

Epstein-Barr Virus

Enzyme immunoassays for the diagnostics of Epstein-Barr virus 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



Diagnostic kits are intended for professional use in the laboratory.



Introduction

Epstein-Barr virus (EBV) is a member of the Herpetoviridae family. An infected human is the source of infection that spreads mainly through air-borne transmission or direct contact. Incubation period is 1-2 months; the majority of infections (up to 90%) occur during childhood. Symptoms of EBV infection are influenced by patient age and immune system status. EBV persists latently in the organism for the rest of the individual's life and can be reactivated.

Diagnosis of infection

Diagnosis of the disease is based on a clinical picture and laboratory tests. Determination of specific IgA, IgG, and IgM class antibodies against particular EBV antigens using ELISA method and confirmatory Immunoblot or Microblot-Array methods is a useful tool for the detection and determination of stage of EBV infection.

Clinical symptoms of EBV disease

Primary infection

- Inapparent infection
- Non-specific fever disease
- Complex of infectious mononucleosis (pharyngitis, lymphadenopathy, splenomegaly, fever)

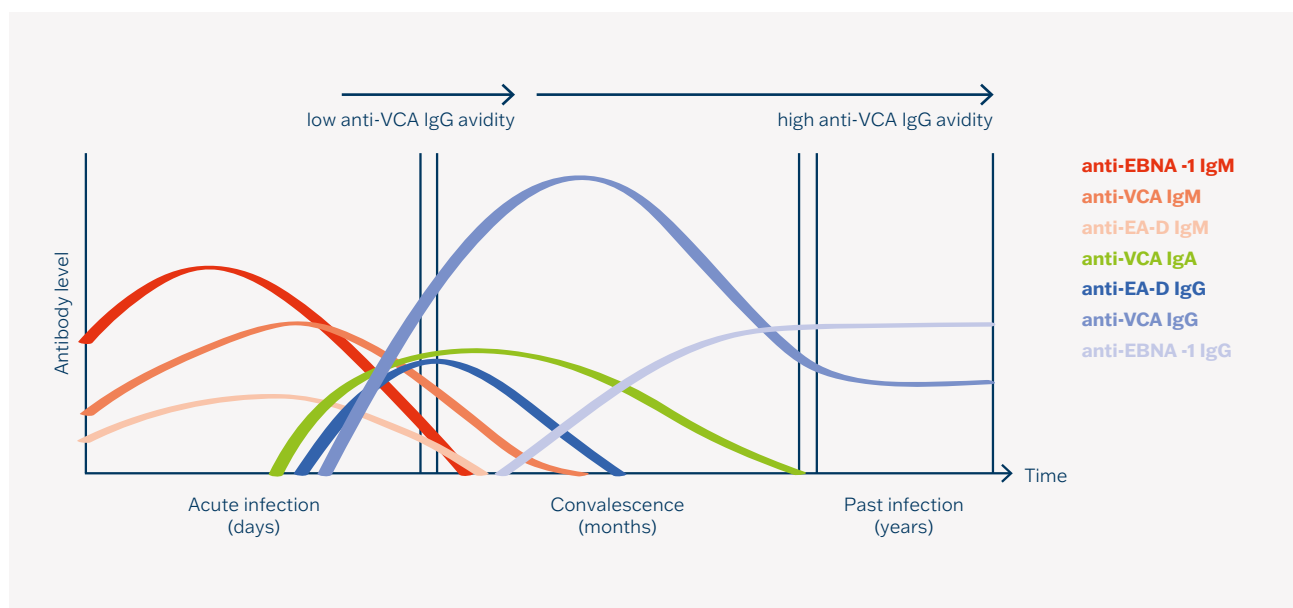
Latent chronic infection

- Asymptomatic salivary excretion of virus

Reactivation

- Asymptomatic in general
- At immunodeficiency (e.g. malignant lymphoproliferative disorders, tongue leucoplakia, lymphoid pneumonia, etc.)
- In certain geographic areas: Burkitt's lymphoma, nasopharyngeal carcinoma

