VIROTECH EBV IgG LINE Immunoblot

(EBV IgG LINE-32)

Order No.: WE102G32

(EBV IgG LINE-96)

Order No.: WE102G96

VIROTECH EBV IgM LINE Immunoblot

(EBV IgM LINE-32)

Order No.: WE102M32

(EBV IgM LINE-96)

Order No.: WE102M96

FOR IN VITRO DIAGNOSIS ONLY

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Contents

1. Intended Use

LINE Immunoblot for the qualitative detection of Epstein Barr Virus (EBV) specific IgG- resp. IgM-antibodies in human serum. The kit is intended to differentiate or prove seronegativity, primary infection or past infection in expanded EBV diagnosis.

2. Diagnostic Meaning

The Epstein Barr virus belongs to the family of the Herpesviridae and is transmitted mainly by saliva, when it infects the epithelial cells of the oropharynx initially and then the B-lymphocytes. The virus is the cause of infectious mononucleosis (IM) and chronic active EBV infection. There is also an association between EBV infections and Burkitt's lymphoma and also nasopharyngeal carcinomas in Africa and Asia. According to serological investigations, approx. 95% of adults are seropositive for EBV.

Primary EBV infections are normally asymptomatic but can be the cause of infectious mononucleosis in adolescents and young adults. IM is a self-limiting illness and is characterised by lymphadenopathy, fever, hepatosplenomegaly and leukocytosis with atypical lymphocytes (1-6).

The role of EBV serology is making a differential diagnosis from clinically similar symptomatic diseases caused by CMV, Rubellavirus, Mumpsvirus, HIV, HAV, HBV, HCV and neurotropic viruses as well as brucellosis, listeriosis, leptospirosis, toxoplasmosis and neoplastic diseases like lymphoma and leukemia (7).

3. Principle of Test

Proteins of the pathogen-antigen are transferred to the nitrocellulosis membrane by a micro-dispensing method. The nitrocellulosis membrane is then cut into single strips.

Incubation of the antigen-coated nitrocellulose strips with samples of human serum or plasma permits the detection of specific antibodies. These antibodies develop immuncomplexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alcalic phosphatasis conjugated anti-human IgG- respectively IgM-antibodies. After unbound conjugated antibodies have been removed by a further washing step, a visualisation of the antigen/antibody-complex (of the bound antibodies) is accomplished by the addition of a non-coloured substrate, which forms blue-violet precipitates at each site („antigen bands") where the conjugated anti-human antibodies have bound. The enzyme/substrate-reaction is stopped through washing the nitrocellulose-strips with aqua dest./deionised. Depending on the observed band pattern one can interpret the presence of specific IgG- respectively IgM-antibodies.

4. Package Contents

4.1 Kit for 32 determinations

1.	**IgG resp. IgM Nitrocellulose test strips** with applied antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	**1x**	32 strips
2.	**IgG resp. IgM Cut off Control,** human serum, prediluted	**1x**	1.0ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**2x**	50 ml
4.	**IgG- resp. IgM- Conjugate** (100x conc.) anti-human-(goat)-Alcalic Phosphatasis, with preservative	**1x**	0.7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**1x**	57 ml
6.	**Evaluation Record sheet** for the notation and deposit of the results	**1x**	1 pcs.

4.2 Kit for 96 determinations

1.	**IgG resp. IgM Nitrocellulose test strips** with applied antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	**3x**	32 strips
2.	**IgG resp. IgM Cut off Control,** human serum, prediluted	**2x**	1.0ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**4x**	50 ml

4. **IgG- resp. IgM- Conjugate** (100x conc.)
   anti-human-(goat)-Alcalic Phosphatasis, with preservative **3x** 0.7 ml
5. **Substrate** (BCIP/NBT), ready to use **3x** 57 ml
6. **Evaluation Record sheet** for the notation and deposit of the results **3x** 1 pcs.

