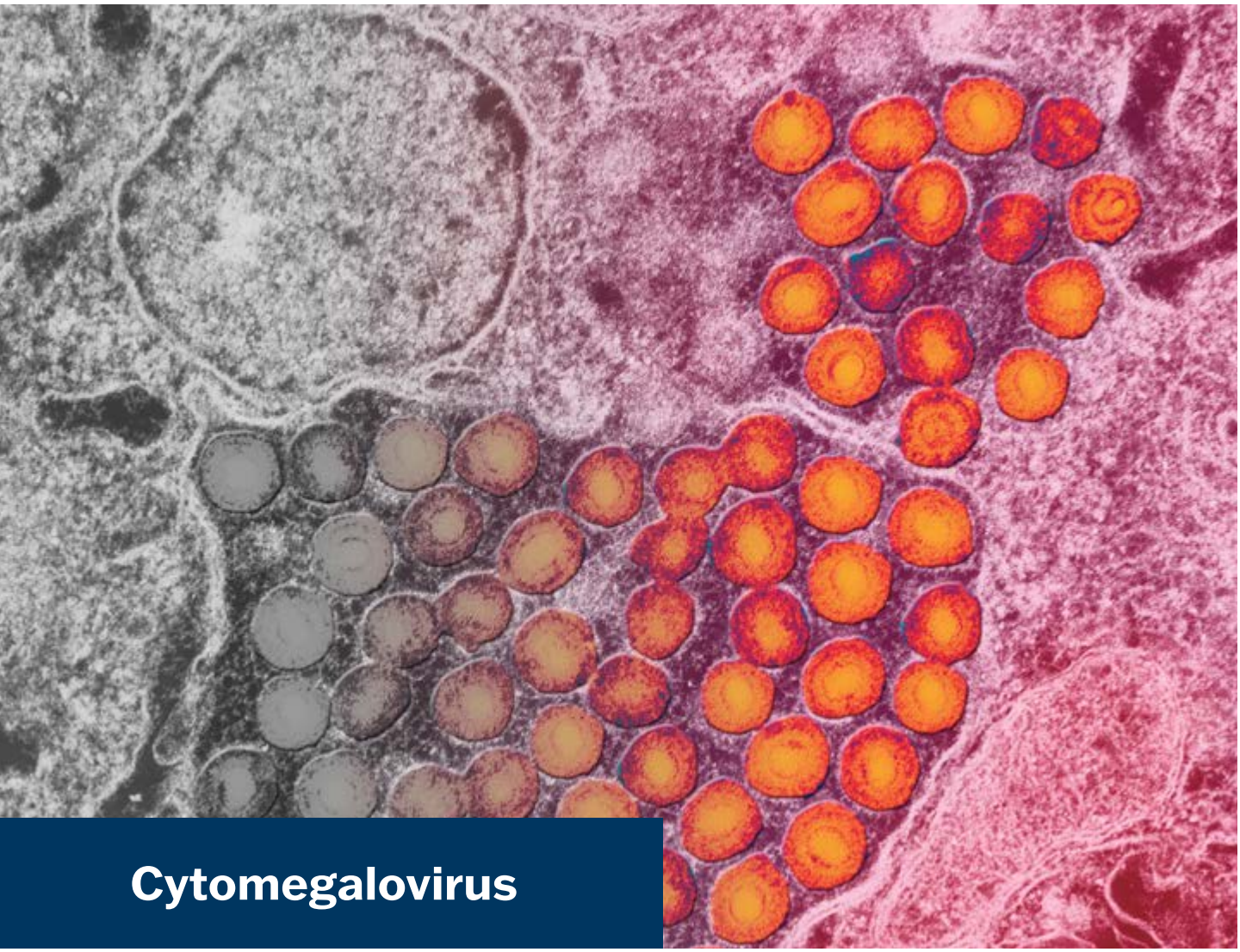


Cytomegalovirus

Enzyme immunoassays for the diagnostics of cytomegalovirus infection

ELISA and **IMMUNOBLOT** kits are optimized and validated for detection of IgA, IgG and IgM antibodies in human serum, plasma or cerebrospinal fluid

