EUROLINE Anti-EBV Profile 2 (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DN 2790-1601-2 G DN 2790-0510-2 G DN 2790-6401-2 G	VCA gp125, VCA p19, EBNA-1, p22, EA-D	IgG	Ag-coated immunoblot strips	16 x 01 (16) 05 x 10 (50) 64 x 01 (64)

Indications: The EUROLINE test kit provides qualitative in vitro determination of human antibodies of the immunoglobulin class IgG to the 5 different EBV antigens **VCA gp125, VCA p19, EBNA-1, p22 and EA-D** in serum or plasma to support the diagnosis of infections with Epstein-Barr virus (infectious mononucleosis, Burkitt´s lymphoma, nasopharyngeal carcinoma).

Application: The EUROLINE Anti-EBV Profile 2 (IgG or IgM) is based on recombinant and native, highly purified antigens and enables differentiation between acute and past EBV infections in one reaction. Serologically rare and difficult constellations can be clearly differentiated using the EUROLINE Anti-EBV Profile 2.

Principle of the test: The test kit contains test strips coated with parallel lines of antigens. In the first reaction step, diluted patient samples are incubated with the immunoblot strips. In the case of positive samples, the specific IgG antibodies (also IgA and IgM) will bind to the corresponding antigenic site. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.
The format DN 2790-0510-2 G belongs to the Immunoblot-PreQ system. The test strips are already placed into the incubation trays (EUROTrays).

Contents of the test kit (DN 2790-####-2 G):

Component	1601	0510	6401	Symbol
1. Test strips coated with antigens VCA gp125, VCA p19, EBNA-1, p22 and EA-D	16 strips	5 x 10 strips in EUROTrays	4 x 16 strips	STRIPS
2. Positive control (IgG, human), 50x concentrate	1 x 0.04 ml	3 x 0.1 ml	4 x 0.04 ml	POS CONTROL 50x
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), 10x concentrate	1 x 3 ml	4 x 3 ml	4 x 3 ml	CONJUGATE 10x
4. Universal buffer, 10x concentrate	1 x 50 ml	1 x 100 ml	3 x 50 ml	BUFFER 10x
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	4 x 30 ml	4 x 30 ml	SUBSTRATE
6. Incubation tray	2 x 8 channels	---	---	
7. Test instruction	1 booklet	1 booklet	1 booklet	

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.
Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)
If using Immunoblot-PreQ (EUROIMMUN order no. DN 2790-0510-2 G), no additional incubation tray is needed.

Updates with respect to the previous version are marked in grey.

For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under:
ZD 2790-0101-2 GM Visual evaluation protocol EUROLINE Anti-EBV Profile 2
If using Immunoblot-PreQ (EUROIMMUN order no. DN 2790-0510-2 G), the strips should stay in the EUROTrays during evaluation. For the evaluation we generally recommend using a EUROIMMUN camera system connected to EUROLineScan software. Strips need to be dry before starting the evaluation.

Preparation and stability of the reagents

Note: This test kit may only be used by trained personnel. Test strips and incubation trays are intended for single use. All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the package with the test strips only when the strips have reached room temperature (+18°C to +25°C) to prevent condensation on the strips. After removal of the strips/Immunoblot-PreQ the package should be sealed tightly and stored at +2°C to +8°C.

- **Positive control:** The control is a 50x concentrate. For the preparation of the working-strength control the amount required should be removed from the bottle using a clean pipette tip and diluted **1:51** with working-strength universal buffer. Example: add 30 µl of control to 1.5 ml of working-strength universal buffer and mix thoroughly. The working-strength control should be used at the same working day.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the working-strength enzyme conjugate the amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with working-strength universal buffer. Example: for 1 test strip dilute 0.15 ml anti-human IgG concentrate with 1.35 ml working-strength universal buffer. The working-strength enzyme conjugate should be used on the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the working-strength universal buffer shake the bottle. The amount required should be removed from the bottle using a clean pipette tip and diluted 1:10 with deionised or distilled water. Example: for 1 test strip add 2 ml concentrate to 18 ml deionised or distilled water. The working-strength universal buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light.

Storage and stability: The test kit must be stored at a temperature between +2°C and +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, controls and incubated test strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

Warning: The control of human origin has tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:51** with working-strength universal buffer using a clean pipette tip. For example, add 30 µl of sample to 1.5 ml working-strength universal buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

If using Immunoblot-PreQ DN 2790-0510-2 G, manual incubation is not possible. Please see options below.

