JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1997, p. 685–690 Vol. 35, No. 3
0095-1137/97/$04.00+0

An Optimized PCR Leads to Rapid and Highly Sensitive Detection of *Borrelia burgdorferi* in Patients with Lyme Borreliosis

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Received 28 August 1996/Returned for modification 8 October 1996/Accepted 12 December 1996

The present study aimed at developing an optimized PCR protocol for the sensitive and specific detection of all three *Borrelia burgdorferi* genospecies pathogenic to humans in Lyme borreliosis patients. A rapid DNA extraction method using alkaline lysis was introduced and was found to be superior to other DNA extraction methods. Nested PCR was performed with primer sets targeting the plasmid-located *ospA* gene and a chromosomal gene segment encoding a 66-kDa protein (p66). In spiked synovial fluid (SF) fewer than three borreliae/sample were detected. The specificities of the amplicons were confirmed by Southern blot analysis with PCR-derived probes. Urine, cerebrospinal fluid (CSF), and SF specimens from 57 patients with Lyme borreliosis and from 58 controls were examined. In clinical samples the diagnostic sensitivity of PCR was 85% with SF samples, 79% with urine samples, and 91% with paired SF-urine samples from patients with Lyme arthritis and was 79% with CSF samples, 45% with urine samples, and 87% with paired CSF-urine specimens from neuroborreliosis patients. One patient each with neuroborreliosis and with Lyme arthritis had PCR-positive urine samples only. In 17% of all cases both primer sets yielded positive results, while the other patients were positive with only one primer set. Among these, more positive results were obtained with the p66 gene primer than with the *ospA* primer. The specificity exceeded 99%. We conclude that DNA from *B. burgdorferi* sensu lato species can sensitively and specifically be detected with the optimized PCR method described. At least two different primer sets should be used, and whenever possible, urine and CSF or SF should be analyzed in parallel to achieve maximum sensitivity of the test. This protocol, therefore, considerably enhances the diagnostic power of PCR in patients with *B. burgdorferi* infection.

Lyme borreliosis is the most prevalent tick-borne disease of the northern hemisphere, with many areas in Europe and the United States having high levels of endemicity. It is a multisystem spirochetosis with dermatologic, neurologic, and rheumatologic manifestations (2, 4, 13, 31). The causative agent of Lyme disease, *Borrelia burgdorferi* sensu lato, is heterogenous. Three different genospecies, *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* have been identified as pathogenic in humans. While in the United States only *B. burgdorferi* sensu stricto has been discovered, in Europe all three genospecies have been isolated from ticks and patients (3, 5, 20, 35, 39). There is evidence that these species possess different organotropisms and therefore may preferentially cause distinct manifestations of Lyme borreliosis. This may to some extent explain the variations in clinical manifestations of Lyme disease in European and American patients (1, 33, 37).

Most skin manifestations of Lyme disease can be recognized clinically. In contrast, patients with neuroborreliosis or Lyme arthritis often present with unspecific symptoms that may vary in severity and often mimic those of other diseases (2, 13, 15, 25, 31). In our experience based on Central European patients, only about one-third of individuals remember having had a tick bite or a preceding erythema migrans, the pathognomonic early skin manifestation of Lyme borreliosis. In these patients, it is often difficult to definitely diagnose Lyme disease on clinical grounds only.

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Laboratory tests, including bacterial culture and serologic methods for the detection of antiborrelial antibodies, are of limited value to support the diagnosis of Lyme disease since they lack both sensitivity and specificity (13, 16, 32). Recently, PCR has increasingly been used in the diagnosis of Lyme disease by direct detection of *B. burgdorferi* DNA in various specimens. However, published protocols appear to be laborious and time consuming, especially concerning the isolation of DNA. Most of the studies have been limited to single clinical manifestations, i.e., skin manifestations, Lyme arthritis, or neuroborreliosis, or to the detection of *B. burgdorferi* sensu stricto in American patients (8–11, 14, 17, 18, 21–24, 30, 34). Standardized and optimal conditions taking into account the heterogeneity among species and strains have not been defined yet. An optimized PCR protocol should be easy to perform and, to be applicable to all patients with Lyme disease, should be able to detect all species and strains with equal sensitivity.

