*recom*Line EBV IgG [Avidität] [IgA]
*recom*Line EBV IgM

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Line EBV IgG [Avidität] [IgA], IgM is a qualitative test for the detection of IgG, IgM or IgA antibodies and the determination of the avidity of IgG antibodies to the Epstein-Barr Virus (EBV) in human serum or plasma. For the determination of anti-EBV IgA, IgA conjugate can be ordered separately. For the determination of IgG antibody avidity, an avidity reagent can be ordered separately.

2 Intended use

The *recom*Line EBV IgG [Avidität] [IgA], IgM is a line immunoassay based upon recombinant Epstein-Barr virus antigens. In contrast with ELISAs, the testing principle allows the identification of specific antibodies to individual antigen classes of the virus due to the separate line-up of individual antigens, thus capturing the entire spectrum of reactivity on one test strip. This helps with the allocation of an EBV infection to the different possible stages.
The *recom*Line EBV IgG, IgM can be used as a confirmatory test for the clarification of unclear screening results or as a screening test.

3 Test principle

Highly purified recombinant EBV antigens are fixed on to nitrocellulose membrane strips. The strips of the *recom*Line EBV IgG [avidity] [IgA] and the strips of the *recom*Line EBV-IgM tests have a different antigen load:
*recom*Line EBV IgG [Avidität] [IgA]: EBNA-1, p18 (VCA), p23 (VCA) BZLF1 (IEA), p138 (EA), p54 (EA)
*recom*Line EBV IgM: p23 (VCA) ZEBRA (IEA), p138 (EA), p54 (EA)

The test strips are incubated with the diluted serum or plasma sample, with specific antibodies attached to the pathogen antigens on the test strip.

1. Unbound antibodies are then flushed away.
2. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG, IgA and/or IgM), which are coupled to horseradish peroxidise (HRP).
3. Unbound conjugate antibodies are then flushed away.
4. Specifically bound antibodies are detected with a staining reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point.

There are control bands at the upper end of the test strips:

a) The reaction control band under the strip number, which must show a reaction for every serum/plasma sample.
b) The conjugate controls (IgG, IgA, IgM) are used for the inspection of the antibody class detected. If, for example, the test strip is used for the detection of IgG antibodies, the conjugate control band IgG shows a clearly defined band.
c) "Cut-off control": The intensity of this band allows the assessment of the reactivity of each antigen band (see 9.2. Evaluation).

4 Reagents

4.1 Package contents

The reagents in one package are sufficient for 20 (200) tests.

Each test kit contains:

WASHBUF A 10 X	**100 ml (10x100ml)** Wash Buffer A (**10 times concentration**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	**40 ml (10x40ml)** Chromogenic substrate Tetramethylbenzidin (**TMB, ready-to-use**)
MILKPOW	**5 g (10x5g)** skimmed milk powder
INSTRU	**1** Instructions for use
EVALFORM	**1 (10)** Evaluation form

4.1.1 *recomLine EBV IgG [Avidität] [IgA]*

In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2 (20)** tubes, each with 10 numbered test strips
CONJ IgG	**500 µl (10x500)** anti-human IgG conjugate **(100-fold concentration, green cap)** From rabbit, containing NaN3 (<0.1%), MIT (0.1%) and chloroacetamide (0.1%)

Determining avidity

For the determination of EBV IgG antibody avidity, an avidity reagent is also available, supplied with instructions for use.

AVIDI Item No. 11010	**1** avidity reagent (solid substance 25g) for 60 ml ready-to-use solution

IgA determination

Also available for the determination of IgA antibodies (in addition to *recom*Line EBV **IgG**):

CONJ IgA Item No. 10016	**500 µl** anti-human IgA conjugate **(100-fold concentration, colourless cap)** From rabbit, containing NaN3 (<0.1%), MIT (0.1%) and chloroacetamide (0.1%)

4.1.2 *recomLine EBV IgM*

In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2 (20)** tubes, each with 10 numbered test strips
CONJ IgM	**500 µl (10x500 µl)** anti-human IgM conjugate **(100-fold concentration, purple cap)** From rabbit, containing NaN3 (<0.1%), MIT (0.1%) and chloroacetamide (0.1%)

