ELISA-Inkubation
ELISA Incubation

Antigen-beschichtete Reagenzgefäße
antigen-coated wells

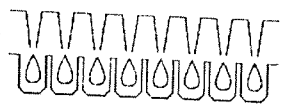
Pipettieren: 100 µl je Reagenzgefäß
Pipette: *100 µl per well*

Kalibratoren, Kontrollen, verdünnte Proben
calibrators, controls, samples

1. **Inkubieren:** 30 min bei Raumtemperatur (18°C bis 25°C)
Incubate: *30 min at room temperature (18°C to 25°C)*

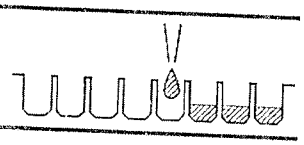
Waschen: 300 µl (man.)/450 µl (aut.) je Reagenzgefäß
Einwirkzeit: 30-60 s je Waschzyklus
Wash: *300 µl (man.)/450 µl (aut.) per well*
residence time: 30-60 s per washing cycle

3x

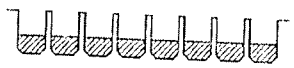


Pipettieren: 100 µl je Reagenzgefäß
Pipette: 100 µl per well

Enzymkonjugat
enzyme conjugate



2. **Inkubieren:** 30 min bei Raumtemperatur (18°C bis 25°C)
Incubate: *30 min at room temperature (18°C to 25°C)*

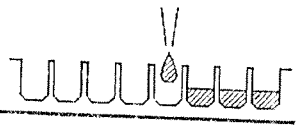


Waschen: 300 µl (man.)/450 µl (aut.) je Reagenzgefäß
Einwirkzeit: 30-60 s je Waschzyklus
Wash: *300 µl (man.)/450 µl (aut.) per well*
residence time: 30-60 s per washing cycle

3x

Pipettieren: 100 µl je Reagenzgefäß
Pipette: *100 µl per well*

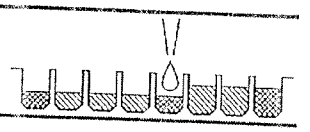
Chromogen/Substrat
chromogen/substrat



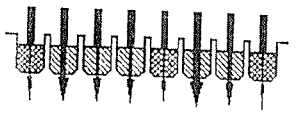
3. **Inkubieren:** 15 min bei Raumtemperatur (18°C- 25°C)
Incubate: *15 min at room temperature (18°C- 25°C)*

Pipettieren: 100 µl je Reagenzgefäß
Pipette: *100 µl per well*

Stopplösung
stop solution



Auswerten: Photometrische Messung (450 nm)
Evaluate: *photometric measurement (450 nm)*



EUROIMMUN Medizinische Labordiagnostika AG

Qualitätskontrollzertifikat
Quality Control Certificate

Produkt: *Product:* Anti-EBV-CA ELISA (IgG)

Best.-Nr.: *Order No:* EI 2791-9601 G

Ch.-B.: *Lot:* E150312AW

Verw. bis: *Exp. Date:* 11-Mar-2016

		Referenzwert *Reference value*		Valider Bereich *Valid range*	
Kalibrator 1 *Calibrator 1*	200 RU/ml	1,939	O.D.	> 0,700	O.D.
Kalibrator 2 *Calibrator 2*	20 RU/ml	0,315	O.D.	> 0,140	O.D.
Kalibrator 3 *Calibrator 3*	2 RU/ml	0,116	O.D.		
Pos. Kontrolle 1 *Pos. Control 1*	quantitativ *quantitative*	124	RU/ml	87 - 161	RU/ml
Pos. Kontrolle 1 *Pos. Control 1*	semiquantitativ *semiquantitative*	4,0	Ratio	2,2 - 5,8	Ratio
Neg. Kontrolle *Neg. Control*	quantitativ *quantitative*	2	RU/ml	0 - 15	RU/ml
Neg. Kontrolle *Neg. Control*	semiquantitativ *semiquantitative*	0,1	Ratio	0 - 0,7	Ratio

O.D. Kalibrator 1 > O.D. Kalibrator 2 > O.D. Kalibrator 3
O.D. Calibrator 1 > O.D. Calibrator 2 > O.D. Calibrator 3

Die Charge wurde von der Qualitätskontrolle getestet und erfüllt alle Spezifikationen.
The lot has been tested by the quality control laboratory and meets the specifications.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
  For example, for 1 microplate strip: 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: The control sera used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents are contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of the patient samples

