CHAPTER IX

CONCLUSIONS

Measurement of MIC for *P. falciparum* by PCR technique requires several procedures 1) performing drug susceptibility test, 2) DNA extraction of parasites, 3) primers sensitivity test and 4) using PCR and RT-PCR technique for evaluating MIC value. Initially, three different methods of DNA preparation from pyrimethamine susceptibility test of *P. falciparum* were compared in order to find out the most compatible approach to perform PCR. The most appropriate technique for DNA preparation was phenol chloroform extraction method because this technique supplied high quality of repeatedly DNA and gene amplification products at reasonable cost. Particularly, this method can be performed in a large number of samples at the same time. PCR and RT-PCR technique were used to detect the parasite tested against antimalarial drugs. The use of PCR technique to detect the low level of parasite requires the sensitivity data of primers used; *rap-1* and *ssrRNA* primers. It was found that the sensitivities of *rap-1* primers were a bit lower than that of microscopic assay (in PCR) and as sensitive as microscopy (in RT-PCR) whereas the sensitivity of *ssrRNA* primers is much higher than that of microscopy (both PCR and RT-PCR). Therefore, the assessment of drug susceptibility test using the microscopic examination could be confirmed by PCR or RT-PCR technique of *rap-1* primers because of its sensitivity.

According to the drug susceptibility results by microscopic examination, the MIC values of *P. falciparum* T9/94RC17 against quinine, mefloquine, chloroquine and pyrimethamine are 5×10^{-7}, 2×10^{-7}, 5×10^{-7}, and 5×10^{-8} M respectively. From the PCR and RT-PCR outcomes, the *ssrRNA* primers could not be used for evaluating the survived parasite during drug susceptibility test because these primers are too sensitive to measure survived parasites as well as the RT-PCR technique of *rap-1* primers. Consequently, this study reveals that the PCR of *rap-1* primers have a potential to be used for detecting the presence of survived parasite during the drug susceptibility test. An attempt to apply the direct PCR and RT-PCR, to further simplify the method, the experiment revealed that both techniques could not be used for evaluating the drug susceptibility test. The direct PCR cannot detect the living parasites even in control

group which may caused by high amount of PCR inhibitors while the direct RT-PCR technique was too sensitive.

From the sensitivity test with other isolates of *P. falciparum*: MH20, TD12 and K160, the PCR of *rap-1* gene has a potential to be develop for parasite detection in drug susceptibility test.

According to all results, the PCR assay using *rap-1* primer is more sensitive than microscopic examination assay. Giemsa-stained thick blood smears may be used to carry out epidemiological retrospective studies, but one limitation to its use is that samples containing a low number of parasites may produce false negative results by PCR. This research demonstrates that PCR can be a practical and effective surveillance tool for screening the MIC values from drug susceptibility test. However, the existing difficulty of this research is the visualization of PCR product band which is depended on contrast of picture, staining of agarose gel with ethidium bromide and photography. These limitations are variable factor of PCR product band detection. Consequently, to solve this problem, the use of real-time PCR may be another practical developed approach for parasite detection because this technique gave in quantitative data that would be more accurate and acceptable.

More works are needed to further clearify some barriers which will be a great benefit for drug susceptibility monitoring system and to public health service to find proper treatment to malaria patients.