PART B
CHAPTER V

Introduction and Review of Literature
Diagnosis and epidemiological surveillance are essential elements in the management of the malaria infection both at the level of treatment of malaria infected patient and to control the spread of the drug resistance strains. The major problem in recent years has been development of drug resistance parasites and appearance of various new strains with in all the pre-existing *Plasmodium* species. The microscopic examination of blood for the diagnosis of malaria parasites is quite sensitive and semiquantitative method in the hands of skilled technician. Traditional diagnosis of malaria which is based on microscopic examination of Giemsa-stained thick and thin blood smears has drawbacks due to its labor intensive nature, inability to identify drug resistance strains, strain heterogeneity and to monitor the efficacy of chemotherapeutic treatment of malaria. The number of slides that can be accurately examined in a day range between 40 to 60.

Alternative methods developed for diagnosis of malaria are based on species specific DNA diagnostic probes which are applicable for large scale screening of field samples (Barker 1990, Barker *et al.*, 1986). Recently serodiagnostic tools based on the dipstick principle for the detection of plasmodial histidine-rich protein 2 (HRP-2) or parasite-specific lactate dehydrogenase (pLDH), have become available for the qualitative detection of *P. falciparum* malaria and it has potential of enhancing speed and accuracy of the diagnosis of *P. falciparum* malaria, especially if nonspecialized laboratories and skilled technician are not available in malaria endemic areas. A *Plasmodium* lactate dehydrogenase (pLDH) dipstick has been designed separately to diagnose *P. falciparum* and *P. vivax* malaria and evaluated in populations where both species are endemic. The dipstick is unable to detect *P. vivax* in the presence of *P. falciparum* because of cross-reactivity in the pan-specific band, so accurate species identification in mixed infections and strain identification still remains a problem in malaria diagnosis (Quintana *et al.*, 1998). Hence the development of new diagnostic methods for identification of low level of infection and of different strains of malaria parasites is of utmost importance.

Molecular genetics is having an important impact on the study of genes in natural populations of malaria parasites. The polymerase chain reaction (PCR) is proving particularly valuable for identifying genes in parasites taken directly from their hosts, without the need to establish them in culture. The polymerase chain
reaction (PCR) has been widely used to monitor treatment response in human immunodeficiency virus infection (Hamed et al., 1993); *P. falciparum* and *P. vivax* infection (Kain et al., 1993, Irion et al., 1998) and other antimicrobial chemotherapeutic treatments (Aurelius et al., 1990). The PCR amplification of highly polymorphic cell surface antigens (Edqh et al., 1997), small-subunit rRNA gene (Ciceron et al., 1999), highly repeated subtelomeric sequence (rep20) (Urdaneta et al., 1998), DHFR-TS (dihydrofolate reductase-thymidylate synthase) gene (Wataya et al., 1991) and p126 gene of *P. falciparum* (Zalis et al., 1996), is another alternative highly sensitive and non radioactive method for diagnosis of the malarial parasite. Due to its high specificity and sensitivity it can detect parasite level which is below microscopic threshold and also is very useful in genotyping of different *P. falciparum* strains from different geographic origin.

Genes that encode for cell surface antigens e.g. Major merozoite surface antigen-1 (MSP1; Mackay et al., 1985), major surface antigen-2 (MSP2; Fenton et al., 1991; Smythe et al., 1991), circumsporozoite surface antigen (CSP; Dame et al., 1984) and ring-infected erythrocyte surface antigen (RESA; Favalaro et al., 1986) which are considered potential vaccine candidates and are generally used to identify different strains of the *P. falciparum*. The two *P. falciparum* antigens located on the merozoite surface (MSP1 and MSP2) exist in numerous alleles in natural population of *P. falciparum* exhibiting both sequence and size polymorphism in tandemly repeated region. The PCR has been used to amplify tandemly repeated segments using primers recognising conserved sequences in the flanking region. In circumsporozoite surface protein (CSP) of *P. falciparum*, size and sequence polymorphism of tandem repeat segment has been observed within *Plasmodial* species. The ring-infected erythrocyte surface antigen (RESA) antigen shows sequence differences but no size polymorphism between different strains has been identified (Kemp et al., 1987). In the present study applicability of polymerase chain reaction (PCR) for strain identification of *P. falciparum* in several field isolates has been worked out.