Antibody response of dominant antigens



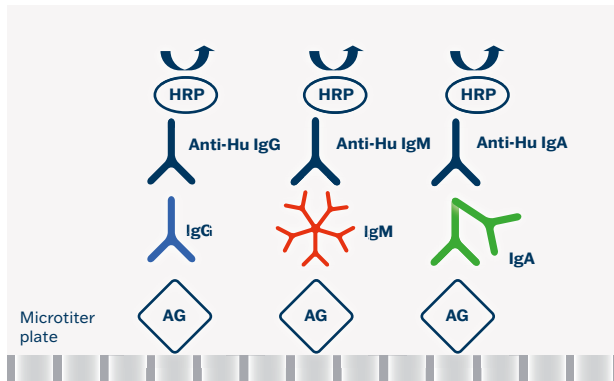
Specific antigens

Antigen	Description
EBNA-1	Epstein-Barr nuclear antigen 1; IgG: an important diagnostic marker of the late phase or reactivation of the infection IgM: the antibodies are detectable 2-4 months after primary EBV infection, they may also appear during reactivation
EBNA-2	Epstein-Barr nuclear antigen 2; IgG: high antibody titres are present during chronic infection or in the post-acute phase The absence of IgG anti-EBNA-2 antibodies and the presence of anti-EBNA-1 antibodies rules out primary infection
VCA p18	Viral Capsid Antigen p18; IgA: marker of primary infection; high titres persist in patients with nasopharyngeal carcinoma IgM: marker of primary infection; they may also be present during infection reactivation IgG: an important marker of the late phase of the infection, antibodies do not occur in primary infections
VCA p23	Viral Capsid Antigen p23; Antibodies against this antigen can be detected during all phases of the infection (both IgG and IgM), they persist in the body for a long time
EA-D p54	Early Antigen Diffuse p54; BMRF1; IgA: produced during primary infection; high titres during reactivation; high titres persist in patients with nasopharyngeal carcinoma An additional marker of acute EBV infection, detectable even in the latent phase of primary infection (both IgG and IgM)
EA-D p138	Early Antigen Diffuse p138; IgA: produced during primary infection; high titres during reactivation; high titres persist in patients with nasopharyngeal carcinoma An additional marker of acute EBV infection, detectable even in the latent phase of primary infection (both IgG and IgM)
EA-R	Early Antigen Restricted protein p85; IgG: antibodies usually occur at a later stage; they are practically absent during the acute phase except in children; high levels in patients with reactivation or in immunocompromised patients
Rta	Replication and transcription Activator (BRLF1); A very early antigen IgG: a potential diagnostic marker of a nasopharyngeal carcinoma
ZEBRA	Z Epstein-Barr replication activator protein; Trans-activator protein BZLF1; IgM: it is a very early indicator of an acute infection IgG: it is an early stage marker but it is also detectable during the late stages of the infection Serological marker of EBV reactivation, marker of EBV-associated diseases
gp85	Probable membrane antigen gp85 (BDLF3);
gp350	Epstein-Barr virus envelope glycoprotein gp350 (BLLF1); IgM: high titres in patients with infectious mononucleosis IgG: the titre increases only a few months after the primary infection Specific immune response for EBV-associated diseases
LMP1	Latent membrane protein 1 Frequent in latent infections Linked to EBV-associated malignancies (nasopharyngeal carcinoma)

ELISA

Test Principle

The assays are based on a sandwich type ELISA method.



Summary Protocol

Step	Test steps
	1. Dilute samples – serum/plasma 1:101 (10 µl + 1 ml)
	2. Pipette controls and diluted samples 100 µl – blank = empty well
	3. Incubate 30 min. at 37 °C
	4. Aspirate and wash the wells 5 times
	5. Add 100 µl Conjugate – blank = empty well
	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 30 min. at 37 °C
	10. Add 100 µl Stopping solution – Including blank
	11. Read colour intensity at 450 nm

Antigens

Recombinant antigens with highly specific immunodominant epitopes.

Clinical Application

- Screening tests for the detection of infection with EBV in humans
- Disease stage diagnosis

User Comfort

- Ready-to-use components
- Colour-coded components
- Interchangeable of 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 immunoreactivity of antibody response
- Long Shelf Life: 15 months from date of production
- Short total assay time
- Avidity test (EIA EBV VCA IgG)
- Detection in cerebrospinal fluid (EIA EBV VCA)
- Ready for automation
- Quantitative evaluation available
- Customer support

