SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO ANTIMALARIAL DRUGS

Report on global monitoring 1996–2004

World Health Organization

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EXECUTIVE SUMMARY

Antimalarial drug resistance

Resistance of *Plasmodium falciparum* to chloroquine appeared almost simultaneously in Colombia and on the frontier between Thailand and Cambodia. In Asia, chloroquine resistance was initially confined to the Indochinese peninsula, until the 1970s, when it spread westwards and towards the neighbouring islands in the south and east. The advent of chloroquine resistance in Africa occurred much later, and it took a decade to cross the continent. Today, only countries in Central America north of the Panama Canal and on the island of Hispaniola have not documented chloroquine-resistant *P. falciparum* malaria. Despite cross-resistance between chloroquine and amodiaquine, amodiaquine remains more effective than chloroquine in areas of chloroquine resistance. Nevertheless, amodiaquine could rapidly lose its efficacy if it is used intensively in areas in which chloroquine resistance is widespread or very high.

The sulfadoxine–pyrimethamine combination was used as a replacement for chloroquine in most countries. At the beginning of the 1980s, however, that treatment became almost totally ineffective in Thailand and neighbouring countries, and resistance to the treatment spread rapidly in South America. In 1993, Malawi was the first country in East Africa to change from chloroquine to the sulfadoxine–pyrimethamine combination as the first-line drug, and other African countries followed this example in the late 1990s. Because of extensive use of the combination, however, resistance also spread in East Africa.

Resistance to mefloquine is found mostly in Cambodia, Myanmar, Thailand and Viet Nam. Sporadic cases of prophylactic failure of mefloquine in travellers and therapeutic failure have been reported in Africa, other Asian countries and South America. Several studies have shown a diminution in sensitivity in vitro, and studies in vitro in West Africa showed the existence of strains with decreased sensitivity to mefloquine even before its introduction into the region for therapeutic use. Resistance to quinine is often overestimated, as the dosage of 24 mg base per kg for 7 days is rarely respected, and the threshold for resistance in vitro has not been clearly defined.

So far, no resistance to artemisinin or artemisinin derivatives has been reported, although some decrease in sensitivity in vitro has been reported in China and Viet Nam.

Effects of resistance

Resistance to antimalarial drugs has increased the global cost of controlling the disease. Therapeutic failure necessitates consultation at a health facility for further diagnosis and treatment, resulting in loss of working days for adults and absence from school for children. Studies in East Africa suggest that ineffective treatment causes anaemia, which renders children's health more fragile. In Central Africa, the appearance of chloroquine resistance led to an increase in hospital admissions because of severe attacks of malaria. Similarly, increasing mortality trends were found at the community level in Senegal. The impact of drug resistance can also be illustrated by the changes in the proportion of *P. falciparum* relative to other species of malaria parasites. For example, in India since the advent of drug resistance, *P. falciparum* accounts for more than 50% of all malaria attacks, instead of the previously reported 23%.

World Health Organization (WHO) activities in response to antimalarial drug resistance

The rapid spread of antimalarial drug resistance over the past few decades has necessitated increased monitoring for further resistance, to ensure proper management of clinical cases and early detection of changing patterns of resistance for revision of national malaria treatment policies. The available testing procedures include therapeutic efficacy testing (also known as in vivo testing), the in vitro sensitivity assay and studies of gene mutations and gene amplifications associated with parasite resistance. In order to interpret and compare results within and between regions and to follow trends over time, these tests must be conducted with similar procedures and standards. Therefore, WHO has placed emphasis on standardizing the available methods. In particular, since 1996, WHO has updated the protocol for assessing antimalarial drug efficacy on the basis of expert suggestions and feedback from the field.

WHO supports national malaria control programmes, including the development and strengthening of national networks for monitoring antimalarial drug resistance. The goal of these networks is to monitor the efficacy of antimalarial drugs (including combination therapy) for the treatment of malaria caused by *P. falciparum*, and, to a lesser extent, *P. vivax*. WHO support includes assistance in choosing appropriate sentinel sites, training, strengthening reference laboratories for quality control, and analysis of data. Updated information on antimalarial drug efficacy is an important factor in making decisions to change drug policy. Between 1996

and 2004, more than 50 countries (nearly 30 in Africa) changed their first-line drug for the treatment of uncomplicated *P. falciparum* malaria on the basis of results from their monitoring systems.