The diagnostic tests used to detect an infection should be simple, rapid, specific, highly sensitive and able to differentiate between closely related parasite species. Improvement in malaria diagnostics should facilitate the identification of patients infected with drug resistance or sensitive *Plasmodial* species, to distinguish
recrudescent malarial infection (treatment failure) from reinfection (new infection), to monitor the efficacy of antimalarial treatment and treatment of such cases with appropriate drug. With the recent advances in the field of molecular biology, highly sensitive and rapid techniques have been developed for parasite diagnosis. The advanced and conventional diagnostic tools currently available for diagnosis of malaria parasites and relative merits of each method are discussed below in detail:

**Microscopic examination:** The microscopy has been essential for the diagnosis of malaria which involves the examination of Giemsa-stained thick and thin blood films. This traditional method allows identification of various parasite development stages, estimation of parasitemia, degree of anemia (density of erythrocytes and reticulocytes) and detection of other parasitic infections. Because of minimal technological requirement and short time lag between sample collection and diagnosis this is a suitable diagnostic tool in acute infection cases or when number of samples to be analyzed are less. An expert microscopist can detect 20-40 parasites per microliter (or a parasitemia of 0.0004%) in standard thick blood film (Bruce-Chwatt 1984). A trained microscopist can accurately examine only 70-80 slides per day and thus due to its labor-intensive and time consuming nature of this method it is not appropriate for large scale screening of the blood samples (Barker et al., 1989, Fraenzen et al., 1984, Lanar et al., 1989). Also by microscopic examination it is difficult to identify mixed *P. falciparum* and *P. vivax* infection if both are present at ring stage. While microscopy continues to be essential tool for malaria diagnosis, there is a growing need for new epidemiological tools that can easily distinguish between different species/strains of parasite and between present and past infection.

**DNA probe diagnostics:** This is another approach based on the detection of DNA sequences unique to a species of malaria causing parasite by using species specific DNA probes. In the genome of parasite there are certain sequences that are repeated tandemly, present in multiple copies and they have no cellular function. The parasite species specific differences have been identified and these differences are valuable for the diagnostic purpose. The repetitive sequences give good sensitivity as compared to single copy sequences when used as hybridization probes for detection of complementary sequence in small test DNA extracted from parasite or in whole organism. The specific DNA probe such as *P. falciparum*-specific probe pPF14’ can
detect 10 picogram of purified *P. falciparum* DNA which is equivalent to 100 parasites (Fraenzen et al., 1984, Barker 1990). With improved samples handling such as DNA extraction and use of non-isotopically labeled probes this method can detect 20-25 parasites/μl of infected blood. The sensitivity of this probe has been further increased through the amplification of the target DNA using PCR and with the PCR amplification, *P. falciparum*-specific probe pPF14' can detect a single organism in a 20 μl blood (Laserson et al., 1994). Microtiter plate-hybridization (MPH) technique has been developed for the detection of human malaria parasite in which the target DNA sequence of the 18S ribosomal RNA gene is amplified by PCR and hybridized with the species-specific probes immobilized on a microtiter well (Miyake et al., 1995). The pPF rep 20 is another DNA probe that recognizes the DNA of *P. falciparum*, but does not hybridize with the DNA of *P. vivax*, *P. cynomolgi*, *P. knowlesi* and *P. yoelii*. The rep 20 probe was able to detect parasitemia level of 0.001% in 20 μl blood from culture and 10 pg DNA of *P. falciparum* isolates (Diao et al., 1991, Delves et al., 1989). A simple procedure has been developed for spotting blood samples directly onto nylon membrane filter, without the necessity to treat samples with pronase or proteinase K, followed by hybridizing with *Plasmodial* species specific radiolabelled DNA probe (Pollack et al., 1985, Barker et al., 1986). This DNA probe based diagnosis method also requires expert technician and handling of radioactive isotopes. This makes it impractical under clinical or field conditions in endemic areas. Another disadvantage of this method is less sensitivity of *P. vivax* DNA probes to detect *vivax* malarial infection as compared to *P. falciparum* specific DNA probes (Barker 1990, Barker et al., 1992).

