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Article

Diagnostic Relevance of the Detection of *Legionella* DNA in Urine Samples by the Polymerase Chain Reaction

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Abstract Urine samples from 317 patients with pneumonia and from 242 patients without pneumonia were tested using a polymerase chain reaction (PCR) system for detection of the *Legionella* 5S rRNA gene. The results were compared with findings obtained using the established methods for diagnosis of legionellosis. Of the 317 patients with pneumonia, 58 had confirmed legionellosis, 35 had a presumptive *Legionella* infection, and 224 had no evidence of *Legionella* infection as determined by conventional methods using published criteria. The PCR was positive for 42 patients with confirmed infections, yielding a sensitivity of 72.4%. Furthermore, 16 (47%) patients with presumptive legionellosis and five (2.2%) patients without other evidence of *Legionella* infection had positive results. All samples from 242 patients without pneumonia were PCR-negative. When the results for all patients were considered, the specificity of the assay was ≥98.9%. The results demonstrate that the sensitivity and specificity values of urinary PCR are in the same range as those of established methods. The use of PCR in urine complements the repertoire of rapid diagnostic methods, especially for infections caused by legionellae not belonging to *Legionella pneumophila* serogroup 1, in which tests for detection of urinary antigen often fail.

Introduction

Bacteria of the genus *Legionella* are a common cause of both community-acquired and nosocomial pneumonia. Presently, the genus *Legionella* comprises 41 species [1], of which 18 have been recognised as pathogenic to humans [2]. The remaining 23 species have been isolated only from environmental sources. Approximately 50–75% of *Legionella* infections are caused by *Legionella pneumophila* serogroup 1, 20–30% by other serogroups of *Legionella pneumophila*, and 5–20% by other species within the genus *Legionella* [3]. Several studies have demonstrated that pneumonia caused by legionellae cannot be differentiated from other types of pneumonia by clinical, radiographic, or nonspecific laboratory findings [4, 5]. Therefore, the specific etiologic diagnosis must be based on microbiologic laboratory tests.

However, none of the diagnostic tests presently available fulfills all expectations with respect to sensitivity and specificity. Culture is the method of choice and has a sensitivity of about 70%, but special media and selective techniques are needed [2]. Furthermore, isolation of legionellae frequently requires more than 3 days, and broad-spectrum antibiotic therapy decreases the culture yield [4]. The detection of antibodies in patient sera often provides only a retrospective diagnosis and has a sensitivity of about 70% [4]. The value of the detection of *Legionella* antigens in urine and of the detection of legionellae by direct fluorescent antibody (DFA) testing in respiratory tract secretions depends

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much on the pool composition and the quality of antisera used [3]. Because of the antigenic diversity of legionellae, the sensitivity and specificity of DFA assays may be unsatisfactory, especially for infections caused by species other than *Legionella pneumophila*.

Since the discovery of legionellae, many attempts to improve and expand the spectrum of diagnostic methods have been undertaken. The most recent advances have been made in diagnostic tests based on the detection of *Legionella* nucleic acids by the polymerase chain reaction (PCR). In order to detect legionellae at the species or genus level, PCR systems have been developed to amplify conserved regions of the 5S rRNA, the 16S rRNA, and the macrophage infectivity potentiator (*mip*) genes [6–8]. Several studies have reported the use of PCR for detection of legionellae in clinical specimens, usually specimens from the respiratory tract [6, 7, 9–13]. *Legionella* DNA has also been detected by PCR in serum samples [14–16].

In 1995 we reported the presence of *Legionella* DNA in urine samples of laboratory-infected guinea pigs as well as of patients with legionellosis [17]. More recently, Murdoch et al. [16] were able to detect *Legionella* DNA in both serum and urine samples. However, both studies [16, 17] evaluated a relatively small number of patients. Therefore, a larger sample size was required to more accurately determine the sensitivity and specificity of the test. Here, we present the results of a PCR assay used to test urine samples from 317 patients with pneumonia and 242 patients without pneumonia.