In light of the experience of the East African Network for Monitoring Antimalarial Treatment, WHO is supporting additional subregional networks for monitoring antimalarial drug resistance. Information on drug efficacy derived from these networks and experience with new treatment strategies can be shared among WHO Member countries in order to assist ministries of health in making appropriate policy changes to provide access to the most effective antimalarial treatments available. In addition to the East African Network for Monitoring Antimalarial Treatment, five additional subregional networks have been established in Africa, one in Central Africa (Réseau d'Afrique Centrale pour le Traitement Antipaludique), two in West Africa (Réseau d'Afrique de l'Ouest pour le Traitement Antipaludique), one in the Horn of Africa (Horn of Africa Network for Monitoring Antimalarial Treatment) and one in the Indian Ocean (Réseau d'Etude de la Résistance aux antipaludiques dans la sous région Océan Indien). Two networks outside Africa are operating and receiving technical or financial support from WHO: the Mekong network and the Amazon network (Red Amazónica para la Vigilancia de la Resistencia a las Drogas Antimaláricas).

Conclusion

The results of tests for treatment efficacy can be used in deciding whether an antimalarial drug is still effective. The protocol for monitoring the efficacy of first- and second-line drugs is designed to meet specific programmatic objectives, and all national malaria control programmes should set up a monitoring network, with trained teams and sentinel sites, so that surveillance becomes routine. Countries that have not yet changed national policies based on monotherapy should start studying combination therapies with or without artemisinin derivatives in order to comply with WHO's treatment policy recommendation. They should adopt the standardized protocol to allow comparison of study results with those in neighbouring countries or within subregional or regional networks. The monitoring period should be at least 28 days. The objective of antimalarial treatment is to obtain a radical cure, regardless of the transmission in an area. Standard antimalarial doses (the maximum tolerated within the usually accepted therapeutic dose) should be used both as part of national treatment policy and in treatment efficacy studies. WHO will maintain a database in order to meet the challenge of antimalarial drug resistance.

In vitro tests and molecular markers are key tools, the use of which should be encouraged on a wider scale so that each drug can be studied independently, in particular when combination therapies are used. These tools are part of an early warning system, which helps to guide testing for treatment efficacy. Use of in vitro tests and molecular markers is demanding in terms of training and resources, and reference centres should be set up at national or regional level. It is essential to standardize methods and implement quality control to ensure continuity and quality of data.

INTRODUCTION

The malaria parasite, because of its genetic diversity, has demonstrated an almost uncanny ability to evade the unfavourable conditions imposed by drug therapy. Hardy genotypes escape unharmed and pass along their resistance to progeny, as sensitive organisms die off. Thus, to date, the vast majority of antimalarial therapies widely used have lost their usefulness over time.

Chloroquine – the mainstay of antimalarial treatment for decades – is an instructive example. The drug's mode of action is to accumulate in the parasite's food vacuole, interfering with the polymerization of toxic haem produced by the digestion of haemoglobin and leading to parasite death by haem poisoning. Due to acquired mutations (*pfct* gene), the chloroquine resistant strains accumulate less of the drug and are therefore unharmed by it. Under continued drug pressure those strains become dominant over time. Chloroquine-resistant parasites first emerged around 1960 in South-East Asia and South America and have since spread all over the world. The result is chloroquine is no longer an effective treatment against falciparum malaria except in a few regions of the Americas.

Similarly, sulfadoxine-pyrimethamine, which kills the malaria parasite through specific inhibition of two successive enzymes in the biosynthesis of folic acid, has lost its effectiveness in many parts of the world because of several mutations in the *dhfr* and *dhps* genes that allow the parasite to circumvent this effect.

It is impossible to predict the potential therapeutic lifespan of the latest generation of antimalarial medicines, artemisinin-based combination therapies (ACTs). To date no treatment failures due to artemisinin drug resistance have been documented, but there is evidence from *in vitro* tests of decreased sensitivity in South-East Asia – the region that has traditionally been the birthplace of anti-malarial drug resistance.

That ACTs could lose their potency is a sobering prospect, because they are the only consistently effective drugs available today. As these medicines become more and more widely used, careful monitoring of their therapeutic efficacy and of any emerging resistance will be crucial.

This report, which presents a broad overview of antimalarial drug efficacy worldwide, sets forth clear monitoring criteria, while stressing rational use of available tests for evaluating resistance and showing how therapeutic efficacy test results help in updating national malaria treatment policies. It also draws attention to the need for countries to use standardized WHO protocols. With WHO coordination, countries in all malaria endemic regions are conducting therapeutic efficacy measurements using standardized methodologies.

It is hoped the report will serve as a useful resource for policy makers, scientists and developers of antimalarial medicines.