Types of kits

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

EIA



SmartEIA



Test Characteristics

<u>ELISA</u>	<u>Diagnostic sensitivity</u>	<u>Diagnostic specificity</u>
EIA EBV EA-D IgG	95.5%	97.7%
EIA EBV EA-D IgM	95.1%	94.4%
EIA EBV EBNA-1 IgG	96.9%	98.9%
EIA EBV EBNA-1 IgM	97.8%	97.7%
EIA EBV VCA IgA	97.7%	97.9%
EIA EBV VCA IgG	98.8%	98.9%
EIA EBV VCA IgM	98.6%	98.4%

Interpretation of Results

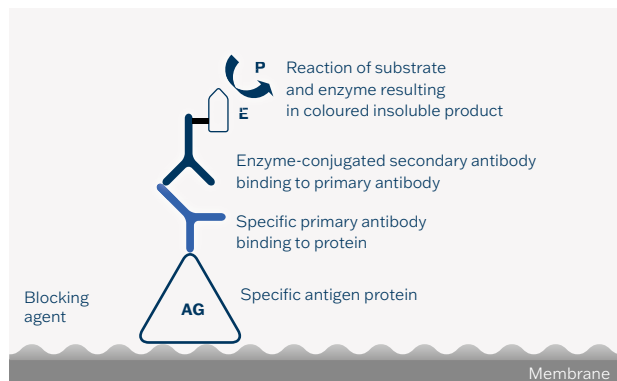
Model situations for evaluation of results

<u>Evaluation</u>	<u>VCA</u>			<u>EA-D</u>		<u>EBNA-1</u>	
	<u>IgM</u>	<u>IgA</u>	<u>IgG</u>	<u>IgM</u>	<u>IgG</u>	<u>IgM</u>	<u>IgG</u>
Seronegativity	-	-	-	-	-	-	-
	+	-	-	+	-	+	-
	+	+	-	+	+	(+)	-
Primary infection	+	+	+	+	+	(+)	-
	+	(+)	+	-	(+)	-	-
	-	(+)	+	-	(+)	-	+
Postacute stage	-	-	+	-	-	-	+
Previous infection	-	-	+	-	-	-	+
Reactivation	+	(+)	+	(+)	(+)	(+)	+

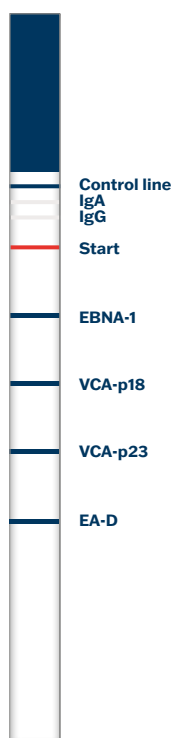
IMMUNOBLOT

Test Principle

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



Antigens



EBNA-1 – Epstein-Barr nuclear antigen 1; in the IgG class, an important diagnostic marker of late phase or reactivation of the infection; in IgM class detectable antibodies for 2-4 months after primary EBV infection, may also appear also in reactivation

VCA-p18 – Viral Capsid Antigen p18; an important marker of late phase of infection; IgG antibodies do not appear in primary infections; IgM antibodies can be detected in early phase of the infection

VCA-p23 – Viral Capsid Antigen p23; antibodies to this antigen can be detected at all stages of infection in IgG and IgM class, IgG antibodies persist in the body for a long time

EA-D – Early Antigen Diffuse p54; BMRF1; an additional marker of acute EBV infection, antibodies detectable in IgG and IgM class, even in the latent phase of primary 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 antigens of Epstein-Barr virus
- Confirmation of ambiguous results
- Confirmation for ELISA tests

User Comfort

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

Test Characteristics

<u>Parameter</u>	<u>Diagnostic Sensitivity</u>	<u>Diagnostic Specificity</u>
EBV IgA	95.4%	96.6%
EBV IgG	100.0%	92.5%
EBV IgM	92.7%	95.7%

Correlation of Methods

Comparison with IMMUNOBLOT

Number of corresponding results: 97.6%

		<u>BLOT-LINE EBV IgG</u>	
		negative	positive
Reference kit	+	0	119
	-	40	4

Number of corresponding results: 92.5%

		<u>BLOT-LINE EBV IgM</u>	
		negative	positive
Reference kit	+	3	38
	-	36	4

Comparison with ELISA

<u>Parameter</u>	<u>Total conformity</u>
EBNA-1 IgG	92.2%
VCA IgG	93.3%
EA-D IgG	94.8%

