

**Evaluation of the „Zeckentest Borreliose“
- a Lateral Flow Test Strip for the
detection of *Borrelia burgdorferii***

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By

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1. Objective & Goal

To evaluate independently the lateral flow test strip for the detection of *Borrelia burgdorferii* regarding sensitivity and specificity

2. Methods

First, the „Zeckentest Borreliosis“ were examined regarding his analytical sensitivity.

The test runs were carried out as described in the instruction manual. The specificity was tested, using the enclosed buffer solution.

For the positive controls and the determination of the analytical cut off, different dilutions of *Borrelia burgdorferii* B31 lysate were prepared.

For the determination of the functional sensitivity and specificity, 50 ticks were collected by the cloth-dragging method and frozen until they were examined in the laboratory.

The test runs were carried out as described in the instruction manual.

The confirmations of the test results were carried out by polymerase chain reaction (PCR) with a following restriction fragment length polymorphism.

3. Background

Borrelia is a genus of bacteria of the spirochete phylum. It causes borreliosis, a zoonotic, vector-borne disease transmitted primarily by ticks and some by lice, depending on the species. There are 36 known species of *Borrelia*.

Of the 36 known species of *Borrelia*, 12 of these species are known to cause Lyme disease or borreliosis and are transmitted by ticks. The major *Borrelia* species causing **Lyme disease** are *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia garinii* and *Borrelia valaisiana*.

Lyme disease is the most common tick-borne disease in the Northern Hemisphere. *Borrelia* is transmitted to humans by the bite of infected ticks belonging to certain species of the genus *Ixodes* (the hard-bodied 'hard ticks').^[2] Early manifestations of infection may include fever, headache, fatigue, depression, and a characteristic skin rash called erythema migrans. Left untreated, late manifestations involving the joints, heart, and nervous system can occur. In most cases, the infection and its symptoms are eliminated with antibiotics, especially if diagnosis and treatment occur early in the course of illness. Late, delayed, or inadequate treatment can lead to late manifestations of Lyme disease which can be disabling and difficult to treat.^[3]

4. Test principle

The TICKTEST is an immunologic rapid test for the fast and qualitative detection of Borreliosis pathogens (*Borrelia (B.) garinii*, *Borrelia afzelii* und *Borrelia burgdorferi sensu stricto*) **directly in the tick.**

The new TICKTEST enables you and the doctor a reliable assistance for a faster diagnosis and an immediate treatment, if necessary.

Within **10 minutes** you can read out the result.

The TICKTEST is an immunologic „sandwich assay“ for the detection of *Borrelia burgdorferi sensu lato*. Anti-Borrelia antibodies are fixed on the testline and canine anti-chicken antibodies are fixed on the control line. Next to the membrane is located a coloured fleece, which is impregnated with a mouse anti-Borrelia-antibody-goldconjugate.

By using the extraction reagents and the pipette, the Borrelia-antigen from the tick is dropped on the test cassette during the test procedure. Now, the Borrelia-antigen reacts with the coloured goldconjugate-particles to an aggregate.

During the test performance the samples is running over the membrane, which is caused by capillary power, and binds onto the testline.

If there is Borrelia-antigen present in the sample, a red line in the „T“ area is formed . If there is no Borrelia-antigen present in the sample, you will see no red testline.

5. Results

Analytical sensitivity

Detection of the analytical sensitivity was obtained by analysis of the standards which were prepared by diluting a stock solution of Borrelia burgdorferii antigen with a proper buffer solution to 5 concentration; 50, 100, 200, 1000, and 5,000 ng/ml. 200 ng/ml of the the lysate solution correspond to 300- 500 Borrelia/tick. 200 µl of standard solution was pipetted into the test cassette and the solution flow to the top of nitrocellulose membrane. The reaction between the conjugate and target analyte took place immediately and carried through the next membrane with the immobilized binder. The target analyte and the immobilized anti- Borrelia burgdorferii complete their binding with gold antibody probe. As soon as all solution reached to the top of the IC strip test, usually within 5 minutes, the control line was clear visible. The test line was read out after 10 minutes.