1. ANTIMALARIAL DRUG RESISTANCE

1.1 Definition

Drug resistance has been one of the major obstacles in the fight against malaria for decades. The World Health Organization (WHO) defines "drug resistance" as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject". Drawn up in 1965 and 1973, this definition was modified in 1986, in the light of improved understanding of human metabolism of sulfonamide, to include the phrase "the form of the drug active against the parasite must be able to gain access to the parasite or the infected erythrocyte for the duration of the time necessary for its normal action" (1). The definition was made more exact in view of the observation that some individuals metabolize sulfonamides and sulfones differently and the fact that these drugs bind strongly to plasma proteins (2). A further reason for refining the definition is the antagonistic effect of folic acid on the efficacy of sulfadoxine–pyrimethamine administered concomitantly (3).

This definition was drawn up, however, when neither the technique for culture of *P. falciparum* in vitro nor high-performance liquid chromatography had been developed and molecular biology was still an emerging science. It is therefore not surprising that the definition of drug resistance given above, which is still the "official" one, is based on clinical observation. In fact, confirmation of resistance to an antimalarial drug requires proof that the parasites are recrudescent in a patient who recently received treatment and demonstration that an effective blood concentration of the drug or its metabolites has been maintained for at least four parasitic cycles (4). The results of the in vitro test and the existence of mutation(s) in the gene implicated in resistance to the drug are additional indications of resistance, although these tests (in particular, a profile of pharmacokinetics) are seldom conducted simultaneously with an in vivo test. Accordingly, failure of parasitological clearance is conventionally considered to be an indicator of resistance. The concept of resistance is associated with treatment failure rather than with prophylactic failure, although the latter is often a good marker of reduced sensitivity to an antimalarial drug. Nevertheless, some well-documented cases of prophylactic failure have been treated successfully with the same drug at a therapeutic dose (5).

1.2 Treatment failure and drug resistance

1.2.1 Limits of the in vivo test

Studies in vivo with drugs to which there is proven resistance (e.g. chloroquine or sulfadoxine–pyrimethamine) or for which interindividual variations in pharmacokinetics are not large are seldom complemented by studies of pharmacokinetics. Apparent resistance to some drugs, however, such as atovaquone, mefloquine, halofantrine and lumefantrine, to which there is marked interindividual variation in pharmacokinetics, even at standard doses, can often be explained in terms of insufficient blood concentrations. A clear distinction must therefore be made between treatment failure, i.e. the absence of resolution of clinical signs after antimalarial treatment, and true resistance to an antimalarial drug. In daily practice, many factors can contribute to treatment failure: incorrect dosage, poor patient compliance in respect of either treatment dose or duration of treatment, poor drug quality, drug interactions, interindividual variation in pharmacokinetics including poor absorption, rapid elimination (diarrhoea, vomiting) and insufficient or poor biotransformation of pro-drugs because of human genetic characteristics. Most of these factors are excluded in controlled tests of therapeutic efficacy, as drug administration is supervised, the results of microscopic examinations of blood films are validated and the origin and quality of the drugs used in the test are verified. The outcome of a therapeutic efficacy test is an amalgam of three factors: the human factor (immunity), the parasite factor (drug resistance) and pharmacokinetics (interindividual variation). Owing to the combination of the anti-parasitic effect of the drugs and acquired immunity, an adult African living in an area of high transmission might be able to eliminate resistant parasites despite ineffective treatment. Conversely, a non-immune child with severe gastrointestinal problems associated with malaria might respond with therapeutic failure, even if he or she is infected with drug-sensitive parasites (6).

1.2.2 Usefulness of studies of pharmacokinetics–pharmacodynamics

Complete information on the correlation between antimalarial drug concentrations in plasma or whole blood and treatment response is currently lacking. Extrapolation of dose–effect curves derived from in vitro models is neither sufficiently exact nor reliable.

A single blood sample taken during the first few days of monitoring is inadequate to confirm that the drug has been properly absorbed. In conventional studies of pharmacokinetics, several blood samples are required, and this is feasible only for a restricted number of patients or healthy volunteers. When complete data are available on a population, a limited number of blood samples is sufficient to determine, with the appropriate software programs and models, the pharmacokinetics of the drug and its characteristics in individuals that give rise to interindividual variations (population-based method of analysis) (7).

In the event of failure, monitoring the concentration of an antimalarial drug (or metabolite) with a sufficiently long elimination period is useful in differentiating between resistance and the pharmacokinetic variation in the patient. In treatment failure, the drug concentration is bound to be less than the minimal inhibitory concentration (MIC) of the parasites that are proliferating. At the day of failure, a blood concentration higher than the MIC that is generally sufficient to eliminate a sensitive parasite is a strong argument for resistance. The MIC values of most of the antimalarial drugs are not known. Furthermore, a very low drug concentration at the time of failure does not necessarily mean that the strain is sensitive: the blood sample might have been taken some days after the reappearance of clinical signs, and malaria itself might modify the volume of distribution of the drug, with a resulting reduction in its concentration.