The objective of the present study was to establish such a PCR protocol by evaluating different methods, including the newly introduced DNA extraction by alkaline lysis. Different primer sets, including the combination of primers specific for the plasmid-located outer surface protein A (*ospA*) gene (21) and a chromosomal gene segment encoding a 66-kDa protein (p66) (26, 28), were also compared. We feel that the protocol described herein considerably enhances the diagnostic potential of PCR for patients with *B. burgdorferi* sensu lato infections.

MATERIALS AND METHODS

Patients and control subjects. Fifty-seven patients with different manifestations of Lyme borreliosis were examined. Thirty-five patients suffered from Lyme arthritis and 22 patients had neuroborreliosis. A third patient group consisted of

TABLE 1. Demographic characteristics of patients and control subjects

Pathology of patients or controls	*n*	Sex (male/female)	Mean age (yr) (range)	No. of patients or controls with:	
				Positive Lyme serology (IgG/IgM)[a]	Previous antibiotic therapy
Patients with Lyme arthritis	35	20/15	44 (7–82)	35/2	7
Patients with neuroborreliosis	22	11/11	39 (20–58)	22/0	6
Patients with symptoms suggestive of Lyme borreliosis	11	4/7	45 (28–62)	11/0	4
Controls[b]	58	22/40	50 (25–75)	0/0	

[a] IgG, immunoglobulin G; IgM, immunoglobulin M.
[b] Pathology of controls: rheumatoid arthritis, 10; collagen vascular diseases, 16; HLA-B27-associated arthritis, 7; osteoarthritis, 4; inflammatory CNS diseases, 14; noninflammatory neurologic diseases, 7.

11 patients with histories and serologic results suggestive of Lyme disease who presented with unspecific symptoms including headache, subtle neurologic impairment, myalgias, or constitutional symptoms. All patients were from areas of high-level endemicity in Germany and remembered having had a tick bite and/or an erythema migrans. Lyme serology was performed by using full-antigen enzyme-linked immunosorbent assay (ELISA) and Western blotting (DPC Biermann, Bad Nauheim, Germany). Only patients who were seropositive by both ELISA and immunoblotting, thus fullfilling the criteria proposed by Engstrom et al. (6), were enrolled in the study. Neuroborreliosis was diagnosed in patients who had a clinical picture indicating Lyme borreliosis like Bannwarth's syndrome and a cerebrospinal fluid (CSF) pleocytosis or elevated CSF protein levels or when an autochthonous intrathecal production of specific antiborrelial antibodies could be demonstrated (17, 25). Lyme arthritis was diagnosed in patients with oligoarthritis and in whom other rheumatic diseases, including reactive arthritis, seronegative spondylarthropathy, and rheumatoid arthritis had been excluded (23, 25). Seventeen patients had been treated with oral antibiotics prior to this investigation.

Lumbar punctures and arthrocenteses were performed for diagnostic purposes only, and a small part of the specimens obtained were used for PCR. All patients gave their informed consent to participate in this study.

The group of control subjects consisted of 37 patients with various rheumatic diseases including rheumatoid arthritis, reactive arthritis, systemic lupus erythematosus, and osteoarthritis and 21 patients with different inflammatory and noninflammatory central nervous system (CNS) diseases, including multiple sclerosis, myelitis, and meningitis. All control subjects were seronegative. The patients' and control subjects' demographic characteristics are outlined in Table 1.

***B. burgdorferi* strains and cultivation.** *B. burgdorferi* was grown in BSK-H medium supplemented with 6% rabbit serum (Sigma, St. Louis, Mo.) under microaerophilic conditions at 37°C and was subcultured twice a week (15). The following pathogenic *B. burgdorferi* sensu lato strains were used to evaluate our PCR protocol: ZS7 (kindly provided by M. M. Simon, Max Planck Institute, Freiburg, Germany) and LW2 (both *B. burgdorferi* sensu stricto), 387 and A (both *B. garinii*; kindly provided by U. Goebel, Institute of Microbiology, Charité, Berlin, Germany), and PKo (*B. afzelii*; kindly provided by V. Preac-Mursic, Max von Pettenkofer Institute, Munich, Germany) (35, 39). For spiking experiments, spirochetes were enumerated by dark-field microscopy.

Sample preparation and DNA isolation. For the preparation of clinical specimens and borrelia cultures, alkaline lysis without phenol-chloroform DNA extraction was used as previously described by Rolfs et al. (27) for amplification of human DNA or DNA from infectious agents, and as previously described by Jiwa et al. (12) for the amplification of cytomegalovirus DNA. Samples were processed as indicated below.

