Cytomegalovirus IgG ELISA

Catalog No. E-CVG-K01

CE 0483

IVD

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INTENDED USE

The RD-RatioDiagnostics E-CVG-K01 Cytomegalovirus IgG Elisa test system is an Enzyme-Linked Immunosorbent Assay (ELISA) kit providing material for the semi-quantitative detection of IgG-class antibodies to Cytomegalovirus in human serum or plasma. This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

Cytomegalovirus (CMV) belongs to Herpes virus family and is transmitted through saliva, sexual contact, perinatally, or through blood transfusions or organ transplantation. Infection with CMV appears to be worldwide and common despite the relative rarity of clinical disease. CMV causes most of the congenital virus infections in humans, with an incidence ranging from 0.2 to 2.2% of live births in different populations. Intrauterine transmission of the virus can occur at any time during gestation, but most infants are probably infected during birth or after birth from ingesting CMV-infected maternal milk. Disease of newborn with CMV infection is an often severe, fatal illness, usually affecting the salivary glands, brain, kidneys, liver and lungs.
After the primary infection, CMV can persist in a dormant state as a latent infection.
During immunosuppressive treatment of patients (e.g. recipients of organ transplants), latent infection can be activated and appear as a secondary infection. CMV is one of the most serious and frequent pathogens in AIDS patients. CMV pneumonia, a life threatening infection may occur in about 20% cases BMT (Bone Marrow Transplant) patients. The ability to distinguish primary from latent infection is of great importance inasmuch as primary maternal infections have greater pathological potential for fetus.
Diagnosis is made mainly by serological findings of antibodies (IgG and IgM classes) to CMV.

PRINCIPLE OF THE TEST

The E-CVG-K01 Cytomegalovius IgG kit is based on the ELISA technique. In the assay, calibrators and unknowns are incubated in microtitration wells coated with purified and inactivated CMV antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-CMV IgG antibodies present.

REAGENTS

The RD-RatioDiagnostics Cytomegalovirus IgG ELISA kit contains sufficient reagents for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED	QUANTITY	CATALOG NO.
CMV Antigen-Coated Microtitration Strip	One Plate	E-CVG-10
Wash Concentrate	One Bottle	E-WSL-30
Sample Diluent	One Bottle	E-DLB-40
TMB-Substrate	One Bottle	E-TMB-08
Calibrator 0	One Vial	E-CVG-01
Calibrator 1	One Vial	E-CVG-02
Calibrator 2	One Vial	E-CVG-03
Calibrator 3	One Vial	E-CVG-04
Calibrator 4	One Vial	E-CVG-05
2nd Antibody Conjugate	One Bottle	E-CVG-20
Stopping Solution	One Bottle	E-STP-09

MATERIAL NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator

REAGENTS PROVIDED:

Antigen-Coated Microtitration Strips:
One stripholder containing 12x8 (96) microtitration wells coated with purified inactivated *Cytomegalovirus* antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate:
One bottle, 100 ml, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent:
One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

***Cytomegalovirus* IgG Calibrators**
Five vials , each 2 ml of human serum, *calibrated according to PEI reference standards Anti-CMV IgG, Germany*, in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. *This PEI anti-CMV IgG reference standard has been prepared from one positive donor (not treated, no additives).* The value for Calibrator 1 represents the Cut-Off control, values are reported on the labels of the vials. Store at 2-8°C until expiration date.

2nd Antibody Conjugate:
One bottle, 12 ml, containing anti-human IgG monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

TMB-Substrate:
One bottle, 12 ml, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

Stopping Solution:
One bottle, 15 ml, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS
For *in vitro* use
The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain some reagents made with human and animal sources material (e.g. serum, plasma or bovine albumin*)* or used in conjunction with human and animal source material. The material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HbsAg; the material has no record of any animal infection. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:
Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.*Sample diluent and controls contain diluted BSA.*

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING
Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY
A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS:

Wash Solution:

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 ml of the Wash Concentrate into a clean container and dilute by adding 900 ml of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips:

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Assay Procedure:

All specimens and reagents to reach room temperature (~25°C) before use. Samples and Calibrators should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µl of serum into 1 ml of Sample Diluent.
3. Pipette 100 µl of each diluted serum sample and ready to use calibrators to the appropriate wells. Leave one well for the blank.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 ml of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
6. Add 100 µl of ready to use Enzyme-Labeled 2nd Antibody-conjugate into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µl of TMB Chromogen Solution to each well (including the blank) using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µl of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

RESULTS

Calculate the mean absorbance for each calibrator and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Calibrator 1, the sample is positive for the presence of specific IgG.
Calculate the ratio between the average OD value of the sample and that of the Calibrator 1. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

Semi-Quantitative results:

The IgG anti-*CMV* concentration of each sample can be expressed in *international units/mL (IU/mL).* A graph can be constructed by plotting the U/mL against the average OD of the controls; when the OD of the sample is reported on the graph, the U/mL contained in the serum sample can be calculated. A standard curve must be performed for each run.

Positive/Negative results can be expressed in U as follows:

Positive : sample concentration > 10 U/mL

Negative: sample concentration < 9 U/mL

Equivocal: sample concentration ranges between 9 and 10 U/mL.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the acute phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.
- Serological data of immunocompromised patients and newborn children have restricted value.

QUALITY CONTROL

Subtract the value of the blank from all the other readings. The OD values of Calibrator 1 must be at least 0.2. Calibrator 4 must have an OD at least 3 times that of Calibrator 1.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

86 well selected human sera, collected from a clinical laboratory in Frankfurt, Germany, were analyzed by this Cytomegalovirus IgG Elisa and reference Elisa method based on quantitative detection. Out of 86 samples, 63 were positive for the presence of IgG antibodies to CMV by RD-RatioDiagnostics Elisa, and reference method showed 61 of them as positive, two as brd line. The RD-RatioDiagnostics Kit E-CVG-K01 has sensitivity 100% and specifity of 100 % when brd lines samples are considered as positive. An analytical comparison between two assays showed R^2=0.811 which is acceptable considered a serological assay. The results are briefly summarized in Tab1.

Tab1.

Assay Comparison	*RD-RatioDiagnostics*		
TESTB	Positive	Negative	Brd. Line
Positive	61	0	0
Negative	0	23	0
Brd Line	2	0	0

2. Precision

Tab. 2

2. Intra-assay Study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	1,82	0.361	0,019
SD	0.06	0,014	0.005
CV%	**3,08**	**4,1**	**26,5**

Tab. 3

3. Inter-assay study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	1,4	0,946	0,039
SD	0.23	0,49	0,0029
CV%	**1,61**	**5,2**	**7,4**

3. Interferences

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

4. Analytical sensitivity

The analytical sensitivity of RD-RatioDiagnostics CMV IgG ELISA has been carried out by serial dilution of high positive sample; it showed that the assay has an analytical sensitivity (detection limit) up to 1.8 Units/mL.

REFERENCES

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