CHAPTER VIII

Discussion
Historically microscopy has been used as a traditional method for the
diagnosis of malaria. Its main limitation is the expertise required for optimal staining
and accurate interpretation of blood films for different Plasmodial species. The
difficulty in identifying various strains commonly used in laboratories and in
epidemiological field studies of *P. falciparum*, has been an impediment to understand
their origin and inter-relationship between geographically different strains. Due to
limitations of microscopic diagnosis of malaria, now more advanced diagnostic
methods have been developed, which are based on the amplification of nucleic acids
by polymerase chain reaction (PCR) (Edoh et al., 1997, Tirasophon et al., 1994),
detection of trophozoite derived histidine-rich protein-2 antigen (ParaSight F antigen
capture assay; Dipstick Test) (Genton et al., 1998, Humar et al., 1997, Schiff et al.,
1993) and parasite-specific lactate dehydrogenase (pLDH, OptiMAL® assay) (Makler
et al., 1993, Makler et al., 1998) by monoclonal antibodies have become available for
sensitive detection, speciation, and quantitation of all human *Plasmodium* infections.

PCR amplification of parasite DNA represents alternative method for
detecting low level of DNA that could be used to identify subpatent parasitemia. Due
to high specificity and sensitivity it is also very useful in genotyping of parasites from
individuals with non-clinical low level infections. The applicability of PCR in
understanding the diversity in certain Plasmodial genes, determining characters such
as drug resistance and antigens in field populations of *P. falciparum* has been
investigated. Different isolates of *P. falciparum* and of other Plasmodial species
exhibit a high degree of biological diversity in MSP-1, MSP-2, CSP and RESA cell
surface antigens. The allelic diversity that exits in certain genes of *P. falciparum* is
due to extensive degree of recombination in parasite population which generates
different strains with variable characters (Walliker et al., 1994, Walliker, 1989). In this
study we have described a simple, rapid and non-radioactive method for identifying
size polymorphism in PCR products from MSP-1, MSP-2, CSP and RESA of *P.
falciparum*. The PCR genotyping of the genes encoding the highly polymorphic loci
MSP-1, MSP-2, CSP and RESA of *P. falciparum* provides a highly sensitive and
specific method for the detection of malaria infection and strain identification of *P.
falciparum*. For detection and strain identification of *P. falciparum*, DNA was
extracted from Giemsa stained thick smear and blood of infected patients by chelex-
100 method (Masatsugu et al., 1995, Wooden et al., 1993). PCR was performed with parasite DNA extracted from different blood samples and Giemsa-stained thin and thick smears using cell surface antigen specific primers. The amplified products from various field isolates from patients were analyzed on agarose gel.

The variations observed in size of amplified products of MSP-1 and MSP-2 surface antigens in the present study, illustrates their extensive allelic diversity in natural *P. falciparum* population, however no size polymorphism was detected in these isolates in relation to CSP and RESA antigens. One *P. falciparum* infected blood sample which was identified by microscopic examination as single strain was diagnosed by the PCR method as mixed infection of two different strains of *P. falciparum*.

A multiplex PCR approach using primer pairs for MSP-1 and MSP-2 simultaneously with different *P. falciparum* infected isolates showed different banding pattern for MSP-1 and MSP-2 due to genetic polymorphism in different alleles of these surface antigens. This difference in banding pattern obtained could be used to identify different strains of *P. falciparum*.

The primer pairs used in this study are highly specific for surface antigens MSP-1, MSP-2, CSP and RESA of *P. falciparum*, still they diagnosed one *P. vivax* infected blood sample when amplified using MSP-2 specific primers. This difference in results from microscopy and PCR method is due to incorrect speciation. By microscopic examination of Giemsa-stained blood smears it is difficult to distinguish *P. falciparum* infection from *P. vivax* if only ring stage is present.

Out of a total of 52 isolates, 47 were identified positive by using different primer pairs for surface antigens MSP-1, MSP-2, CSP and RESA of *P. falciparum*. The five microscopically positive but PCR negative samples may be due to degradation of DNA during extraction or storage, inhibition of the PCR amplification by sample, insufficient amount of target DNA due to inadequate cellular lysis and most importantly incorrect species identification by microscopic examination. The simultaneous application of PCR method with microscopy for diagnosis of malaria
infection provided convincing evidence that PCR detects specimens containing \textit{P. falciparum} DNA when parasite densities are below microscopic threshold.