4.2 Materials required but not supplied

- Incubation trays (can be purchased as needed from MIKROGEN)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotators
- Vacuum pump or similar device
- Volumetric cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for bio-hazardous materials

5 Shelf life and handling

- Store reagents at +2 °C to 8 °C before and after use, **do not freeze**.
- Subject all ingredients for at least 30 minutes to room temperature (+18 °C to 25 °C) before beginning the test. The test procedure is carried out at room temperature.
- Washing Buffer, Milk Powder, Dilution Buffer, Conjugate and TMB can be interchanged between the different *recom*Line and *recom*Blot test systems, if these components carry the same symbols. Consider the shelf life of these components.
- Mix the concentrated reagents and samples thoroughly before use. Avoid a build up of foam.
- Only open the tube containing the test strip immediately before use to avoid condensation. Leave unused strips in the tube and continue to store at +2 °C to +8 °C (reseal tube tightly, test strips must not become moist before the test!).
- The strips are marked with the serial number, as well as the test code.
- The packages bear an expiration date. After this has been reached no guarantee of quality can be offered.
- Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.
- The test should only be carried out by trained and authorised personnel.
- In case of significant changes by the user to the product and/or the instructions for use, application may be beyond the purpose specified by MIKROGEN.
- Cross-contamination of patient samples or conjugates can lead to inaccurate test results. Add patient samples, test strips and conjugate solu-

tion carefully. Make sure that incubation solutions do not flow over into other wells. Carefully drain liquids.
- The strips must be completely wetted and immersed throughout the entire procedure.
- Automation is possible; you will receive further information from MIKROGEN.

6 Warnings and precautions

- For *In vitro* diagnostic use only.
- MIKROGEN has not validated these tests for screening of blood, blood components, cells, tissues, organs or any of their derivatives in order to assess the suitability for transfusion, transplantation or cell administration.
- All blood products must be treated as potentially infectious.
- The test strips were manufactured with inactivated whole cell lysates and/or recombinant produced bacterial, viral or parasitic antigens.
- After the addition of patient or control specimens the strip material must be considered infectious and treated as such.
- Suitable disposable gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion and chloroacetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide can form an explosive azide upon contact with heavy metals such as copper and lead azide.
- All siphoned liquids must be collected. All containers must include appropriate disinfectants for the inactivation of pathogenic human viruses and other pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to your hygiene regulations. The concentrations and incubation periods of the manufacturer must be observed.
- Use incubation trays only once.
- Handle strips carefully using plastic forceps.
- Do not substitute or mix the reagents with reagents from other manufacturers.
- Read through the entire instructions for use before carrying out the test and carefully follow the directions. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

7 Sampling and preparation of reagents

7.1 Samples

The sample can be serum or plasma (EDTA, citrate, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling so as to avoid haemolysis. Avoid Microbial contamination of the samples. Insoluble substances must be removed from the sample prior to incubation. The use of heat-inactivated, icteric, haemolysed, lipemic or turbid samples is not recommended.

Caution!
If tests are not carried out immediately, the samples can be stored for up to 2 weeks at +2 to +8 °C. Long-term storage of the samples is possible at -20 °C or below. Repeated freezing and thawing of samples is not recommended due to the risk of producing inaccurate results. Avoid more than 3 cycles of freezing and thawing.

7.2 Preparation of solutions

7.2.1 *Preparation of ready-to-use wash buffer A*

This buffer is required for serum and conjugate dilution as well as washing stages.
Prior to dilution, the volume of wash buffer A must be determined for the corresponding number of tests.
First, the skimmed milk powder is dissolved in wash buffer A concentrate, and then deionised water is added to bring the solution up to the final volume (dilution: 1 + 9). The quantities required for a defined number of test strips are to be mathematically determined according to the following formula (device-specific dead volume is not considered):

Reagent	Formula	Example: 5 strips
Skimmed milk powder [g]	= number of strips x 0.1	0.5 g
Wash buffer A concentrate [ml]	= number of strips x 2	10 ml
Deionised water [ml]	= number of strips x 18	90 ml
Ready-to-use wash buffer A [ml]	= number of strips x 20	100 ml

Ready to use wash buffer A can be stored at +2 C – +8°C for up to four weeks. The ready to use wash buffer A is odourless and easily marred.

