TestLine®

Instruction for use

EIA CMV IgA

REF CMA096

Σ 96

Kit for professional use

IVD CE 2265

TestLine Clinical Diagnostics s.r.o.
Křižíkova 68, 612 00 Brno, Czech Republic
Tel.: +420 541 248 311
FAX: +420 541 243 390
E-mail: info@testlinecd.com
www.testlinecd.cz
www.testlinecd.com

CONTENT

1 Intended Use

Enzyme immunoassay provides the detection of IgA antibodies to Cytomegalovirus in human serum, plasma or cerebrospinal fluid.

2 Introduction

Human cytomegalovirus (CMV, Human Herpesvirus 5, HHV 5) belongs to the group of herpesviruses.

Primary infection with human CMV most commonly occurs in childhood or adolescence via various modes of transmission (respiratory, urogenital). The clinical course is usually asymptomatic or mild (fever, fatigue, symptoms of mononucleosis). After primary CMV infection, the virus enters a latent phase and subsequent reactivation (secondary infection) occurs depending on changes in the host-virus relationship (pregnancy, severe illness, stress, immunosuppressive therapy). Reinfection may occur with another CMV strain. Primary CMV infections during pregnancy pose a serious risk (congenital infections with varying degrees of damage occur in 1/3 to 1/2 of newborns, while only in 1% in case of reactivations). Immunocompromised people (AIDS, transplants, etc.) usually develop symptoms with involvement of various organs, often with fatal consequences.

The diagnosis of the disease is based on clinical picture and laboratory tests. Serological methods for the determination of specific IgA, IgM and IgG class antibodies and their avidity by ELISA are most commonly used in the laboratory diagnosis of CMV.

IgA antibodies are an indicator of active infection, i.e. primary infection and reactivation. Reactivation produces antibodies of the IgA class, which may or may not be accompanied by specific IgM. Thus, IgA class antibodies are of great importance for the confirmation of CMV reactivation in the presence of IgG antibodies.

IgM antibody levels usually increase several weeks after the infection and then slowly decrease over 4 to 6 months. In immunodeficient patients, they may be present at low levels for up to two years after infection. The detection of IgM antibodies alone cannot distinguish between primary and secondary infection, as reactivation can also induce their synthesis.

Specific IgG antibodies are detectable approximately 1 week after the increase of IgM and IgA levels. Their seroconversion (titre increase) indicates primary infection. The differentiation of primary infection from reactivation is based on the avidity of IgG antibodies, which is important for the assessment of the risk of congenital infection.

Detection of IgG antibodies is also established as a standard method for identifying CMV positive individuals in donor screening.

3 Test Principle

The kit is intended for detection of specific IgA antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen – antibody from the analysed sample – labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to human IgA conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific IgA antibodies in the sample.

Antigen Used

Purified and inactivated antigen isolated from CMV AD 169 strain with a high content of specific immunodominant epitopes

4 Materials Provided

MICROPLATE	**Microtitre Plate** coated with antigen, 12 x 8 wells in bag with desiccant	1 pc
CONTROL -	**Negative Control** Solution containing no specific human antibodies, ready to use	1 × 2 ml
CUTOFF	**CUT-OFF** Solution containing specific human antibodies in cut-off concentration, ready to use	1 × 3 ml
CONTROL +	**Positive Control** Solution containing specific human antibodies, ready to use	1 × 2 ml
CONJUGATE	**Conjugate** Solution containing peroxidase labelled animal immunoglobulin to human IgA, ready to use	1 × 15 ml
DILUENT 2	**Sample Diluent 2** Buffer with protein stabilisers, ready to use	1 × 105 ml
SUBSTRATE 2	**TMB-Complete 2** Chromogenic substrate solution containing TMB/H_2O_2, ready to use	1 × 15 ml
WASH 20x	**Wash Solution** 20× concentrated buffer	1 × 75 ml
STOP	**Stop Solution** Acid solution, ready to use	1 × 15 ml
	Instructions for use	1 pc

5 Other Material Required for Manual Test Performance

Single and multichannel pipettes

Disposable tips

Microplate washer

Timer

Incubator (37°C)

Microplate reader

6 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

Samples Preparation and Storage

The following human body liquids can be used for testing: serum, citrate plasma and cerebrospinal fluid. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C. Diluted samples should be used as soon as possible.

7 Preparation of Reagents

Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 ml of the concentrated Wash Solution + 1425 ml of distilled water.

Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.

The Controls (positive, negative and CUT-OFF) are ready to use, do not dilute further!

The Conjugate is ready to use, do not dilute further!

TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

Interchangeability of reagents

The Sample Diluent, TMB-Complete and the Avidity Solution are interchangeable in EIA kits of TestLine Clinical Diagnostics s.r.o., provided they have the identical numeric marking (e.g. Sample Diluent 2, Sample Diluent 3, etc.). The Stop Solution and the Wash Solution are universal in all kits.

8 Preparation of Samples

Mix gently the Sample Diluent prior to use.

Dilution of sera and plasma samples

Dilute well mixed samples 1:101 with the Sample Diluent:

E.g.: 10 µl of sample + 1 ml of the Sample Diluent

Mix well.

Dilution of cerebrospinal fluid samples (CSF)

Dilute well mixed CSF 1:3 with the Sample Diluent:

E.g.: 50 µl of CSF + 100 µl of the Sample Diluent

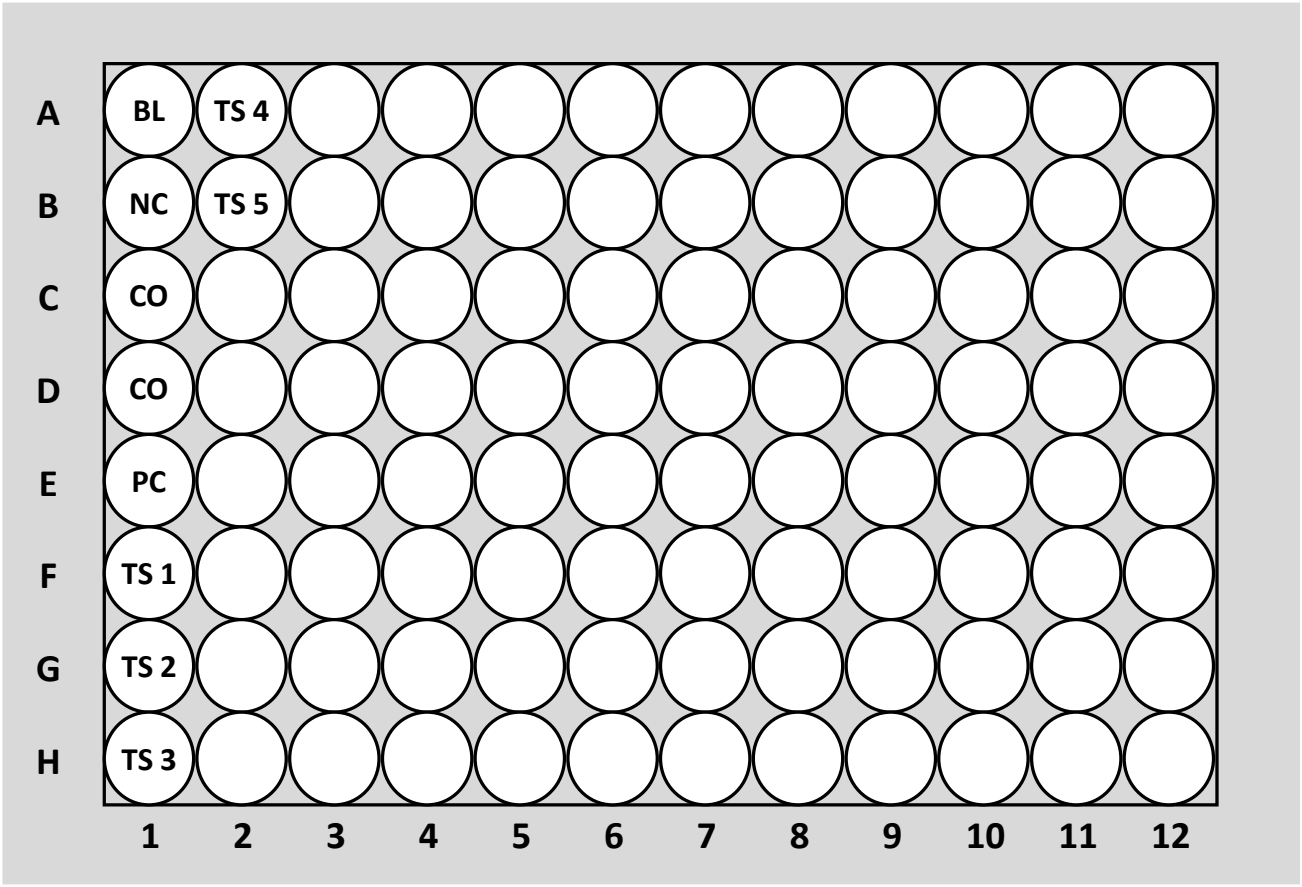
Mix well.

