Clinical Use of Capillary PCR To Diagnose Mycoplasma Pneumonia

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Mycoplasma pneumoniae is a common respiratory tract pathogen that can lead to the development of pharyngitis, tracheobronchitis, and pneumonia. *M. pneumoniae* is the cause of 15 to 20% of cases of community-acquired pneumonia (9) among older children and adults and has also been implicated in a variety of respiratory tract infections. These cases tend to be relatively mild; however, this pathogen can lead to severe, even fatal, cases of pneumonia (15, 20). Therefore, the development of rapid, sensitive, and specific diagnostic techniques is necessary. The laboratory diagnosis of *M. pneumoniae* infections presently relies upon conventional serological methods. However, these methods provide only retrospective diagnosis and require paired serum samples to demonstrate a significant increase in antibody titer; in addition, false-negative results have frequently been reported for immunocompromised hosts (11, 13). Recently developed PCR techniques show high specificity and sensitivity (4, 16, 17). Various studies have compared PCR techniques with serological diagnosis of *M. pneumoniae* infection (3, 5, 8) and have shown the former to be superior to serological diagnosis with respect to speed, sensitivity, and specificity. However, these results must be validated in the number of times that the PCR is performed necessary to reduce the rate of false-negative results.

**MATERIALS AND METHODS**

**Patients.** Clinical specimens were routinely obtained from patients with signs of community-acquired respiratory tract infection and admitted to the First Department of Internal Medicine, Kurume University School of Medicine, and to the National Kyushu Medical Center between August 1996 and November 1998. A total of 325 samples (98 throat swabs, 120 sputa, and 107 BALF) were obtained from 197 patients and examined in the present study.

**Preparation of DNA specimens.** Patient sputum samples were incubated with the same volume of Sputatyme (semialkaline proteinase, 2.5 mg/ml, Nippon基因, 45 mM; KH₂PO₄, 21 mM [pH 7.2]; Kobayashi Pharmaceutical Co., Tokyo, Japan) at 37°C for 10 min. The samples were centrifuged at 1,600 × g for 15 min. The sediments were resuspended in 0.5 ml of TE buffer (10 mM Tris-Cl, 1 mM EDTA [pH 7.5]) and then spun for 10 s at 13,000 × g. This procedure was repeated twice, and the final pellet was resuspended in 100 μl of proteinase K buffer (50 mM KCl, 10 mM Tris-Cl, 2.5 mM MgCl₂, 0.5% Tween 20, 100 μg of proteinase K per ml [pH 8.3]). The mixture was incubated for 45 min at 50°C and then for 10 min at 95°C to inactivate the proteinase. BALF samples were centrifuged at 1,600 × g for 10 min. The sediments were resuspended in 1 ml of phosphate-buffered saline and then incubated with the same volume of Sputatyme. These samples were treated in the same manner as the sputum samples. Throat swabs were twisted in 1 ml of TE buffer, and aliquots were centrifuged at 1,600 × g for 10 min. The pellets were treated with 100 μl of proteinase K buffer. We used 2 μl of this mixture as the DNA sample.

**PCR.** Primers for amplification of the 250 bases in the region of the ATPase operon, MP5-1 (5'-TTGCCCTTAAAGGTGTTGACTTC-3') and MP5-2 (5'-CCATGTAGCTGATAGC-3'), were used for *M. pneumoniae*-specific amplification (18). Human DNA, DNA from species generally found in the respiratory tract, *M. genitalium* DNA, *M. salivarium* DNA, *M. orale* DNA, and *Escherichia coli* DNA gave negative results in the PCR with these primers (data not shown). DNA amplification for capillary PCR was performed with 50 mM Tris (pH 8.5)–3 mM MgCl₂–20 mM KCl–50 μg of bovine serum albumin per ml–0.5 μM each primer–0.5 μM each dideoxynucleotide triphosphate–2 μl of DNA sample–0.4 U of Taq polymerase (Promega Co.) per 10 μl unless specified otherwise. The reaction mixture was placed in the capillary tube by capillarity. The mixture was placed in the center of a 10.8-cm length of microcapillary tubing.
shown that capillary PCR is approximately 1,000 times more rapid than conventional PCR (6, 19, 21). Our studies have demonstrated that mycoplasma detection time can be rapidly achieved, and reaction times can be shortened (15 to 30 min from the start to the completion of the reaction). Thus, capillary PCR is more sensitive, specific, and rapid than conventional PCR (6, 19, 21). Our studies have shown that capillary PCR is approximately 1,000 times more sensitive than conventional PCR (10). The present study was designed to evaluate the usefulness of capillary PCR in detecting Mycoplasma pneumoniae.