Also available on request:

IgG or IgM- Positive control, human serum, prediluted, 0.5 ml.
The positive bands > For the cut--off band, refer to the certificate supplied with the kit.
(Order No.: IgG: WE102P60 or IgM: WE102P80)

IgG/IgM- Negative control, human serum, prediluted, 0.5 ml.
The negative control shows no bands or no bands relevant to the evaluation. > Cut-off band.
(Order No.: IgG/IgM: WE102N10)

5. Storage and Stability

Store test kit at 2-8°C. The shelf life of the single components is mentioned on the relevant label; for shelf life of the Kit please refer to the Quality Control Certificate.

1. Do not expose the single kit components to high temperature nor freeze them.
2. Do not use the kit reagents after their expiring date.
3. Do not expose reagents to strong light during storage or incubation.
4. The BCIP/NBT-substrate solution is sensitive to light and has to be stored in dark.
5. **Nitrocellulose test strips**: Use strips immediately after taken out of the bag. Close bag with the not required strips again savely and store at 2-8°C. When putting the results into archives please take care that the nitrocellulose test strips and templates are protected against direct sunlight, to avoid fading of the bands.

Material	Status	Storage	Shelflife
Test Samples	Undiluted	+2 to +8°C	1 week
Test Strips	After Opening	+2 to +8°C (stored in supplied bag)	3 months
Controls	After Opening	+2 to +8°C	3 months
Conjugate	After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	ca. 6h
Substrate	After Opening	+2 to +8°C (protect from light)	3 months
Washing Solution	After Opening	+2 to +8°C (protect from light)	3 months
	Final Dilution (ready-to-use)	+2 to +8°C	4 weeks
	Final Dilution (ready-to-use)	*or* room temperature	2 weeks

6. Precautions and Warnings

1. Only sera, that have been tested and found to be negative for HIV1-ab, HIV2-ab, HCV-ab and Hepatitis-B-surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls and conjugate as well as the antigen strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Use plastic foreceps and wear protective gloves when handling the Immunoblot.
3. Please follow the local valid waste disposal regulations.

4. The incubation baths are designed by the manufacturer for a single use. The reuse of the incubation baths is at the risk of the user. If they are to be reused we recommend that after use the incubation baths be disinfected for several hours in 1% sodium hypochlorite solution and then rinsed thoroughly with tap water followed by distilled or deionized water.

7. Additionally required material (not supplied)

1. Incubation tray (if required available with order no.: WE300.08)
2. Rocking platform (vertical not centrifugal)
3. A wash bottle for stopping
4. Pipette or handwasher
5. Micro-pipettes 5 µl - 1500 µl
6. Pipette filler
7. Test tubes, 2-20 ml volume
8. Plastic foreceps
9. Aqua dest. or deionised water
10. Filter paper

8. Examination Material

Either serum and plasma may be used as test materials, even when the package leaflet only mentions serum. Plasma samples may contain any anticoagulant.

9. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

9.1 Preparation of Samples

1. 15 µl serum or plasma are needed for each patient sample.
2. Blood samples should be taken separately by venous puncture. Serum is separated after complete coagulation (not applicable to plasma). If they are to be stored longer sera have to be frozen at -20°C.
3. Repeated freezing and thawing should be avoided.
4. Sera that are heat-inactivated, lipaemic, haemolytic or microbiologically contaminated, may lead to faulty results and shall therefore not be used.
5. Do not use turbid samples (especially after thawing), centrifuge if necessary (5 minutes at 1000sg), pipette clear supernatant and use in testing.

9.2 Preparation of Reagents

1. To facilitate routine laboratory work, all LINEs and EcoBlots can be processed in a single test run with the same incubation times and the same component - when these are independent of the parameters and batches. The cut off controls now have parameter and batch specific values.
2. Bring the corresponding concentrate to room temperature (20-25°C) before preparing the dilution. Use only high quality Aqua dest./deionised and bring up to room temperature (20-25°C) before usage.
3. Mix dilutions well before starting the test.
4. **Dilution-/Washbuffer:**
   The dilution-/washbuffer is provided as a 10-fold concentrate. Dilute the dilution-/washbuffer concentrate 1:10 with distilled or deionised water (10ml/50ml/100ml concentrate + 90ml/450ml/900ml A distilled or deionised water), mix well. Both the concentrated and the dilute dilution/washing buffer may exhibit a yellow colouration. This colouration does not influence the stability of the dilution/washing buffer or the function or the reliability of the diagnostic test.

