Px-conjugate

* Use of identical pipette tip for different solutions

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