Advantages

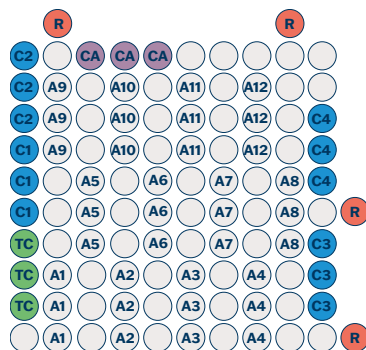
- Identical assay procedure
- Easy interpretation and reproducibility of results
- Sophisticated evaluation software
- High diagnostic efficiency
- Ready for automation
- Customer support



<u>Parameter</u>	<u>Total conformity</u>
EBNA-1 IgM	94.4%
VCA IgM	91.9%
EA-D IgM	96.1%

MICROBLOT-ARRAY

Distribution of antigens and control spots

















Description of antigens

- A1** – EBNA-1
- A2** – EBNA-2
- A3** – VCA p18
- A4** – VCA p23
- A5** – EA-D p54
- A6** – EA-D p138
- A7** – EA-R
- A8** – Rta
- A9** – ZEBRA
- A10** – gp85
- A11** – gp350
- A12** – LMP1

Description of control spots

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

Summary Protocol

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)
	5. Pipette Controls and diluted samples 100 µl
	6. Incubate 30 min. at room temperature
	7. Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	8. Pipette Conjugate 100 µl
	9. Incubate 30 min. at room temperature
	10. Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	11. Pipette Substrate solution (BCIP/NBT) 100 µl
	12. Incubate 15 min. at room temperature
	13. Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
	14. Dry and evaluate strips

User Comfort

- Low sample consumption
- Antigens spotted in triplicate – minimizing statistical variation
- Fully automatic assay processing and results evaluation using spot intensity (AU), IP or quantitative (U/ml)
- Parallel testing of multiple markers simultaneously
- High sensitivity
- Ready-to-use reagents
- Short total assay time

Test Characteristics

<u>Pathogen</u>	<u>Diagnostic Sensitivity</u>	<u>Diagnostic Specificity</u>
EBV IgA	98.3%	96.7%
EBV IgG	98.8%	95.0%
EBV IgM	96.4%	92.9%



Correlation of Methods

Comparsion with EIA



Reactivity in different stages of EBV disease

		<u>Clinical classification of the sample</u>				
		Seronegative (n=18)	Primary infection (n=43)	Postacute infection (n=34)	Previous infection (n=135)	Reactivation (n=17)
MBA EBV IgM	pos	0 (0%)	42 (98%)	5 (15%)	4 (3%)	12 (71%)
	co	0 (0%)	0 (0%)	1 (3%)	3 (2%)	1 (6%)
	neg	18 (100%)	1 (0%)	28 (82%)	128 (95%)	4 (23%)
MBA EBV IgG	pos	0 (0%)	36 (84%)*	34 (100%)	135 (100%)	17 (100%)
	co	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	neg	18 (100%)	7 (16%)	0 (0%)	0 (0%)	0 (0%)
MBA EBV IgA	pos	0 (0 %)	17 (39%)	24 (18%)	15 (44%)	4 (23%)
	co	0 (0%)	2 (5%)	10 (7%)	4 (12%)	2 (12%)
	neg	18 (100 %)	24 (56%)	101 (75%)	15 (44%)	2 (12%)