5. **IgG resp. IgM conjugate**
   Dilute the conjugate 1 + 100 with finally diluted dilution/washing buffer and mix thoroughly. 1.5 ml conjugate working solution is required for each serum sample. See conjugated dilution table (item: "Test Procedure").
6. **Substrate Solution**
   The substrate solution is delivered ready-to-use.

9.3 Immunoblot Test Procedure

Attention: **The antigenstrips must only be tested in the released Ig-class.**
(pls. refer to the label on the blot booklet and the marking on each single test strip).

For the correct performance and evaluation of the EBV LINEs, each test run should include the appropriate parameter and batch-specific cut off controls.

For a secure EBV diagnostic the LINE shall be proceeded in IgG and IgM.

1. Test has to be proceeded at room temperature.
2. For each sample put 1 strip into the channel of a clean incubation tray. Hold strip only at the marked upper end.
3. Pipette 1,5ml ready to use **dilution-/ washbuffer** each and put onto the rocking platform. Take care that the antigen strips are consistently covered with liquid, the strips must not dry out during the whole test procedure.
4. The solid antigen strips are being moistured completely within one minute and can be incubated in supine, lateral position or face-down position.
5. **15 µl patient serum or plasma** or **100 µl of the cut-off or positive / negative control** added by pipetting, if at all possible at the upper marked end of the strip. Incubate patient serum and control for **30 minutes** on the rocking platform. Take care that during pipetting and following pour away no cross-contamination of the single patient samples occur.
6. Aspirate or carefully pour away the liquid out of the channels completely. During the pour away of the liquid, the antigen strips remain at the bottom of the channel. Drain the remaining liquid onto a cellulosis paper.
7. **Washing** of strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate washing buffer always completely. Before ending of the last washing step, prepare the needed amount of fresh conjugate dilution (refer to table).
8. Aspirate or pour away the liquid completely out of the channels (please refer to point 6).
9. Pipette 1,5 ml of the prepared **conjugate dilution** each into the corresponding incubation channel and incubate for **30 minutes** on the rocking platform.
10. Aspirate or pour away the liquid completely out of the channels (please refer to point 6).
11. **Washing** of the strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate the washbuffer always completely. Afterwards rinse **1 x 1 minute** with **Aqua dest./deionised.**
12. Pour away or aspirate the liquid completely out of the channels (refer to point 6).
13. Pipette 1,5 ml ready to use **substrate solution** each into the channels and allow to develop **10** ± **3 minutes** on the rocking platform.
14. **Stop** the color reaction by pouring away the substrate solution. Afterwards wash the strips without incubation in between for **3 x** with 1,5 ml **Aqua dest./deionised** each.
15. Pour away the aqua dest./deionised and let the strip dry on a clean cellulosis paper. The background-coloring, that may be observed on the moistured antigen strips disappears completely when the strips are completely dry. Solid antigen strips need a little longer than the conventional antigen strips until they are completely dry.
16. Use the included calculation protocol for the interpretation. The inscription of the high-specific band on the protocol sheet make the interpretation of the patient samples easier for you.

For test procedure scheme pls. refer to last page

9.4 Use of Immunoblot-processors

The following instruments have been validated for the automatic processing of the Blots and LINEs: Apollo and Profiblot. All commercially available Blot machines are suitable in principle.

10. Interpretation of Results

For a secure interpretation each LINE is fitted out with two controls:

1. **Serum control:**
   Only after the incubation with patient serum the serum incubation band appears below the markline.
2. **Conjugate control:**
   The LINE strip is fitted out with a conjugate control band which appears after incubation with the respective conjugate.