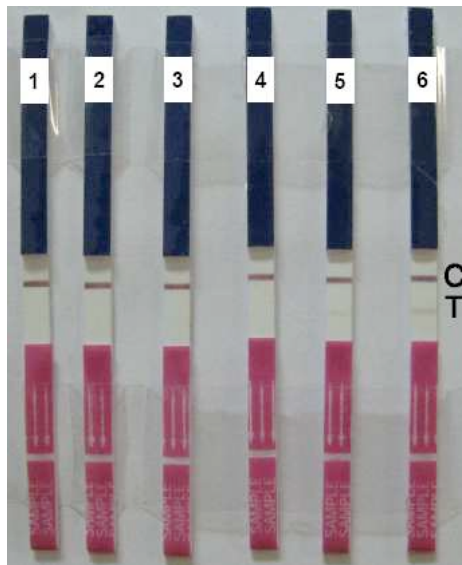


Figure 4: Immunochromatographic test strip results at *Borrelia burgdorferi* concentration of 0, 50, 100, 200, 1000, and 5000 ng/ml. C = control line; T= test line

Functional sensitivity

The reference method were carried out by PCR-RFLP detection. For DNA purification the residual test solution of the "Zeckentest Borreliose" were used.

DNA purification. The DNA of the samples was isolated using a DNA isolation kit (Quiagen). This procedure is based on cell lyses by sarkosyl and chaotropic ions and subsequent binding of DNA to silica particles. The DNA was eluted from silica particles in 20 μ l of TE buffer (10mM Tris-Cl, 1mM EDTA). Volume of 5 μ l of this preparation was used for amplification.

PCR assay. The PCR assay is based on the specific flagellin sequence amplification for detection of *B. burgdorferi* sensu lato was performed (Picken et al. 1996). The 50 μ l PCR mixture contained: 1x HotStarTaq Master Mix (Qiagen, Germany), 15 pmol of each FL3 primer (5'-MGA GCT TCT GAT GAT GCT GCT GGY ATG GGR G-3') and FL5 primer (5'-GRG GAA CTT GAT TAG CYT GYG CAA TCA TTG CC-3'), 100 μ M of dUTP (Sigma), and 5 μ l of template DNA received after standard DNA isolation. All PCR runs were performed on a thermocycler (Perkin Elmer) with the following profile: an initial activation

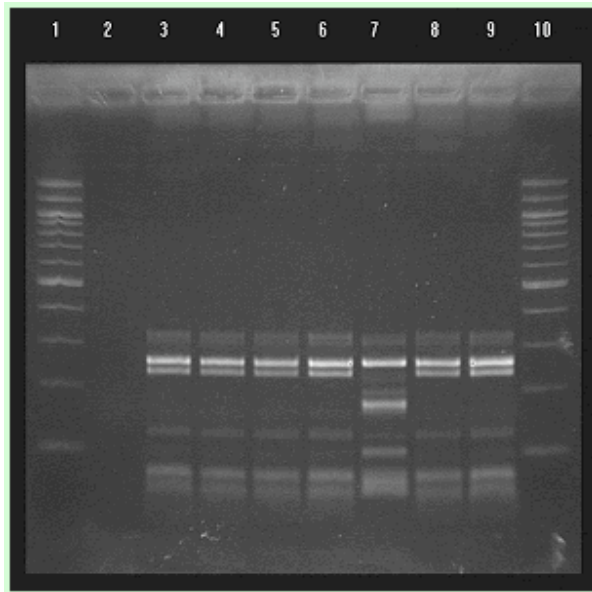
step at 96°C for 12 min, thirty cycles consisting of a denaturation step for 10 sec at 96°C, an annealing step for 10 sec at 65°C, an extension step for 40 sec at 72°C and the final extension at 72°C for 4 min.



PCR analysis results

Restriction fragment length polymorphism (RFLP). The sequences coding flagellin gene (*fla*) of *Borrelia burgdorferi sensu stricto*, *Borrelia afzelii* and *Borrelia garinii* were gathered from GeneBank database (NCBI). The multiple alignments were by the CLUSTALW program (Thomson, Higgins and Gibson 1994) done and the restriction fragment length polymorphism (RFLP) patterns, obtained after *AluI* endonuclease digestion, were predicted using the WEBCUTTER 2.0 program. These predicted RFLP patterns were proved on reference *Borrelia burgdorferi sensu lato* strains achieved from German Collection of Microorganism and Cell Cultures (DSMZ). For identification of *Borrelia* strains only fragments longer than 100 bp were taken into account.