Blocking: Fill the channels of the incubation tray according to the number of serum samples to be tested with 1.5 ml working-strength universal buffer each. Remove the required amount of test strips from the packaging using a pair of tweezers and place them one by one in the channels containing the buffer (Make sure that the surface of the test strips is not damaged!). The number on the test strip should be visible.
Use of Immunoblot-PreQ: Set up the required incubation trays according to the work protocol and insert into the incubation device.
Incubate at room temperature (+18°C to +25°C) for **15 minutes** on a rocking shaker. Afterwards aspirate off all the liquid.

Sample incubation: (1st step) Fill each channel with 1.5 ml of the diluted serum samples using a clean pipette tip.
Incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.

Wash: Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working-strength universal buffer on a rocking shaker.

Conjugate incubation: (2nd step) Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase-conjugated anti-human IgG) into each channel.
Incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.

Wash: Aspirate off the liquid from each channel. Wash as described above.

Substrate incubation: (3rd step) Pipette 1.5 ml substrate solution into the channels of the incubation tray.
Incubate at room temperature (+18°C to +25°C) for **10 minutes** on a rocking shaker.

Stop: Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionised or distilled water.

Evaluate: Place test strip on the evaluation protocol, air dry and evaluate.
Immunoblot-PreQ: The evaluation of the test strips is realised exclusively via the EUROIMMUN camera systems.

For automated incubation with the **EUROBlotMaster** select the program **Euro02 Inf WB30**.

For automated incubation with the **EUROBlotOne** select the program **Euro 01/02.**

For automated incubation of Immunoblot-PreQ with the **EUROBlotOne** see instruction manual EUROBlotOne (YG_0153_A_UK_CXX).

EUROLINE Anti-EBV Profile 2 (IgG)

Incubation protocol

Blocking
(Manual incubation)
Pipette 1.5 ml working-strength universal buffer into the incubation channel and insert test strip

15 min — **Shake**

1. Step: Sample incubation
Aspirate off, pipette 1.5 ml of diluted serum sample (1:51) into the incubation channel

30 min — **Shake**

Wash
Aspirate off, wash 3 x 5 min with 1.5 ml working-strength universal buffer each

2. Step: Conjugate incubation
Aspirate off, pipette 1.5 ml working-strength enzyme conjugate into the incubation channel

30 min — **Shake**

Wash
Aspirate off, wash 3 x 5 min with 1.5 ml working-strength universal buffer each

3. Step: Substrate incubation
Aspirate off, pipette 1.5 ml substrate into the incubation channel

10 min — **Shake**

Stop
Aspirate off, rinse three times with 1.5 ml distilled water

Evaluation
EUROLineScan (digital)

Interpretation of results

Handling: For the evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN) and evaluated with **EUROLineScan**. For general information about the EUROLineScan program please refer to the EUROLineScan user manual (YG_0006_A_UK_CXX, EUROIMMUN). The code for entering the **test** into EUROLineScan is **EBV2.1 EL_IgG**.
The EUROLineScan software determines the probability of a fresh EBV infection (see page 7).

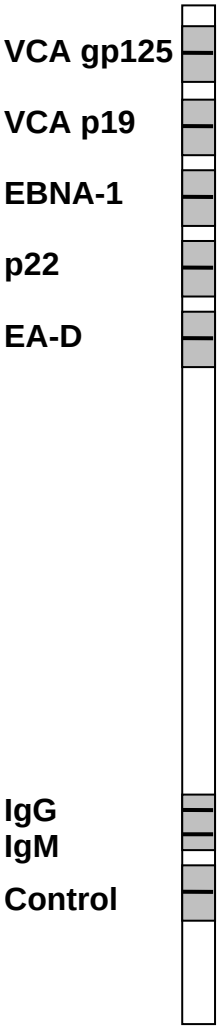
If a visual evaluation must be performed, place the incubated test strips onto the respective work protocol for visual evaluation. This protocol is available at EUROIMMUN under the order no. ZD 2790-0101-2 GM.

If using Immunoblot-PreQ (EUROIMMUN order no. DN 2790-0510-2 G), the strips should stay in the EUROTrays during evaluation. For the evaluation we generally recommend using a EUROIMMUN camera system connected to EUROLineScan software. Strips need to be dry before starting the evaluation.