9 Assay Procedure

Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls and the diluted samples according to the working schedule.
   - Leave A1 well empty (blank).
   - Pipette 100 µl of the Negative Control into 1 well.
   - Pipette 100 µl of CUT-OFF into 2 wells.
   - Pipette 100 µl of the Positive Control into 1 well.
   - Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.
2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Conjugate into all wells except A1 well.
5. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
6. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
8. Cover the microplate with the lid and incubate at 37°C for 30 minutes. Keep out of light.
9. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

10 Working Schedule



BL	Blank (empty well)	
NC	100 µl	CONTROL -
CO	100 µl	CUT-OFF
PC	100 µl	CONTROL +
TS 1-x	100 µl	diluted tested sample

11 Quality Control

The test id valid if:

The absorbance of blank is lower than 0.150.

$$BLANK < 0.150$$

The absorbance of the Negative Control is lower than half of the mean absorbance of CUT–OFF.

$$\boxed{CONTROL\ -} < 0.5 \times \boxed{CUT\text{-}OFF}$$

The mean absorbance of CUT-OFF is within a range of 0.150 – 0.900.

$$0.150 < \boxed{CUT\text{-}OFF} < 0.900$$

The absorbance of the Positive Control is higher than the mean absorbance of CUT-OFF.

$$\boxed{CONTROL\ +} > \boxed{CUT\text{-}OFF}$$

12 Results Interpretation

Calculation of Index of Positivity (IP)

Divide the absorbance of a tested sample by the mean absorbance of CUT-OFF measured in the same test run:

$$IP = \frac{\text{Absorbance of sample}}{\text{Mean absorbance of CUT-OFF}}$$

Interpretation of the test results is described in the table (Table 1).

Table 1 Interpretation of results

Index of Positivity (IP)	Evaluation
lower than 0.9	negative
0.9 to 1.1	borderline
higher than 1.1	positive

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Serological finding can be interpreted only in the context of results of other laboratory tests and patient clinical picture.

13 Test Performance

13.1 Specificity and Sensitivity

Diagnostic specificity was determined in the panel of negative sera. Diagnostic sensitivity was determined in the panel of positive sera. The number of sera tested and the results obtained are described in the table (Table 2).

13.2 Reproducibility

Reference control samples were tested in a statistically significant number of replicates, either in one or several analyzes. Acquired data was used for Intra assay and Precision within the laboratory. The obtained results are described in the table (Table 2).

13.3 Analytical Sensitivity – maximum threshold sensitivity

The analytical sensitivity is the maximum binary dilution of CUT-OFF-like or low positive sample, possibly international recognized standards, giving absorbance significantly different from the background. The value is expressed in units of U/ml. This value is a minimum limit of detection and quantification. The obtained results are described in the table (Table 2).

13.4 Intra-homogeneity

The intra-homogeneity is expressed as the amount of agreement among 100 replicates of CUT OFF-like or low positive serum in one analyse. The value is expressed as a coefficient of variation. The obtained results are described in the table (Table 2).

13.5 Measuring range of the kits

The measuring range of the kit lies between values where the lower limit is determined by the analytical sensitivity value and the upper limit depends on the measuring capability of the equipment used.

Table 2 Test Performance

Parameter	Value
Specificity (n 55)	95.83%
Sensitivity (n 19)	94.74%
Intra-assay	4.71%
Within-laboratory precision	4.93%
Analytical sensitivity limit – index of positivity (IP)	0.06
Intra-homogeneity	5.63%

13.6 Interference

Two samples (one negative plasma pool and one positive plasma pool) were spiked with potentially interfering endogenous substances. Results of interference testing are shown in the table (Table 3).

Table 3 Interference Results

Interfering substance	The result was not affected up to concentration:
Bilirubin	0.4 mg/ml
Triacylglycerols	20 mg/ml
Haemoglobin	5 mg/ml

13.7 Cross-reactivity

The assay was evaluated for potential cross-reactivity using samples positive for selected pathogens and factors. Results of testing are shown in the table (Table 4).