Materials and Methods

Case Definitions for Legionellosis. The definition of legionellosis was made in accordance with the guidelines of the World Health Organization [18], supplemented by newer definitions proposed both by Plouffe et al. [19] and the European Working Group on *Legionella* Infections (Eurosurveillance Weekly, reported by C. Joseph [PHLS, Communicable Disease Surveillance Center, London, UK] on behalf of the European Working Group on *Legionella* Infections), which include criteria for detection of urinary antigen. Accordingly, a confirmed *Legionella* infection was defined by isolation of the pathogen in culture, by a fourfold or greater rise of the specific antibody titre against *Legionella pneumophila* serogroup 1 in the indirect fluorescent antibody (IFA) test, or by a positive urinary antigen test for *Legionella pneumophila*. A presumptive *Legionella* infection was defined by a positive result of DFA testing performed with respiratory specimens, by a single elevated antibody titre ($\geq$256) against *Legionella* spp., or by a rising antibody titre to legionellae other than *Legionella pneumophila* serogroup 1.

Origin of the Urine Samples. A total of 559 urine samples from 559 patients were examined by PCR and an enzyme immunoassay (EIA) for detection of urinary antigen. The study included 317 patients with pneumonia for whom physicians requested diagnostic tests for Legionnaires' disease. The first urine sample had been collected between day 4 and day 33 after the onset of symptoms and was used for diagnoses. The samples were stored frozen at –20 °C until testing by PCR. The EIA for detection of urinary antigen was repeated at the same time the PCR was performed, and these data were used for evaluation. Furthermore, 150 samples from patients with culture-diagnosed urinary tract infections and 92 specimens from patients in a transplant unit, both groups without symptoms of pneumonia, were tested by both methods as controls.

Preparation of Urine Samples for the Polymerase Chain Reaction. Urine samples were prepared for PCR using the Geneclean II kit (Bio 101, USA) as recommended by the manufacturer and described previously [17]. Briefly, 350 µl of urine and 1050 µl of NaI stock solution were mixed, and 2 µl of glass milk (Bio 101) was added. The solution was left to stand for 15 min at room temperature with intermittent vortexing. After centrifugation and washing three times, the DNA was eluted from the matrix with 25 µl of water at 55 °C. In order to confirm the absence of inhibitory substances to the PCR, 100 PCR-negative urine samples from pneumonia patients were re-examined after addition of *Legionella* control DNA (EnviroAmp *Legionella* Kit; Perkin-Elmer, USA). Each 350 µl sample was spiked with 5 pg of DNA prior to DNA extraction. *Legionella* DNA was amplified from all of these controls, suggesting that either no inhibitors were present or they were removed by sample preparation.

Amplification of Legionella DNA. For amplification of the *Legionella* 5S rRNA gene by PCR, the primer sequences of the Perkin-Elmer EnviroAmp *Legionella* Kit (PT 87: 5′-GGCGAC TATAGCGATTTGGAA, PT 161: 5′-GGCGACTATAGCGG TTTGGAA, PT163: 5′-GCGATGACCTACTTTCGCATGA, and PT 165: 5′-GCGATGACCTACTTTCACATGA) were used. Based on the data in the manufacturer's package insert, of the 26 *Legionella* spp. tested, all except *Legionella israelensis* yielded positive results using these primers. Oligonucleotides were custom synthesised by Biometra, Germany. The PCR was performed in a volume of 50 µl containing 50 mM KCl, 50 mM Tris (PH 9.0), 2 mM $MgCl_2$, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 400 µM dUTP (all deoxyribonucleosides from Perkin-Elmer), 50 pmol of each primer, 2 units of *Taq* polymerase (Ampli*Taq* DNA Polymerase, Perkin-Elmer), and 0.5 units of uracil *N*-glycosylase (Amp*Erase*; Perkin-Elmer). Twenty microliters of sample was added and the PCR was performed in a model 9600 thermal cycler (Perkin-Elmer) using the following protocol: preincubation at 45 °C for 10 min and initial denaturation at 95 °C for 10 min followed by 35 cycles of denaturation at 95 °C for 15 s, annealing and extension at 65 °C for 45 s, and final extension at 72 °C for 2 min.