**Immunodiagnosis:** The essential element in immunodiagnosis is identification and purification of species-specific test antigens or highly specific antibodies. Recombinant DNA techniques permit the cloning and expression of genes encoding synthesis of antigens of diagnostic interest. Recently immunodiagnostic tools based on detection of plasmodial histidine-rich protein 2 (HRP-2) and parasite-specific lactate dehydrogenase (pLDH) have become available for sensitive detection, speciation, and quantitation of all human *Plasmodium* infections (Piper et al., 1999, Makler et al., 1998). The ParaSight-F test and the ICT Malaria Pf test are commercially available kits marketed for the diagnosis of *P. falciparum* malaria and
both tests are antigen-capture assays based on the detection of *P. falciparum* histidine-rich protein 2 in peripheral blood (Genton *et al.*, 1998, Humar *et al.*, 1997, Schiff *et al.*, 1993). The quantitative microplate method, immunocapture pLDH (ICpLDH) assay, has been developed that utilizes monoclonal antibodies (mAbs) to capture the pLDH and then to measure the captured enzyme by its ability to reduce 3 acetyl pyridine adenine dinucleotide (APAD) (Makler *et al.*, 1993, Makler *et al.*, 1998). A rapid immunochromatographic method, the OptiMAL® assay, has been formatted to capture pLDH as an antigen, and then to signal the presence of this captured antigen (enzyme) with a colloid conjugated antibody (Makler *et al.*, 1998). The dipstick test has high sensitivity and specificity as compared with microscopy in the diagnosis of *P. falciparum* infection and semi-quantitative assessment of parasite density can be done by analyzing intensity of the band. The serological assays are simple, more accurate and rapid (<10 min/test) for the detection of malarial parasite but due to cross-reactivity in the pan-specific band this method is unable to identify mixed infection by *P. falciparum* and *P. vivax* (Quintana *et al.*, 1998). The Parasight F test has been reported positive in patients with rheumatoid factor (Laferi *et al.*, 1997). The reason for these false positive results may be the presence of the HRP-2 antigen in the bloodstream after the the clearance of peripheral parasite or sequestered parasites or immunocomplexes and all these observations require further evaluation.

**Polymerase Chain Reaction (PCR) diagnostic:** PCR amplification of *Plasmodial* genes with a genus-specific primer set, and species-specific primer set to differentiate between *P. falciparum* and *P. vivax* by multiplex PCR has been evaluated for the diagnosis of malaria infections with laboratory strains and different field samples. The PCR amplification of the various genes e.g., highly polymorphic cell surface antigens (Edoh *et al.*, 1997), small-subunit 18S rRNA gene (Ciceron *et al.*, 1999, Das *et al.*, 1995), highly repeated subtelomeric sequence (rep20) (Urdaneta *et al.*, 1998), DHFR-TS (dihydrofolate reductase-thymidylate synthase) gene (Wataya *et al.*, 1991) and p126 gene of *P. falciparum* (Zalis *et al.*, 1996) has been employed to check the diagnostic potential of the malaria parasite. From various investigations it is implied that PCR is a specific, sensitive and valuable diagnostic tool in epidemiologic field studies of malaria infection, to monitor the response of patient during drug treatment, immunization trials and to distinguish recrudescent from a new infection in areas with active transmission. Based on the sequence diversity of the *Plasmodium* genes,
species-specific oligonucleotide primers cocktail for *P. falciparum* and *P. vivax* allows identification of the two human malaria species in a single amplification reaction and as compared to thick blood film microscopy it can detect one *P. falciparum* and/or *P. vivax* parasite per microliter of blood (Tirasophon *et al.*, 1994). The rapidity and simplicity of PCR for malaria detection has further increased by development of easy sampling and parasite DNA extraction methods from dried blood spotted on filter paper or blood from a finger prick or Giemsa stained thin and thick blood smears (Masatsugu *et al.*, 1995 and Wooden *et al.*, 1993). The use of Giemsa stained blood smears for PCR analysis is more convenient as they are made routinely in the hospital, easy to transport from health station in endemic areas and old smears can also be used for the extraction of parasite DNA. The reverse transcriptase-polymerase chain reaction (RT-PCR) method for the detection of malaria based on amplification of 18S ribosomal RNA gene can detect 0.3 parasites per microliter of blood (Abdullah *et al.*, 1996). The PCR-RFLP analysis during efficacy trial of a new compound drug CGP 56697 and of chloroquine against acute, uncomplicated *falciparum* malaria can distinguish between recrudescent from a new infection (Irion *et al.*, 1998). The nested PCR is another modification for detection of the malaria parasite *P. falciparum* by two set of oligonucleotide primers specific for the junction region of the gene coding for the DHFR-TS and intensity of the results by the nested PCR can be correlated with parasite densities (Arai *et al.*, 1994). The point mutation at nucleotide 323 within the DHFR-TS gene of *P. falciparum* causes resistance to pyrimethamine and these drug-resistant isolates can be identified by the PCR using mutation-specific primers (Zolg *et al.*, 1990). The potential disadvantage of PCR diagnostic is its labor-intensive nature, requires skilled expert, chances of the PCR contamination and well equipped laboratory in malaria endemic areas. Due to these major limitations it is unlikely that PCR will replace conventional microscopic diagnosis method but it is important diagnostic tool for identification and control of multidrug resistance parasite strains.