7.2.2 *Preparation of conjugate solutions*

The conjugate solution must be prepared immediately before use. It is not possible to store the ready for use conjugate solution.
One part of the conjugate concentrate is diluted with 100 parts of the ready to use wash buffer A (1 + 100).
The quantities required for a defined number of test strips are to be calculated according to the following formula:

Reagent	Formula	Example: 5 strips
Conjugate concentrate [µl]	= number of strips x 20	100 µl
Ready-to-use wash buffer A [ml]	= number of strips x 2	10 ml

The conjugate quantities are calculated without dead volume. Depending on handling (manual or on a device), please mix additional conjugate for 1 to 3 strips.

8 Test procedure

No.	Execution	Note
1	Temper all reagents for at least 30 minutes at 18°C - 25° (room temperature) before beginning the test.	The test procedure is carried out at room temperature.
2	Prepare test strips Place the strips in **2 ml** of ready-to-use **wash buffer A**. **Important:** IgG and IgM strips are not interchangeable!	Do not touch the strips with bare hands - use the forceps. The strip number points upward. A well is required in the incubation tray (see 4.2) for each strip. The strips must be completely immersed.
3 a) b)	Incubation of samples **20 µl** of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 100) Incubate for **1 hour** with gentle shaking	 Pipette the sample at one end of the immersed strip in the wash buffer A and mix as quickly as possible by carefully shaking the tray. Cover the incubation tray with plastic cover and place in the shaker.
4 a) b) c)	Washing Carefully remove the plastic cover from the incubation trays. Gently siphon serum dilution from the individual wells. Pipette **2 ml** of ready to use **wash buffer A** into each well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A.	Carry out washing stages 8.4a-8.4c three times in total. Avoid cross-contamination The manufacturer's instructions must be followed during automatic processing.
5	Incubation with conjugate Add **2 ml** of ready-to-use **conjugate solution** and incubate for **45 minutes** while shaking gently.	Cover the incubation tray with plastic cover and place in the shaker.
6	Washing see under 8.4	Carry out washing stages three times in total (see 8.4a-8.4c)
7	Substrate reaction Add **1.5 ml** of ready-to-use **substrate solution** and incubate for **8 minutes** while shaking gently.	
8	Stopping the reaction Wash at least three times **briefly** with **deionisied water**.	
9	Drying the strips Dry the strips between 2 layers of absorbent paper for **2 hours** prior to analysis.	Carefully remove strips from water using plastic forceps. Store strip away from light.
Caution! **Incubation solutions must not flow into other wells. Splashing must be avoided especially when opening and closing the lid.**		

9 Results

Caution:
Please do not use automated interpretation without consideration of the information on interpretation given below.

9.1 Validation – Quality Control

An analysis of the test can be carried out if the following criteria have been fulfilled:
1. Reaction control band (top line) with clearly visible stain, dark band.
2. Antibody class:
   LEBG strips (second and third bands): the corresponding conjugate control band must show a clear staining. The other conjugate band may show weak, unspecific staining on the test strips.

LEBM strip (second band): the conjugate control band must show a clear staining.
3. "Cutoff control" (LEBG: fourth band, LEBM: third band): weak, but visible staining.

9.2 Evaluation

The analysis of the test strips can be visual or computer-assisted - using the test strip analysis software *recom*Scan. The *recom*Scan software is designed to support the evaluation of test strips. Further information and related instructions for the computer-assisted analysis is available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 *Assessment of band intensity*

1. Note the date and batch number, as well as the detected antibody class, on the attached evaluation form.
2. Enter the sample identification numbers to the evaluation sheet.
3. Now stick the corresponding test strip onto the appropriate fields on the evaluation form using a glue stick. Align the test strip with the reaction control bands along the marked lines. Then use a transparent adhesive tape to attach the test strip to the left of the marked lines (do not tape over the reaction control band!). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.
4. Now identify the bands of the developed test strip using the control strip printed on the evaluation sheet and enter them in the evaluation sheet. For each corresponding immunoglobulin class, assess separately the intensity of the bands occurring on the basis of Table 1.