Detection of Amplification Products. PCR products were electrophoresed on vertical 8% polyacrylamide gels, stained, visualised, and denatured for Southern blotting as described previously [17]. The DNA was then transferred onto positively charged nylon membranes (Qiabrane Membrane; Qiagen, Germany) using capillary blotting in a TBE (0.089 M tris, 0.089 M boric acid, 0.002 M EDTA) buffer gradient overnight. The membranes were baked at 80 °C for 1 h, prehybridised at 55 °C for 1 h in Gold Hybridisation Buffer (Amersham Life Science, UK), and hybridised at 55 °C for 3 h with 2.5 pmol/ml of the biotinylated oligonucleotide PT 125 (5′-GCGCCAATGATAGTGTG, sequence from the Perkin-Elmer EnviroAmp *Legionella* Kit). After washing, the hybridised probe was incubated for 90 min with streptavidin-horseradish peroxidase complex (Amersham Life Science) and detected with chemiluminescence (ECL Detection Kit, Amersham Life Science). When 350 µl urine samples were spiked with *Legionella* control DNA (Perkin-Elmer) prior to sample preparation, the analytical sensitivity of the PCR was 5–50 fg DNA, which is in the same range as that previously published using the ^{32}P-labelled oligonucleotide PT 125 [17].

Conventional Methods for Diagnosis of Legionellosis. Cultures of respiratory tract specimens were performed as described previously [17]. Sputa were processed prior to culturing by heat treatment (for 20 min at 50 °C). Identification and serotyping of isolates were done as described elsewhere [20]. Antibodies in

patient sera were determined by indirect immunofluorescence antibody (IFA) testing according to Wilkinson [21], using *Legionella pneumophila* serogroups 1 to 14, *Legionella bozemanii*, *Legionella dumoffii*, *Legionella jordanis*, *Legionella longbeacheae* serogroups 1 and 2, and *Legionella micdadei*. DFA testing for the detection of legionellae in respiratory tract specimens was performed according to the methods of Harrison and Taylor [22], using a fluorescent monoclonal antibody specific for *Legionella pneumophila* (Fresenius; Oberursel, Germany). The detection of urinary antigen by EIA was performed as described previously [23, 24]. All urine specimens were tested using rabbit anti-*Legionella pneumophila* (serogroup 1) IgG [23]. If the results of culture or the indirect immunofluorescence antibody test suggested or confirmed legionellosis caused by other serogroups of *Legionella pneumophila* or other *Legionella* spp., the urine samples were tested by five additional EIAs using rabbit anti-*Legionella pneumophila* (serogroups 2, 3, 5, 6) IgG as well as rabbit anti-*Legionella micdadei* IgG [25]. In one case of culture-proven *Legionella pneumophila* serogroup 10 infection, the antigenuria was detected by an EIA using *Legionella pneumophila*-specific IgG from goat (m-Tech, USA) as capture antibody and a monoclonal antibody (designated 11/9) recognising a common epitope of *Legionella pneumophila* serogroups 2–6, 8–10, and 12–14 [25]. Positive results of EIA to detect urinary antigen were confirmed by repeating the test with boiled urine samples. All 451 urine samples from the 317 patients with pneumonia were examined by both PCR and urinary antigen testing, whereas the number of samples examined by the other methods varied, depending on the clinical materials submitted.

Statistical Analysis. The P value for differences in the rate of positive results was derived from chi-square 2×2 contingency tables.

Results

Patients without Pneumonia. All 92 urine samples from transplant patients and all 150 urine samples from patients with urinary tract infections were negative by PCR. All urine samples from patients with urinary tract infections contained more than 10^5/ml colony-forming units; the following urinary tract pathogens were identified: *Acinetobacter* spp. ($n=9$ samples), *Candida* spp. ($n=3$), *Citrobacter* spp. ($n=6$), *Enterococcus* spp. ($n=46$), *Escherichia coli* ($n=50$), *Klebsiella* spp. ($n=10$), *Morganella* spp. ($n=5$), *Proteus* spp. ($n=11$), *Pseudomonas* spp. ($n=6$), *Staphylococcus* sp. ($n=1$), and *Streptococcus* spp. ($n=3$).

Patients with Confirmed Legionella Infection. Altogether, 58 of the 317 patients with pneumonia met the definition of having confirmed *Legionella* infection as given in Materials and Methods [18, 19]. In this group of patients, legionellosis was confirmed by (i) culture (*Legionella pneumophila* serogroup 1 [21 patients], serogroup 3 [2 patients], serogroups 2, 5, 6, 10 [1 patient each], *Legionella hackeliae* [1 patient]) and partly by seroconversion or urinary antigen detection in 28 cases, by (ii) seroconversion and partly by urinary antigen detection in 14 cases, and by (iii) urinary antigen detection alone in 16 cases. Of the 58 patients with confirmed legionellosis, 42 (72%) were positive by PCR. The highest rate of positive results (79%) was found for patients with antigenuria. The rate for confirmed cases without antigenuria was less (53%, $P=0.07$). The results of PCR, listed according to the diagnostic method by which legionellosis was confirmed, are given in Table 1.

