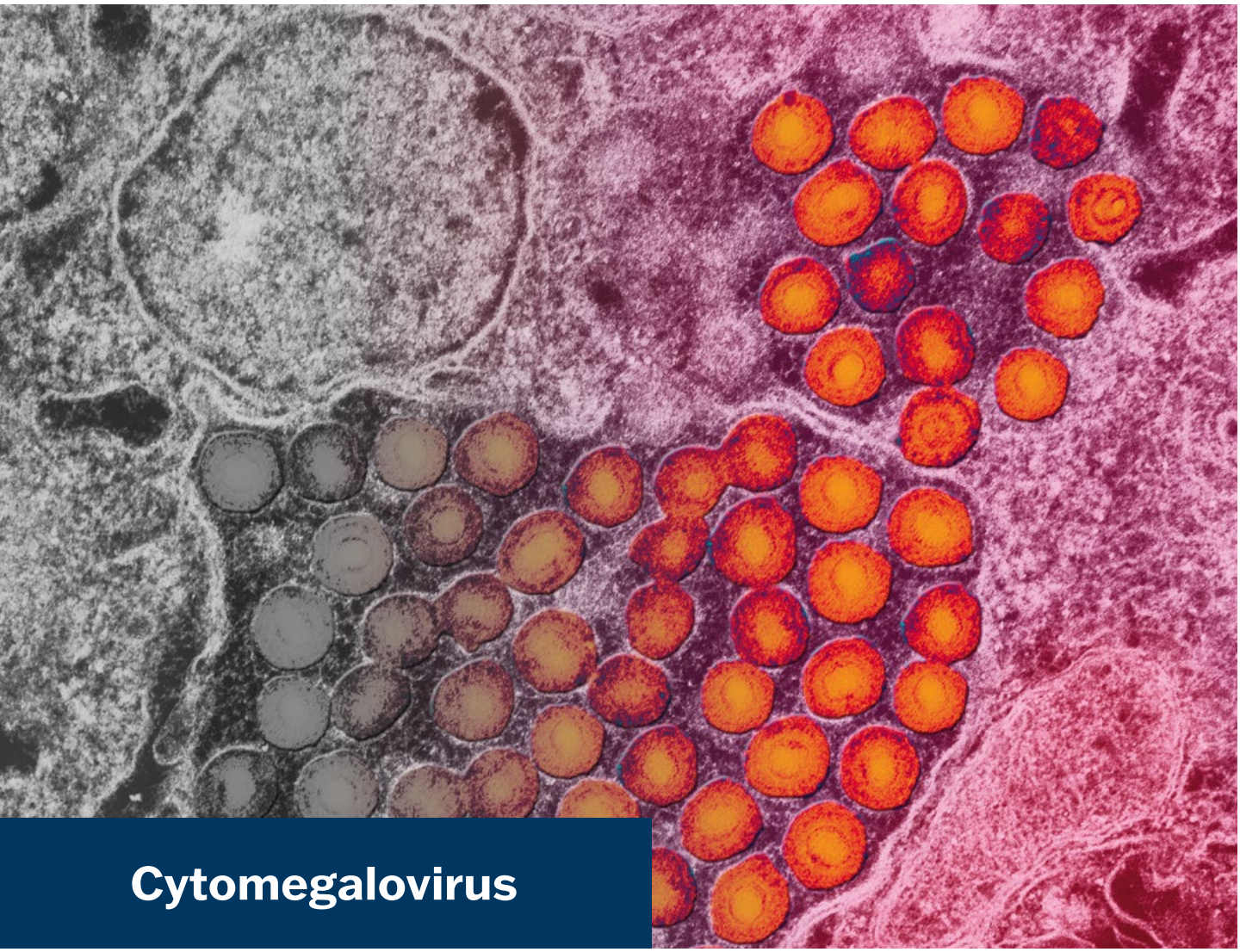


Cytomegalovirus

Enzyme immunoassays for the diagnostics of cytomegalovirus infection

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

IVD CE 2265

Diagnostic kits are intended for professional use in the laboratory.