Table 1: Assessment of band intensity in relation to the cut-off band

Stain intensity of the bands	Assessment
No reaction	-
Very low intensity (lower than the cut-off band)	+/-
Low intensity (equivalent to the cut-off band)	+
High intensity (higher than the cut-off band)	++
Very strong intensity	+++

Caution!
The band patterns in the *recom*Line EBV IgG, IgM and IgA tests may show different intensities. It is possible that the *recom*Line EBV IgG shows stronger and darker bands than the *recom*Line EBV IgM and/or the *recom*Line EBV IgA. The intensity of the protein bands depends on the concentration of the EBV-specific antibodies.
Assessment of avidity: See Chapter 9.5.

9.3 Interpretation of test results

- IgG testing is required in either case.
- A serum is EBV reactive, as soon as a strip shows at least one antigen band with an intensity of "+".
- No band or isolated bands with an intensity of "±" indicate a negative result.

In order to assess the EBV immune status, the results of the IgG test should always be viewed in conjunction with the IgM test results.
In most cases, the EBV status can be determined by antibody reactions to a number of key antigens and by the typical antigen band constellations. These EBV antigens and their importance in the determining of EBV infection status are summarised in Table 2. However, the biology of EBV and individual immune responses make it difficult to set up strict guidelines for all antigens as regards essential reactivity patterns during classification. Frequently occurring antigen band constellations are listed in Table 3.

Table 2: Key antigens of different stages of infection

Antigen	Classification	Assessment of EBV status
EBNA-1	p72 Epstein-Barr nuclear antigen 1	High diagnostic significance, key marker for past infections, EBNA-1 IgG confirms a past infection and excludes any acute primary infection
p18	VCA (virus capsid antigen)	p18^{Mikrogen} is a modified p18, IgG titres are a second key marker for past EBV infections
p23	VCA (virus capsid antigen)	Frequently, antibodies in IgG and IgM can already be detected at the beginning of an infection. IgG antibodies remain detectable after past infections.
ZEBRA	IEA (immediate early antigen); Immunodominant partial sequence of the ZEBRA protein	Good IgM marker for the detection of an acute EBV infection
BZLF1	IEA (immediate early antigen); Complete ZEBRA protein	IgG and IGA antibodies can be found in the early stages, IgG reactivity can often be found after past infections
p138 p54	EA (early antigens)	With acute infection, IgG, IgM and IgA reactivity likely in all antibody classes

Table 3: Interpretation of reactivity - typical reaction patterns

IgG: EBNA-1 and p18 negative (p23, BZLF1, p138, p54 possible) IgM: ZEBRA, EA (p54 and/or p138) positive, p23 possible	**Primary infection - Infectious Mononucleosis**
IgG: IgG: EBNA-1 and/or p18 positive, p23 and BZLF1 frequently positive; weak EA titres possible IgM: negative	**Past infection**
IgG: as with past infection, also weak early antigen (EA) reaction IgM: weak reactivity to EA and/or ZEBRA p23	**Secondary reactivation (not clinically relevant)**
IgG: Strong reaction with all antigens, in individual cases, loss of anti-EBNA IgM: weak reactivity possible IgA: clear reactivity with EA and/or BZLF1 and/or VCA	**Reactivation***
Serologic picture as with reactivation (high IgA titres!)	**NPC and EBV-associated lymphomas**
No EBV-specific bands in IgG, IGM and IgA determination	**EBV negative**
IgG/IgM/IgA: isolated bands (except for IgG-EBNA-1 or IgG-VCA) in only one class of antibody, if all other classes are negative	**EBV status unclear** (test should be repeated after approx. 2-3 weeks)

* EBV reactivation is the resumption and completion of lytic replication in a person with a latent infection. The extent of this increase can be very low and of short duration, e.g. with a temporary immune system malfunction due to other infections or any other cause; but may also be of a longer duration and serious in nature, e.g. in patients with immune disorders/on immunosuppressive medication; in these cases, there is the risk of EBV-associated lymphomas. All intermediate stages are possible. A not clinically relevant, short-term EBV proliferation is known as a "secondary reactivation".