Restriction analysis of amplified PCR products was performed by *AluI* endonuclease digestion (New England BioLabs). The restriction DNA fragments were analysed by agarose gel electrophoresis through 2% agarose gel, visualized by ethidium bromide staining, detected using UV transillumination (312 nm), and analysed by ULTRA LUM gel detection and analysis system.



PCR-RFLP analysis results

Summary of the results

	PCR/ RFLP		
		±	=
Zeckentest Borreliose	±	15	1
	=	1	33

Sensitivity: 94% (15/16)

Spezifität: 97% (33/34)

Accuracy: 96%

Positive predictive value: 94% (15/16)

Negative predicitive value: 97% (33/ 34)

6. Summary of the evaluation

The determination of the analytical and functional sensitivity and specificity, the performance and the handling of the lateral flow test was evaluated.

This evaluation showed, that the "Zeckentest Borreliose" is a very good alternative method for detection of *Borrelia burgdorferi*. The accuracy of the lateral flow assay is 96 % in comparison to PCR. The analytical detection limit of 300-500 *Borrelia*/tick was confirmed in the laboratory.

The "Zeckentest Borreliose" is an "easy to handle" test device, which can be used everytime. The test result is read out after 10 minutes.

7. References

1. Ryan KJ, Ray CG (editors) (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. pp. 434–437. [ISBN 0838585299](#).
2. Johnson RC (1996). "[Borrelia](#)". *Baron's Medical Microbiology* (Baron S et al, eds.) (4th ed.). Univ of Texas Medical Branch. [ISBN 0-9631172-1-1](#). <http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=mmed.section.1965>.
3. Cairns V, Godwin J (2005). "[Post-Lyme borreliosis syndrome: a meta-analysis of reported symptoms](#)". *Int J Epidemiol* **34** (6): 1340–1345. [doi:10.1093/ije/dyi129](#). [PMID 16040645](#). <http://ije.oxfordjournals.org/cgi/content/full/34/6/1340>.
4. BARANTON, G.; POSTIC, D.; SAINT-GIRONS, I. et al - Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp nov., and group VS461 associated with Lyme borreliosis. **Int. J. system. Bact., 42:** 378-383, 1992.
5. CRAFT, J.E.; GRODZICKI, R.L. & STEERE, A.C. - Antibody response in Lyme disease: evaluation of diagnostic tests. **J. infect. Dis., 149:** 789-795, 1984.
6. GRODZICK, R.L. & STEERE, A.C. - Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen

preparations for diagnosing early Lyme disease. **J. infect. Dis.**, **157**: 790-797, 1988.

7. MAGNARELLI, L.A.; ANDERSON, J.F.; JOHNSON, R.C.; NADELMAN, R.B. & WORMSER, G.P. - Comparison of different strains of *Borrelia burgdorferi* sensu lato used as antigens in enzyme-linked immunosorbent assays. **J. clin. Microbiol.**, **32**: 1154-1158, 1994.
8. STEERE, A.C. - Lyme disease. **New Engl. J. Med.**, **321**: 586-596, 1989.
9. Canica, MM, Nato, F, du Merle, L, Mazie, JC, Baranton, G, Postic, D. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme Borreliosis. *Scand J Infect Dis* 1993; 25:441-448.
10. Gern L. *Borrelia burgdorferi* sensu lato in Europe and North America: two different ecological situations. In: International conference on Lyme borreliosis, Bruxelles: Rily group, Ottignies, Belgium, 1-5, 2001.
11. Picken MM, Picken RN, Han D, Cheng, Y, Strle F. Single-Tube Nested Polymerase Chain Reaction Assay Based on Flagellin Gene Sequences for Detection of *Borrelia burgdorferi* sensu lato. *Eur J Clin Microbiol Infect Dis* 1996; 15:489-498.
12. Postic D, Assous MV, Grimont PAD, Baranton G. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of rrf (5S) rrl (23S) intergenic spacer amplicons. *Int J Syst Bacteriol* 1994; 44:743-752.
13. Wilske B, Preac-Mursic V, Göbel UB, Graf B, Jauris-Heipke S, Soutschek E, Schwab E, Zumstein G. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and OspA sequence analysis. *J Clin Microbiol* 1993; 31:340-350.