CHAPTER I

INTRODUCTION

The chlamydiae are among the more common pathogens throughout the animal kingdom. They are nonmotile, Gram-negative, obligate intracellular bacteria that we once regarded as viruses. They differ from viruses by possessing both RNA and DNA and have cell walls quite similar in structure to those of Gram-negative bacteria. They are susceptible to many broad-spectrum antibiotics, possess a number of enzymes, and have a restricted metabolic capacity. None of these metabolic reactions results in the production of energy. Thus, they have been considered as energy parasites that use the ATP produced by the host cell for their own requirements. Chlamydiae have a unique developmental life cycle, with an intracellular growth, replicative form, the reticulate body (RB) and an extracellular metabolically inert, infective form, the elementary body (EB) (1,2).

Chlamydia pneumoniae is the recently recognized third species of the genus *Chlamydia*. It was formerly known as *Chlamydia psittaci*, strain TWAR. The strain name TWAR came from the laboratory identifying letters of the first two isolates: TW-183 and AR-39. TW-183 was isolated from the eye of a child in Taiwan during a trachoma vaccine trial in 1965. AR-39 came from a throat swab of student at University of Washington who had pharyngitis in 1983. At first, the TWAR organism was considered to be *C. psittaci* because it clearly did not belong to the *C. trachomatis* species. In 1987, the TWAR strain was established as a separate species of *Chlamydia* by DNA homology studies and the unique morphology of its elementary body (3-6). At present, *C. pneunoniae* is a common and important cause

of respiratory infection worldwide. Seroepidemiologic studies indicate that *C. pneumoniae* is one of the most widespread infectious agent. Approximately 40% to 60% of adult population around the world have antibodies to *C. pneunoniae*, which suggests that the infection is extraordinry prevalent and reinfection is common(7-12). In Thailand, the seroprevalence to *C. pneumoniae* has been reported to be approximately 40 - 70% (13-15). It is known to be the cause of both upper respiratory tract infections such as pharyngitis, sinusitis, otitis media, and lower respiratory tract infections such as bronchitis, mild to severe pneumonia and asthma. *C. pneumoniae* has been associated with both epidemic and endemic occurrence of acute respiratory disease. It is the cause of about 6 - 20 % of all cases with community acquired pneumonia either in outpatient clinics or in hospitals and 5% of all bronchitis cases. In most studies of pneumonia, *C. pneumoniae* has been the third or fourth most common cause of pneumonia identified. The patient's age at the time of infection influences the clinical response. Pneumonia due to *C. pneumoniae* is more common among the elderly and less common among persons less than 20 years of age (16-23). *C. pneumoniae* has a tendency to cause chronic and persistent infection. In addition to its role in respiratory diseases, there is growing evidence that *C. pneumoniae* may be involved in the pathogenesis of atherosclerosis, as several studies have demonstrated the presence of the organism in atherosclerotic lesions (24-30).

Since *C. pneumoniae* can cause severe clinical disease, correct diagnosis and therapy are important issues. However, conventional assays for the detection of *C. pneumoniae* have limitation, thus there is a need for more accurate diagnostic methods. Laboratory diagnosis of *C. pneumoniae* infection is made by isolation of

the organism in cell culture, or serologically by the microimmunofluorescence (MIF) test. However, in contrast to *C. trachomatis*, it is difficult to recover *C. pneumoniae* from clinical specimens. Despite efforts to improve the sensitivity of cell culture, only a few laboratories have been able to isolate and propagate *C. pneumoniae* from clinical specimens (1,18) . MIF test is the only sensitive and specific serologic assay for *C. pneumoniae* but it requires the detection of rising antibody titers in both acute and convalescent phase sera, resulting in a delay in definitive diagnosis. Furthermore, the value of MIF serology has been questioned since the lack of specific antibody was observed in sera of patients from whom the organism could be isolated(24,25). ELISA test may be used for diagnosis of acute respiratory chlamydial infections, even though there is a cross-reactivity among the *Chlamydia* species(31). Thus, alternate methods of identification should be helpful in improving the detection of *C. pneumoniae* infection.

Recently, the polymerase chain reaction (PCR) techniques have been developed for detecting *C. pneumoniae* by using DNA primers derived from different DNA target sequences (31-35). The results of these studies showed that the PCR technique provided high sensitivity and specificity for detection of *C. pneumoniae.* In addition, it is not time consuming and comparatively easy to perform. Therefore, it is important to set up a PCR-based protocol which is sensitive, specific and rapid for routine diagnosis of *C. pneumoniae* infection. In this study, the DNA target for amplification will be compared between the major outer membrane protein gene (*ompl*) and the 16S rRNA gene. However, because of the high sensitivity of the PCR technique, false positive from carry over contamination of the previous amplified products often occurs. Thus, a uracil-N-glycolylase (UNG) is used to

degrade contaminating amplification products from previous PCRs. In order to identify inhibitors of the PCR, we construct the DNA control for the amplification of *C. pneumoniae* DNA with the same primer pairs used for the target DNA.