CHAPTER I

INTRODUCTION

Mycoplasma pneumoniae is the smallest and simplest self-replication prokaryotes that lacks cell wall and resists to cell wall-active antibiotics. The morphology of mycoplasmas show a marked polymorphism (1). They are rounded, elongated, fusiform, bottle-shaped or filamentous. The organism is 0.2-0.3 μm in size. Colonies on agar media are usually small (50 to 500 μm) and show the typical fried-egg shape, consisting of an opaque, granular central zone on the agar surface.

M. pneumoniae is an important respiratory tract pathogen. It is a leading cause of atypical pneumonia and other respiratory infections such as tracheobronchitis, bronchiolitis, croup, and less severe upper respiratory tract infections (2). The incidence rate varies greatly according to age and is highest among primary school children (3-5). Epidemics take place at intervals of 4 to 7 years (3,6). Infections with this organism are endemic in densely populated areas; cyclic increases occur at long intervals and result in prolong epidemics (6). Epidemics also occur in military recruits, public schools, university students and day-care centers (7-11). Approximately 10% of the cases of community-acquired pneumonia that occur in endemic periods and up to 50% of the

cases that occur in epidemic periods are caused by *M. pneumoniae* (3). Several studies have found an *M. pneumoniae* infection rate of 10% to 20% in hospitalized pediatric patients and 20% to 40% in ambulatory pediatric patients with pneumonia (3,12,13). In Thailand definite *M. pneumoniae* pneumonia was first diagnosed in 1975 (14).

M. pneumoniae pneumonia may be self-limited within 1-3 weeks. Serious pneumonia caused by *M. pneumoniae* infection and its complication, i.e. pericarditis, hemolytic anemia and meningoencephalitis frequently occurs (15-16). Clinically, *M. pneumoniae* pneumonia cannot be differentiated from pneumonia caused by other bacteria or viruses. A specific diagnosis is important because treatment of *M. pneumoniae* infection with β-lactam antibiotics is ineffective, whereas treatment with macrolides or tetracycline may markedly reduce the duration of the illness (13,17).

The diagnosis of *M. pneumoniae* still relies on classical culture and serology, both of which are impractical clinically. Culture is time-consuming, labor-intensive, successful in only 30%-60% of serologically diagnosed cases (18-20), and although the presenting of *M. pneumoniae* in the oropharynx is usually associated with disease, the organism is frequently cultured from throat specimens of convalescent patients for as long as 6-8 weeks after clinical recovery (21). Serologic methods are easier but are generally nonspecific, insensitive, and retrospective (22-27). In patients with primary infections, immunoglobulin M (IgM) can be detected from 7 days after the onset of symptoms but in patients with reinfection, the demonstration of four-fold

rise in Immunoglobulin G (IgG) antibodies is required. However, the recently available microparticle agglutination (MAG) assay has been marketed and shown to be specific and sensitive (28). Rapid direct tests such as antigen detection and hybridization with DNA probes were developed. DNA probes and antigen-detecting ELISA have good specificity but low sensitivity (29). The use of radioactive labeling of DNA probe makes it inappropriate for routine diagnosis (30-34).

Recently, polymerase chain reaction (PCR) has been developed for detecting *M. pneumoniae*. It seems to be the most promising direct technique because of its high sensitivity and specificity (35-38). However, different primer sets for the detection of *M. pneumoniae* have been described (39-44); detection of the amplification product is usually performed by hybridization with a specific probe, which is very time-consuming (45); and none had protocols to prevent false positive results due to contamination of previous PCR products. Therefore, it is important to set up a PCR-based protocol which is more suitable and reliable for routine diagnostic procedures to detect *M. pneumoniae* in clinical specimens. The target of amplification will be compared between P1 gene which codes for P1 cytadhesin on cell surface (46), and 16S rDNA (47). A rapid alternative method for sensitive detection of *M. pneumoniae* DNA is a two-step PCR (nested PCR) with the use of uracil-N-glycosylase (UNG) in first step PCR to prevent false-positive from product carry over (48).