Several studies in which therapeutic efficacy tests were combined with sampling of plasma or whole blood for the drug at various times during follow-up have shown that children have lower drug concentrations and cured persons have higher drug concentrations than those in whom treatment failed (8–13). There are two possible explanations for the latter finding. First, failures are associated with inadequate drug concentrations rather than resistance; secondly, there is a statistically higher likelihood that a resistant strain will emerge if the drug is present at a suboptimal concentration.

1.3 Objective of the report

The aim of this document is to describe WHO's work in monitoring antimalarial drug resistance, in particular in setting up a database and standardizing therapeutic efficacy tests, promoting more rational use of the available tests for evaluating resistance and, most importantly, showing how therapeutic efficacy test results help in updating national malaria treatment policies. The objective of this document is not to describe the

molecular mechanisms of resistance, its origin or the factors that contribute to its appearance, or its epidemiological or socioeconomic consequences. Several recent publications can be consulted for information on those aspects (14–19).

The importance of monitoring for further resistance has increased with the rapid spread of antimalarial drug resistance over the past few decades. Monitoring is necessary to allow for proper management of clinical cases and for early detection of changing patterns of resistance in order to revise national malaria treatment policies. The available procedures include therapeutic efficacy testing (also known as in vivo testing), which involves repeated assessment of clinical and parasitological outcomes of treatment during a fixed period of follow-up to detect any reappearance of symptoms or signs of clinical malaria and to detect parasites in the blood as an indication of reduced sensitivity to a particular drug. Alternative testing methods include assay of parasite susceptibility to drugs in culture and studies of gene mutations or gene amplifications associated with parasite resistance (Table 1).

2. THERAPEUTIC EFFICACY TEST

Note. The therapeutic efficacy test remains the gold standard for determining antimalarial drug efficacy. In spite of its limitations, it provides decision-makers with a straightforward, readily understandable indicator of the efficacy of an antimalarial drug or combination therapy for treating the disease in a given population at risk. The therapeutic efficacy test is not sufficient on its own to confirm drug resistance.

2.1 Historical background

2.1.1 In vivo test

The first test systems for evaluating the response of *P. falciparum* to drugs in vivo were developed in 1965, shortly after the first occurrences of chloroquine resistance were observed in this species. These test systems were revised in 1967 and remained largely unchanged until the WHO Scientific Group on the Chemotherapy of Malaria and Resistance to Antimalarials modified them in 1972 (20). The standardized tests were originally developed for chloroquine. Performance of these tests relied on adherence to set criteria for administration of a standard treatment regimen of the appropriate drug and regular examination of blood for the stipulated period, i.e. 7 or 28 days for chloroquine. Their use in the field was constrained by the need for daily blood sampling during the first week, followed by weekly tests if follow-up was extended beyond 7 days. In addition, these tests were conceived primarily for assessing the parasitological response of *P. falciparum* in areas with low-to-moderate malaria transmission (ideally in a setting free of malaria transmission, in particular for the extended 28-day field test). Therefore, little attention was paid to the clinical response to the drugs or to the immunity of patients. In view of the lack of clinical information, which is generally required by policy-makers, it was decided to introduce a simplified test system, in which the number of parasitological observations was reduced and the test was complemented by standardized clinical observations.

2.1.2 Simplified protocols

Several attempts to simplify the 1973 WHO in vivo test did not result in a satisfactory test. Excessive simplification introduced biases. Thus, the brief surveillance period of 7 days tends to result in an underestimate of the actual percentage of therapeutic failures, especially for drugs with a long half-life. Two studies, one with mefloquine in Thailand and the other with chloroquine in East Africa (Kenya, Malawi and Zambia) showed that the presence of parasites on day 2 or day 3 was predictive of treatment failure (21, 22). A similar association had not been demonstrated for other antimalarial drugs, in particular sulfadoxine–pyrimethamine, and these short-term tests have not been used widely. Nevertheless, an increase in fever or parasitaemia clearance time is still considered an early indicator of reduced efficacy of a tested drug.

A standardized protocol has been developed by the Centers for Disease Control and Prevention, Atlanta, Georgia (United States of America), and WHO to assess the therapeutic efficacy of antimalarial drugs against clinically manifest infections with *P. falciparum* in infants and young children in areas of high transmission. A modified version of the protocol was reviewed and endorsed in August 1996, at an inter-country workshop on malaria treatment and resistance in Kenya, Malawi (Mangochi) and Zambia. Although it is considered the standard method for monitoring the therapeutic efficacy of antimalarial drugs for the treatment of children suffering from uncomplicated *P. falciparum* malaria in areas of high transmission, there are large areas of the South-East Asia, Western Pacific and Eastern Mediterranean regions, South and Central America and sub-Saharan Africa where malaria transmission is of low intensity or shows large cyclical variations verging on epidemics (23). In these areas, the level of immunity is generally low. As these areas are also affected by drug-resistant *P. falciparum* and the clinical consequences of resistance are even more marked than in areas with stable malaria, the protocol still required adaptation for areas with moderate or low endemicity.