BG TestLine®

Introduction

Human cytomegalovirus (CMV, Human Herpesvirus 5, HHV 5) is a member of the Herpetoviridae family. Primary infection of CMV occurs mainly in childhood or adolescence. The infection can be transmitted in different ways (e.g. respiratory apparatus, digestive or urogenital tract). Clinically, the disease process is usually asymptomatic or mild (fever, fatigue, mononucleosis symptoms). Dormant infections where the virus survives can be reactivated – generally by changes in host -virus relations (pregnancy, serious disease, stress, immunosuppressive treatment). Reinfection is possible with a different strain of CMV. During the acute stage of infection, convalescence and reactivation of the disease, the virus is exuded in saliva and urine. CMV infection during pregnancy causes developmental defects (virus is easily transmitted through the placenta and affects the fetus). During primary infection mother-to-infant transmission of the virus (i.e. via placenta) occurs in 1/3 to 1/2 of cases; however, during the reactivation stage, transplacental transmission occurs in only 1% of cases.

Diagnosis of infection

Diagnosis of the disease is based on a clinical picture and laboratory tests.
ELISA method used for detection of specific antibodies IgA, IgG or IgM in human serum or plasma is the most frequent laboratory method used in serological diagnostics of CMV infection.

Diagnostic significance of antibody classes

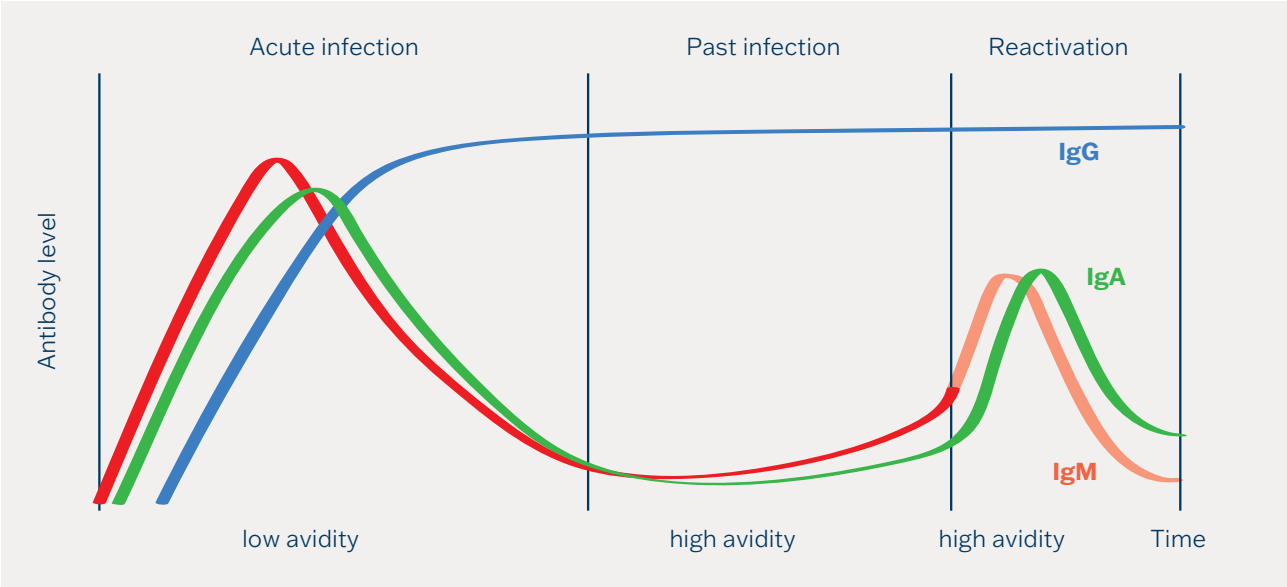
IgA: Antibodies of IgA class are a sign of an active infection – primary infection as well as reactivation. The IgA antibodies are produced during reactivation and they can rarely be produced with specific IgM class antibodies. Specific IgA antibodies are very important for confirmation of CMV infection reactivation, when they are present with IgG antibodies.

IgM: Production of IgM antibodies usually increases a few weeks after infection and then (during 4–6 months) decreases slowly. In immunosuppressed patients the IgM antibodies can be present at low levels even two years after infection. As IgM antibodies can also be produced during reactivation, IgM determination alone cannot discriminate primary infection from reactivation.