Note: A correctly performed test for class IgG antibodies against EBV antigens is indicated by a positive reaction of the control band and the IgG band. If one of these bands shows a very weak reaction or none at all, the result must not be used for evaluation.

Antigens and their arrangement on the strips:

Antigen	Source
VCA	
VCA gp125	Native VCA gp125, purified by affinity chromatography
VCA p19	Recombinant VCA p19 antigen
EBNA	
EBNA-1	Recombinant EBNA-1 antigen
p22	Recombinant p22 antigen. p22 is a capsid antigen. Antibodies against p22, like anti-EBNA-1 antibodies, are formed in the late phase of infection.
EA	
EA-D	Recombinant EA-D antigen
Control bands: **IgG or IgM** **Control:** Incubation control indicating a correctly performed incubation.	



Based on signal intensity, the results can be divided into:

Signal	**Signal intensity EUROLineScan Flatbed scanner**	**Result**	
No signal	0-5	o	Negative
Very weak band	6-10	(+)	Borderline
Medium to strong band	11-25 or 26-50	+, ++	Positive
Very strong band with an intensity comparable to the control band	>50	+++	Strong positive

The table above contains **values** for the evaluation using a flatbed scanner. The **values** for other instruments supported by EUROLineScan can be found in the EUROLineScan program. To do so mark the corresponding assay in the test list (main menu: "Help" → "Test") and click on details and select **the corresponding instruments** in **"image source"**.

Antibodies of class IgG against Epstein-Barr virus

VCA	Negative	Both VCA bands (VCA gp125, VCA p19) negative or only very weakly positive.
	Positive	At least one of the VCA bands (VCA gp125, VCA p19) moderately or strongly positive.
EBNA-1	Negative	EBNA-1 band negative or only very weakly positive. **Note:** When the EBNA-1 band is negative and a fresh infection is excluded (VCA IgM negative), a moderate or strong reaction of the p22 band indicates a late phase of infection with loss of anti-EBNA-1 (see interpretation, page 7).
	Positive	EBNA-1 band moderately or strongly positive.
EA	Negative	EA band negative or only very weakly positive.
	Positive	EA band moderately or strongly positive.

Antibodies of class IgM against Epstein-Barr virus

VCA	Negative	Both VCA bands (VCA gp125, VCA p19) negative or only very weakly positive.
	Positive	At least one of the VCA bands (VCA gp125, VCA p19) moderately or strongly positive.

Interpretation of EUROLINE Anti-EBV Profile 2 results

Infection status	Typical results in the EUROLINE Anti-EBV Profile 2
Negative	Antibodies of class IgG against EBNA-1, VCA and EA negative **and** antibodies of class IgM against VCA negative
Acute infection	Antibodies of class IgG against EBNA-1 negative and VCA positive (often antibodies of class IgG against EA positive) **and** antibodies of class IgM against VCA positive *** Serological complications may occur due to the lack of formation of IgM class anti-VCA antibodies in acute EBV infections.**
Late phase of infection	Antibodies of class IgG against EBNA-1 and VCA positive **and** antibodies of class IgM against VCA negative
Late phase of infection with loss of anti-EBNA-1	Antibodies of class IgG against EBNA-1 negative**, but p22 band moderately or strongly positive**, VCA positive and EA negative **and** antibodies of class IgM against VCA negative *** Serological complications may occur due to persisting IgM class anti-VCA antibodies.**
Reactivated infection	For the characterisation of **reactivated EBV infections** in immuno-suppressive individuals or in persons with weakened immune system, the determination of the viral load is recommended since the serological results often do not correlate with the results of direct tests.

Statistical analysis of the signal intensity of bands VCA gp125, VCA p19, EBNA-1, p22 and EA-D allows a statement about the probability of a primary (acute) EBV infection. The signal intensity is evaluated using the EUROLineScan software and the calculated probability is shown for the respective patient in the details window. With values from 0% to 30% primary infection is unlikely. With values between 31% and 69% no statement can be made. Values from 70% to 100% indicate a high probability of primary infection. In every case, the clinical symptoms must correlate with the serological findings.