We performed PCR analysis for Mycoplasma DNA on a total of 325 samples from 197 patients with community-acquired pneumonia and in whom Mycoplasma infection was suspected. There were 68 PCR-positive specimens. Review of the differences in PCR positivity rates based on the site of specimen collection showed the highest rate of detection (28.6%) from throat swabs. However, there were some problems with proper collection of throat swab specimens. For example, inadequate scraping often resulted in a false-negative result because of insufficient amounts of DNA. We recommend scraping the pharyngeal mucosa strongly and extensively. We attributed the low positivity rate (14.2%) from sputum samples to the fact that very few patients with Mycoplasma pneumonia have productive sputum. We then evaluated the results for patients with a definitive diagnosis of Mycoplasma pneumonia based on the measurement of serum Mycoplasma antibody titers. Among the 31 patients with significantly elevated titers of serum Mycoplasma antibodies, the PCR results were positive for 25. Thus, capillary PCR had a sensitivity of 80.6% (25 of 31). These results are in agreement with those reported by other authors. Of the PCR-positive cases in this study, only three showed no rise in serum antibody titers (i.e., false positives). Accordingly, capillary PCR had a specificity of 89.3% (25 of 28). This finding has been attributed to the persistence of Mycoplasma pneumoniae in the respiratory tract after acute infection or to the existence of an asymptomatic carrier state.

Among the patients with a definitive diagnosis of Mycoplasma pneumonia based on elevated serum antibody titers, the PCR results were negative for six (false negatives). To examine the cause of these false-negative results, we compared the results for throat swab specimens and BALF specimens (generally with high PCR positivity rates) from patients diagnosed with Mycoplasma pneumonia based on serum antibody titers (Table 2). One false-negative result was from a BALF sample. We considered the reasons for the PCR-negative result for this sample, including the possibility that the actual pneumonia lesion site had not been washed and/or that the DNA of M. pneumoniae was lost during the sample treatment process. BALF contain large amounts of substances inhibiting PCR. However, because additional bronchoalveolar lavage could not be performed, we cannot say with certainty why this negative result occurred. The other five false-negative results were from throat swab specimens. Testing (PCR) had been performed only once for all these cases. Sometimes we could obtain positive results for PCR-negative patients when additional scrapings of the pharyngeal mucosa were tested. We have already discussed potential problems in the collection of throat swab specimens. It is essential to scrape the pharyngeal mucosa strongly and extensively.

### TABLE 1. Results of PCR for M. pneumoniae

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Total no. of specimens</th>
<th>No. (%) of specimens that were PCR positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat swab</td>
<td>98</td>
<td>28 (28.6)</td>
</tr>
<tr>
<td>Sputum</td>
<td>120</td>
<td>17 (14.2)</td>
</tr>
<tr>
<td>BALF</td>
<td>107</td>
<td>23 (21.5)</td>
</tr>
</tbody>
</table>

### RESULTS

Table 1 shows the distribution of PCR results by specimen type. The PCR for M. pneumoniae was positive in 68 samples (20.9%) overall. The positivity rates were 28.6% for throat swab samples, 14.2% for sputum samples, and 21.5% for BALF samples. The PCR was most sensitive with throat swab samples. A fourfold or greater increase in serum antibody titers or standing titers of 1:128 or higher were observed for 31 patients, indicating the presence of M. pneumoniae in these patients. However, only 25 (80.6%) of the 31 patients had positive results in the PCR. The six patients (19.4%) who had negative PCR results showed rising serum antibody titers (false negative). Three patients (1.8%) who did not show rising serum antibody titers had positive PCR results (false positive).