The test procedure is valid, if the serum control as well as the internal conjugate control appears clearly visible on the developed antigen strip.
Please refer to the protocol sheet for the information of the exact position of the serum- and the conjugate control.

10.1 Interpretation of the patient samples

Please refer to the protocol sheet for position and denotation of reactive bands.
IgM bands: gp125, p18, EA-D
IgG bands: EBNA1, gp125, p18, EA-D

10.2 Usage of the Cut-Off Control

Bands with an intensity weaker than the cut-off band of the cut-off control are not considered for the interpretation.

IgG cut-off bands:	1. **p18** for assessment:	of gp125-, p18- and EA-D-band of EBNA1-band, with pos. IgM-serology
	2. **EBNA1** for assessment:	of EBNA1-band, with neg. IgM-serology
IgM cut-off band:	**p18** for assessment:	of gp125-, p18- and EA-D-band

10.3 Meaning of the Antigens

List of the synthetic antigen peptides used (EBNA1, p18, EA-D) as well as the affinity purified gp125 antigen of the Epstein Barr virus antigen.

Antigen / Description	**Meaning of the antigens**	**Specificity of the antibodies with the LINE**
EBNA1	**E**pstein-**B**arr **N**uclear **A**ntigen, a viral protein, which is expressed in the nucleus of latent infected cells. IgG antibodies against EBNA1 are regarded as reliable marker for a past EBV infection. In rare exceptional cases the IgG immune response against EBNA1 (primary or secondary) may not appear. In immunosuppressed patients IgG antibodytiters against EBNA1 may greatly decrease (secondary EBNA1 loss).	**IgG:** central highly specific marker for past EBV infection
VCA-gp125	Several ñ**V**irus **C**apsid **A**ntigensò have been described. Among them the proteins gp125 and p18 are regarded as immunodominant.	**IgG-gp125:**

	As a rule IgM antibodies against VCA-gp125/p18 disappear again several weeks after infection, IgG antibodies against VCA-gp125/p18 are kept lifelong. Sometimes IgM antibodies against VCA-gp125/p18 are built again in reactivations.	highly specific general marker for EBV infections **IgM:** highly specific for an EBV primary infection
VCA-p18	Please refer also to notes to VCA-gp125 in ñmeaningò The p18-peptide is a high specific marker for primary infection in IgM-serology. As a rule in primary infections first a significant anti-p18 IgM antibody response is found followed by an increase of anti-p18 IgG antibodies. This IgG increase reaches its maximum when the IgM titers decrease and before an IgG immune response against EBNA1 appears.	**IgG-p18:** highly specific marker for contact with EBV in advanced stage **IgM:** highly specific for an EBV primary infection
EA-D	ñ**E**arly **A**ntigen-**D**iffuseò belongs to the early antigens, which are synthesised in the viral replication cycle (acute phase of infection). IgG and IgM antibodies against EA-D appear with negative EBNA-IgG at the same time in primary infections typically. IgG antibodies against EA-D decrease during convalescence but may greatly increase again during EBV reactivations. But this antibody increase is no statement about the clinical relevance of an EBV reactivation.	**IgG:** **1.)** specific for EBV primary infection **2.)** serological marker for EBV reactivation **IgM:** specific for EBV primary infection

10.4 Interpretation criteria

The interpretation of the serological result shall always include the clinical picture, epidemiological data and further diagnostical parameter.

Recommended IgM assessment

Band Pattern	Meaning	Assessment
no bands or band(s) < cut-off-band	negative	No IgM antibodies against EBV antigens detectable.
gp125 ≥ cut-off-band (p18)	positive	Notice to a primary infection especially with missing IgG immune response against EBNA1 (please refer also to IgG assessment for EBNA1)
p18 ≥ cut-off-band (p18)	positive	Notice to a primary infection especially with missing IgG immune response against EBNA1 (please refer also to IgG assessment for EBNA1)
EA-D isolates reactively ≥ cut-off-band (p18)	negative	IgM antibodies against EA-D are frequently developed in primary infections but always appear together with IgM antibodies against p18 and/or gp125 and are therefore not considered for assessment.

