VIROTECH Borrelia Vet. + OspA IgG LINE Immunoblot

(Borrelia Vet. + OspA IgG LINE Hund/Dog)

Order No.: DE226G32

Borrelia Vet. + OspA IgG LINE Set Pferd/Horse

Order No.: DE226K62

ONLY FOR IN VITRO DIAGNOSTIC TESTING in dogs and horses

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1. Intended Use

The VIROTECH Borrelia Veterinary plus OspA Line is a LINE Immunoblot Testkit for the qualitative detection of *Borrelia* (*B.*) *burgdorferi* sensu lato specific IgG antibodies in dog or horse serum. This test kit can distinguish a wild type infection from a vaccination in the dog.

2. Diagnostic Meaning

General

The pathogen of Lyme borreliosis (LB), the spirochete *B. burgdorferi*, was discovered in 1981 by Burgdorfer and Barbour and classified as a species in the genus *Borrelia* (1).

Lyme borreliosis (LB) is a systemic disease, caused by infection with the spirochete *B. burgdorferi* (7,8). The disease is transmitted in the bite of an infected tick. The tick *Ixodes ricinus* has been identified as the main vector in Europe (2, 5). The following species have been idenified as (human) pathogens in Europe: *B. burgdorferi* sensu stricto, *B. afzelii, B. garinii*, *B. bavariensis,* and *B. spielmanii* (5, 6, 9, 10, 11). These are subsumed under the term *B. burgdorferi* sensu lato (s.l.)

It is currently unclear to what extent animals (dog and horse) also suffer from LB after infection with *B. burgdorferi* s.l.. It is currently assumed that most infected animals (initially) develop no clinical changes. The owner of the animal generally only contacts the vet once symptoms (such as paralysis) have developed. Serological determination of IgG is then indicated (12,13).

Clinical characteristics in the dog:

The best indications of LB in the dog are impaired general condition with anorexia and fever, together with variable lameness from arthritis. Aside from these symptoms, lymphadenopathies have been observed in ca. 5% of cases and severe impairment of renal function in ca. 2% of cases (3).

Clinical characteristics in the horse:

In a German study, the symptoms of 50 horses were examined after infection with *B.burgdorferi*. The frequency of eye diseases (conjunctivitis, keratoconjunctivitis, retinitis) was striking. On the other hand, the general less specific symptoms, such as weight loss and impaired performance (24%), joint inflammation (12%) and lameness (10%) were the most frequent symptoms leading to the visit to the vet. Polyarthritis was often found and this can occur in almost all joints of the extremities (4).

3. Principle of Test

Antigenic pathogen proteins are coated on a nitrocellulose membrane using a special spraying procedure. The nitrocellulose membrane is then cut into single strips.

The antigen-coated nitrocellulose strips are then incubated with dog or horse serum samples, in order to detect specific antibodies. These antibodies develop immune complexes with the antigen fixed on the test strip. Unbound antibodies are removed by washing. The individual nitrocellulose strips are then incubated with alkaline phosphatase-conjugated anti-dog or anti-horse IgG conjugates. After unbound conjugated antibodies have been removed by a further washing step, a visualisation of the antigen/antibody-complex is accomplished by the addition of a non-coloured substrate, which forms blue-violet precipitates at each site 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-antibodies.

4. Package Contents

4.1 Kit for 32 determinations

1.	**Nitrocellulose test strips** with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	**1x**	32 strips
2.	**IgG Cut off Control,** dog serum, prediluted	**1x**	0,5ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**2x**	50 ml
4.	**Anti dog IgG Conjugate** (100x conc.) **Anti-dog**-(rabbit)-Alcalic Phosphatasis, with preservative	**1x**	0,7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**1x**	57 ml

6. **Evaluation record sheet for dog**
   for recording and archiving the results **1x** 1 pcs.

4.2 Horse Set

Also available on request (DE226K62)

IgG Cut-off control, horse serum, prediluted	**1x**	0.5 ml
Anti-horse IgG conjugate (100x konz.)		
Anti-horse, (rabbit)-alkaline phosphatase, with preservative	**1x**	0.7 ml
Evaluation record sheet for horse		
for recording and archiving the results	**1x**	1 pcs.

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 label of the kit box.

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 safely 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	Stability
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. Control sera, samples, diluted samples, conjugates and the nitrocellulose test strips should be regarded as being potentially infectious and treated accordingly. Please handle products in accordance with laboratory directions.
2. Use plastic forceps 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 (for order numbers please refer to the product catalogue)
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. Test Procedure

Precise adherence to the user manual is the prerequisite for obtaining correct results.

8.1 Preparation of the Samples

1. 15µl serum are required per sample.
2. Blood samples should be taken aseptically by venipuncture. After complete coagulation the serum should be separated. The samples may be stored at 2-8°C for one week. If they are to be stored longer sera have to be frozen at -20°C.
3. Repeated freezing and thawing should be avoided.
4. Do not use turbid samples (especially after thawing), centrifuge if necessary (5 minutes at 1000sg), pipette clear supernatant and use in testing.