A modified protocol was therefore presented and reviewed at an inter-regional meeting on malaria control with emphasis on drug resistance in Manila, the Philippines, in October 1996, and at an expert meeting in Manaus, Brazil, in March 1998 on the efficacy of antimalarial drugs (24). During an informal consultation on monitoring drug resistance to antimalarial drugs in the Mekong region, in Phnom Penh, Cambodia, in October 2000, several modifications were suggested and included in a draft protocol adapted for areas with low-to-moderate transmission rates. These took into consideration in particular the need for a common classification

both for areas of high transmission and for those of low-to-moderate transmission. The final version of the protocol was adopted by experts at an informal consultation in Geneva in 2001 (25).

2.2 Standard WHO protocol for testing the therapeutic efficacy test protocol

2.2.1 Objectives

The results of tests for therapeutic efficacy are the most important information for determining whether an antimalarial drug is still effective. Surveillance of therapeutic efficacy over time is an essential component of malaria control. Although this test does not provide all the scientific data necessary for understanding drug resistance in a given environment, it provides basic data on the efficacy of first- and second-line drugs and, where necessary, replacement drugs, thereby allowing ministries of health to develop rational treatment strategies and policies. With a few exceptions, all policy changes have been based on the results of in vivo tests. In South Africa, data on drug sensitivity in vitro were also taken into consideration when chloroquine was abandoned as a first-line drug. In Mali, the results of use of molecular markers were determinant in deciding to use sulfadoxine–pyrimethamine instead of chloroquine during an epidemic outbreak (26). The therapeutic efficacy test can also give a better understanding of the epidemiology of resistance, which is necessary for identifying and evaluating strategies for the use of antimalarial drugs and for reducing and preventing the selection of resistant strains.

2.2.2 Method

Studies of therapeutic efficacy are relatively straightforward to set up, but they tend to be lengthy and may be costly; in particular, medical and technical personnel (especially microscopists) must be trained. In order to interpret the results and to analyse them over a long period or to compare those for different regions, studies must be carried out with the same standardized protocol, at the same sentinel sites, in the same age groups and, if possible, at the same time of year in a given site.

The standard protocol for the therapeutic efficacy test consists of evaluating the efficacy of first- and second-line drugs or drug combinations administered over 1–3 days. The WHO protocol is not suitable for evaluating slow-

acting drugs or rapidly eliminated drugs that must be given frequently for more than 3 days. The design is simple: a one-armed prospective study of clinical and parasitological responses after administration of antimalarial treatment to children aged 6–59 months with a degree of immunity that is unlikely to have much impact on the outcome of the test. In areas of low or moderate transmission in which it is difficult or time-consuming to enrol children in this age group, children over 5 years old and adults can be included, although it should be borne in mind that the results will be biased, as adults always respond better than children. In order to avoid the inclusion of asymptomatic carriers, only patients with 2000 parasites per µl (or 1000 parasites per µl in areas of low or moderate transmission) are included. Inclusion and exclusion criteria, calculation of sample size, length of follow-up, assessment criteria, data analysis and management, ethical considerations and quality controls are described in detail elsewhere (25). Patients included in the study and not lost to follow-up or excluded (because of e.g. self-medication, development of concomitant febrile infections, refusal to continue participation) are classified in one of the following categories: early treatment failure, late clinical failure, late parasitological failure and adequate clinical and parasitological response. Between 1996 and 2004, two WHO protocols were in widespread use, with different classifications as a result of the subdivision of adequate clinical response in the 2001 protocol into late parasitological failure and adequate clinical and parasitological response (Annexes 1 and 2). A table of the principal modifications made to the protocol between 1996 and 2001 is given in Annex 3. The modifications to the protocol apply essentially to areas of high transmission and concern follow-up, sampling and classification. In spite of these modifications, studies carried out with the 1996 and the 2001 protocol are still comparable.