Recommended IgG assessment for a negative IgM assessment

	Occurring band(s) in IgG ÓCut off band				IgG assessment	Exemplary text for findings
IgM assessment	EBNA1 Ó EBNA1 Cut off band	gp125 Ó p18 Cut off band	p18 Ó p18 Cut off band	EA-D Ó p18 Cut off band		
Negative	neg.	neg.	neg.	neg.	Negative	No antibodies against EBV antigen detected
	pos.	neg./pos.	neg./pos.	neg./pos.	Positive	Indication of past EBV infection
	neg.	pos.	pos.	neg./pos.	Positive	Indication of past EBV infection
	neg.	pos.	neg.	neg.	Positive	Indication of contact with EBV. Distinction between primary and past infection is not possible. Control recommended
	neg.	neg.	pos.	neg.	Positive	Indication of past EBV infection. Control recommended
	neg.	neg.	neg.	pos.	Positive	Indication of contact with EBV. Control strongly recommended.
	neg.	pos.	neg.	pos.	Positive	Suspected primary EBV infection. Control strongly recommended
	neg.	neg.	pos.	pos.	Positive	Indication of contact with EBV. Distinction between primary and past infection is not possible. Control recommended

Recommended IgG assessment for a positive IgM assessment

	Occurring band(s) in IgG ÓCut off band				IgG assessment	Exemplary text for findings
IgM assessment	EBNA1	gp125	p18	EA-D		
Positive	neg.	neg.	neg.	neg.	Negative	Indication of a primary infection
	pos.	neg./pos.	neg./pos.	neg./pos.	Positive	Indication of past EBV infection
	neg.	pos.	neg./pos.	neg./pos.	Positive	Indication of primary infection
	neg.	neg./pos.	pos.	neg./pos.	Positive	Indication of primary infection
	neg.	neg./pos.	neg./pos.	pos.	Positive	Indication of primary infection

10.5 Limits of the Test

1. A negative Blot result does not completely exclude the possibility of an EBV infection.
2. In rare cases patients may show „inverse" bands (dark background, white bands), these are not to be considered, means the Immunoblot can not be assessed in such cases. The serum should be checked using other serological methods.
3. EBV serology on its own does not allow a secure statement about clinical relevance of an EBV reactivation (9).
4. A negative anti-EBNA1 is not conclusively a notice to a primary infection. In immunosuppressed patients it may come to a secondary anti-EBNA1 loss and in 5% of EBV infected persons (EBNA1-nonresponder) no anti-EBNA1 is built (7).
5. A negative VCA-IgM result does not exclude the possibility of a primary infection, as in some cases no VCA-IgM is built during an acute infection (IgM-nonresponder) (7).
6. Antibodies which were passively transmitted shortly before examination may influence the EBV serological result. This might be e.g. by blood transfusion or maternally transmitted antibodies to the infant.

11. Performance Data

11.1 Sensitivity

Following sera collectives were tested to determine the sensitivity:

1. sera from patients with EBV primary infection (n=10, sera from proficiency testings and PanBio, Australia)
2. sera from donors with past EBV infection (n=40, sera from Dr. Gärtner, Homburg/Saar)

ELISAs and/or IFA were used as reference tests (analytical finding).

Sera collective (n=50)		**LINE IgG + IgM overall result**	
		Primary Infection	**Past Infection**
Finding	**Primary Infection**	10	0
	Past Infection	0	40

The results of the EBV LINE correspond with the reference findings in all cases.

11.2 Specificity

Following sera collectives were tested to determine the specificity:

1. sera from pregnant women (n=15)
2. sera from children (n=10)
3. EBV seronegative sera (n=20, sera from Dr. Gärtner, Homburg/Saar)

ELISAs and/or IFA were used as reference tests (analytical finding).