Patients with Presumptive Legionella Infection. An additional 35 patients with pneumonia met the definition of presumptive *Legionella* infection. In this group of patients, presumptive legionellosis was diagnosed by the DFA test and the IFA test in four cases, by the DFA test alone in 12 cases, and by the IFA test alone in 19 cases. The PCR was positive for 16 patients, yielding a rate of 47% for presumptive cases (Table 1). The percentage of positive PCR findings was significantly greater ($P=0.029$) in cases characterised by positive DFA test results (67%) than in cases with a single elevated titre (26%).

Pneumonia Patients Without Evidence of Legionella Infection by Conventional Methods. Of the 224 pneumonia patients without any positive results by conven-

Table 1 Detection by polymerase chain reaction (PCR) of *Legionella* DNA in urine samples from patients with pneumonia

	No. (%) of patients positive/no. tested
Method by which legionellosis was confirmed	
Culture (and partly by other methods)	21/28 (75)
Seroconversion to *Legionella pneumophila* serogroup 1 (and partly by other methods)	12/18 (66.7)
Urinary antigen detection (and partly by other methods)	34/43 (79.1)
Culture and/or seroconversion without antigenuria	8/15 (53.3)
Total (all confirmed cases)	42/58 (72.4)
Method by which legionellosis was diagnosed	
DFA test only	8/12 (67)
IFA test only	5/19 (26)
DFA and IFA tests	3/4 (75)
Total (all presumptive cases)	16/35 (47)
Pneumonia patients with no evidence of *Legionella* infection, as determined by other methods	5/224 (2.2)

DFA, direct fluorescent antibody; IFA, indirect fluorescent antibody

tional methods, urine samples from 219 patients were negative by PCR. Only five specimens were positive (Table 1). From three of these patients, only urine samples were available for testing. From the other two, specimens from the respiratory tract were available and were examined by DFA testing and culture. Examinations by culture for other common pneumonia pathogens yielded negative results.

Comparison Between Urinary Antigen Detection and Polymerase Chain Reaction in Urine Samples. As detailed above, the percentage of PCR-positive patients among those with confirmed *Legionella* infections was markedly higher for patients with antigenuria than for those without antigenuria. Nevertheless, nine (21%) patients with antigenuria were negative by PCR (Table 1). On the other hand, *Legionella* PCR in urine samples was positive for 19.9% of all pneumonia patients tested, whereas the percentage of patients in whom antigenuria was detected (13.6%) was significantly smaller ($P = 0.033$; Table 2). Seven urine samples from patients with confirmed legionellosis were obtained from day 4 to day 6 after the onset of symptoms. All of these were positive by both urinary antigen testing and PCR (data not shown).

Relative Importance of the Methods for Diagnosis of Legionella Infection. Five different methods for recognising or precluding *Legionella* infections in a series of 317 pneumonia patients were used. Altogether, 58 (18.3%) patients were diagnosed with confirmed legionellosis and 35 (11%) patients with presumptive legionellosis. *Legionella* infection was confirmed by culture in 14.7%, by rising antibody titre to *Legionella pneumophila* serogroup 1 in 10.5%, and by detection of urinary antigen in 13.6% of all cases (Table 2). The percentage of PCR-positive patients was greater ($P \leq 0.17$) and amounted to 17.8%. The highest rates of positive results were found for patients who tested positive by the IFA test (19.2% for results other than seroconversion to *Legionella pneumophila* serogroup 1) and by the DFA test (24.2%). However, DFA-positive and PCR-positive cases were confirmed by accepted methods to be a *Legionella* infection in only 22 of 182 (12.1%) and in only 42 of 317 (13.2%) cases, respectively.

Diagnostic Accuracy of the Polymerase Chain Reaction for Detection of Legionella in Urine Samples. The sensitivity of amplification of *Legionella* DNA by PCR in urine samples was 72.4% for patients with confirmed *Legionella* infection and 62.4% for patients with confirmed or presumptive *Legionella* infection. With the exception of urinary antigen testing of confirmed cases, the sensitivities of all other methods used were lower (Table 3). The specificity of the assay was checked by testing urine samples from 150 patients with urinary tract infection and 92 patients in a transplant unit, all without symptoms of pneumonia. All of these specimens were negative. In addition, urine samples from 224 pneumonia patients without any results indicating a confirmed or presumptive *Legionella* infection were tested. Five of these patients were positive. However, it is not possible to exclude legionellosis in these patients (see above). Taking these results together, the specificity of the assay is $\geq 98.9\%$.