2.2.3 Networks

Application of the standard WHO test for determining the therapeutic efficacy of antimalarial drugs involves either teams working centrally, which then conduct tests across the country, or teams working at the district level with backing from central level teams. In both cases, the initial training of central teams is of crucial importance to avoid major blunders, such as sampling errors, incorrect patient follow-up, poor quality of microscopic examinations or interpretative errors in classifying the therapeutic response. Since 1997, WHO, with support from regional offices, has conducted training in Africa: in Burkina Faso (for nine French-speaking countries in West Africa), in Cameroon (for seven French-speaking countries in Central Africa) and in the Gambia (for four English-speaking countries in West Africa). Furthermore, the WHO

protocol has been introduced in 11 African countries by consultants or national experts attached to national malaria programmes or scientific institutions. Training courses were also conducted in Sri Lanka in 1997 (for seven countries in South-East Asia), in Brazil in 1998 (for seven countries in South America), in Viet Nam in 1997 and in India in 1998. After the introduction of the modified protocol in 2001, several refresher sessions were held in Thailand and Viet Nam in 2001 and 2004 (for countries of the Mekong region), in Yemen and the Islamic Republic of Iran in 2002 (for countries in the Middle East region), in India and Myanmar in 2003 and 2004 (for countries in South-East Asia), in Bolivia in 2002 (for countries in the Amazon region) and in Egypt in 2004 (for countries in the Horn of Africa).

WHO encourages those responsible for national malaria control programmes to promote the development and reinforcement of national networks that include the national programme, the ministry of health, universities, research institutes and the national reference laboratory. The role of national networks is to monitor the efficacy of antimalarial drugs (and combinations) in the treatment of *P. falciparum* malaria and, to a lesser degree, *P. vivax* malaria. Updated data on therapeutic efficacy is a key element in deciding whether to change a national malaria treatment policy. The network is also responsible for transmitting data and forwarding recommendations to the people who decide national drug policies.

In addition to training, WHO helps countries in choosing sentinel sites, in improving quality control in reference laboratories and in data analysis. In order to facilitate the collection and analysis of data and to improve its quality, two software programs have been developed: one by WHO, which requires the commercial software Excel developed by Microsoft®, and the other by the United States Centers for Disease Control and Prevention, which requires EpiInfo. WHO also provides free of charge, on request, quality-controlled drugs for use in national malaria control programmes for conducting therapeutic efficacy tests.

Taking the experience of the East African Network for Monitoring Antimalarial Treatment as its guide, WHO has lent support to the creation of subregional networks for monitoring antimalarial resistance (27, 28). The information on therapeutic efficacy generated by these networks and experience acquired with new drug combinations can be shared between countries in order to provide the best possible advice to ministries of health in the selection of a new policy. The creation of networks also allows more effective management of problems in border areas where population movement is intense. In these regions, health conditions are often precarious, access to health care is difficult and many poor-quality drugs are

circulating in the unofficial sector. These factors lead to the rapid development and spread of drug resistance. Accordingly, countries within the same network can decide to set up sentinel sites on both sides of their common border. Apart from the East African Network for Monitoring Antimalarial Treatment, five further networks have been established in Africa, one in Central Africa (Réseau d'Afrique Centrale pour le Traitement Antipaludique), two in West Africa (Réseau d'Afrique de l'Ouest pour le Traitement Antipaludique), one in the Horn of Africa (Horn of Africa Network for Monitoring Antimalarial Treatment) and one in the Indian Ocean (Réseau d'Etude de la Résistance aux antipaludiques dans la sous région Océan Indien). Outside Africa, two networks are operational and receive technical or financial assistance from WHO: the Mekong network and the Amazon network (Red Amazónica para la Vigilancia de la Resistencia a las Drogas Antimaláricas) (Annex 4).

3. IN VITRO TESTS

Note. The results of therapeutic efficacy tests remain the basis for antimalarial drug use policies. Tools such as in vitro tests and molecular analysis can, however, help to clarify or complement the results of therapeutic efficacy tests. Nonetheless, the correlation between therapeutic response and ex-vivo measures such as the in vitro test or the presence of mutant genes remains to be defined.

3.1 Definition

Assays for sensitivity in vitro can be used to measure the intrinsic sensitivity of *P. falciparum* parasites to antimalarial drugs, without the confounding factors from the host that influence tests in vivo. Parasites are exposed to a precisely known concentration of antimalarial drug and observed for inhibition of maturation into schizonts. Several in vitro tests exist, which differ primarily in how their results are interpreted. These include microscopic examination of blood films for the WHO mark III test, the radioisotopic test and the enzyme-linked immunosorbent assay with antibodies directed against *Plasmodium* lactate dehydrogenase or histidine-rich protein II. They also differ in their end-points (appearance of schizonts with at least three nuclei, fixed incubation period, arbitrary optical density reading in control wells) and metabolic measures (incorporation of nucleotide precursor, production of parasite-specific enzyme, secretion of soluble antigen).