**CELL SURFACE ANTIGENS**

Genes encoding cell surface antigens has been considered as potential vaccine candidates and are generally used to study diversity in different strains of *Plasmodium* species. The difficulty in identification of various strains commonly used in
laboratories and in malaria endemic areas has been great obstacle to understand their origin and interrelationship. 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). The genetic variations in the malarial parasite genes can be analyzed by PCR and this polymorphism can be considered as genetic marker for the genotyping of *P. falciparum* population in field studies. Three genes for highly polymorphic antigens have been used widely as genetic markers: merozoite surface antigens 1 and 2 (MSA1 and MSA2) and the glutamate-rich protein (GLURP) (Snounou *et al.*, 1998, Björkman *et al.*, 1998). Beside these two more surface antigens, circumsporozoite surface antigen (CSP) and ring-infected erythrocyte surface antigen (RESA) also exhibit polymorphism but size polymorphism has not been observed in RESA antigen. The genes that encode for surface antigens have conserved 5' and 3' ends but contain a region with blocks of tandemly repeated sequences which vary in size and DNA sequence from strain to strain of *P. falciparum* (Kemp *et al.*, 1987). The degree of allelic diversity in many antigens of *P. falciparum* can be studied by amplification of tandemly repeated region, by selecting the primers from highly conserved 5' and 3' regions flanking the variable repeats. The species and strain specific allelic diversity in these three surface antigens i.e MSP1, MSP2, CSP and RESA are discussed below:

**Merozoite Surface Antigens:** The MSP1 and MSP2 are two integral membrane proteins associated with the surface of merozoites and are promising vaccine candidates against blood stage malarial parasite. The MSP1 of *P. falciparum* is a 195 kDa glycoprotein which undergoes proteolytic cleavage into smaller polypeptide 83 kDa, 42 kDa and 19 kDa fragments during the maturation of the merozoites (Mackay *et al.*, 1985, Kemp *et al.*, 1987). All the fragments are shed from the surface of the merozoites except 19 kDa fragment, it remains on the surface of the merozoite after invasion (Kemp *et al.*, 1987). The MSP2 is a smaller polypeptide of 45 kDa that is not processed like MSP1 during merozoite maturation and remains attached to merozoite surface by glycosylphosphatidylinositol (GPI) moiety (Fenton *et al.*, 1991, Smythe *et al.*, 1991). The MSP1 and MSP2 in the natural population of *P. falciparum* are present in antigenically diverse forms and this diversity has arisen due intragenic recombination between different alleles of merozoite surface antigens. The studies on the reactivities of MSP1 and MSP2 with strain specific monoclonal antibodies has
shown the presence of numerous serological variants of each antigen which is encoded by different alleles in different strain of *P. falciparum* (Fenton et al., 1991). The tandemly repeated region in MSP1 and MSP2 shows polymorphism in sequence and length in different alleles. The MSP1 gene has been divided into 17 discrete blocks depending upon the amino acid homology. The block 1 and block 2 are most highly conserved regions that encode N- and C-terminal sequences respectively. The schematic representation of conserved, repetitive and variable nonrepetitive regions of MSP2 gene are also shown in plate-1. The variable blocks 2, 4a, 4b, 6, and 10 of the MSP-1 gene of *P. falciparum* were typed by allelic type-specific PCR in northern Tanzania and numerous MSP-1 gene types were identified by unique combinations of allelic types detected in each variable block (Ferreira et al., 1998). Barnwell et al., 1999 identified the 185 kDa MSA from *P. vivax* which is posttranslationally not modified and remains bound to the merozoite membrane by a glycosylphosphatidylinositol (GPI) lipid anchor. Also similar MSA protein homologue is also present in *P. cynomolgi* and *P. knowlesi* which are expressed as 150-kDa and 110-kDa proteins. The extent of genetic polymorphism in highly polymorphic parasite genes encoding the merozoite surface antigens MSP1 and MSP2 of *P. falciparum* field isolates can be studied by the PCR which can be further used for genotyping, diagnosis and strain identification of *P. falciparum* parasite populations circulating in a particular region (Björkman et al., 1998). The characterization of allelic variants is achieved by length polymorphism analysis or by the use of methods with higher resolution such as restriction fragment length polymorphism (RFLP) and single-stranded conformational polymorphism (SSCP). The amplification of polymorphic domains and its subsequent (RFLP) fingerprint pattern analysis has been used to distinguish a recrudescence from a new infection during antimalarial treatment (Irion et al., 1998, Snounou et al., 1998). The PCR-RFLP analysis has been used to differentiate extensive polymorphism in MSP2 alleles in malaria endemic area in Papua New Guinea and classify MSP2 alleles on the basis of distinct digestion patterns of different alleles (Felger et al., 1994). The SSCP analysis is another strategy to identify minor sequence polymorphisms, which depends on the altered electrophoretic mobility of the denatured DNA. It can detect differences of as little as one base substitution between different allelic variants and is a field applicable method to distinguish re-infections from treatment failures (Kain et al., 1994). Furthermore different alleles of the same merozoite surface antigens in
multiple concurrent \textit{P. falciparum} infections get amplified and because of different length and/or sequence composition, yield individual products of different size which can be used to detect presence of multiple, mixed \textit{P. falciparum} infections in the sample. The other merozoite surface antigen MSA-4 protein located on the merozoite surface can be determined by immunofluorescence assays and contains hydrophobic signal sequences, glycosylphosphatidylinositol (GPI) attachment signals and a single epidermal growth factor-like (EGF-like) domain at their carboxyl termini (Marshall \textit{et al.}, 1997). The merozoite surface antigen-3 (MSP-3) is a secreted polymorphic antigen of molecular mass 48 kDa associated with erythrocytic schizonts and merozoites of \textit{P. falciparum} asexual blood stages (McColl \textit{et al.}, 1997). A distinct structural feature of MSP-3 is a domain composed of three blocks of tandemly-repeated heptads. Antibodies raised in mice against 48 kDa MSA-3, as well as human antibodies immunopurified on this peptide provides clinical protection against \textit{P. falciparum} malaria and the ability of specific anti-MSA-3 antibodies to block the parasite schizogony in the antibody-dependent cellular inhibition (ADCI) assay suggests that this molecule is involved in eliciting protective immunity (Oeuivray \textit{et al.}, 1994).

\textbf{Circumsporozoite Surface Protein}: The circumsporozoite protein (CSP) covers the surface of the sporozoite and has been identified from several species of the \textit{Plasmodium} e.g. \textit{P. falciparum}, \textit{P. vivax}, \textit{P. malariae}, \textit{P. knowlesi}, \textit{P. berghei}, \textit{P. yoelii} and \textit{P. cynomolgi}. It ranges from 40-60 kDa in these species (Kemp \textit{et al.}, 1987). The structural distinct features of CSP gene in all \textit{Plasmodium} species have a putative hydrophobic signal and anchor sequences at the NH$_2$ and COOH termini, respectively, two small regions (regions I and II) that are conserved in all CSP genes studied to date, and a central region containing the immunodominant, tandemly repeated peptide sequence (Plate-1). Different species of \textit{Plasmodium} exhibiting polymorphism in CSP is due to variation in the number, length and sequence of tandem repeat units but overall size of protein remains same. The central tandemly repetitive region of the CSP in \textit{P. malariae} consists of 45 copies of the sequence Asn-Ala-Ala-Gly and 6 copies of the sequence Asn-Asp-Ala-Gly (Lal \textit{et al.}, 1988) whereas the \textit{P. falciparum} CSP has 37 copies of tandem repeats of tetrapeptide Asn-Ala-Asn-Pro and 4 copies of Asn-Val-Asp-Pro (Lockyer \textit{et al.}, 1987). The immunodominant repeat region of CSP in \textit{P. yoelii} is composed of two distinctly
different types of tandem repeats, one tandem repeat unit is of six amino acids \text{Gln-Gly-Pro-Gly-Ala-Pro} in length while the other is four \text{Gln-Gln-Pro-Pro} residues long (Lal et al., 1987). The polymorphism in tandemly repeated domain of CSP in different strains within species has been observed but degree of polymorphism varies from species to species. The tandemly repetitive region of CSP in different strains of \textit{P. falciparum} is relatively conserved in the \text{Asn-Ala-Asn-Pro} and \text{Asn-Asp-Ala-Gly} tetrapeptide repeat unit sequence but number of repeat units can vary (Kemp et al., 1981). Immunization with irradiated-attenuated malaria sporozoites has been shown to protect both rodents and humans against a homologous sporozoite challenge but due to limited supply of sporozoites and its instability this approach is not applicable in large scale immunization. Irradiated-attenuated sporozoites retain their capacity to invade hepatocytes and transform into trophozoites without undergoing complete schizogony. Upon sporozoite challenge, in the immunized animals, the rate of transformation of sporozoites into hepatic parasites is less than 2%, with severe morphological changes in the exoerythrocytic stages of the parasite (Zechini et al., 1999). The protective immunity against \textit{Plasmodium} induced by immunization with irradiated sporozoites depends on both humoral and cellular response that induces IL-2 and IFN gamma but not IL-4 lymphokines (White et al., 1994).