Sample material: 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: Patient samples are diluted **1:101** sample buffer. For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st)	Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).
Washing:	Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash. Automatic: Wash reagent wells 3 times with 450 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus"). Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer. Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values. Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.
Conjugate incubation: (2nd)	Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to 25°C).
Washing:	Empty the wells. Wash as described above.
Substrate incubation: (3rd)	Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to 25°C) (protect from direct sunlight).
Stopping the reaction:	Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.
Measurement:	**Photometric measurement** of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the micro-plate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using the analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry.
Automated test performance using other fully automated, open system analysis devices is possible, however, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 2	P 6	P 14	P 22			C 1	P 4	P 12	P 20		
B	pos.	P 7	P 15	P 23			C 2	P 5	P 13	P 21		
C	neg.	P 8	P 16	P 24			C 3	P 6	P 14	P 22		
D	P 1	P 9	P 17				pos.	P 7	P 15	P 23		
E	P 2	P 10	P 18				neg.	P 8	P 16	P 24		
F	P 3	P 11	P 19				P 1	P 9	P 17			
G	P 4	P 12	P 20				P 2	P 10	P 18			
H	P 5	P 13	P 21				P 3	P 11	P 19			

The pipetting protocol for microtiter strips 1-4 is an example for the semiquantitative analysis of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the quantitative analysis of 24 patient sera (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The reagent wells are break off format. Therefore, the number of tests performed can be matched to the number of samples, minimizing reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according the following formula:

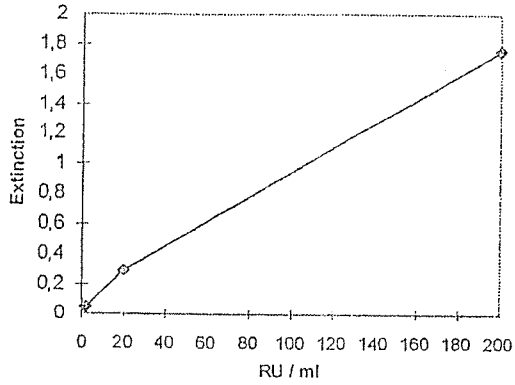
$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

In cases of borderline test results, an additional patient sample should be taken 7 days later and re-tested in parallel with the first patient sample. The results of both samples allow proper evaluation of titer changes.

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction of a serum sample lies above the value of calibrator 1 (200 RU/ml). The result should be given as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **20 relative units (RU)/ml**. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

Test characteristics

Calibration: As no international reference serum exists for antibodies against EBV-CA, the calibration is performed in relative units (RU).

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibration sera are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The microplate wells were coated with the purified Epstein-Barr virus capsid antigens. The antigen source is provided by inactivated cell lysates of human B cells infected with the "P3HR1" strain of Epstein-Barr viruses.

Linearity: The linearity of the Anti-EBV-CA ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95. The Anti-EBV-CA ELISA (IgG) is linear at least in the tested concentration range (4 RU/ml to 141 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The detection limit of the Anti-EBV-CA ELISA (IgG) is 0.9 RU/ml.

Cross reactivity: The quality of the antigen and the source of antigen (P3HR1-Cells EBV infected) used ensures a high specificity of the ELISA. No cross reactivities with Herpes viruses were determined. The test is anti-EBV specific.

Antibodies against	n	Anti-EBV-CA ELISA (IgG)
Adenovirus	10	0%
Chlamydia pneumoniae	5	0%
CMV	3	0%
Influenza virus A	4	0%
Influenza virus B	9	0%
Measles virus	9	0%
Mumps virus	9	0%
Mycoplasma pneumoniae	3	0%
Parainfluenza virus Pool	10	0%
RSV	8	0%
Rubella virus	10	0%
VZV	5	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0,4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	Mean value (RU/ml)	CV (%)
1	47	7.4
2	90	5.8
3	93	4.2

Inter-assay variation, n = 4 x 6		
Serum	Mean value (RU/ml)	CV (%)
1	47	8.2
2	90	3.2
3	93	5.4

Specificity and sensitivity: 111 clinically and serologically precharacterized sera (Interlaboratory test samples of INSTAND, Germany / Labquality, Finland) were examined with this EUROIMMUN ELISA. The test showed a specificity and a sensitivity of 100% each.

n = 111		INSTAND / Labquality		
		positiv	borderline	negativ
EUROIMMUN Anti-EBV-CA-ELISA (IgG)	positive	92	0	0
	borderline	3	1	0
	negative	0	0	15

Reference range: The levels of the anti-EBV-CA antibodies (IgG) were analyzed with this EUROIMMUN ELISA in a collective of 500 healthy blood donors. With a cut-off of 20 RU/ml, 93.4% of the blood donors were anti-EBV-CA positive (IgG) which reflects the known percentage of infections in adults.