9.4 Significance of IgG antibodies to the p18 antigen of MIKROGEN

Extensive new evaluations were carried out for the interpretation of the validity of the r-p18 antigen. The results can be summarised as follows:

- It can take up to three weeks following the start of the illness before positive anti-p18 IgG antibodies can be detected.
- With the detection of anti-EBNA-1 and/or anti-p18 IgG antibodies, past EBV infections can be determined.
- Anti p18-IgG is a second key marker in EBV serology, in addition to anti-EBNA-1 IgG. Its key advantage is that patients without anti-EBNA-1 antibodies after an EBV infection or secondary loss of anti-EBNA-1 have these markers and a post-acute or past EBV infection can thus be clearly established. Such cases have been reported in patients with immunosuppression and immune deficiency.

9.5 Extended diagnosis by determining the avidity

IgG antibodies undergo a maturation process, with the antibodies of early stages showing low avidity, while the antibodies of past infections show high avidity. In contrast to high avidity antibodies, low avidity antibodies can be removed from the binding site on the test strip with an avidity reagent. By parallel processing 2 IgG assays - of which one must be treated with the avidity agent - it can be established whether the IgG antibodies are of low (acute events) or high avidity (past infection).

9.5.1 *Test principle and test execution*

The avidity of EBV IgG antibodies can be determined with the avidity reagent, Item No. 11010. The instructions for performing the test are given in the instructions for the use of the avidity reagent.

9.5.2 *Assessment and interpretation of avidity for recomLine EBV IgG*

- The avidity is only determined when the overall IgG results have been positive.
- Bands on the IgG strip that have a lower reactivity than the cut-off are not taken into account when determining the avidity.
- Compare the intensities of the corresponding bands on the two test strips (IgG strips and avidity strips) incubated with the same patient sample. Check whether there have been any changes in intensity.
- A reduction in VCA band intensity (p18, p23) of more than 60% can be considered to be low avidity, a reduction between 40 and 60% as intermediary.
- With high avidity, the band intensity of the avidity strips shows a reduction of less than 40% and the IgG antibodies are regarded as being highly avid.
- A selective reduction in the intensity of EA / IEA bands (p54, p138 and BZLF1) is not an indication of a new infection, as the maturation of these antibodies does not make it possible to reach definite conclusions on the infection status. Reactivation and/or secondary reactivation can result in the formation of new low avidity antibodies.
- In general, it is not possible to make absolute rules for avidity evaluation. As maturation of activity may be delayed, past infection cannot be excluded with low avidity in individual cases. Avidity should always be interpreted within the context of all other examination results.

Table 4: Interpretation notes to assess the infection status when determining avidity. Assessment of VCA p18 and p23 bands.

IgG Reaction patterns	IgG Determining avidity	EBV infection status Interpretation
negative	Not suitable	No serological evidence of infection
positive BZLF1, p138, p54	Avidity of antibodies to BZLF1, p54, p138 does not make it possible to reach conclusions concerning the stage of infection	Suspected acute infection
positive p23, BZLF1, p138, p54	p23: low/intermediary	Suspected acute infection
positive p23, BZLF1, p138, p54	p23: high	Suspected recent infection
positive p18, p23, BZLF1, p138, p54,	p18: low-intermediary p23: high	Suspected recent infection
positive p18, p23, BZLF1, p138, p54	p18: high p23: high	past infection
positive EBNA-1, p18, p23, BZLF1, p138, p54	p18: high p23: high	past infection