\textbf{Ring-infected erythrocyte surface antigen (RESA):} The ring-infected erythrocyte surface antigen (RESA) in \textit{P. falciparum} is a 125-kDa polypeptide which is present in dense granules of merozoites and becomes associated with the erythrocyte membrane after phosphorylation by an erythrocyte membrane kinase at the time of merozoite invasion (Aikawa et al., 1990, Foley et al., 1990). The gene encoding RESA of \textit{P. falciparum} consists of two exons separated with a short intron located near the 5' end of the coding region and immediately upstream of intervening sequence is a short hydrophobic stretch of 13 amino acids which acts as a trafficking signal sequence (Favaloro et al., 1986). Like other cell surface antigens of \textit{Plasmodium} RESA contains two blocks, (5' and 3' tandem repeat) of tandemly repeated sequence in exon 2 as shown in plate-1. Both these blocks contain closely related acidic amino acids in their tandem repeat unit. The PCR amplification of 3' repeated region of exon 2 of RESA gene were studied in 150 \textit{P. falciparum} isolates from Thailand which demonstrated extensive degree of allelic polymorphism with substitution ranging from one to six bases in this region (Sueblinvong et al., 1996). The polymorphism in
RESA tandemly repeated domains are not due to change in repeat number or length that typify gene from different strains but as a result of allelic sequence variation. Immunization of Aotus monkeys with 5' repeat peptide of RESA protected against overwhelming infection with \textit{P. falciparum}. In this protection trial correlation between antibody responses to two of three repetitive sequences in 5' repeat block of RESA was documented (Collins et al., 1991). The amplification of RESA yields the amplified fragments of the same length from all the strains of \textit{Plasmodium} species and therefore provides a convenient control marker to ensure that all the lanes are running in the equivalent manner in agarose gel electrophoresis.

In the present study detection and typing of \textit{P. falciparum} strains by PCR was evaluated and this approach can be further used for molecular epidemiology studies of malaria, species diagnosis, assessment of intraspecies diversity, and transmission studies in areas of high endemicity. The feasibility, high sensitivity and high resolution of PCR based genotyping of \textit{Plasmodium} species proves its applicability for differentiating between true recrudescence and reinfection during efficacy trials of novel antimalarial compounds. The \textit{P. falciparum} strain specific polymorphic genetic markers merozoite surface antigen 1 (MSP-1), merozoite surface antigen 2 (MSP-2), circumsporozoite protein (CSP) and ring-infected erythrocyte surface antigen (RESA) were used in field genotyping of \textit{P. falciparum}. The efficacy and reproducibility of PCR protocols for detection and genotyping of \textit{P. falciparum} depends upon DNA extraction methods, template DNA, primer design, primer combination in multiplex PCR, genetic markers and product analysis. The advantages of simple DNA extraction by chelex method from Giemsa stained slides and presence of high degree of polymorphism in malarial genes were taken for rapid and sensitive typing of different \textit{P. falciparum} field isolates.