CHAPTER I

INTRODUCTION

Mycoplasma pneumoniae is the smallest and simplest self-replicating procaryotes 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 um in size. Colonies on agar media are usually small (50 to 500 um) and show the typical fried-egg shape, consisting of an opaque, granular central zone embedded in the agar, and the flat translucent peripheral zone on the agar surface.

M. pneumoniae infections occur most frequently in school-aged children and young adults (2,3). Infections with *M. pneumoniae* are endemic in densely populated areas ; cyclic increases occur at long intervals and result in prolong epidemics(4). Epidemics also occur in military recruits, public schools, university students and day-care centers (5-9). In Thailand definite *Mycoplasma pneumoniae* pneumonia was first diagnosed in 1975 (10).

M. pneumoniae is a common cause of community-acquired respiratory tract infections. 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* (2). *M. pneumoniae* pneumonia may be self-limitted within 1 - 3 weeks. Serious pneumonia caused by *M. pneumoniae* infection and its complication frequently occurs(11,12). Therefore,

the correct diagnosis is the most important for treatment and prevention of complication. Since clinical features of *M. pneumoniae* infections present from subclinical to upper respiratory tract infection and broncopneumonia, clinical diagnosis alone may not be enough and laboratory diagnosis must share the meaning role for treatment advantage.

A major obstacle in diagnosing infections caused by *M. pneumoniae* is the lack of a rapid and sensitive method for detecting the organism in clinical specimens (13). Culture is relatively insensitive and generally requires a week or more for recovery of the organism (100% specificity and 81.1% sensitivity)and thus is not practical in most laboratories (14). A DNA probe that provides rapid results has recently been marketed. However, the use of radioactive labelling and the low sensitivity of the test makes it unappropriate for routine diagnosis(15-19).

Several different serological tests for diagnosis of infections due to *M. pneumoniae* have been described, but these also have limitations in terms of sensitivity and specificity (20-26). Serological diagnosis is also time-consuming since Immonoglobulin M (IgM) can be detected from 7 days after the onset of symptoms in patients with primary infections and the demonstration of fourfold rise in Immunoglobulin G (IgG) antibodies in patients with reinfection. However, the recently available microparticle agglutination (MAG) assay has been marketed and shown to be specific and sensitive for diagnosis of *M. pneumoniae* infections(27).

Recently, polymerase chain reaction(PCR) has been developed for detecting *M. pneumoniae* by using DNA primers derived from

different DNA target sequences. The targets are specific cloned DNA segment (28-30), cytadhesin P1 gene (31,32), 16S rRNA gene (32-34), the gene encoding elongation factor *Tu* (35) and the 16S rRNA(34). These studies showed that the use of PCR for detecting *M. pneumoniae* was rapid and specific but the detection limit was varied from as low as 1 - 10 to as high as 1,000 organisms. However, most of these studies need radioisotope and none had protocols to prevent false-positive results due to the contamination of previous PCR products. It is thus proposed to set up a PCR-based protocol which is more suitable and reliable for routine diagnostic procedures to detect *M. pneumoniae*. The target of amplification wiil be P1 gene which codes for P1 cytadhesin on cell surface and is present in multiple copies in the *M. pneumoniae* genome (36)

RESEARCH OBJECTIVE

The purposes of this study were as follows :

- develop PCR-based method which is suitable and reliable for detection of *M. pneumoniae* in routine diagnostic laboratory.
- evaluate the use of developed PCR in simulated clinical samples.