Discussion

Pneumonia caused by legionellae has a case-fatality rate of approximately 15–20%, which can be much higher in nosocomial cases in patients with severe

Table 2 Diagnosis of *Legionella* infections in 317 patients with pneumonia

Diagnostic method used	No. of patients examined	No. (%) of *Legionella* infections diagnosed
Culture	190	28 (14.7)
IFA test	172	18 (10.5)[a] 33 (19.2)[b]
Urinary antigen detection	317	43 (13.6)
DFA test	182	44 (24.2)[c]
PCR with urine samples	317	63 (19.9)[d]

[a] Seroconversion to *Legionella pneumophila* serogroup 1
[b] Single elevated titre (≥ 256) against *Legionella* spp. or rising titre to legionellae other than *Legionella pneumophila* serogroup 1 (patients with seroconversion to *Legionella pneumophila* serogroup 1 excluded)
[c] DFA-positive cases were confirmed by accepted methods to be *Legionella* infections in only 22 of 182 (12.1%) cases
[d] PCR-positive cases were confirmed by accepted methods to be *Legionella* infections in only 42 of 317 (13.2%) cases
IFA, indirect fluorescent antibody; DFA, direct fluorescent antibody; PCR, polymerase chain reaction

Table 3 Sensitivity of the polymerase chain reaction (PCR) in urine specimens versus other methods used for the diagnosis of *Legionella* infection

Diagnostic method used	No. (%) of patients positive/no. tested	
	Confirmed cases	Confirmed and presumptive cases
PCR in urine samples	42/58 (72.4)	58/93 (62.4)
Culture	28/47 (59.6)	28/74 (37.8)
IFA test[a]	15/32 (46.9)	15/42 (35.7)
Urinary antigen detection	43/58 (74.1)	43/93 (46.2)
DFA test	20/37 (54.1)	31/53 (58.5)
IFA test[b]	11/17 (64.7)	19/40 (47.5)

[a] Seroconversion to *Legionella pneumophila* serogroup 1
[b] Single elevated titre (≥ 256) against *Legionella* spp. or rising titre to legionellae other than *Legionella pneumophila* serogroup 1 (patients with seroconversion to *Legionella pneumophila* serogroup 1 excluded)
DFA, direct fluorescent antibody; IFA, indirect fluorescent antibody

underlying diseases [26]. Therefore, it is important to obtain laboratory results that point to legionellosis as accurately and quickly as possible in order to initiate appropriate antimicrobial therapy. However, none of the laboratory methods presently available is optimal with respect to both diagnostic sensitivity and specificity [3]. The main reasons for this relate to (i) difficulties with the isolation of the pathogen by current culture methods, (ii) delayed or absent immune response in the host that affect the reliability of serological tests, and (iii) the diverse number of species and serogroups of legionellae that cause the major difficulties of antigen detection in urine samples or respiratory specimens [3]. As a consequence, *Legionella* infections appear to be significantly underdiagnosed [19]. Attempts have therefore been made to improve the diagnostic situation by the detection of *Legionella* DNA using PCR technology [3]. Based on our previous finding that *Legionella* DNA is excreted in the urine of affected patients [17], we examined urine samples from 317 patients with pneumonia as well as from 242 patients without pneumonia to evaluate the diagnostic accuracy of the detection of *Legionella* DNA in urine.

The sensitivity and specificity of PCR for the detection of *Legionella* DNA in clinical specimens are difficult to judge at present, as most of the published studies have dealt with relatively small numbers of patients and samples [6, 9–12, 16]. For PCR in urine samples, we previously estimated a specificity of 88% [17]. In the present study, we took measures to avoid carryover contamination of the PCR by utilising uracil *N*-glycosylase. The specificity, as determined by testing urine samples from 242 patients without pneumonia and from 224 pneumonia patients without positive results by other methods, was 98.9%. For the five patients with positive PCR results only, it is not possible to exclude *Legionella* infection. Therefore, with the exception of culture, all other established methods have specificity values that are not noticeably higher than that of PCR in urine [2, 4].