8.2 Preparation of Reagents

1. 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.
2. Mix dilutions well before starting the test.
3. **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. The dilution-/wash buffer, concentrated or already diluted, may eventually show a yellow dye. This yellow dye has no influence to the shelf life of the dilution-/wash buffer nor does it influence the functionality or diagnostic meaning of the test run.
4. **IgG 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").
5. **Substrate Solution**
   The substrate solution is delivered ready-to-use.

8.3 Immunoblot Test Procedure

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

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. Pipette **15µl dog or horse serum** (gives a dilution of 1+100) or **100µl of the cut-off** control, if possible at the upper marked end of the strip. Incubate dog or horse 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. Pour away or aspirate liquid completely out of the channels.
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. Evaluation of the dog or horse samples is facilitated if the specific bands are labelled on the protocol sheet.

For test procedure scheme pls. refer to last page

8.4 Use of Immunoblot-processors

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

9. Interpretation of Results

To facilitate interpretation, each LINE strip is provided with a test function control (serum control):

1. **Serum control**

The test kit has a common serum control band for both dog and horse:
The serum incubation band appears under the marking line after incubation with serum.
The test performance is valid if the serum control is clearly recognisable on the developed nitrocellulose test strip. The position of the serum control band is taken from the protocol sheet.

9.1 Interpretation of the dog and horse samples

The position and designation of the reactive bands are taken from the protocol sheet.
IgG Bands: VlsE-Mix-Dog, OspA-Mix, DbpA-Mix, OspC-Mix, BmpA (p39), p58, p83, VlsE-Mix-Horse

9.2 Usage of the Cut-Off Control

Bands of intensity less than the cut-off band in the cut-off control are excluded from the interpretation.
IgG dog cut-off band: OspA-Mix
IgG horse cut-off band: VlsE-Mix

9.3 Meaning of the Antigens

List of the highly purified recombinant *B. burgdorferi* antigens in the test:

1. The **VlsE-Mix** consists of two recombinant antigens of the genospecies *B. burgdorferi* sensu stricto (B31) and *B. garinii* (IP90).
2. The **OspA-Mix** consists of three recombinant antigens of the genospecies *B. afzelii* (PKo), *B. garinii ZQ1* and *B. burgdorferi s.s.ZS7*.

3. The **OspC-Mix** consists of three recombinant antigens of the genospecies *B. afzelii* (PKo), *B. bavariensis* (PBi) and *B. burgdorferi* sensu stricto (ZS7).
4. The **DbpA-Mix** consists of two recombinant antigens of the genospecies *B. bavariensis* (PBi) and *B. garinii* (PBr) and highly purified *B. afzelii* (PKo).

Antigen/ Designation	**Significance of the antigens**	**Specificity of the Antibodies in the LINE**
VlsE-Mix recombinant	**V**ariable major protein **l**ike **s**equence **E**. VlsE is a lipoprotein expressed in vivo. It contains highly immunogenic epitopes which are conserved over different genospecies. VlsE is a 35 kDa antigen, coded on lp28-1. Biological significance: *B. burgdorferi* s.l. can persist in infected mammals, in spite of an active immune response. It is though that combinational antigen variation in the VlsE surface protein acts as an immune escape mechanism and contributes to this persistance. Marker for pathogen contact or wild infection with *B. burgdorferi* s. l.	Specific
OspA-Mix recombinant	**O**uter **s**urface **p**rotein **A** • OspA antibody titres are particularly found after vaccination.	Highly specific
DbpA-Mix Highly purified/ recombinant	**D**ecorin **b**inding **p**rotein **A** (also Outer surface protein 17 or p17). Plasmid-coded lipoprotein. The DbpAs from various isolates of the species *B. burgdorferi*, *B. afzelii*, *B. garinii, B. bavariensis* and *B. spielmanii* have been described as sensitive and specific antigens with complementary activity. • DbpA antibodies tend to be found to advanced or disseminated Lyme borreliosis infections.	Highly specific
OspC-Mix (p23) recombinant	**O**uter **s**urface **p**rotein **C**. Plasmid coded lipoprotein Surface protein • OspC are found in both wild infections and occasionally after vaccination.	Highly specific
BmpA (p39) Recombinant *B.afzelii* (PKo)	**B**orrelial **m**embrane **p**rotein **A**. Chromosomally coded, central marker in IgG serology for disseminated Lyme borreliosis infections	Highly specific
p58 recombinant *B.bavariensis* (PBi)	**O**ligopeptide **p**ermease **p**rotein **A**-2 (OppA-2). Chromosomally coded lipoprotein, conserved between species • p58 antibodies tend to be found to advanced or disseminated Lyme borreliosis infections.	Highly specific
p83 recombinant *B.afzelii* (PKo)	Chromosomally coded, antigen associated with the protolasmic cylinder, conserved within *B. burgdorferi* sensu lato. Central marker in the IgG serology of advanced Lyme borrelioses.	Highly specific

9.4 Interpretation Criteria

9.4.1 Interpretation for the dog:

Recommended IgG interpretation for the dog
Information generally for bands ≥ cut off band intensity. Exception: isolated VlsE

dog	Finding	Interpretation
0 Band or Band < cut off	negative	No evidence for contact with pathogen