IgG: Specific IgG antibodies can be detected approx. 1 week after the increase of IgM and IgA antibodies. Their seroconversion (increase of titre) indicates primary infection. The IgG antibodies persist in low levels for the entire life of the person. The method of IgG avidity detection is used for discrimination between primary infection and reactivation. It is important for the risk assessment of congenital transmission.

Detection of IgG antibodies to CMV is also used as a standard method for screening at blood donors

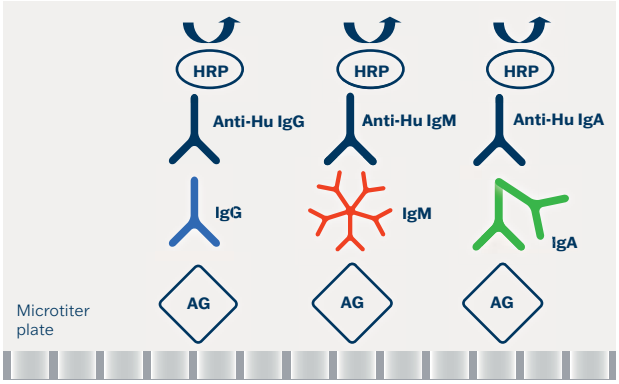
Antibody response



ELISA

Test principle

The assays are based on a sandwich type ELISA method.



Summary protocol

Step		Test steps
	1.	Dilution of samples – serum/plasma 1:101 (10 µl + 1 ml) – cerebrospinal fluid 1:3 (50 µl + 100 µl)
	2.	Pipette Controls and diluted samples 100 µl – Including blank
	3.	Incubate 30 min. at 37 °C
	4.	Aspirate and wash the wells 5 times
	5.	Add Conjugate 100 µl – Including blank
	6.	Incubate 30 min. at 37 °C
	7.	Aspirate and wash the wells 5 times
	8.	Add 100 µl Substrate (TMB-Complete) – Including blank
	9.	Incubate 15 min. at 37 °C
	10.	Add 100 µl Stopping solution – Including blank
	11.	Read colour intensity at 450 nm

Antigens

Purified and inactivated antigen isolated from AD 169 strain of Cytomegalovirus enriched with highly specific immunodominant epitopes.

Clinical application

- Screening test for the detection of specific IgA, IgG and IgM antibodies in human serum, plasma or cerebrospinal fluid
- Evaluating results of therapy using the semiquantitative determination
- Disease stage diagnosis

User comfort

- Ready-to-use components
- Colour-coded components
- Interchangeable components
- Breakable colour-coded microplate strips
- CUT-OFF and calibrators included
- Semiquantitative evaluation of results (Index of Positivity) or quantitative evaluation of results (U/ml)
- Easy assay procedure

Advantages

- Identical assay procedure
- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Long shelf-life: 15 months from date of production
- Short total assay time
- Avidity test (EIA CMV IgG)
- Sample diluent with RF-sorbent (EIA CMV IgM)
- Quantitative evaluation available
- Ready for automation
- Customer support

Test characteristics

ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA CMV IgA	94.7%	95.8%
EIA CMV IgG	98.8%	98.9%
EIA CMV IgM	98.5%	98.9%

The kits are validated with the BBI Diagnostics Panel, A Boston Biomedica Company, Boston, USA.

Results interpretation

IgA	IgM	IgG	Avidity	Interpretation
–	–	–	non done	Seronegative
+	+	–	non done	Acute infection
+	+	+	low	Acute infection
+	(+)	+	high	Convalescence or reactivation
–	–	+	high	Past infection

Types of kits

SmartEIA kits are designed for automated processing using the Agility® analyser.

