VIROTECH Borrelia Vet. Hund/Dog-Pferd/Horse IgG ELISA
(Borrelia Vet. Hund/Dog-Pferd/Horse IgG ELISA)

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FOR IN VITRO DIAGNOSIS ONLY

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Content

1. Intended Use

The VIROTECH Borrelia burgdorferi (Species B. burgdorferi sensu stricto) Veterinary ELISA is an indirect ELISA for the semiquantitative and qualitative detection of specific IgG antibodies in dog or horse serum.

2. Diagnostic Relevance

General

The pathogen for borreliosis, the spirochaete Borrelia burgdorferi, was discovered in 1981 by Burgdorfer and Barbour and classified as a species of the genus Borrelia (1).

3 species of Borrelia burgdorferi sensu lato have now been identified: Borrelia burgdorferi sensu stricto, Borrelia garinii and Borrelia afzelii (2).

Epidemiology:

Borreliosis occurs endemically in all temperate zones (3). There are however differences in the relative frequency of the 3 pathogens in different regions. Thus, only B. burgdorferi sensu stricto has been observed in North America, but all three species have been found in Europe (2).

Transmission:

B. burgdorferi is predominantly transmitted by hard ticks of the genus Ixodes when they bite or suck blood.

Ixodes scapularis is the vector known in the USA; Ixodes ricinus is the main vector in Europe 4).

Clinical picture in the dog:

It is generally accepted that the most characteristic symptoms of Lyme borreliosis in the dog are impaired well-being, with anorexia and fever, with fluctuating lameness and arthritis. Lameness and arthritis are really pathognomic symptoms. Aside from these symptoms, lymphadenopathies are observed in about 5% of cases and impairment of renal function in about 2% of cases. The latter may be severe (5).

Clinical picture in the horse:

Horses are often bitten by ticks when they are grazing on the meadow. In western and central Europe, these are almost always of the species Ixodes ricinus, which sucks the bloody of horses in all three stages – the adult, the nymph and the larva (5).

In a German study (Hannover), the symptoms of the disease were determined in 50 horses. The high proportion of eye diseases - conjunctivitis, keratoconjunctivitis, retinitis – was striking; this is consistent with observations in the USA. On the other hand, the horses were most often taken to the vet because of general and less specific symptoms – such as loss of weight and energy (24%), inflamed joints (12%) and lameness (10%). Polyarthritis was often found and this can occur in almost any joint in the extremities (6).

3. Test Principle

The test antibodies in the animal serum form an immune complex with the antigen fixed on the microtitre plate. The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtitre-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents

1. **1 Microtitre-Plate [MTP]** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer, (blue, ready to use), 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution, 50ml**, (20x concentrated), pH 7,2, with preservative and Tween 20
4. **IgG negative control dog [NEG IgG Dog], 1300µl,** dog serum with protein stabilizers and preservative, ready-to-use
5. **IgG cut-off control dog [CO IgG Dog], 1300µl,** dog serum with protein stabilizers and preservative, ready-to-use
6. **IgG positive control dog [POS IgG Dog], 1300µl**, dog serum with protein stabilizers and preservative, ready-to-use
7. **Anti-Dog-IgG conjugate, 11ml,** rabbit horseradish peroxidase conjugate, contains preservative, ready-to-use
8. **IgG negative control horse [NEG IgG Horse], 1300µl,** horse serum with protein stabilizers and preservative, ready-to-use
9. **IgG cut-off control horse [CO IgG Horse], 1300µl,** horse serum with protein stabilizers and preservative, ready-to-use
10. **IgG positive control horse [POS IgG Horse], 1300µl,** horse serum with protein stabilizers and preservative, ready-to-use
11. **Anti-Horse-IgG conjugat,e 11ml,** goat horseradish peroxidase conjugate, contains preservative, ready-to-use
12. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all compoments is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Samples, diluted samples, controls, conjugate and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If involved body parts are contacted, immediately wash under flowing water and possibly look up a doctor.
3. The disposal of the used materials is effected referring to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

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

8.1 Examination Material

Sera are used as examination material.
Always prepare samples-dilution freshly.
For a longer storage the sera must be frozen. Repeated defrosting shall be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The ready-to-use controls (positive control, cut-off control, negative control) are parameter- and species-specific and are to be used exclusively with the plate batch in the kit.
The ready-to-use conjugates are also parameter- and species-specific, but may be used for different plate batches.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.

8.3 VIROTECH ELISA Test Procedure

1. For each test batch, pipette 100µl each of the ready-to-use dilution buffer (blank), the negative, positive and cut-off IgG controls, together with the diluted animal sera. We recommend that adjacent duplicates should be performed for the blank, controls and sera samples; it is absolutely essential that the cut-off control should be performed in duplicate. Working dilution of the animal sera: 1:400; e.g. predilution: Mix 10µl serum + 90µl dilution buffer (1:10); Second dilution: Take 10µl of this mixture and pipette 390µl dilution buffer into it (1:40). This corresponds to a dilution of 1:400.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of the ready-to-use animal-specific conjugate into all wells.

5. Incubation of the conjugate: 30 min. at 37°C (with cover)
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping the substrate reaction: Pipette 50µl citrate stop solution into each cavity. Carefully and thoroughly shake the plate until the fluids are well mixed and the colour in each well is homogenous.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Schemata

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

This procedure ensures the accuracy of your ELISA processor and serves additionally for the Quality Assurance of your laboratory.

