

## 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.

**B  
G** **TestLine**®

## Introduction

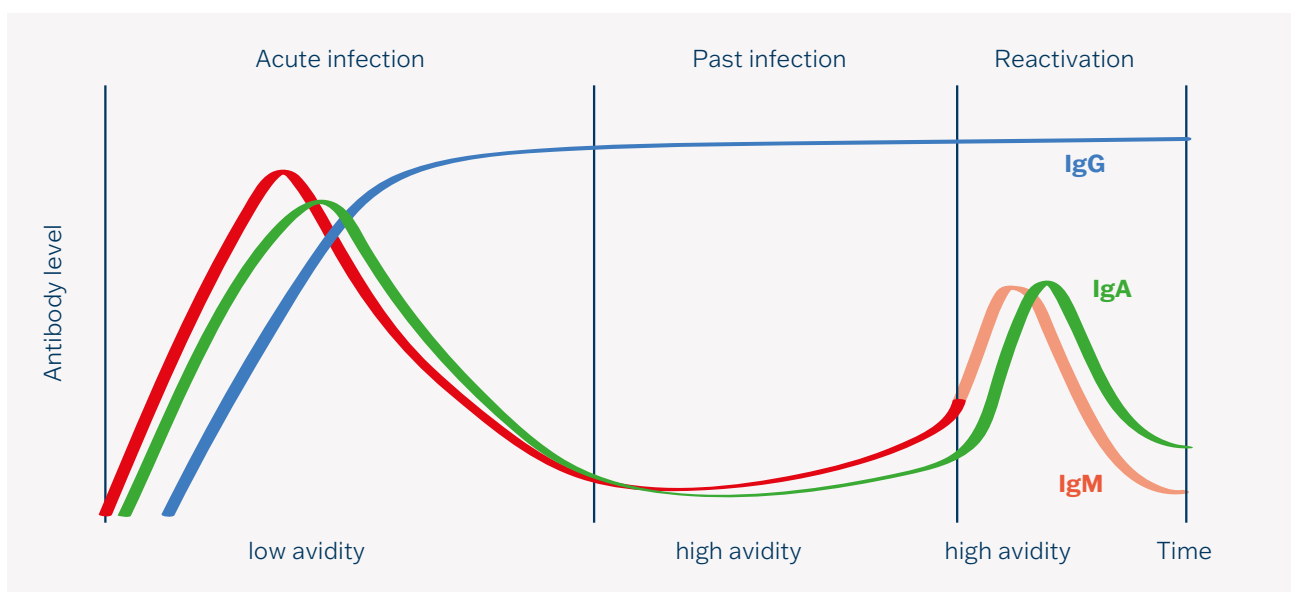
Human cytomegalovirus (CMV, Human Herpesvirus 5, HHV 5) is a member of the Herpesviridae family. Primary infection of CMV occurs mainly in childhood or adolescence. The infection can be transmitted in different ways (e.g. respiratory apparatus, digestive or urogenital tract). Clinically, the disease 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.

## Antibody response



## 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

## Results interpretation

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

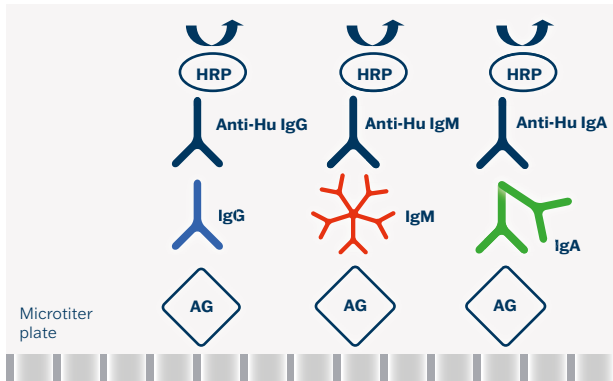
## Specific antigens

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

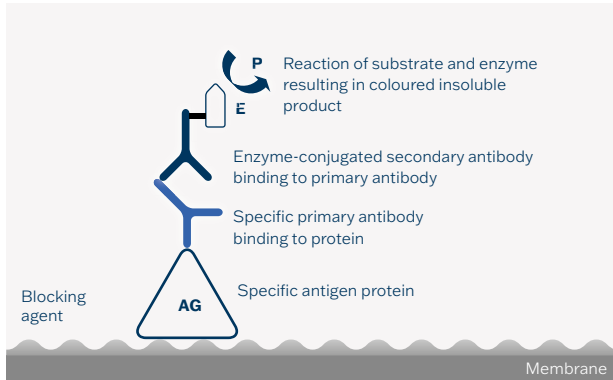
ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA CMV IgA	94.7%	95.8%
EIA CMV IgG	98.8%	98.9%
EIA CMV IgM	98.5%	98.9%

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

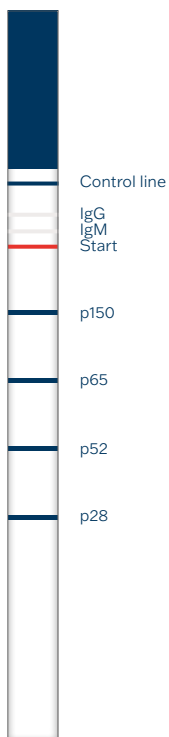
# IMMUNOBLOT

## 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

<b>Immunoblot</b>	<b>Diagnostic Sensitivity</b>	<b>Diagnostic Specificity</b>
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 lines 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

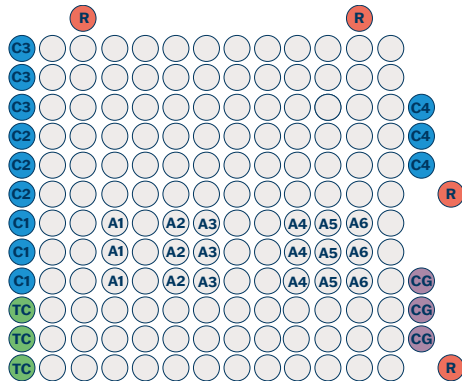
## Interpretation of results

	<b>IgM</b>				<b>IgG</b>			
	<u>p150</u>	<u>p65</u>	<u>p52</u>	<u>p28</u>	<u>p150</u>	<u>p65</u>	<u>p52</u>	<u>p28</u>
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

<u>Pathogen</u>	<u>Diagnostic Sensitivity</u>	<u>Diagnostic specificity</u>
Microblot-Array CMV IgG	98.1%	99.9%
Microblot-Array CMV IgM	96.9%	99.1%

## Protocol Summary

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

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



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## Ordering information

### ELISA

<b>Cat. No.</b>	<b>Product</b>	<b>Units</b>
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

<b>Cat. No.</b>	<b>Product</b>	<b>No. of Tests</b>
CMGL20	BLOT-LINE CMV IgG	20
CMML20	BLOT-LINE CMV IgM	20

### MICROBLOT-ARRAY

<b>Kód</b>	<b>Product</b>	<b>Units</b>
CMGMA48	Microblot-Array CMV IgG	48
CMMMA48	Microblot-Array CMV IgM	48

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