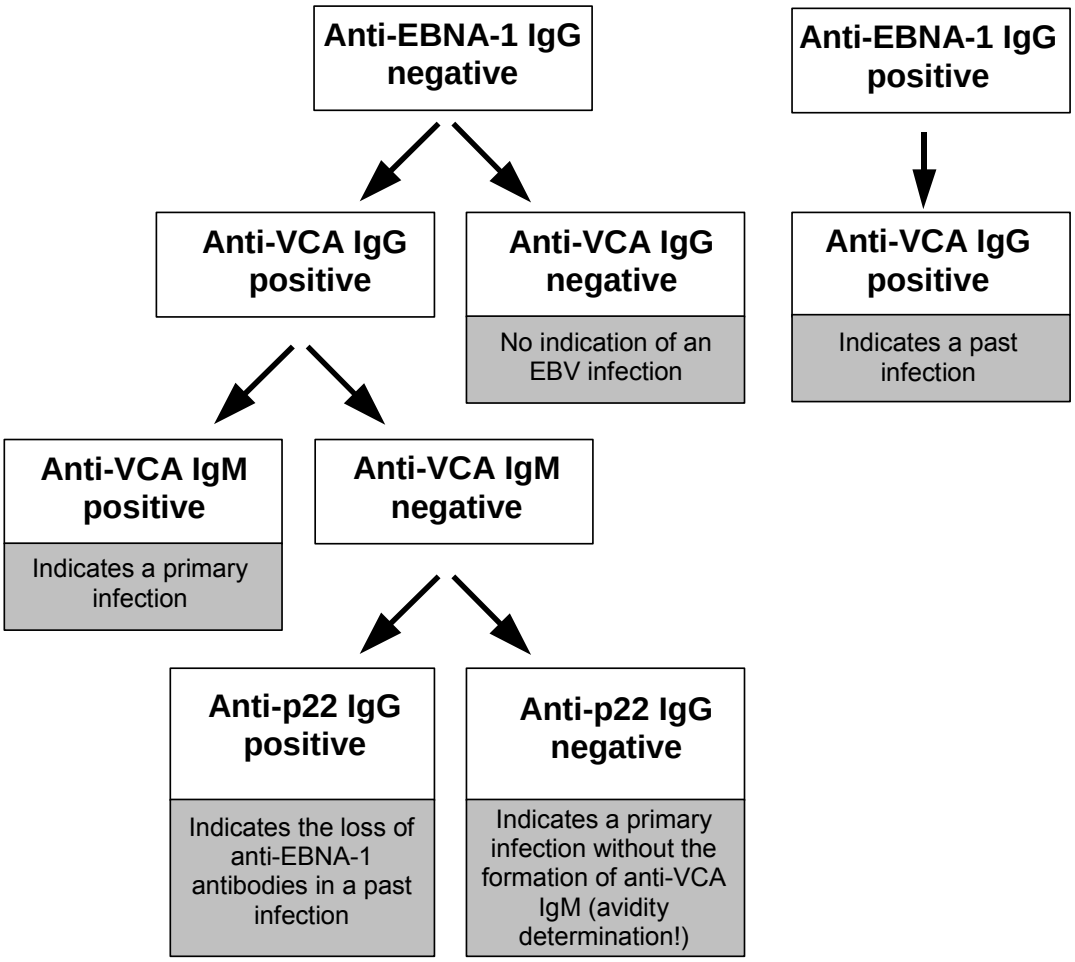
Overview:

	Associated antibodies *				
Infection status	**Anti-VCA (IgG)**	**Anti-VCA (IgM)**	**Anti-EBNA-1 (IgG)**	**Anti-p22 (IgG)**	**Anti-EA-D (IgG)**
Negative	-	-	-	-	-
Acute infection	+	+	-	-	+
Late phase of infection	+	-	+	+	-
Reactivated infection	For the characterisation of reactivated EBV infections in immuno-suppressive individuals or in persons with weakened immune system, the determination of the viral load is recommended since the serological results often do not correlate with the results of direct tests.				