The sensitivity of PCR in urine, as calculated for 58 patients with confirmed *Legionella* infections, was 72.4% (Table 3). One factor that may have negatively influenced sensitivity is the prolonged storage of the samples before testing and, in some cases, the occurrence of one or two freeze-thaw cycles. These conditions can reduce the availability of DNA for PCR [27]. In addition, the first urine sample was obtained more than 10 days after the positive respiratory specimen from seven of the 28 culture-positive patients. Only two of these seven patients were positive by PCR, none by urinary antigen testing. Therefore, it appears reasonable to assume that the sensitivity of PCR may be underestimated. Nevertheless, the sensitivity is in the same range as that reported by Murdoch et al. [16], who reported a value of 64% for DNA amplification from urine and serum samples. The value improved to 73% if interpretation was restricted to samples taken within 4 days of the onset of symptoms [16]. A comparison with other studies of *Legionella* PCR with respiratory specimens is difficult, as the numbers of samples tested were small or the classifications of *Legionella* infections were different from those used in our study [6, 7, 10–13, 15].

Due to sensitivity problems of the three methods that are used to confirm a *Legionella* infection, or due to the lack of specimens, it is often only possible to make the diagnosis of a presumptive *Legionella* infection. Of the 35 patients with presumptive infection in our study, 16 (47%) excreted *Legionella* DNA in the urine (Table 1). In presumptive cases diagnosed solely by IFA testing patients excreted *Legionella* DNA significantly less frequently than DFA-positive patients ($P=0.029$), which is conceivable because both PCR in urine samples and the DFA test become positive in an early stage of the illness, whereas the IFA test becomes positive later, when antibiotic treatment is likely to have been initiated. Although neither method can confirm a *Legionella* infection, concurring positive results substantially increase the likelihood that a patient actually has legionellosis.

Theoretically, the detection of antigen in urine should be the method that offers the best comparability to PCR performed on urine samples. This is because the samples are identical and are obtained from the same phase of the illness. Accordingly, the highest rate of PCR-positive cases was found among patients with antigenuria (Table 1). It is conceivable that both components (DNA and antigen) may be processed and excreted with similar kinetics. Nevertheless, nine of the 43 (21%) patients with antigenuria had negative PCR results. It is likely that DNA in urine samples is not as stable as lipopolysaccharides, which are the target of our urinary antigen assays.

In summary, among 317 patients with pneumonia who were clinically suspected to have a *Legionella* infection, we found 18.3% to have confirmed cases of infection and 11% to have presumptive cases. Unfortunately, specimens from only about 60% of the patients were tested by all diagnostic methods, including culture, IFA testing, and DFA testing in addition to the detection of both DNA and antigen in the urine samples. This, however, represents the characteristics of routine submission of specimens by physicians. Compared to cases proven by both culture and seroconversion (Table 2), the PCR in urine samples yielded more positive findings ($P=0.17$ and $P=0.045$, respectively). Ninety-two percent of all PCR-positive cases represent confirmed or presumptive infections as determined by other methods. Keeping in mind that the specificity of the *Legionella* DNA detection is $\geq 98.8\%$ as well as that the sensitivities of all other methods used does not exceed 60–80% [4], it appears likely that the remaining

five pneumonia patients for whom only the PCR was positive also had a *Legionella* infection.

Due to the known limitations of each of the tests presently available, it is advisable to examine different types of clinical specimens and to use a combination of diagnostic methods for each case of suspected Legionnaires' disease. The results of this study demonstrate that the sensitivity and specificity of PCR performed on urine samples are in the same range as those of the established methods. The oligonucleotide primers used for the *Legionella* 5S rRNA gene detect almost all members of the genus [17]. This avoids the problems of antigen-based methods, which require a pool of specific antisera. In this study, we were able to amplify *Legionella* DNA from urine samples from culture-proven cases of infection due to *Legionella pneumophila* serogroups 1–3, 5, 6, and 10 and *Legionella hackelie*. Furthermore, two samples from patients showing seroconversion to *Legionella micdadei* and one sample from a patient with seroconversion to *Legionella longbeacheae* were positive by PCR. In addition, this test is valuable early in the course of infection. All of the seven urine samples from patients with culture-proven cases taken from day 4 to day 6 after the onset of symptoms were positive by PCR. Therefore, including urinary PCR in the repertoire of methods applied on a routine basis could enhance the overall ability to diagnose *Legionella* infections. Additional studies are warranted to obtain data concerning the duration of DNA excretion in the course of the illness as well as the influence of antibiotic therapy.

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