3.2 Scope

In vitro tests can be used to monitor drug resistance in a country or region. In view of their technical difficulty and their cost, it is recommended that, in countries endemic for malaria, in vitro tests be carried out solely by central reference laboratories with the requisite skills and resources. Links should be forged between malaria control programmes and research programmes to promote use of this system. The tests should be performed on a sample that is sufficiently large (50–100 people per sentinel site) and should, when possible, be conducted in several sentinel sites within a country.

3.3 Indications

The indications for use of in vitro tests in epidemiological monitoring are described below.

3.3.1 Cross-resistance pattern

Resistance levels vary from one region to another, and the degree of cross-resistance is often characterized by a high level of resistance to a drug. Cross-resistance occurs essentially to drugs belonging to the same chemical family or to drugs with similar modes of action (chloroquine and amodiaquine; quinine and mefloquine; pyrimethamine and cycloguanil). Although mefloquine and artemisinin belong to different chemical families, cross-resistance between these two drugs might arise from their similar mode of action or mode of transportation (29–34). Conversely, a negative correlation, implying that one drug is more active when another has less activity in vitro, has been reported in West Africa and in Thailand between chloroquine and mefloquine (N. White, unpublished data; 35, 36). This negative correlation suggests that a mefloquine-resistant strain tends to be sensitive to chloroquine and vice versa. Such data might be important in changing a first-line drug, especially if in vitro observations are confirmed by clinical responses.

3.3.2 Baseline drug sensitivity

In vitro tests are useful for obtaining baseline data on the sensitivity to a drug before it is introduced in national policy (as monotherapy or combination therapy) (37–41). Nevertheless, ad-hoc studies conducted at a single site are of little use from an epidemiological standpoint; studies should ideally be incorporated into routine long-term surveillance, especially when the resistance threshold to the drug has not been determined in vitro (42–45).

3.3.3 Temporal and spatial monitoring of parasite susceptibility to drugs

In vitro tests conducted over time can provide early warning of impending resistance before it becomes clinically apparent, and can help guide therapeutic efficacy studies (9, 46–56). In vitro tests are also useful for monitoring changes over time in susceptibility to a drug that has been withdrawn (57, 58). The usefulness of these tests has become evident with the ever-increasing use of combination therapy. It is often impossible to conduct therapeutic efficacy tests for each component, owing to ethical problems, non-availability of the drug as a single therapy and the need to study a large number of patients. In vitro tests can be used to monitor susceptibility to

each drug in a combination (59). Nevertheless, despite strict adherence to a standardized protocol, the long follow-up period might raise problems of data comparability. Investigators can encounter variations in some parameters (human serum batch, pretreated plate batch, drug batch), which are beyond their control (L. Basco, unpublished data, 2004; 60).

The in vitro test also allows comparison of the sensitivity of strains at different sites in the same or different countries, provided that an identical protocol is used (61–66). Such comparative tests are useful for quality control of in vitro tests.

3.3.4 Validation of molecular markers

Most of the mutations related to antimalarial drug resistance were identified in references clones and validated subsequently in field isolates by in vitro tests (67–73). Such studies have also shown a marked correlation between in vitro data and molecular markers. Molecular markers can therefore replace in vitro tests in some circumstances. This is of particular interest for drugs requiring special in vitro test conditions or for which the results are difficult to reproduce (pyrimethamine, cycloguanil, sulfadoxine) (74–76).

3.4 Advantages and drawbacks

3.4.1 Advantages

From the research standpoint, in vitro tests obviate many of the confounding host factors that characterize the therapeutic efficacy test. In vitro tests allow a more objective approach to parasite resistance, as they are based on direct contact between parasites and incremental drug concentrations. Several tests can be carried out with the same sample, and several drugs can be studied at the same time, including drugs that are still at the experimental stage (with the exception of pro-drugs).

3.4.2 Drawbacks

It is often difficult to compare results, even from laboratories where the same type of test is used, as the results, which are usually expressed as the 50% inhibitory concentration (IC50), the 90% inhibitory concentration (IC90) or the MIC, are the expression of about 20 factors (including erythrocyte volume fraction, initial parasitaemia and volume distributed in the wells), which are rarely identical in different laboratories.

There is inconsistent evidence of a correlation between the results of therapeutic efficacy tests and in vitro tests (77). This is because of, firstly, non-adherence to the standard method for therapeutic efficacy tests and, secondly, lack of validation of the threshold of resistance in vitro. In order to validate the in vitro cut-off point for resistance, the results of in vitro tests and of therapeutic efficacy tests conducted in a non-immune population (children or travellers) must be compared in a sufficiently large sample. Furthermore, patient follow-up must be sufficiently long, and every effort must be made to confirm that failures are not due to insufficient drug absorption, reinfection or other causes unrelated to drug resistance. To date, few thresholds of resistance have been correctly validated. Moreover, a cut-off point validated with a given test is valid only for that assay system, with its specific in vitro factors. Another reason for the inconsistent results of in vitro–in vivo comparisons is infection with two or more parasite populations; a predominantly sensitive parasite population might mask the phenotype of a population of resistant parasites present in the isolate.