9. Test Evaluation

The ready-to-use controls serve as quantification controls for the semiquantitative determination of specific igG antibodies; the concentration can be given in VIROTECH Units = VE. The method can correct for fluctuations in the test procedure, leading to high reproducibility. The mean OD value is used to calculate the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The mean is calculated from the duplicate OD values.

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$VE_{\text{(positive control)}} = \frac{OD_{\text{(positive control)}}}{OD_{\text{(cut - off control)}}} \times 10$$

$$VE_{\text{(animalserum)}} = \frac{OD_{\text{(animalserum)}}}{OD_{\text{(cut - off control)}}} \times 10$$

9.3 Interpretation Scheme IgG

Result (VE)	Evaluation
< 8,0	negative
8,0 - 12,0	borderline
> 12,0	positive

1. If the VE value measured for the sample is greater than 12 VE, the sample is considered to be positive.
2. If the VE value measured lies within the borderline range, there is no significant increase in antibody concentration and the samples are regarded as threshold.
   If an infection is to be reliably detected, the antibody concentration must be determined in two serum samples: a serum sample directly

after the start of the infection and a second sample two weeks later. The antibody concentrations in the two samples must be measured in parallel, in a single test batch. A correct diagnosis cannot be made on the basis of a single serum sample.

3. If the measured values are under the defined borderline range, there are no measurable antigen-specific IgG antibodies in the sample. The sample is classified as negative.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.

10. Performance Data

10.1 Sensitivity and Specificity

IgG Detection in the Dog

In order to determine the performance of the Borrelia burgdorferi Veterinary ELISA, 351 sera were tested in the VIROTECH Diagnostics ELISA and in the reference tests (ELISA and Western blot). The tests were performed in the laboratories of VIROTECH Diagnostics GmbH, Rüsselsheim, and of Maastricht University.
35 sera were positive in both tests and 278 sera negative in both tests. For 6 sera, the results of the two tests did not agree. Threshold results (32) were excluded from the calculation.

The result is shown in the following table.

		Reference Test	
		+	-
VIROTECH	+	35	6
	-	0	278

Specificity = 98%
Sensitivity = 100%

IgG Detection in the Horse

In order to determine the performance of the Borrelia burgdorferi Veterinary ELISAs, 119 sera were tested in the VIROTECH Diagnostics ELISA and in the reference test (Western Blot). The tests were performed in the laboratories of VIROTECH Diagnostics GmbH, Rüsselsheim.
3 sera were positive in both tests, 69 sera negative in both tests and 13 sera gave threshold values in both tests. For 3 sera, the results of the two tests did not agree. Threshold results (34) were excluded from the calculation.

The result is shown in the following table.

		Reference Test	
		+	-
VIROTECH	+	3	3
	-	0	69

Specificity = 96%
Sensitivity* = 100%

*This is calculated as 100% sensitivity, However, this value is statistically unreliable, as only 3 positive Borrelia IgG sera were available for testing.

10.2 Cross-Reactivity

There has been much discussion about cross-reactions between B. burgdorferi and other spirochaetes, particularly Leptospira (5).
In a study, a number of Leptospira-positive dog and horse sera were tested on the Borrelia burgdorferi Veterinary ELISA.

Borrelia burgdorferi Veterinary ELISA			
Collective	**Negative**	**Equivocal**	**Positive**
Lepospira-positive dog sera (n=50)	46	2	2
Leptospira-positive horse sera (n=42)	27	6	9

11. Literature

1. Burgdorfer, W., Barbour, A.G., Hayes S.F. et al. (1982); Lyme disease – a tick-borne spirochetosis?; Science 216:1317-19.
2. Dressler, F., Ackermann, R. and Steere, A.C. (1994); Antibody responses to the three genomic groups of Borrelia burgdorferi in European Lyme Borreliosis; J. Infect. Dis. 169:313-318.
3. Sigal, L.H. and Curran, A.S.; Lyme disease: a multifocal worldwide epidemic; Annu. Rev. Publ. Health (1991); 12:85-109.
4. Barbour, A.G. and Hayes, S.F. (1986); Biology of Borrelia species; Microbiol. Rev. 50(4):381-400.

5. Horst, H.; Einheimische Zeckenborreliose (Lyme-Krankheit) bei Mensch und Tier; 3. überarbeitete Auflage; Demeter-Verlag im Spitta Verlag; 1997: 173-174, 176-178.
6. Liebisch, G.; Der Nachweis von Borrelien bei Haus und Wildtieren: Patienten oder Reservoir der Lyme-Borreliose?; 22. Kongress der Deutschen Veterinärmedizinischen Gesellschaft; 8.-11. April 1997; Bad Nauheim.

Preparation of Dog- /Horse Serum and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ **Samples Dilution**
1:400

z.B.:
Mix 10 µl serum + 90µl dilution buffer (serum dilution buffer is ready-to-use)
Mix 10µl of this mixture with 390µl dilution buffer.

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Dog- /Horse Serum** blank value (Dilution Buffer) and controls
⇩		
Wash 4times		**350 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG
⇩		
Wash 4times		**350 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrat**e
⇩		
Stopping		**50 µl Stopping Solution** shake carefully
⇩		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**