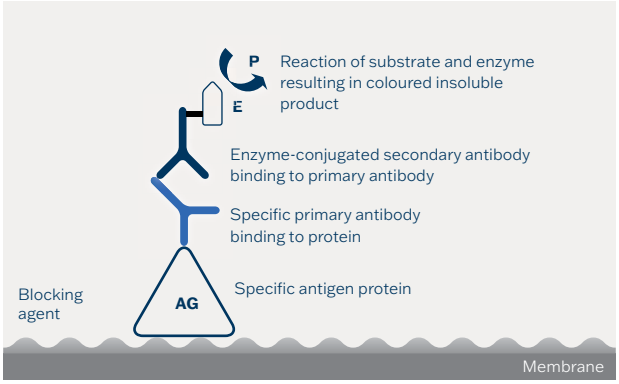
EIA

SmartEIA

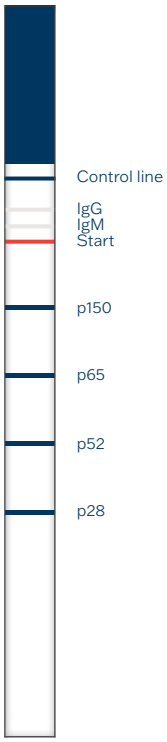
IMMUNOBLOT

Test principle

Recombinant antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



Antigens



p150 Tegument protein UL32
A strong immunogen of the late stage of infection (late antigen), it does not develop in the early stage. Detectable in the IgG class in higher titres even in reactivation.

p65 Tegument protein UL83
In the IgM class – one of the markers of the early stage of infection
In the IgG class – rather typical for the late stage or infection reactivation

p52 CM2 protein; UL44
In the IgM class – an important marker of the early stage of primary infection
In the IgG class – reactivity rather in the late stage, or infection reactivation

p28 Tegument protein UL99
A strong immunogen; it may develop in late stages of infection.

Summary protocol

Step		Test steps
	1.	Pipette Universal solution 2.5 ml
	2.	Strips soaking 10 min. at room temperature – Shaker
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (30 µl + 1,5 ml)
	5.	Pipette Controls and diluted samples 1.5 ml
	6.	Incubate 30 min. at room temperature – Shaker
	7.	Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	8.	Pipette Conjugate 1.5 ml
	9.	Incubate 30 min. at room temperature – Shaker
	10.	Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	11.	Pipette Substrate solution (BCIP/NBT) 1.5 ml
	12.	Incubate 15 min. at room temperature – Shaker
	13.	Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. – Shaker
	14.	Sticking and evaluation of strips

Clinical application

- Detailed determination of the presence of antibodies against specific CMV antigens
- Confirmation of ambiguous results
- Confirmatory to ELISA tests

User comfort

- Ready-to-use components
- Colour-coded strips
- Interchangeable components
- Positive and Negative controls
- Control lineis present on the strip
- Possibility of software evaluation

Advantages

- Identical assay procedure
- Easy interpretation and reproducibility of results
- Sophisticated evaluation software
- High diagnostic specificity and sensitivity
- Ready for automation
- Complex customer support

Test characteristics

Immunoblot	Diagnostic Sensitivity	Diagnostic Specificity
CMV IgG	95.9%	99.0%
CMV IgM	96.5%	99.0%

Interpretation of results

	IgM				IgG			
	p150	p65	p52	p28	p150	p65	p52	p28
Early primary infection	–	+	+	–	–	–	–	–
Primary infection	(+)	+	+/–	(+)	–	(+)	(+)	(+)
Late primary infection	+	+/–	+/-	(+)	+	+	+	(+)
Persistence of infection	–	–	–	–	+	+	+	(+)
Reactivation	+/–	+	+	(+)	+	+	+	(+)

Ordering information

ELISA

Cat. No.	Product	Units
CMA096	EIA CMV IgA	96 wells
CMG096	EIA CMV IgG	96 wells
CMM096	EIA CMV IgM	96 wells
SK-CMA096	SmartEIA CMV IgA	96 wells
SK-CMG096	SmartEIA CMV IgG	96 wells
SK-CMM096	SmartEIA CMV IgM	96 wells
xxxTLN	CKS negative	3.5 ml
xxxTLP	CKS positive	3.5 ml

SmartEIA kits are designed for automated processing using the Agility® analyser.

IMMUNOBLOT

Cat. No.	Product	No. of Tests
CMGL20	BLOT-LINE CMV IgG	20
CMML20	BLOT-LINE CMV IgM	20

TestLine Clinical Diagnostics Ltd.
Krizikova 68, 612 00 Brno, Czech Republic
+420 549 121 203
sales@testlinecd.com
www.testlinecd.com

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

V11/2021