Clinical significance

Epstein-Barr virus (EBV) and herpes simples virus types 1 and 2 belong to the most ubiquitous human herpes viruses in adults [1]. Epstein-Barr virus is the causative agent of infectious mononucleosis (glandular fever), a febrile disease usually accompanied by pharyngitis and lymphadenopathy, frequently by hepatosplenomegaly and more rarely by exanthema. EBV infections are also found in connection with the pathogenesis of Burkitt's lymphoma, nasopharyngeal carcinoma, and, as current research results show, multiple sclerosis [1, 2, 3, 4, 5, 6, 7, 8]. Infectious mononucleosis must be differentiated from cytomegalic inclusion body disease and toxoplasmosis and, in the case of atypical progress, also from HIV or other infections [1, 9, 10].

In pregnancy Epstein-Barr virus can cause infection of the placenta with damage to the foetal heart, eyes and liver [3, 11]. In children, accompanying infections of the kidney have been observed with symptoms from microscopic haematuria to acute kidney failure [1, 3, 12].

The immune response to an EBV infection is characterised by formation of antibodies against the EBV **capsid antigen** (EBV-CA), against **EBV nuclear antigens** (EBNA-1 to EBNA-6) and against **EBV early antigens** (EBV-EA) [1, 2, 3, 4, 5, 6, 7, 8, 13, 14, 15, 16, 17, 18, 19].

In 90% of cases an acute EBV infection can be characterised serologically by the detection of EBV-CA IgM and an increase in titer of EBV-CA IgG using ELISA [13, 14, 15, 16].

A titer increase of at least two-fold for anti-EBV-CA IgG with simultaneous lack of antibodies against EBNA-1 demonstrates the early phase of an acute EBV infection [16]. Of importance in this respect is that in an EBV infection EBNA-1 to EBNA-6 are synthesised earlier than the other EBV antigens (EBV-CA and EBV-EA), but they are only presented to the immune system after the destruction of B cells, so that, timewise, antibodies against EBV-CA and EBV-EA are detectable before antibodies against EBNA [6, 19, 20].

An IgM immune response to EBV-CA with a titer increase of EBV-CA IgG antibodies is considered a reliable indicator of the presence of an acute EBV infection [13, 15, 17]. An early EBV infection can be confirmed using **EBV-CA IgG avidity determination** [15].

IgA antibodies against early EBV proteins are detectable in primary infections, and rarely in reactivations [6]. IgG antibodies against early EBV proteins occur in 70% to 80% of patients with infectious mononucleosis, although only temporarily during the acute phase.

High antibody titers of class IgA against EBV-CA, and also class IgG against EBV-EA can be evaluated as an indication of Burkitt's lymphoma or nasopharyngeal carcinoma, which extends the diagnostic potential of this test [1, 3, 4, 5, 6, 7, 8, 18].

In considering the significance of the detection of antibodies against EBV-EA, it should be taken into account that these can, but don't have to, occur in acute infections and during an inapparent disease course. Differentiation between a primary EBV infection and the more rare reactivation is hardly possible serologically [13, 15, 19].

With the ELISA test for the quantitative in vitro determination of human **IgG antibodies against EBV-CA in cerebrospinal fluid (CSF)** the CSF/serum quotient of the agent-specific antibodies $CSQ_{path.-spec.}$ (IgG) can be measured. The quotient can indicate EBV antibody production in the central nervous system and thus enable diagnosis of a cerebral EBV infection [21].

Literature references

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Anti-EBV-CA ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG-CLASS	SUBSTRATE	FORMAT
EI 2791-9601 G	Epstein-Barr virus capsid antigen (EBV-CA)	IgG	Ag-coated microplate wells	96 x 01 (96)

Principles of the test: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against EBV-CA in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with EBV-CA. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. **Microplate wells** coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. **Calibrator 1** 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. **Calibrator 2** 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. **Calibrator 3** 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. **Positive control** (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. **Negative control** (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. **Enzyme conjugate** peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. **Sample buffer** ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. **Wash buffer** 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. **Chromogen/substrate solution** TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. **Stop solution** 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. **Test instruction**	---	1 booklet	
13. **Quality control certificate**	---	1 protocol	

LOT Lot — CE — Storage temperature
IVD In vitro determination — Unopened usable until

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

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.