(i) *B. burgdorferi* cultures. Spirochetes were pelleted, resuspended in 0.9% saline, and counted. Cells were then centrifuged again, and the pellet was subjected to alkaline lysis. For sensitivity experiments, 10-fold serial dilutions of spirochete suspensions were prepared in pooled synovial fluids (SF) from patients with arthritides other than Lyme borreliosis (mostly rheumatoid arthritis), with spirochete concentrations between 1,000/ml and 1/ml. One milliliter of the spiked SF samples was centrifuged at 350 × *g* for 20 min in a 1.5-ml Eppendorf reaction tube, and alkaline lysis of the pellets obtained was performed.

(ii) Urine. Ten to fifty milliliters of urine was centrifuged directly at 350 × *g* for 20 min at 10°C. After the pellet was washed with 0.9% saline in a 1.5-ml Eppendorf reaction tube, the cells were pelleted again and DNA extraction was performed from the pellet.

(iii) CSF. CSF was obtained by lumbar puncture and was centrifuged immediately at 350 × *g* for 20 min at 4°C in a 1.5-ml Eppendorf reaction tube. If no pellet was visible, the supernatant was removed, leaving about 20 μl at the bottom of the tube; otherwise, the supernatant was decanted. Subsequently, alkaline lysis was performed.

(iv) SF. One to ten milliliters of SF was centrifuged directly after closed-needle arthrocentesis at 350 × *g* for 20 min at 10°C. Pellets were washed with 0.9% saline in a 1.5-ml Eppendorf reaction tube, and cells were pelleted again and subjected to alkaline lysis.

(v) Alkaline lysis. Alkaline lysis was performed by overlaying the pellets with 25 to 500 μl (at least equaling the volume of the pellet) of 50 mM NaOH in a 1.5-ml Eppendorf reaction tube. Samples were vortexed vigorously and spun down briefly. After having been overlaid with 150 μl of light mineral oil (Sigma), samples were heated at 95°C for 15 min. Subsequently, neutralization was achieved by adding 1 M Tris-HCl (pH 7.0) and 4 μl of Tris-HCl for each 25 μl of 50 mM NaOH. Samples were either directly utilized for PCR or stored at −70°C until further use.

PCR. We used four primer sets (TIB Molbiol, Berlin, Germany), two each targeting *B. burgdorferi*-specific segments of chromosomal genes and plasmid-located genes. Primer set 1 (p66 gene primer) (28) targeted a sequence of a chromosomal gene encoding a 66-kDa protein (26), and primer set 2 (flagellin gene primer) (36) targeted a segment of the chromosomal flagellin gene. Primer set 3 (*ospA* primer) (21) was specific for a portion of the *ospA* gene, and primer set 4 (*ospA*/*B* primer) was derived from similar sequences of the *ospA* and *ospB* genes which are located on the same linear 49-kb plasmid of *B. burgdorferi* (38). In previous studies these primers were shown to specifically target *B. burgdorferi* gene segments. Southern blotting with PCR-derived probes and nested PCR were carried out in parallel. The sequences of the outer and nested primer pairs and the oligonucleotide probes are shown in Table 2.

In each experiment, an external amplification control (positive control) with boiled *B. burgdorferi* suspensions, an internal amplification control with primers specific for the pyruvate dehydrogenase (*PDH*) gene, and negative controls with water, urine, or SF were run. The PCR reaction mixture (total volume, 25 μl) contained 2.5 μl of the isolated DNA, 2.5 μl of 10-fold PCR buffer (Perkin-Elmer Cetus, Norwalk, Conn.; final concentrations, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% [wt/vol] gelatin), 0.3 μM concentrations of each primer (TIB Molbiol), 200 μM concentrations of each nucleotide (Boehringer Mannheim GmbH, Mannheim, Germany), and 0.8 U of *Taq* polymerase (AmpliTaq; Perkin-Elmer Cetus).

The outer PCR was carried out with a total of 40 cycles in an automated DNA thermal cycler (PTC 100; Biozym, Hessisch Oldendorf, Germany). DNA was denatured at 94°C for 1 min, primer annealed at 42°C for 1 min (*ospA*/*B* primer at 52°C), and extended at 72°C for 1 min.