*Sera collective (n=45)		**LINE IgG + IgM overall result**	
		Past Infection	**Seronegative**
Finding	**Past Infection**	19	0
	Seronegative	0	25

(*) It was not possible to compare one child serum as the ELISA did not show a clear serological finding. This unclear finding was not considered in the calculation of the specificity.

The results of the EBV LINE correspond with the reference findings in all cases.

11.3 Diagnostical Sensitivity

Clinically characterized sera from patients with EBV primary infection (n=30) and past infection (n=36) were tested to determine the diagnostical sensitivity (sera from Dr. Gärtner, Homburg/Saar).

Sera collective (n=66)		LINE IgG + IgM overall result	
		Primary Infection	**Past Infection**
Diagnostytical Finding	**Primary Infection (*)**	29	0
	Past Infection	0	36

(*) One unclear finding was not considered when calculating the diagnostical sensitivity. It is a serum from an one year old child with sickle-cell anemia who received a blood transfusion. This serum was assessed as past infection with the LINE and also reacted borderline for EBNA1 in the reference IFA.

From the above table it is apparent that all clinically defined sera (except the above unclear finding) were recognized in the overall judgement.

11.4 Diagnostical Specificity

20 seronegative sera were tested to determine the diagnostical sensitivity (sera from Dr. Gärtner, Homburg/Saar).

Sera collective (n=20)		LINE IgG + IgM overall result	
		Neg.	**Pos.**
Diagnostical Finding	**Neg.**	20	0
	Pos.	0	0

11.5 Cross-Reactivity

The cross-reactivity was examined by testing 47 sera from patients:

1. with a CMV infection (n=20, sera from Dr. Gärtner, Homburg/Saar)
2. with a disease from the rheumatic milieu (n=20, sera from Dr. Gärtner, Homburg/Saar)
3. with an autoimmune disease, ANF-positive (n=7, sera from Dr. Schäfer, Heidelberg)

An IFA was used as reference test for the sera in 1. and 2. and for the sera in 3. the ELISAs EBNA1-IgG, VCA-IgM, VCA-IgG and EA-D.

The results of the EBV-LINE correspond with the reference findings in all cases and no cross-reactivities were recognizable.

11.6 Prevalence (Expected Values)

80 blood bank sera were tested to determine the prevelance rate of 95% in adults (past EBV infection) as described in literature (8).

Sera collective (n=80)	**LINE IgG + IgM overall result**
Past Infection	78
Seronegative	2

11.7 Intra-Assay-Precision (Repeatability)

For each batch release, a strip with a specific human serum in the IgG and IgM is tested in the quality control from each individual Immunoblot. There is then a 100% control of all Immunoblots.

The intensities of the bands may deviate maximum one intensity level from the mean value on a scale of 1-5.

11.8 Inter-Assay-Precision (Reproducibility)

To determine reproducibility, 3 serums were tested in the IgG. The determination has been effected in 10 different test runs proceeded by 3 different test persons. One serum showed no antigen bands, one serum all antigen bands with strong intensity and one serum bands with weak intensities. In all testings, the serological allegations have been exactly reached.

