

CHAPTER VI

CONCLUSION

The PCR for detection of *Mycoplasma pneumoniae* was set by using the primers specific to P1 gene. When the amplified products were analysed by agarose gel electrophoresis, the PCR had a sensitivity of 10 fg of *M. pneumoniae* purified DNA. The sensitivity of the PCR was increased to 1 fg when the amplified products were analysed by fluorescein-labelled dot blot hybridization and nested PCR. The efficiency of the PCR for detection of *M. pneumoniae* in simulated specimens was evaluated and found that sensitivity was 100 fg of serial dilution of spiked *M. pneumoniae* DNA. 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 had not found in all PCR tested because the negative controls were always negative. Consideration of sensitivity and specificity, the use of nonradioisotope-labelled probe, the use of enzymatic degradation, the developed PCR-based protocol was more suitable and reliable to routine diagnostic of *M. pneumoniae*.