Nested PCR was performed with 2.5 μl of the amplification products used as templates. Twenty-five cycles were carried out with the temperature profile described above.

Amplicons were visualized on a 3% agarose gel stained with ethidium bromide and were documented with a gel documentation system (Appligene imager; Appligene Oncor, Heidelberg, Germany).

For Southern blotting, digoxigenin-labelled probes were synthesized according to the procedure described by Finckh et al. (7). With outer-PCR amplicons as templates, a reamplification as described for the nested PCR, with digoxigenin-labelled dUTP (Boehringer Mannheim GmbH) instead of dTTP and with the nested primers (Table 2), was performed. Nucleotide concentrations were 13 μM digoxigenin–dUTP, 27 μM dTTP, and 40 μM (each) dATP, dGTP, and dCTP.

Southern blotting and nonradioactive hybridization were performed to confirm the specificities of the PCR amplification products and to test the sensitivity of the method in comparison to that of nested PCR. Amplicons were transferred onto nylon membrane (positively charged; Boehringer Mannheim GmbH) by capillary blotting, and DNA fragments were cross-linked by UV irradiation (Stratagene, Heidelberg, Germany). Hybridization was done overnight at 62°C with approximately 25 ng of the PCR-generated, digoxigenin-labelled probes in a hybridization oven (Bachofer, Reutlingen, Germany). The hybridization buffer was composed of 5× SSC (20× SSC is 3 M NaCl, 0.3 M sodium citrate [pH 7.0]), 0.1% (wt/vol) *N*-laurylsarcosine, 0.02% (wt/vol) sodium dodecyl sulfate (SDS), and 2% (wt/vol) blocking reagent (Boehringer Mannheim GmbH). After the blots were washed twice in 2× SSC–0.1% (wt/vol) SDS at room temperature and twice in 0.1× SSC–0.1% (wt/vol) SDS at 62°C, the amplicons were detected colorimetrically with the DIG DNA labelling and detection kit (nonradioactive) (Boehringer Mannheim GmbH) including an alkaline phosphatase-conjugated antidigoxigenin antibody. To minimize the risk of cross-contamination and DNA carryover, the established precautionary measures were taken (27). Pre- and

TABLE 2. Oligonucleotide primer sequences

Target gene	Primer	EMBL accession no.	Base no.	Sequence[a]	Amplicon length (bp)	Reference
ospA	Outer primer 1	X66065	18–39	ggg aat agg tct aat att agc c	665	
	Outer primer 2		660–682	cac taa ttg tta aag tgg aag t		21
	Nested primer 1		54–75	gca aaa tgt tag cag cct tga t	392	
	Nested primer 2		423–444	ctg tgt att caa gtc tgg ttc c		21
p66	Outer primer 1	M58429	9–26	cga aga tac taa atc tgt	371	
	Outer primer 2		362–379	gat caa ata ttt cag ctt		28
	Nested primer 1		30–49	tgc aga aac acc ttt tga at	236	
	Nested primer 2		248–263	aat cag ttc cca ttt gca		28
Flagellin	Outer primer 1	X15660	128–147	ctg ctg gca tgg gag ttt ct	729	
	Outer primer 2		838–857	tca att gca tac tca gta ct		35
	Nested primer 1		280–300	gca gtt caa tca ggt aac ggc	410	
	Nested primer 2		671–690	aga agg tgc tgt agc agg tg		
ospA/*B*	Outer primer 1	X69606	122–140	ttg taa gca aag aaa aaa a	701	
	Outer primer 2		803–822	tta aaa acg ctt taa aat aa		38
	Nested primer 1		146–467	gac ggc aag tac gat cta gct g	414	
	Nested primer 2		540–559	tta aag aag gaa ctg taa ct		38
PDH	Primer 1	J03576	142–161	ggt atg gat gag gag ctg ga	185	
	Primer 2		218–237	cag ccc tcg act aac ctt gt		27

[a] Sequences are shown from 5′ to 3′.

post-PCR sample processings were carried out in separate rooms on different floors.

Nucleotide sequence accession numbers. The EMBL accession numbers for the oligonucleotide primers used are X66065, M58429, X15660, X69606, and J03576.

