CHAPTER VI

DISCUSSION

Implementation of PCR technique in the detection of *C. pneumoniae* from clinical specimens to identify *C. pneumoniae* infection requires several issues to be considered, including the selection of gene target for amplification, appropriate primers, specimen collection, nucleic acid preparation, detection and attenuation of inhibitors of the amplification reaction, appropriate detection of amplification products, and the use of controls for detection of contamination and nonspecific reactions (108).

The sensitivity of the two PCR-based protocols were initially evaluated by using tenfold dilutions of a purified preparation of DNA from *C. pneumoniae* elementary bodies. The sensitivity of *ompl*-based PCR was 10 IFU of *C. pneumoniae* DNA when the products were analyzed by gel electrophoresis and increased to 1 IFU when the products were analyzed by dot blot hybridization or nested PCR. In contrast to *ompl*-based PCR, the sensitivity of 16S rDNA-based PCR was 1 IFU of *C. pneumoniae* DNA when the products were analyzed by gel electrophoresis and increased to 0.1 IFU when detected by dot blot hybridization or nested PCR. The result is similar to the results in the study by Metogho et al. that demonstrated the higher sensitivity of 16S rDNA amplification than that of *ompl* amplification (105).

Recombinant plasmid DNA controls corresponding to *ompl*-based PCR and 16S rDNA-based PCR were spiked into part of each sample instead of *C. pneumoniae* elementary bodies. This is due to the expensive cost of purified *C. pneumoniae*

elementary bodies. In four samples the positive control was not amplified unless the sample was diluted ten-fold, but none of these samples was positive for *C. pneumoniae* after dilution.

In this study, *C. pneumoniae* was detected in 15 throat swab samples from 114 patients with community-acquired pneumonia only by PCR. 16S rDNA nested PCR detected *C. pneumoniae* in all 15 positive samples. Ten out of 15 were detected by single-step PCR of both genes followed by dot blot hybridization, whereas *ompl* nested PCR could not detect *C. pneumoniae* in positive samples. The reason for no detectable product in these samples may be explained by the hypothesis that *C. pneumoniae* in our patients had heterogeneity in the DNA sequences of *ompl* gene corresponding to nested primers especially at the 3' end. To prove this hypothesis, the products of first amplification should be reamplified and sequenced. The organism was not detected by IFA. The reasons for negative result of IFA are unknown. The sensitivity of IFA depends on many factors; such as the number of the organism in clinical specimens, the type of specimens and the skill of the interpreter. In all positive samples, products of first amplification could not be detected by gel electrophoresis. Positive results were detected with dot blot hybridization or nested PCR. This might be the result of very low target organisms in the samples. Hahn et al. found that DFA could be used to detect *C. pneumoniae* if there were at least 12 inclusions of *C. pneumoniae* in the sample (109).

In contrast to IFA results, *C. pneumoniae* was detected by PCR in total of 15 out of 115 throat swab samples (Table 3). Twelve of these samples could be confirmed as true positive by MIF test (Table 5). Three PCR positive samples which

were negative by MIF test were considered to be false positive even though they were positive by both *omp1*- and 16S rDNA-based PCR. The use of an expanded gold standard with the second amplification test that targets a different gene has been purposed for validating of the new nucleic acid-based techniques to detect *C. pneumoniae* (33,108). One of the difficulties in evaluating nucleic acid amplification tests for the diagnosis of *C. pneumoniae* infections is the choice of the reference or gold standard. Because culture is relatively insensitive, many studies refer to serologic results, considering the presence of IgM, a fourfold increase in antibody titers during and after the acute disease episode, or an IgG titer of at least 1:512 to be significant. The presence of clinical symptoms cannot be taken into account, since asymptomatic infections by *C. pneumoniae* have been documented by culture and PCR (110).

The percentages of PCR positivity (12.3 %) that were observed in CAP patients in our studies corresponded well to the previous studies (33,18). *C. pneumoniae* has been accounted to be the cause of 6 – 20 % of case of CAP. Of 15 throat swab samples with PCR positive obtained from 14 patients, 11 had MIF antibody levels considered to be diagnostic of acute infection with *C. pneumoniae* (Table 4). The lack of diagnostic antibody titers among 2 patients who were PCR positive indicates a low sensitivity of serological test with a single sera specimen. If convalescent sera had been obtained, more of these patients might have had diagnostic titers. It has been suggested that a third sample obtained 2 months after onset may be useful to detect late rises in antibody titer (22). One patient who had PCR positive result without serological diagnostic titer, the convalescent sera showed a stable IgG level of 1:256 which was defined as preexisting antibody, or it may be

caused by past infection following with chronic or persistent infection with the organism. Forty eight patients (48.0 %) with PCR negative results had antibody levels considered to be diagnostic of *C. pneumoniae* infection. Hyman et al. reported significant antibody titers against *C. pneumoniae* in almost 20 % of culture and PCR negative, subjectively healthy adults (111). The failure to detect *C. pneumoniae* by PCR or IFA in these patients with serologic evidence of infection may be caused by an inadequate sample or to sampling site. To date it is unknown which specimens are most useful for the detection of *C. pneumoniae*. Boman et al. compared different respiratory sampling sites, sputum samples seem to be superior to nasopharyngeal and throat swabs for the detection of *C. pneumoniae* (34) whereas, Norman et al. found a higher percentage of positive PCR tests when specimens were collected with throat swabs (112). The sputum produced in *C. pneumoniae* pneumonia is usually of poor quality and not purulent. Some patients cannot produce sputum at all, therefore nasopharyngeal and throat swab samples are of value for patients who cannot produce a sputum sample. However, the possibility that chlamydial serology was problematic cannot be excluded, because some authors have reported a high seroprevalence of IgG and IgA antibodies to *C. pneumoniae*, sometimes with elevated titers (113). Some high anti-*C. pneumoniae* IgG antibody detected by MIF may be heterotypic, either due to infection with other chlamydial species or other organisms including *Bartonella* and *Bordetella pertussis* could also have occurred (114).

There were 2 throat swab samples with PCR positive obtained from the same patient. The second throat swab and serum samples were collected 15 months later. Results of MIF test revealed fourfold rise titer in IgG and an IgM antibody titer of 1:16. The patient had only IgG antibody titer of 1:512 on the follow-up serum sample.

Hammerschlag et al. suggested that persistent respiratory infection with *C. pneumoniae* may frequently follow acute infection and that the infection may be present for many months. *C. pneumoniae* may be very difficult to eradicate from the respiratory tract with use of currently available antibiotics even if there is a clinical response to therapy (73). The seroprevalence to *C. pneumoniae* in CAD patients in these studies was 92.1 %. In general adult population, the prevalence of antibody against *C. pneumoniae* is varied from 50 – 90 % (70,71).

This study demonstrated that the choice of the gold standard remains difficult and has a major impact on the sensitivities and specificities of the test validated in this study. Additional studies are needed to evaluate further especially on the sensitivity and specificity of PCR for the detection of *C. pneumoniae*. We detected *C. pneumoniae* DNA in the specimens of CAD patients who had no evidence of acute *C. pneumoniae* infection by serology and we found *C. pneumoniae* DNA negative patients but serological showed the evidence of acute infection. However, we found that 16S rDNA nested PCR is a rapid, simple and sensitive method for detection of *C. pneumoniae* in clinical specimens. Therefore, the combination of PCR with MIF test are suggested for diagnosis and study of *C. pneumoniae* infection.