12. References

1. Evans, A.S., J.C. Niedermann, L.C. Cenabre, B. West and V.A. Richards. (1975). A prospective evaluation of heterophile and EBV specific IgM antibody tests in clinical and subclinical infectious mononucleosis. J. Infect. Dis. 132:546-554.
2. Nikoskelainen, J., J. Leikola and E. Klemola. (1974). IgM antibodies specific for EBV in IM without heterophile antibodies. Br. Med. J. Oct 12.4(5936)72-5.
3. Klemola, E., R. von Essen, G. Henle and W. Henle. (1970). Infectious-mononucleosis-like disease with negative heterophil agglutination test. Clinical features in relation to Epstein-Barr virus and cytomegalovirus antibodies. J. Infect. Dis. Jun :121(6) :608-614.
4. Sumaya, C.V. and Y. Ench. (1985). Epstein-Barr virus infectious mononucleosis in children. II. Heterophil antibody and viral-specific responses. Pediatrics Jun: 75(6)1011-9.
5. Schmitz, H., D. Volz, C. Krainick-Riechert and M. Schere. (1972). Acute Epstein-Barr virus infections in children. Med Microbiol Immunol. 158(1):58-63.
6. Inoue, N. et al. (1992). Use of enzyme-linked immunosorbent assays with chimeric fusion proteins to titrate antibodies against Epstein-Barr virus nuclear antigen 1. J Clin Microbiol. Jun;30(6):1442-8.
7. Bauer, G. (2001). Simplicity through complexity: immunoblot with recombinant antigens as the new gold standard in Epstein-Barr virus serology. Clin Lab. 47(5-6):223-30.
8. Modrow, S. und D. Falke (2010). Das Epstein-Barr Virus. S. 572-577. In: Molekulare Virologie, Spektrum Verlag, ISBN: 978-3-8274-1833-3.
9. Gärtner BC et al. (2000). No correlation in Epstein-Barr virus reactivation between serological parameters and viral load. J Clin Microbiol. Jun;38(6): 2458

13. Test Procedure Scheme

Test Procedure in short version

Samples Incubation	**30 minutes**	15 µl patient serum/ plasma/ 100 µl control in 1,5 ml dilution-/washbuffer each
Washing	**3 x 5 minutes**	with 1,5 ml dilution-/washbuffer each
Conjugate incubation	**30 minutes**	with 1,5 ml working dilution (1 + 100)
Washing	**3 x 5 minutes** **1 x 1 minute**	with 1,5 ml dilution-/washbuffer each with Aqua dest./deionised
Substrate incubation	**10 ± 3 minutes**	with 1,5 ml ready to use substrate solution each
Stopping	**3 x without incubation in between**	with 1,5 ml Aqua dest./deionised each

Conjugate Dilution table (rounded)

Number of strips	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Dilution-/washbuffer	1,5ml	3,0ml	4,5ml	6,0ml	7,5ml	9,0ml	11,0ml	12,0ml	14,0ml	15,0ml
Conjugate-concentrate	15µl	30µl	45µl	60µl	75µl	90µl	110µl	120µl	140µl	150µl
Final volume	1,515m	3,03ml	4,545m	6,06ml	7,575m	9,09ml	11,11m	12,12m	14,14m	15,15m

Number of strips	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Dilution-/washbuffer	17,0ml	18,0ml	20,0ml	21,0ml	23,0ml	24,0ml	26,0ml	27,0ml	29,0ml	30,0ml
Conjugate-concentrate	170µl	180µl	200µl	210µl	230µl	240µl	260µl	270µl	290µl	300µl
Final volume	17,17m	18,18m	20,2ml	21,21m	23,23m	24,24m	26,26m	27,27m	29,29m	30,3ml

Number of strips	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Dilution-/washbuffer	32,0ml	33,0ml	35,0ml	36,0ml	38,0ml	39,0ml	41,0ml	42,0ml	44,0ml	45,0ml
Conjugate-concentrate	320µl	330µl	350µl	360µl	380µl	390µl	410µl	420µl	440µl	450µl
Final volume	32,32m	33,33m	35,35m	36,36m	38,38m	39,39m	41,41m	42,42m	44,44m	45,45m

Number of strips	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
Dilution-/washbuffer	47,0ml	48,0ml	50,0ml	51,0ml	53,0ml	54,0ml	56,0ml	57,0ml	59,0ml	60,0ml
Conjugate-concentrate	470µl	480µl	500µl	510µl	530µl	540µl	560µl	570µl	590µl	600µl
Final volume	47,47m	48,48m	50,5ml	51,51m	53,53m	54,54m	56,56m	57,57m	59,59m	60,6ml