RESULTS

Detection of *B. burgdorferi* DNA in spiked SF. To evaluate the sensitivity of our PCR assay, pooled SF spiked with 10-fold serial dilutions of different *B. burgdorferi* strains were analyzed with the four primer sets. Equal sensitivities were achieved with the plasmid *ospA* primer and the chromosomal p66 and flagellin primers, each detecting ≤3 borreliae/sample. Strains of the three species, *B. burgdorferi* sensu stricto (strains ZS7 and LW2), *B. garinii* (strains 387 and A), and *B. afzelii* (strain PKo), were detected with similar sensitivities. Southern blotting confirmed the specificities of the bands obtained. With the *ospA*/*B* primer borrelial DNA could not be amplified by using our protocol.

Detection of *B. burgdorferi* DNA in clinical samples. Initial experiments were performed with the p66, flagellin, and *ospA* primers. In clinical specimens only the p66 and the *ospA* primers amplified borrelial DNA. However, under the PCR conditions outlined, amplification of the template DNA with the flagellin primer could not be obtained. We therefore decided to continue the investigations of the clinical samples with the p66 and *ospA* primer sets only.

As described before (18), the amount of DNA obtained by single PCR was not sufficient to be visualized by ethidium bromide staining. In all clinical samples Southern blotting or nested PCR had to be performed to reliably detect *B. burgdorferi* DNA. The methods gave comparable results and were equally efficient in enhancing the test sensitivity. Although we did not use phenol-chloroform DNA extraction, impairment of test sensitivity by *Taq* polymerase inhibitors occurred in none of the samples. However, in several samples a massive overload of total DNA completely inhibited PCR, including the *PDH* control. This inhibition could be overcome by further dilution of those samples. Neither in urine, SF, or CSF nor in blood or skin biopsies (data not shown) did the presence of human genomic DNA interfere with the specific DNA amplification.

PCR in samples from patients with Lyme arthritis. Forty-six samples, 33 of urine and 13 of SF, from 35 patients with Lyme arthritis were analyzed. These included paired SF-urine specimens from 11 patients.

PCR with urine samples showed an overall sensitivity of 79%, i.e., PCR yielded positive results with at least one primer in 26 of the 33 samples investigated. In 8 samples (24%) *B. burgdorferi* DNA could be detected with the *ospA* primer only, while in 14 samples (42%) only the p66 PCR was positive. In four specimens (12%) both primers yielded positive results, and seven urine samples (21%) were PCR negative. SF analyses revealed positive results in 11 of 13 samples (85%). In five samples (39%) amplification was obtained with the p66 primer only, while three specimens (23%) were positive with the *ospA* primer and with both primers. Two samples (15%) were PCR negative (Table 3).

Of the 11 paired samples 10 (91%) were PCR positive in at least one fluid, SF or urine. For six patients (55%) both specimens were PCR positive. For three patients (27%) *B. burgdorferi* DNA was detectable only in SF, and for 1 patient (9%) it was detectable only in urine (Table 4).

PCR in samples from patients with neuroborreliosis. A total of 22 patients with neuroborreliosis were included in this study. Nineteen CSF and 11 urine samples, including 8 paired CSF-urine samples, were analyzed.

TABLE 3. PCR results for Lyme arthritis patients (n = 35)

Sample	No. (%) positive with:				No. (%) negative with both primer pairs
	ospA primer only	p66 primer only	Both primers	At least one primer	
SF (n = 13)	3 (23)	5 (39)	3 (23)	11 (85)	2 (15)
Urine (n = 33)	8 (24)	14 (42)	4 (12)	26 (79)	7 (21)

TABLE 4. PCR results for 11 paired SF-urine samples from Lyme arthritis patients and for 8 paired CSF-urine samples from neuroborreliosis patients

Sample	No. (%) positive with:				No. (%) negative with both samples
	Urine only	SF or CSF only	Both samples	At least one sample	
Paired SF-urine samples (n = 11)	1 (9)	3 (27)	6 (55)	10 (91)	1 (9)
Paired CSF-urine samples (n = 8)	1 (13)	5 (62)	1 (13)	7 (87)	1 (13)

For 15 of 19 patients (79%) borrelial DNA could be amplified in CSF with either primer. Among these 15 patients, 6 patients (32%) were positive with either the p66 primer or the *ospA* primer, while 3 patients (16%) were positive with both primer sets. PCR was negative for four samples (21%). In urine samples PCR amplified borrelial DNA for 5 of 11 patients (45%). Two urine samples (18%) showed positive PCR results with either the *ospA* or the p66 primer, while 1 urine sample (9%) was positive with both. Six urine specimens (55%) were PCR negative in our assays (Table 5).