10 Limitations of the method - restrictions

- Serological test results must always be considered in the context of other medical assessments of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- There is a high degree of variability in the individual serological response after a virus infection. In EBV serology, this issue can be resolved by using a combination of different markers. Here the anti-EBNA-1 is of key importance: With a positive anti-EBNA-1, an acute EBV infection can be excluded. Negative anti-EBNA-1 can either indicate a new infection or be secondary negative (e.g. due to anti-EBNA-1 loss in immunosuppression). And approximately 5% of those infected with EBV do not develop anti-EBNA-1 after the infection, thus mimicking a new infection.
- This diagnostic gap can be bridged by a second marker, the Mikrogen VCA p18 antigen. Evaluation data shows that IgG antibodies to p18 exclude new infections. Anti-p18 is thus similar to the EBNA-1 response, but has the distinct advantage that individuals with loss of anti-EBNA-1 do not typically lose this late marker, thus allowing for a proper serological assessment (see also Chapter 9.4).
- Based on this data, a new infection can always be excluded where the IgG shows antibodies to EBNA-1 and/or p18.
- The test results are inconclusive, where IgG or IgM only show isolated bands (excluding EBNA-1 and VCA markers), and all other antibody classes are negative. This may be an indication of an incipient reaction to primary infection or a cross reaction. The test should be repeated after approx. 2-3 weeks with a new serum sample.
- A negative result does not exclude the possibility of an EBV infection. False negative results may occur when serum samples are taken very soon after infection.
- Dark test strips: Some patient samples can produce a dark, uniform or patterned staining across the entire nitrocellulose strip (e.g. on serums from patients with milk protein allergies). Various factors in each patient serum are responsible for this. The evaluation of these strips is usually only partly feasible. Thus, "inverse" bands (white bands on dark background), for example, should be evaluated as negative. The respective serum should always be examined using other serological methods.

11 Test performance

11.1 Serum samples from EBV routine diagnostics

A total of 252 routine diagnostics serum samples were examined in the *recom*Line EBV IgG/M/A. The sera had been sent in with the clinical issue of suspected acute EBV infection. Some serum samples had already been checked for acute infections and EBV negative and preselected with the help of the screening results.

Table 5: Assessment of EBV routine samples

	EBV ELISA screening EBV status n (%)			
***recom*Line EBV Interpretation**	Negative	Acute[1]	Past[2]	**Total**
Negative	64 (26%)	0 (0%)	0 (0%)	64 (26%)
Borderline	8 (3%)	5 (2%)	0 (0%)	13 (5%)
Acute/recent	2 (1%)	115 (46%)	0 (0%)	117 (47%)
Past	3 (1%)	10 (4%)	45 (18%)	58 (23%)
Total	77 (31%)	130 (52%)	45 (18%)	252

[1]EBV ELISA results: EBNA-1-IgG: negative, VCA-G: positive/borderline/negative, VCA-M: positive
[2]EBV ELISA results: EBNA-1-IgG: positive/borderline, VCA-G: positive, VCA-M: negative/borderline

In the screening tests, 15/130 samples were classed as suspected primary infection, which was not subsequently confirmed in *recom*Line EBV. Ten of these samples were classified as past infections (EBNA-1 negative, but with strong, highly avid p18 reactivity in IgG). Five sera had to be classified as EBV-inconclusive, as there was only one antigen response for each immunoglobulin class.
For a further 13 sera, which were initially classified as negative in the screening ELISA, the initial findings were not confirmed. Using the *recom*Line EBV, three samples were classified as past infections (EBNA-1 negative, but with strong, highly avid p18 reactivity in IgG and IgM negative). Two samples were identified as acute and/or recent EBV infection and eight samples had an inconclusive EBV status, as the *recom*Line EBV showed only one antigen response for each immunoglobulin class. With such inconclusive results, the test should be repeated after approx. 2-3 weeks.

11.2 Serum samples from acute EBV infections

A total of five courses of a suspected acute EBV infection, comprising 6-9 samples each, were examined (n=38 serum samples from five courses).
Two courses contained serum samples taken before infection. These were identified as EBV-negative. In all courses, a clear IgG response was identified from the first sample of the primary infection. The early antigens BZLF1, p138 and p54 showed a positive reaction, while for one course it took one month after the first sample to identify antibodies to BZLF1. IgM reactivity was also positive in all courses right from the outset (ZEBRA and p54, as well as p138 and p23). In the further course of the infection, IgG antibodies to p23, p18 and also to EBNA-1 were formed. IgG avidity to p23, p18 and EBNA-1 increased gradually, while at the same time IgM reactivity decreased.