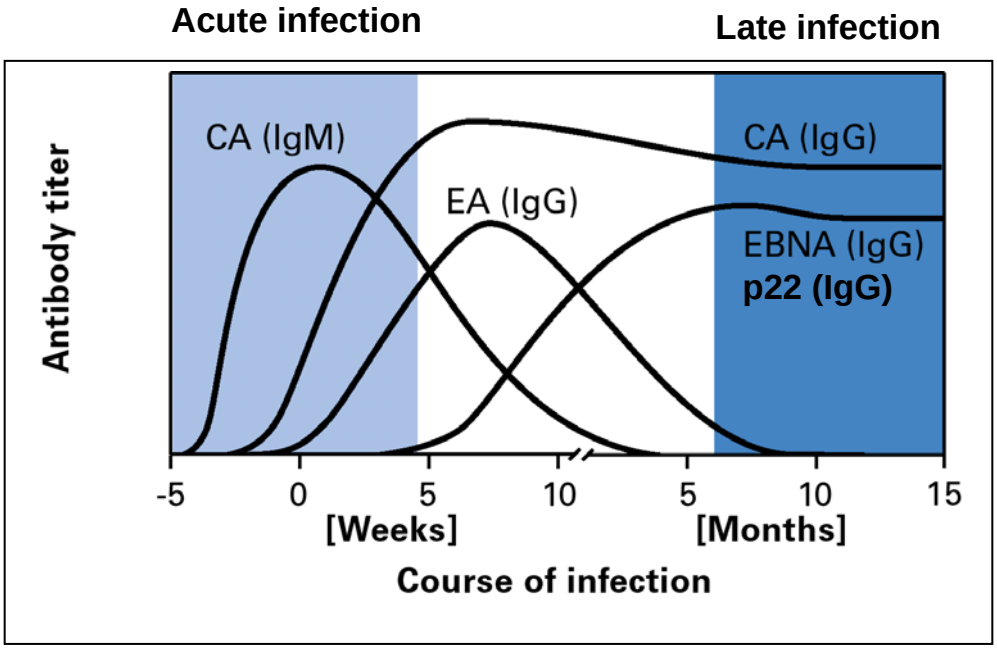
*****(For the prevalence of various antibodies see: Test characteristics/Studies)

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Interpretation scheme for EBV serology:



Time course of antibody formation:



Test characteristics

Measurement range: The EUROLINE is a qualitative method. No measurement range is provided.

Cross-reactions: The high analytical specificity of the test system is guaranteed by the quality of the antigen substrates used (antigens and antigen sources). This EUROLINE specifically detects IgG class antibodies to VCA gp125, VCA p19, EBNA-1, p22 and EA-D. No cross-reactions with other autoantibodies have been found.

Interference: Haemolytic, lipaemic and icteric sera up to a concentration of 5 mg/ml haemoglobin, of 20 mg/ml triglycerides and of 0.4 mg/ml bilirubin showed no effect on the analytical results of the present EUROLINE.

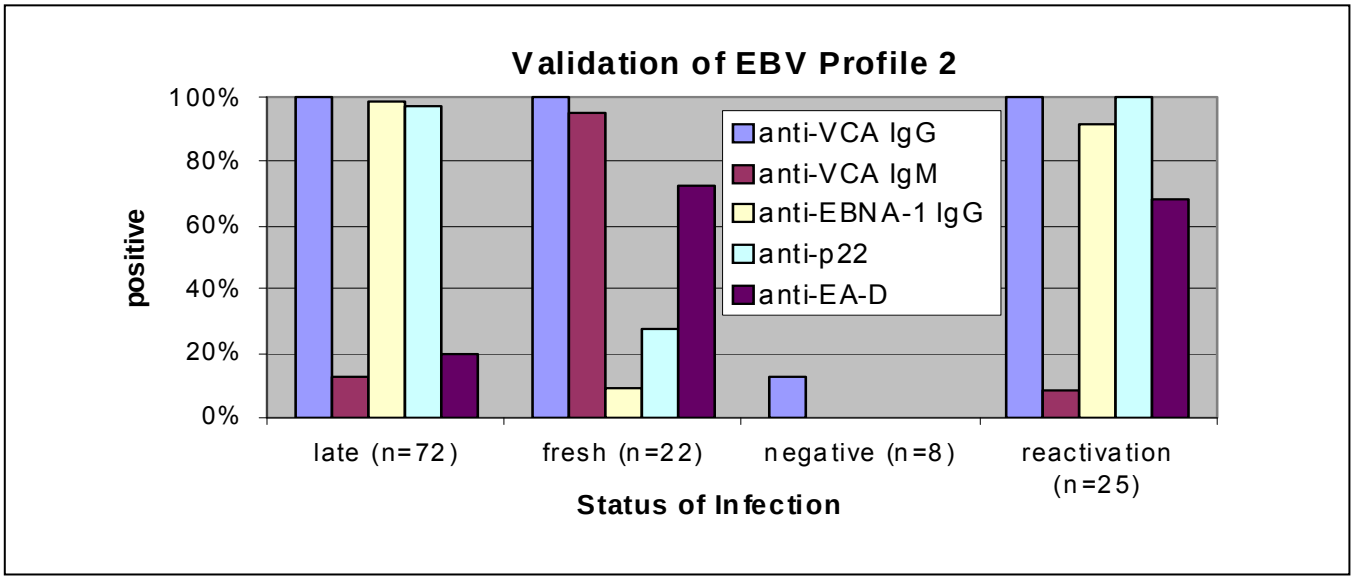
Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This EUROLINE displays excellent inter- and intra-assay reproducibility.