Analyzing eight paired CSF-urine samples revealed that in seven cases (87%) *B. burgdorferi* could be detected in at least one sample. These cases included one patient (13%) who was positive for both fluids, five patients (62%) who were positive for CSF only, and one patient (13%) who was positive for urine only. There was one patient (13%) who was negative for both samples (Table 4).

PCR for patients with symptoms suggestive of Lyme borreliosis. PCR with urine samples was performed for 11 selected patients with positive Lyme serology but with unspecific clinical symptoms. PCR was positive for nine patients (82%) with the p66 primer. Two patients (18%) were also positive with the *ospA* primer. All patients improved clinically after antibiotic treatment, by which time their PCR results were negative.

PCR for control patients. The specificity of the PCR assay was evaluated for 59 clinical specimens from 37 patients with different rheumatologic diseases and 23 specimens from 21 patients with inflammatory and noninflammatory neurologic disorders. In particular, the following specimens were analyzed: 7 SF, 5 urine, and 3 blood samples from 10 patients with rheumatoid arthritis; 12 CSF, 1 SF, 4 blood, and 5 urine samples from 16 patients with collagen vascular diseases (mainly systemic lupus erythematosus); 10 SF, 5 urine, and 3 blood samples from 7 patients with HLA-B27-associated arthritides; 4 SF samples from 4 patients with osteoarthritis; 14 CSF samples from 14 patients with inflammatory CNS diseases, including multiple sclerosis, myelitis, meningitis, and radiculitis; and 7 CSF and 2 urine samples from 7 patients with noninflammatory neurologic diseases. None of these samples showed a positive result with the optimized PCR protocol.

DISCUSSION

The results of the present study on the optimization of a PCR protocol for the detection of *B. burgdorferi* DNA in patients with Lyme borreliosis revealed that (i) the rapid and easy-to-perform DNA extraction by alkaline lysis is a suitable method to isolate *B. burgdorferi* DNA from various clinical specimens and is superior to other DNA isolation protocols and that (ii) by using two primer sets targeting segments of the chromosomal p66 gene and the plasmid-located *ospA* gene and by analyzing paired SF-urine or CSF-urine samples, diagnostic sensitivities of ≥90% for Lyme arthritis and ≥80% for neuroborreliosis could be achieved. In contrast, analyzing single specimens from each patient with one primer set gave sensitivities between 13 and 50%.

The DNA isolation method by alkaline lysis DNA extraction was first described by Rolfs et al. (27) and is now introduced for the molecular diagnosis of Lyme disease by our group. This method has the advantage of avoiding several preparation steps that are used in other protocols and that lead to a considerable loss of DNA material. Moreover, it is inexpensive, and the use of toxic substances can be avoided. Although this simplified protocol does not use phenol-chloroform DNA extraction, no amplification problems due to *Taq* polymerase inhibitors occurred. This may be attributable to the large amount of DNA obtained by alkaline lysis that allows for a sufficient amplification even in the presence of potentially *Taq*-inhibiting substances. Fewer than three borreliae/ml could reliably be detected in spiked specimens. This sensitivity level is superior to those derived from the data reported in most studies, indicating that DNA extraction by alkaline lysis enhances the sensitivity of PCR (11, 17, 18, 30, 38). This is of special importance for Lyme disease for which the concentration of borrelial DNA, especially in CSF but also in other specimens, is frequently near the detection limit of the PCR (14, 17).