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

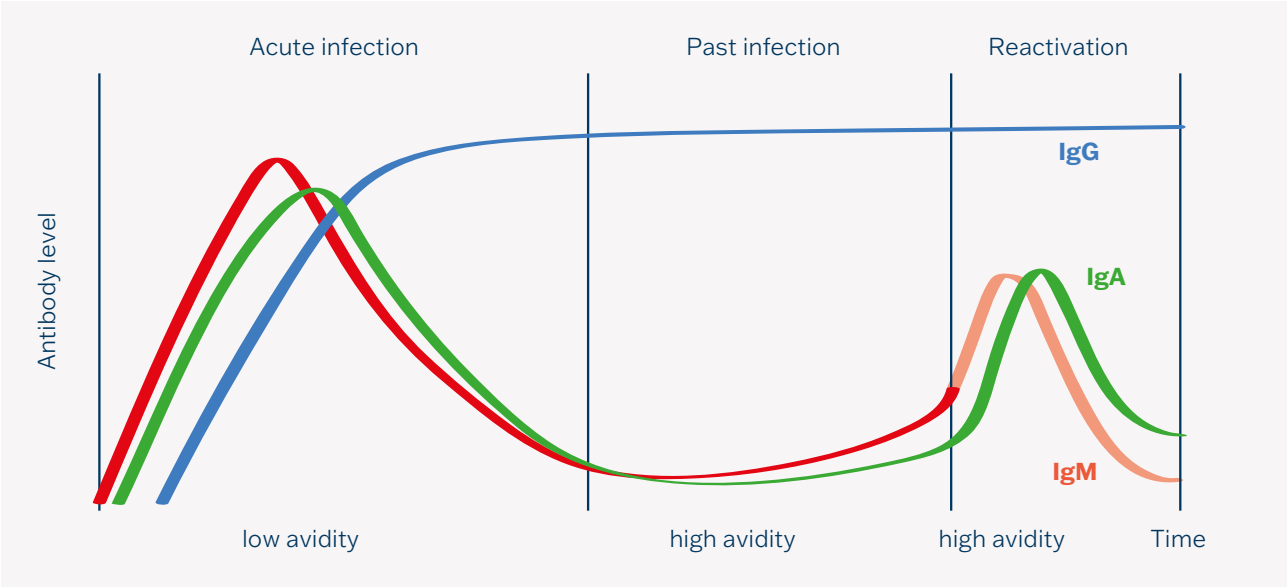
IgA: Antibodies of IgA class are a sign of an active infection – primary infection as well as reactivation. The IgA antibodies are produced during reactivation and they can rarely be produced with specific IgM class antibodies. Specific IgA antibodies are very important for confirmation of CMV infection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



Results interpretation

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

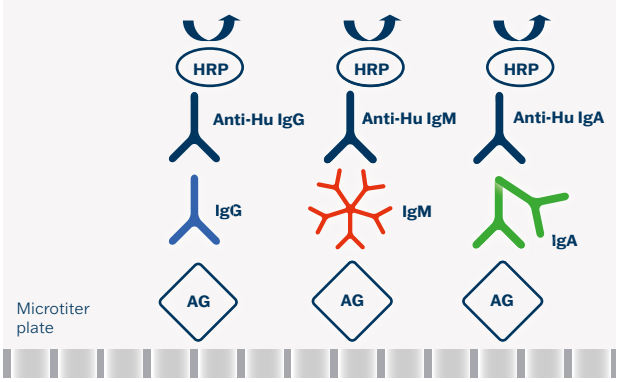
Specific antigens

Antigens	Description
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
IEA (p72)	Immediate early antigen, capsid protein UL123 Plays a role in the early phase of the replication cycle of human CMV Important function in defence mechanisms against CMV infection
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
gB	Membrane glycoprotein B Antibody response in IgG class – approximately 50–100 days after primary infection