* positive especially EA-D p54, ZEBRA

Frequency of anti-EBV antibody capture on clinically defined panels

		<u>Seronegative</u> <u>(n=18)</u>	<u>Primary</u> <u>infection</u> <u>(n=43)</u>	<u>Post-acute</u> <u>stage</u> <u>(n=34)</u>	<u>Previous</u> <u>infection</u> <u>(n=135)</u>	<u>Reactivation</u> <u>(n=17)</u>
EBNA-1	IgM	0 (0%)	11 (26%)	1 (3%)	3 (2%)	3 (18%)
	IgG	0 (0%)	3 (7%)	31 (91%)	131 (97%)	15 (88%)
	IgA	0 (0%)	0 (0%)	2 (6%)	4 (3%)	0 (0%)
VCA p23	IgM	0 (0%)	5 (12%)	0 (0%)	0 (0%)	1 (6%)
	IgG	0 (0%)	10 (23%)	27 (79%)	90 (67%)	9 (53%)
	IgA	0 (0%)	4 (12%)	0 (0%)	4 (12%)	1 (6%)
VCA p18	IgM	0 (0%)	36 (84%)	1 (3%)	1 (1%)	11 (65%)
	IgG	0 (0%)	18 (42%)	30 (88%)	130 (96%)	15 (88%)
	IgA	0 (0%)	10 (29%)	3 (7%)	10 (29%)	3 (18%)
EA-D p54	IgM	0 (0%)	17 (40%)	2 (6%)	1 (1%)	3 (18%)
	IgG	0 (0%)	21 (49%)	18 (53%)	9 (7%)	2 (12%)
	IgA	0 (0%)	9 (21%)	5 (15%)	2 (1%)	1 (6%)
EA-D p138	IgM	0 (0%)	2 (5%)	1 (3%)	0 (0%)	1 (6%)
	IgG	0 (0%)	4 (9%)	10 (29%)	1 (1%)	2 (12%)
	IgA	0 (0%)	3 (7%)	2 (6%)	0 (0%)	0 (0%)
EA-R p85	IgM	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	IgG	0 (0%)	0 (0%)	1 (3%)	1 (1%)	0 (0%)
	IgA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
gp85	IgM	0 (0%)	2 (5%)	0 (0%)	0 (0%)	1 (6%)
	IgG	0 (0%)	0 (0%)	5 (15%)	11 (8%)	2 (12%)
	IgA	0 (0%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)
Rta	IgM	0 (0%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)
	IgG	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	IgA	0 (0%)	1 (2%)	0 (0%)	1 (1%)	0 (0%)
EBNA-2	IgM	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	IgG	0 (0%)	5 (12%)	12 (35%)	32 (24%)	0 (0%)
	IgA	0 (0%)	0 (0%)	1 (3%)	1 (1%)	0 (0%)
ZEBRA	IgM	0 (0%)	8 (19%)	0 (0%)	0 (0%)	1 (6%)
	IgG	0 (0%)	19 (44%)	22 (65%)	52 (39%)	5 (29%)
	IgA	0 (0%)	8 (19%)	3 (9%)	5 (4%)	0 (0%)
gp350	IgM	0 (0%)	4 (9%)	0 (0%)	2 (1%)	1 (6%)
	IgG	0 (0%)	0 (0%)	0 (0%)	5 (4%)	0 (0%)
	IgA	0 (0%)	2 (5%)	2 (6%)	3 (2%)	0 (0%)
LMP1	IgM	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	IgG	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	IgA	0 (0%)	0 (0%)	3 (9%)	3 (2%)	0 (0%)



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Ordering Information

ELISA

Cat. No.	Product	Units
EAG096	EIA EBV EA-D IgG	96 wells
EAM096	EIA EBV EA-D IgM	96 wells
EBG096	EIA EBV EBNA-1 IgG	96 wells
EBM096	EIA EBV EBNA-1 IgM	96 wells
VCA096	EIA EBV VCA IgA	96 wells
VCG096	EIA EBV VCA IgG	96 wells
VCM096	EIA EBV VCA IgM	96 wells
SK-EAG096	SmartEIA EBV EA-D IgG	96 wells
SK-EAM096	SmartEIA EBV EA-D IgM	96 wells
SK-EBG096	SmartEIA EBV EBNA-1 IgG	96 wells
SK-EBM096	SmartEIA EBV EBNA-1 IgM	96 wells
SK-VCA096	SmartEIA EBV VCA IgA	96 wells
SK-VCG096	SmartEIA EBV VCA IgG	96 wells
SK-VCM096	SmartEIA EBV VCA IgM	96 wells

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

IMMUNOBLOT

Cat. No.	Product	No. of Tests
EBAL20	BLOT-LINE EBV IgA	20
EBGL20	BLOT-LINE EBV IgG	20
EBML20	BLOT-LINE EBV IgM	20
BD-EBGL24	BlueBLOT-LINE EBV IgG	24
BD-EBML24	BlueBLOT-LINE EBV IgM	24

The BluBLOT-LINE kits are designed for automatic processing using BlueDiver® analyser.

Microblot-Array

Cat. No.	Product	No. of Tests
EBAMA96	Microblot-Array EBV IgA	96
EBGMA96	Microblot-Array EBV IgG	96
EBMMA96	Microblot-Array EBV IgM	96



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