Table 2 shows the distribution of PCR results by specimen type (BALF and throat swab) among patients with rising serum antibody titers or standing titers of 1:128 or higher were observed for 31 patients, indicating the presence of M. pneumoniae in these patients. However, only 25 (80.6%) of the 31 patients had positive results in the PCR. The six patients (19.4%) who had negative PCR results showed rising serum antibody titers (false negative). Three patients (1.8%) who did not show rising serum antibody titers had positive PCR results (false positive).

### DISCUSSION

DNA diagnostic methods have recently been established to enable the early detection of Mycoplasma infections. DNA diagnostic methods include the use of DNA probe techniques (14) and PCR techniques (1, 2, 12). DNA probe techniques are rapid and highly specific, but they often have insufficient sensitivity and are technically complicated. In contrast, PCR techniques, such as nested PCR and capillary PCR, have better sensitivity. In capillary PCR, a small amount of a sample is placed in a thin, narrow glass tube. This method allows for an improved rate of heat conduction in which air is used as the medium for temperature change. Target reaction temperatures can be rapidly achieved, and reaction times can be shortened (15 to 30 min from the start to the completion of the reaction). Thus, capillary PCR is more sensitive, specific, and rapid than conventional PCR (6, 19, 21). Our studies have shown that capillary PCR is approximately 1,000 times more sensitive than conventional PCR (10). The present study was...
PCR-positive findings from BALF specimens in this study were associated with elevated serum Mycoplasma antibody titers in all cases; there were no false-positive results. Thus, BALF specimens are very useful for the diagnosis of M. pneumoniae pneumonia, but the technical procedures and increased patient burden required to obtain these specimens make their routine clinical use impractical. The use of throat swab specimens is much more suitable for routine clinical practice. However, careful specimen collection is necessary to reduce the rate of false-negative results.

In summary, we have described the usefulness of capillary PCR for the diagnosis of Mycoplasma pneumonia. A capillary tube can be sealed with a gas burner in less than 10 min to overlay a sample with a mineral oil and close a microcentrifuge tube. After amplification, the ends of the glass capillary can be quickly scored with a file and snapped off easily, with the centrifuge tube. After amplification, the ends of the glass capillary tube can be sealed with a gas burner in less time than it takes to overlay a sample with a mineral oil and close a microcentrifuge tube. Moreover, capillary tubes also serve as transfer pipettes. The capillary PCR method used in this study does not require extensive skill and experience and saves time and expense, since a small amount of a reaction mixture can be used for testing. We believe that capillary PCR of throat swab specimens is a method which should be actively applied to patients with Mycoplasma pneumonia and that it could become the most cost-effective method for the diagnosis of M. pneumoniae pneumonia.

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ERRATA

Rapid Typing of *Borrelia burgdorferi* Sensu Lato Species in Specimens from Patients with Different Manifestations of Lyme Borreliosis

JAN D. LÜNEMANN, SILVIA ZARMAS, SUSANNE PRIEM, JULIANE FRANZ, ROLF ZSCHENDERLEIN, ELISABETH ABERER, ROLF KLEIN, LEON SCHOULS, GERD R. BURMESTER, AND ANDREAS KRAUSE

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Volume 39, no. 3, p. 1130–1133, 2001. Page 1132, legend to Fig. 1, lines 10 to 16: “line(s)” should read “row(s)” in every instance. Page 1132: Fig. 1 should appear as shown below.
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Volume 38, no. 4, p. 1382–1384, 2000. Page 1382, column 2, line 11 from the bottom: The sequence for primer MP5-1 should read “5'-GTCAGTGCGAGTGTAAGCA-3'.”