Studies: 127 sera from patients in different stages of the EBV infection (clinically and serologically characterised: Dr Gärtner, Universitätsklinikum des Saarlandes; EUROIMMUN AG) were tested with the EUROLINE Anti-EBV Profile 2 for antibodies of class IgG and IgM against VCA gp125, VCA p19, EBNA-1, p22 and EA-D. The following prevalences were found (in %):

Status of infection	VCA IgG positive	VCA IgM positive	EBNA-1 positive	p22 positive	EA-D positive
late (n = 72)	100	13	99	97	19
acute (n = 22)	100	96	9	27	73
negative (n = 8)	13	0	0	0	0
reactivation (n=25)	100	8	92	100	68

The late phase of the infection is marked by antibodies of class IgG against VCA gp125 and/or p19, as well as against EBNA-1. In case of a secondary loss of the antibodies against EBNA-1 the presence of antibodies of class IgG against p22 gives evidence of a late phase infection.

A primary infection is characterised by antibodies of class IgG and IgM against VCA antigens gp125 and/or p19. Antibodies of class IgG against EA-D may also be present as well.



Clinical significance

Epstein-Barr virus (EBV) is one of the most widely distributed human pathogenic herpes viruses. The virus is transmitted by smear infection, but also by blood transfusions or organ transplants. EBV is the causative agent of infectious mononucleosis (Pfeiffer's disease, kissing disease), a febrile disease usually accompanied by pharyngitis and lymphadenopathy, frequently by hepatosplenomegaly and more rarely by an exanthema. Infections during childhood usually occur without symptoms. In industrial countries mainly adolescents or young adults become infected. These infections often lead to manifest disease. Changed blood counts are characteristic of the disease (mononuclear Pfeiffer cells). More than 90% of the worldwide population are infected with EBV.

EBV infections are also found in connection with the pathogenesis of malignant lymphoma (endemic form of Burkitt's lymphoma in Africa) and nasopharyngeal carcinoma (NPC, especially widespread in South-East Asia). In Southern China, NPC is the third most frequent malignant tumour. EBV is also described as the causative agent of systemic lupus erythematosus SLE, rheumatoid arthritis and Sjögren's syndrome, diseases which are associated with high anti-EBV antibody titers, a large share of EBV-infected B cells, high EBV titers in peripheral blood and an increased risk of lymphoma.

Following a primary infection, the pathogen enters a latency stage, from which it may be reactivated. The immune system of healthy persons can suppress a reactivation. However, in immunosuppressed patients (e.g. with immunosuppressive therapy after organ transplantation or HIV infection), EBV may cause severe lymphoproliferative diseases.

Serology is of special importance in the diagnosis of infectious mononucleosis.

- IgM antibodies against the **viral capsid antigen (VCA)** are characteristic. As the infection progresses, anti-VCA IgG is formed, while anti-VCA IgM levels drop below the detection limit. Anti-VCA IgG persists life-long.
- **Heterophilic antibodies**, which are typical of an EBV infection, appear during the acute phase of infection.
- IgG and IgM against **early antigen (EA)** correlate in 60 to 80% of patients with an active infection, IgG antibodies may persist over a longer time period.
- IgG against **EBNA-1 (Epstein-Barr nuclear antigen)** is detectable after some weeks or months and enables detection of past infections. 5 to 10% of patients with infectious mononucleosis do not produce antibodies against EBNA-1.
- With unclear antibody findings, such as missing anti-VCA IgM in primary infections or missing anti-EBNA-1 IgG in past infections, it is recommended to investigate the **avidity** of IgG against VCA. Low avidity indicates an acute infection.

Infectious mononucleosis must be differentiated from cytomegaly, rubella, fifth disease and toxoplasmosis, as well as HIV and streptococcus infections.

Literature

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