11.3 Seroprevalence

	Blood donors (n=100)		
*recom*Line EBV IgG [IgA], IgM	IgG	IgM	IgA
Seroprevalence	93 %	8 %	26 %

In 93 samples, the EBV immune status, including the results for all immunoglobulin classes, was classified as past infection and in seven as EBV-negative.

11.4 Avidity

Two panels (30 seropositive blood donors, 35 acute/recent infections) were tested with the *recom*Line EBV IgG.
In the blood donor panel (past infections), antibodies to EBNA-1 and/or p18 and/or p23 were highly avid in 29/30 samples. In the acute/recent infections, the avidity of anti-p23 and anti-p18 antibodies was low to intermediate.

In earlier, comprehensive studies with routine sera (n=1577) from EBV serology, the avidity of antibodies to EBNA-1, p18 and p23 was tested extensively, using the precursor version of *recom*Line EBV. A total of 90/1577 (5.7%) were seronegative, 7/1577 (0.4%) showed an inconclusive result and 1480/1577 (93.8%) were positive in the *recom*Line EBV IgG. A total of 1156/1480 of positive patient samples showed the signs of a typical past infection in the *recom*Line EBV IgG. For the remaining 324 samples of the positive IgG results, avidity was determined and the following reactivities were identified:

New EBV infections (42 out of 324 samples):
In most cases, anti-EA antibodies (p138 and p54) were present in different band strengths and avidity maturation. In two-thirds of cases, anti-p23 antibodies in IgG were present at low to intermediate avidity; late markers (anti-p18, anti-EBNA-1) were absent.

Recent infections (12 out of 324 samples):
Antibodies to EBNA-1, antibodies of low and intermediate avidity to p18 and highly avid antibodies to p23 were not detected.

Past EBV infections (270 out of 324 samples):
The samples from this group showed different combinations for the late markers (EBNA-1, p18) and p23, with negative, low positive (+) and positive (2+) band intensities. Existing markers showed constant high avidity and confirmed the evaluation as past infection.

11.5 Analytical specificity

The analytical specificity is defined as the capacity of the test to determine the analytes exactly in the presence of potential interference factors in the sample matrix (e.g. anticoagulants, haemolysis, effects of the sample treatment) or cross reactions with potentially interfering antibodies.

a) Interferences: Control studies on potentially interfering factors have shown that anticoagulants (sodium citrate, EDTA, heparin), haemolysis (up to 1,000 mg/dl haemoglobin), lipaemia, bilirubinaemia (up to 20 mg/dl bilirubin) or three cycles of freezing and thawing do not affect the performance of the test.

b) Cross-reactions: The potential interference of antibodies with other organisms that can produce similar clinical symptoms as in an EBV infection (e.g. CMV, HSV) has been investigated in control studies. Also tested were conditions caused by an atypical activity of the immune system, such as rheumatoid factor. No cross-reactivities were detected. A collective of ANA-positive patient samples showed increased EA IgG and IgA antibody reactivity, this had no impact on the assessment as past EBV infection.

12 Literature

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We will gladly send you further reading on EBV diagnostics on request.

13 Explanation of symbols

Σ	Content is sufficient for <n> applications Number of applications
WASHBUF A 10 X	Wash Buffer A (10 times concentration)
SUBS TMB	Chromogenic substrate Tetramethylbenzidin
MILKPOW	Skimmed milk powder
TESTSTR	Test strips
CONJ IgG	Anti-human IgG conjugate
AVIDI	Avidity reagent (solid substance)
ADD	Additional reagent, available on request
CONJ IgA	Anti-human IgA conjugate
CONJ IgM	Anti-human IgM conjugate
EVALFORM	Evaluation form
INSTRU	Instructions for use
	See instructions for use
CONT	Contents, includes
IVD	In vitro test
LOT	Batch/version number
	Do not freeze
REF	Order number
	Use by Expiry date
x°C y°C	Store at x°C to y°C
	Manufacturer

14 Manufacturer and version information

***recom*Line EBV IgG [Avidität] [IgA]** ***recom*Line EBV IgM**	Item no. **4572 (4576)** Item no. **4573 (4577)**
Instructions for use valid from	GARLEB016EN 2023-05

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