Table 4 Results of Cross-Reacting Pathogens or Factors

Category	n	Positive Result
EBV VCA	9	0
VZV	4	0
Toxoplasma gondii	5	0
Mumps virus	6	0
Mycoplasma pneumoniae	10	0
Chlamydia pneumoniae	8	0
Bordetella pertussis	7	0
RF	6	0
Total	**55**	**0**

14 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.

The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.

It is necessary to observe the local safety rules and regulations.

First aid

In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore, they need to be disposed together with biological waste.

Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

15 Procedural Notes

In order to obtain reliable results, it is necessary to **strictly follow the Instructions for Use**. Always use clean preferably disposable tips and glassware.

Microtitre Plate – in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.

Wash Solution – use high quality distilled water for preparing the working strength Wash Solution.

Washing procedure – keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.

TMB-Complete – the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.

Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps
- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature; bacterial or chemical contamination
- insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- contamination of the well edges with Conjugate or samples
- using reagents from different kit lots
- contact of reagents with oxidants, heavy metals and their salts

The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

The kit might be used in all types of automatic EIA analysers.

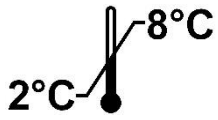
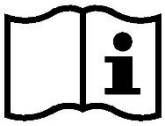
If necessary, TestLine Clinical Diagnostics s.r.o. can offer a certified modification of the Instructions for Use for the specific type of analyser.

The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

16 References

1. Blackburn NK, Besselaar TG, Schoub BD, O'Connell KF. Defferentiation of primary cytomegalovirus infection from reactivation using the urea denaturation test for measuring antibody avidity. *J Med Virol.* 1991, 33(1), 6-9.
2. Bodeus M, Feyder S, Goubau P. Avidity of IgG antibodies distinguishes primary from non-primary cytomegalovirus infection in pregnant woman. *Clin Diagn Virol.* 1999, 12(1), 3-8.
3. Boeckh M, et al. Quantitation of cytomegalovirus: methodologic aspects and clinical application. *Clin Microbiol Rev.* 1998, 11(3),533-554.
4. Dussaix E, Chamtit S, Harzic M, Grangeot-Keros L. CMV-IgG avidity and CMV IgM concentracion in both immunocompromised and immunocompetent patiens. *Pathol Biol.* 1996, 44(5), 405-410.
5. Grangeot-Keros L, Mayaux MJ, Lebon P, Freymuth F, et al. Value of cytomegalovirus (CMV) IgG avidity index for the diagnosis of primary CMV infection in pregnant women. *J Infect Dis.* 1997, 175(4), 944-946.
6. Joassin L, et al. Elimination of nonspecific cytomegalovirus immunoglobulin M activities in the enzyme-linked immunosorbent assay by using anti-human immunoglobulin. *J Clin Microbiol*. 1986, 23(3), 576-581.
7. Lazzarotto T, Varani S, Guerra B, Nicolosi A, et al. Prenatal indicators of congenital cytomegalovirus infection. *J Pediatr.* 2000, 137(1), 90-95.
8. Pappin A, et al. Stability of cytomegalovirus antibodies in plasma during prolonged storage of blood components. *Clin Diagn Lab Immunol.* 1995, 2(1), 25-29.
9. Revello MG, et al. Diagnosis and Management of Human Cytomegalovirus Infection in the Mother, Fetus, and Newborn Infant. *Clin Microbiol Rev.* 2002, 15(4), 680-715.

17 IFU Symbols

	Temperature limitation
	Keep dry
	Expiry date
	Lot number
	Manufactured by
	Consult instructions
	Catalogue number
	Number of tests
	In vitro diagnostic medical device

Notes

Summary of EIA CMV IgA Protocol

Step No.	Symbol	Test steps
1		Dilute samples serum/plasma 1:101 (10 µl + 1 ml) cerebrospinal fluids 1:3 (50 µl + 100 µl)
2		Pipette Controls and diluted samples – 100 µl Blank = empty well
3		Incubate at 37°C for 30 mins
4		Aspirate and wash the wells 5×
5		Pipette Conjugate – 100 µl Blank = empty well
6		Incubate at 37°C for 30 min
7		Aspirate and wash the wells 5×
8		Pipette Substrate (TMB-Complete) – 100 µl Including blank
9		Incubate at 37°C for 30 min
10		Pipette Stop Solution – 100 µl Including blank
11		Read colour intensity at 450 nm