In the PCR studies published so far, various primer sets targeting plasmid-located or chromosomal gene sequences were used. Since it was hypothesized that the amplification of plasmid genes would enhance PCR sensitivity because plasmids are often present in multiple copies (24), portions of the gene coding for the OspA protein were frequently chosen as templates (14, 18, 21, 23). However, the *ospA* gene was shown to be extremely heterogenous, and the sensitivity of PCR depended on the *ospA* genotype of the *B. burgdorferi* strain to be detected (21, 39). Targeting conserved chromosomal genes may be less susceptible to this problem (29). We therefore decided to examine the abilities of four primer sets, two each targeting gene segments on the chromosome and on the 49-kb linear plasmid, to detect *B. burgdorferi* DNA in our patients. The best results were achieved by using the primers for the plasmid-located *ospA* gene (*ospA* primer) (21) and primers for the chromosomal gene segment coding for the 66-kDa protein (p66 primer) (28). With these primers, all three genotypes of *B. burgdorferi* sensu lato could be detected with comparable sensitivities in spiked specimens. Interestingly, using the p66 primer yielded greater sensitivities for most clinical samples tested than using the *ospA* primer. This may indicate that the targeted chromosomal p66 gene segment is more conserved than the *ospA* gene and that the p66 primer binds to the corresponding gene of most *B. burgdorferi* strains (28, 35). *B. burgdorferi* DNA could be detected with the p66 primer in 60% of urine specimens from patients with Lyme arthritis, in 27% of urine specimens from patients with neuroborreliosis, in 72% of SF specimens, and in 60% of CSF specimens. These results significantly exceed the sensitivities reported in other studies on the detection of chromosomal *B. burgdorferi* DNA (11, 23).

TABLE 5. PCR results for neuroborreliosis patients (n = 22)

Sample	No. (%) positive with:				No. (%) negative with both primer pairs
	ospA primer only	p66 primer only	Both primers	At least one primer	
CSF (n = 19)	6 (32)	6 (32)	3 (16)	15 (79)	4 (21)
Urine (n = 11)	2 (18)	2 (18)	1 (9)	5 (45)	6 (55)

Since PCR protocols were otherwise comparable, this enhanced sensitivity may be primarily attributable to the optimized DNA extraction method.

In contrast, PCR with the *ospA* primers resulted in less sensitivity than that reported by others (14, 18, 21, 23), probably due to the heterogeneity of the *ospA* genes of the *B. burgdorferi* strains in our Central European clinical samples. Since the abilities of *ospA* primers to detect different *ospA* genotypes vary (21), some strains may not have been detectable with the primer set utilized in this study. Another explanation would be that not all strains had stable *ospA* gene sequences (11). However, there were specimens in which *B. burgdorferi* DNA could only be detected by *ospA* PCR. It may be speculated that in these samples the concentrations of borreliae were below the detection limit of p66 PCR but that because of the presence of multiple plasmid copies and optimal binding of the *ospA* primers only *ospA* PCR was positive (24). Further characterization of the *B. burgdorferi* species and strains found in the patients included in the study is currently being performed in our laboratory. In any event, since most samples were positive with one or the other primer only, both primers should always be used in parallel to achieve maximum sensitivity.

An analysis of paired SF-urine and CSF-urine samples revealed the greater sensitivity of PCR with SF or CSF samples, as compared to PCR with urine samples. This difference was less evident for Lyme arthritis patients but was significant ($P = 0.03$) for neuroborreliosis patients. For approximately one-third of the patients both specimens were positive, and in both groups one patient each had only a positive urine specimen. However, 91% of the Lyme arthritis patients and 87% of the patients with neuroborreliosis were identified by a positive PCR result with at least one specimen. Thus, PCR with urine specimens is useful in the diagnosis of Lyme borreliosis, especially Lyme arthritis and especially when SF or CSF specimens are not available (9, 17–19). Consistent with these results, in a group of eleven selected patients with a history and serology suggestive of Lyme disease but with unspecific symptoms, PCR with urine specimens was positive for nine patients (82%) with at least one primer set. For maximum sensitivity, however, two or more samples from each patient, including symptom-oriented "lesional" specimens such as SF or CSF, should be analyzed whenever possible.

The above results indicate that with the optimized PCR protocol described herein DNA of the three genospecies *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* can sensitively and specifically be detected in urine, SF, and CSF specimens from patients with Lyme arthritis or neuroborreliosis. DNA extraction by alkaline lysis, the use of two primer sets targeting chromosomal and plasmid-located gene sequences, and the analysis of urine-SF or urine-CSF pairs should be performed to achieve maximum sensitivity of PCR in the diagnosis of Lyme borreliosis.

ACKNOWLEDGMENTS

This study was supported by Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie grants 01 KI 9104 and 01 KI 9503.

We thank K. M. Einhäupl, Director of the Department of Neurology at the Charité University Hospital, Berlin, for providing clinical samples from his patients with neuroborreliosis. We are indebted to Diana Lahn for expert technical assistance.

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