
Evaluation of the Detection of *Borrelia burgdorferi* DNA in Urine Samples by Polymerase Chain Reaction

**Summary:** It is difficult in some cases to identify an infection caused by *Borrelia burgdorferi* and to monitor the effect of therapy. Seropositivity will persist even after successful treatment and therefore may suggest ongoing infection. For direct detection of *B. burgdorferi* DNA in human urine samples, the polymerase chain reaction (PCR) was evaluated. A published primer system was selected, which amplifies a 259 bp fragment from the gene encoding the 23S rRNA. The lower detection limit of the primer system was 10 fg of extracted *B. burgdorferi* DNA. Several methods for the pretreatment of urine samples were tested. Of these, the Gene-clean® kit (Bio 101, USA) showed the best results. A total of 114 urine samples from 74 patients belonging to three clinical groups was investigated: (i) 51 samples from 26 patients with active Lyme disease, (ii) 36 samples from 27 patients with previous infection but no symptoms at the time the urine was collected, and (iii) 27 samples from 21 seronegative control patients without Lyme disease. *B. burgdorferi* DNA was detected in 25 urine samples of 17 patients with active disease, whereas 26 samples from this group of patients were negative. Only one asymptomatic case with previous infection showed a positive result, and the urine samples of the patients without Lyme disease were uniformly negative. Two of four patients from whom samples before and directly after onset of therapy were available converted from negative to positive PCR results after initiation of therapy, accompanied by the symptoms of a Jarisch-Herxheimer reaction. It can be concluded from these results that a positive PCR from urine is with high probability an indicator of active Lyme disease. On the other hand, as only 17 of the 26 patients with active infection were positive, a negative PCR result does not exclude active infection.

**Introduction**

Since the discovery of *Borrelia burgdorferi* as the etiologic agent of Lyme disease [1], Lyme borreliosis has attracted increasing attention both in Europe and in the United States. Clinical symptoms of Lyme disease are variable, but generally, early and late manifestations can be differentiated. By analogy with syphilis, the division into three stages has been widely accepted [2]. Symptoms of stage one are the characteristic erythema migrans as well as nonspecific systemic complaints, the second stage is characterised by neurologic and cardiac sequelae, and stage three includes chronic skin, neurological and arthritic symptoms. Most important for diagnosis are clinical history and examination. Because microscopic or cultural detection of *Borrelia burgdorferi* are difficult and unreliable, serology is the most commonly applied tool of laboratory diagnosis. However, serology for *B. burgdorferi* has some inherent problems, such as the delayed rise of antibody titres in early stages of Lyme disease, and in some instances, the occurrence of false positive results due to the presence of cross-reacting epitopes with other bacteria [2,3].

A clear limitation of serology for *B. burgdorferi* is the distinction between current and previous infection. This issue becomes critical when the effect of therapy must be monitored, and when Lyme disease must be distinguished from illnesses with similar symptoms, especially in endemic regions, where the prevalence of positive antibody titres is high. After successful therapy, it takes at least several months before a significant decrease of antibody titres can be observed. Non-specific symptoms, such as chronic fatigue, may persist in some patients despite effective therapy. IgM antibodies, which are often a valuable aid in other infectious diseases, are not helpful for the monitoring of therapy in Lyme borreliosis, because only a fraction of the patients with active disease exhibit positive IgM titres, especially in the late stage of Lyme borreliosis [4].

The culture of *B. burgdorferi* is generally considered to be difficult and has a low yield from samples from infected patients [2]. Therefore, it is desirable to have a method for the direct detection of the pathogen in patient specimens that is more sensitive than culture. The polymerase chain reaction (PCR) has been proposed as such a method. It is very sensitive, detecting as few as 5–10 spirochetes in one reaction, and it has been applied to ticks [5], biopsies from rodents [6], as well as cerebrospinal fluid [7,8], blood samples [9], urine samples [10,11], and skin biopsies [12,13] from patients. However, the practical value of the PCR as...
Materials and Methods

Culture of B. burgdorferi and extraction of DNA: B. burgdorferi strain B31 (ATCC 35210) and strain TN [15] were routinely cultured in BSK II medium [16]. Genomic DNA from the bacteria was isolated according to standard procedures [17]. Briefly, cells were lysed by treatment with proteinase K (100 μg/ml) and sodium dodecyl sulphate (SDS, 1%), DNA was extracted with phenol/chloroform, then precipitated with isopropanol, and contaminating RNA was digested with 50 μg/ml of DNase free RNase. The optical density was measured at 260/280 nm to determine the various prepared of urine samples:

Preparation of urine samples: Three different methods were evaluated for the preparation of urine samples. These included a centrifugation method [19], the purification of DNA with SpinBind™ tubes (FMC, Rockland, ME, USA), and the GeneClean II® kit (Bio 101, La Jolla, CA, USA). Each method was performed according to the specifications of the authors or manufacturers. Briefly, for preparation of urine samples by centrifugation, 1,600 μl of urine was added to 400 μl of EDTA-formaldehyde solution (0.1 M EDTA, pH 8, 0.5% formaldehyde) in a 2ml-Eppendorf-tube, centrifuged at 18,000 xg for 20 min, and the pellet was washed once with EDTA solution (1 mM) and once with distilled water. The final pellet was resuspended in 20 μl of water and subjected to PCR amplification. For sample preparation with SpinBind™ tubes, 150 μl of urine was added to 300 μl of binding solution, applied to the binding matrix, centrifuged and washed three times with 400 μl of ethanol wash buffer, and eluted two times with 20 μl of water. For sample preparation with the GeneClean II® kit, 350 μl of urine was added to a mixture of 1,050 μl of NaI stock solution and 1.5 μl of glass milk. The suspension was vortexed and left to stand for 15 min at room temperature with intermittent vortexing every 2 min. The suspension was centrifuged briefly for 30 sec, and the pellet was washed three times with 500 μl of New Wash buffer. The DNA was eluted from the glass milk with 20 μl of water at 55°C for 5 min. The preparation of a negative control urine sample from a healthy volunteer was included in every series of PCR experiments. Sample preparation and the preparation of PCR reaction mixes were performed in a strictly separated area from that for amplification and detection.

PCR amplification and detection: For amplification of B. burgdorferi DNA, the primer and probe system of Schwartz et al. [13] was used. A fragment of 259 bp from the gene coding for 23S rRNA was amplified by the primers JS1 (5'-AGAAGTGCCTGAGTCGA) and JS2 (5'-TAGTGCCTACCTCTATTAA). The resulting PCR product was detected with the probe FSI (5'-AGTCTGTTAAGGGA). Oligonucleotides were synthesized using an Applied Biosystems oligonucleotide synthesizer and purified by HPLC. The PCR amplification was performed in a volume of 100 μl containing 50 mM KCl, 50 mM Tris (pH 9.0), 2.0 mM MgCl₂, 200 μM of each deoxynucleoside triphosphate, 50 pmol of each primer, and 2.5 units of Taq polymerase (AmpliTaq® DNA polymerase, Perkin Elmer). Ten μl of sample was added, the solution was covered with 70 μl of mineral oil, and PCR was performed in an automated thermal cycler using the following protocol: initial denaturation at 95°C for 3 min followed by 45 cycles of denaturation at 95°C for 45 sec, annealing and extension at 50°C for 105 sec, and final extension at 72°C for 2 min. For the detection of amplified DNA fragments, 30 μl was electrophoresed on vertical 8% polyacrylamide gels, transferred to nylon membranes, hybridized and exposed exactly as described previously [20], except that hybridization and washings were performed at 47°C.

Selection of patients and urine samples: A total of 114 urine specimens were collected from 74 patients attending a general practice in Kraichtal, Northern Baden, Germany. Kraichtal is a region in which Lyme borreliosis has a high prevalence. The rate of seropositivity is 16.9% in the general population [21]. Samples were collected and stored frozen at −20°C until tested. Fifty-one samples originated from 26 patients having active Lyme disease at the time the urine was collected (group A). Thirty-six urine samples were from 27 patients with previous B. burgdorferi infection but successful treatment according to clinical criteria and no symptoms indicating active disease at the time of urine collection (group B). Twenty-seven samples were from 21 serologically negative patients with other diseases than Lyme borreliosis (group C). Among these were patients with rheumatoid arthritis, gout, arthrosis, and chronic bronchitis.

Lyme disease in groups A and B was diagnosed by a combination of clinical and serological criteria. Clinical criteria leading to the diagnosis were the presence of at least one major symptom of Lyme borreliosis, such as arthritis, neuritis, or carditis [22], combined with a history of preceding erythema migrans (EM) and/or tick bite. Conventional serology for B. burgdorferi using an immunofluorescence assay (IFA) for IgG (borderline titre: 256) and an IFA for IgM (borderline titre: 32) was positive in all patients. Furthermore, all patients of groups A and B showed typical band patterns on Western immunoblots. Criteria for the positivity of Western immunoblots were either a strong immune response against at least the 41 kDa antigen of B. burgdorferi, when the patient had a typical history of tick bite and/or EM and symptoms compatible with early Lyme borreliosis, or the presence of an immune response against B. burgdorferi specific antigens on the blobs [23], such as the 100 kDa-range antigen, the outer surface antigens A (31 kDa) and B (34 kDa), or the 21 kDa antigen (pC). Markers for disease activity were the presence of arthritis, neuritis, or carditis, in combination with general symptoms, such as sweating, fatigue, palpitations, arthralgias, or myalgias.

Of the 26 patients with active disease (group A), one had erythema migrans (stage one), five patients had stage two borreliosis, and 20 suffered from late stage (stage three) disease. Of these 26 patients, one had a cutaneous manifestation only (EM), three had cardiac symptoms (one acute carditis with second degree atrioventricular block and heart failure, one acute carditis with first degree atrioventricular block and intermittent atrial tachycardia, and one chronic cardiomyopathy with first degree atrioventricular block and subsequent response to antibiotic ther-