VlsE-dog		Finding	Interpretation
isolated	= cut off	negative	No evidence for contact with pathogen
	> cut off	Infection	Infection indicated
+ ≥ 1 Band (except OspA)		Infection	Infection indicated

Without OspA and without VlsE-dog	Finding	Interpretation
0 - 1 Band	Negative	No evidence for contact with pathogen
2 - 3 Bands	Borderline	Evidence for contact with pathogen
≥ 4 Bands	Infection	Infection indicated

OspA		Finding	Interpretation
Isolated or + ≥ 1 Bands (exception VlsE)		Vaccination	Vaccination
+ VlsE-dog isolated	= cut off	Vaccination	Vaccination
	> cut off	Vaccination + Infection	Vaccination and evidence for infection
+ VlsE-dog + ≥ 1 Band		Vaccination + Infection	Vaccination and evidence for infection

Do not consider the VlsE-band horse for the IgG interpretation dog.

9.4.2 Interpretation for the horse:

Recommended IgG interpretation for the horse
Information generally for bands ≥ cut off band intensity.

horse	Finding	Interpretation
0 Band or Band < cut off	negative	No evidence for contact with pathogen

VlsE-horse	Finding	Interpretation
+ 0 - 2 Bands	Borderline	Evidence for contact with pathogen
+ ≥ 3 Bands	Infection	Evidence for infection

Special case	Finding	Interpretation
VlsE-horse + DbpA + 1 Band	Infection	Evidence for infection

Without VlsE-horse	Findings	Interpretation
0 - 2 Bands	Negative	No evidence for contact with pathogen
3 Bands	Borderline	Evidence for contact with pathogen
≥ 4 Bands	Infection	Evidence for infection

Do not cosider the VlsE-band dog for the IgG interpretation horse.
OspA should not be regarded as a specific band in equine immune reactions to *B. burgdorferi* s.l..

After a negative or borderline finding and if there is still clinical suspicion of Lyme borreliosis, a furtehr test should be performed after ca. 4-6 weeks. This applies to both dogs and horses.

9.5 Limits of the Test

1. When interpreting serological results, the clinical presentation and any other laboratory findings must **always** be considered.
2. A negative Blot result does not totally exclude the possibility of an infection with Borrelia, as the antibodies may still be under the limit of detection. If there is still clinical suspicion, a second blood sample should be taken after ca. 4-6 weeks.
3. IgG antibodies may be detectable even years after clinical remission.
4. It is known that there may be cross-reactions between *B. burgdorferi* s.l. and other spirochetes, particularly leptospires.

10. Performance Data

Origin of the sera:
217 defined dog sera and 149 defined horse sera were obtained Professor Reinhard Straubinger DVM from the Faculty of Veterinary Medicine, Department of Veterinary Science, Institute for Infectious Diseases and Zoonoses.
A comparison was performed with the two stage test of the Faculty of Veterinary Medicine, defined here as the standard test. The comparison was performed under the management of Professor Straubinger in the Institute for Infectious Diseases and Zoonoses in the Department of Veterinary Science. The results are as follows:

10.1 Dog

Test or Interpretation Procedure	Dog Sera (n=217)			
Interpretation with the **two-step** test Prof. Straubinger	49 negative	46 vaccination	94 infection 8 borderline	20 vaccination + infection
Borrelia Veterinary plus OspA LINE	49 negative	42 vaccination 3 vaccination + infection 1 negative	95 infection 2 borderline 3 vaccination + infection 2 negative	18 vaccination + infection 1 vaccination 1 infection

The negative sera were all correctly identified.
98% of the vaccinations were detected, with 3 additional cases of evidenec for infection. The only negative was identified as “weak vaccination” in the two stage test. There was a clear OspA band in the Line blot, although this was < cut off.
The number of borderline cases was clearly reduced in the infections. 3 additional vaccinations were detected.
18 cases of vaccination + infection were confirmed in this way. 1 case of pure vaccination and 1 case of pure infection were found.

10.2 Horse

Test or Interpretation Procedure	Horse Sera (n=149)		
Interpretation with the **two-step** test Prof. Straubinger	50 negative	41 borderline 9 borderline/negative	45 infection 4 infection/ antigen contact
Borrelia Veterinaray plus OspA LINE	50 negative	16 borderline 7 infection 27 negative	33 infection 11 borderline 5 negative

This table shows that more sera were classified as clearly negative with the Borrelia Veterinary plus OspA LINE test which had mostly previously been interpreted as positive / threshold.
This also tends to agree with the reality of diagnostic testing. According to Professor Straubinger’s laboratory in the Faculy of Veterinary Medicine, the available reports indicate that there tend to be many positive results with current test systems and interpretation criteria which must be seen as unreliable for conclusive diagnosis.

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12. Test Procedure Scheme

Test Procedure in short version

Samples Incubation	**30 minutes**	15 µl dog- / horse serum / 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 for dog and horse conjugate (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