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

Test characteristics

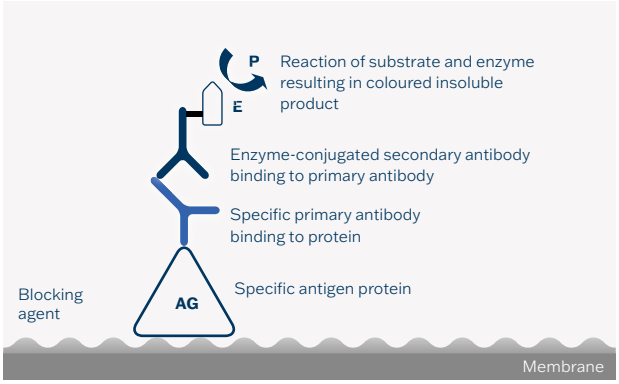
ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA CMV IgA	94.7%	95.8%
EIA CMV IgG	98.8%	98.9%
EIA CMV IgM	98.5%	98.9%

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

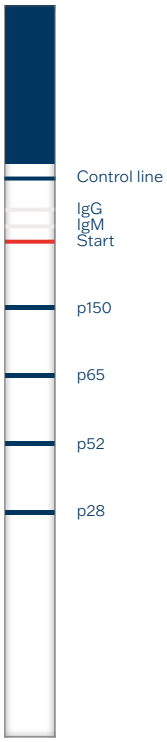
IMMUNOBLOT

Test principle

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



Antigens



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

Test characteristics

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

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

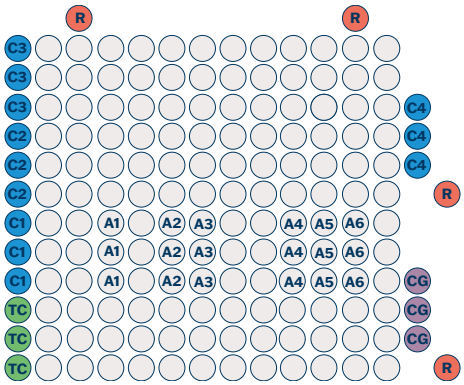
- Identical assay procedure
- Easy interpretation and reproducibility of results
- Sophisticated evaluation software
- High diagnostic specificity and sensitivity
- Ready for automation
- Complex customer support

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	+/–	+	+	(+)	+	+	+	(+)

MICROBLOT-ARRAY

Distribution of antigens and control spots



Description of antigens

A1 – p150
A2 – IEA (p72)
A3 – p65
A4 – p52
A5 – p28
A6 – CMV gB

Description of control spots

R – Reference
TC – Test control
CM – Conjugate control IgM
CG – Conjugate control IgG
C1 – Calibration 1
C2 – Calibration 2
C3 – Calibration 3
C4 – Calibration 4

User Comfort

- Low sample consumption
- Antigens spotted in triplicate – minimizing statistical variation
- Possibility of automatic assay processing and results evaluation
- Parallel testing of multiple markers simultaneously
- High sensitivity and specificity

Test Characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic specificity
Microblot-Array CMV IgG	98.1%	99.9%
Microblot-Array CMV IgM	96.9%	99.1%

Protocol Summary

Step	Test steps
1.	Pipette Universal solution 150 µl
2.	Strips soaking 10 min. at room temperature
3.	Aspirate
4.	Dilute samples – serum/plasma 1:51 (10 µl + 500 µl) – cerebrospinal fluid 1:3 (50 µl + 100 µl)
5.	Pipette Controls and diluted samples 100 µl
6.	Incubate 30 min. at room temperature
7.	Quick wash with Universal Solution*
8.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
9.	Pipette Conjugate 100 µl
10.	Incubate 30 min. at room temperature
11.	Quick wash with Universal Solution*
12.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
13.	Pipette Substrate solution (BCIP/NBT) 100 µl
14.	Incubate 15 min. at room temperature
15.	Quick wash using the distilled water *
16.	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
17.	Dry and evaluate strips

* if using a washer, fill the wells to the brim and aspirate immediately after filling the last well

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

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

MICROBLOT-ARRAY

Kód	Product	Units
CMGMA48	Microblot-Array CMV IgG	48
CMMMA48	Microblot-Array CMV IgM	48

TestLine®

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CERTIFIED LR ISO 9001 · ISO 13485

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

V05/2025