CHAPTER VII

CONCLUSION

The PCR for detection of *Mycoplasma pneumoniae* was set to the use of two sets of primers which one specific to P1 gene (MP-PCR) and the other specific to 16S rRNA gene (16S rDNA PCR). The amplified products were analysed by agarose gel electrophoresis, the sensitivity of detection of MP-PCR and 16S rDNA PCR was 10 fg of *M. pneumoniae* DNA. Two-step PCR (nested PCR) was developed MP-nested PCR, and 16S rDNA nested PCR. The sensitivity of detection was increased to 1 fg and 0.1 fg of *M. pneumoniae* DNA by MP-nested PCR, and 16S rDNA nested PCR, respectively. The efficiency of the nested PCR for detection of *M. pneumoniae* in clinical specimens was evaluated and found that six patients were positive for *M. pneumoniae* detection, which correlated with serology test (MAG assay) and one patient was suggestive case. The false-positive due to amplicon carryover was prevented by using incorporation dUTP instead of dTTP and adding the UNG in reaction mixture prior to PCR. (For this experiment, amplicon carryover was not found in all PCR tested because the negative controls were always negative). None normal controls were amplified by PCR, it proved to be reliable method. Consideration of sensitivity and specificity, the developed 16S rDNA nested PCR-based protocol was suitable and reliable for rapid detection of *M. pneumoniae*. However, combination of serological test and culture should also be performed for higher confidence of the results.