Most of the parasites sampled from patients practising self-medication fail to grow or grow poorly with a diminished IC50, owing to the presence of drugs in the blood (78). This technical constraint eliminates many samples. Similarly, samples with low rates of parasitaemia (‹0.1%) should be eliminated when the radioisotope method is used. New techniques involving monoclonal antibodies are sensitive enough to detect a parasitaemia of 0.01%.

The in vitro test has been used to determine whether two drugs act synergistically, additively or antagonistically against reference clones. Multiple combinations of drugs at different concentrations are required, and the sensitivity of the clone to both drugs must be known in advance in order to plot an isobologram. The in vitro test is not, however, the best model for studying a fixed-dose combination such as sulfadoxine–pyrimethamine: the selected fixed ratio of 1:80 does not allow simulation of drug interaction at varying ratios of concentrations of the two drugs in blood over time (79).

3.4.3 Standardization

Standardization of the method used, especially culture methods and notification of results, is of growing importance. Results should not be expressed as percentage resistance, especially when the thresholds of resistance are not validated. They should rather be expressed as a geometric mean of the IC50 or MIC, which allows a more precise quantitative comparison of sites in a given country and over time. Two networks have been created with the objective of using a standardized in vitro assay

method: the Paludisme network of laboratories in French-speaking countries and the Red Amazónica para la Vigilancia de la Resistencia a las Drogas Antimaláricas.

Since 2000, WHO has been working with the University Sains Malaysia in Penang, Malaysia, which manufactures pre-dosed plates for in vitro tests. Quality control of the plates has been supervised since 2002 by the Institut de Médecine Tropicale du Service de Santé des Armées in Marseille, France.

4. MOLECULAR MARKERS

4.1 Implementation

Molecular markers can also be used for monitoring drug resistance in a country. Moreover, they can be used to distinguish between reinfection and recrudescence in treatment efficacy tests. These markers allow the study of many isolates within a short time. Collection, storage and transport of specimens for subsequent molecular analysis are far easier than for in vitro tests. It is essential to establish close collaboration between malarial control programmes and research groups working in the field of molecular analysis.

4.2 Genetic markers of resistance

Recent advances in molecular biology have made possible the identification of genetic markers that are linked to *P. falciparum* resistance, although molecular markers of resistance have not been identified for all antimalarial drugs.

4.2.1 Genetics of *P. falciparum* resistance

The genetic mechanisms of *P. falciparum* drug resistance have not been completely elucidated. Five genes that appear to play a role in regulation of resistance to the principal chemical families of antimalarials in current use have been identified.

Dihydrofolate reductase (*dhfr*)

Several studies on the *dhfr* gene have provided solid proof of the fundamental importance of a point mutation at the Ser108Asn codon in the pyrimethamine-resistant phenotype of *P. falciparum*. Additional point mutations at the Asn51Ile, Cys59Arg or Ile164Leu positions increase the level of resistance to antifolates. Not only is there an almost perfect correlation between the presence of a mutant codon 108 and resistance to pyrimethamine in vitro, but also the level of resistance increases with the number of mutations (71).

Triple mutations at codons 108, 51 and 59 are seen in South-East Asia and Africa. A quadruple mutant represents the severest form of resistance and is responsible for a high level of resistance to the sulfadoxine–pyrimethamine

combination as well as to chlorproguanil–dapsone (80, 81). The Ile164Leu mutation is relatively uncommon but has been reported in Bangladesh, Bolivia, Brazil, Cambodia, China, India, Malaysia, Peru, Thailand and Viet Nam (82–93). A few rare strains carry the quadruple mutations in Africa: in Ghana, Kenya and the United Republic of Tanzania (94–98). In South America, mutation at codon 59 is less common and is replaced by the Cys50Arg mutation and repeated insertion of five amino acids between codons 30 and 31, which was not found to play a role in resistance (99). More recently, rare mutations Asn188Lys, Ser189Arg and Val213Ala, which are associated with high resistance comparable with that observed when the Ile164Leu mutation is present, have been demonstrated in a yeast-based model (100).

Cycloguanil resistance appears to be associated with the double mutations Ser108Thr and Ala16Val. The Ser108Thr mutation associated with an Ala16Val mutation does not appear to modify sensitivity to pyrimethamine (101). The presence of mutations specific to cycloguanil resistance is extremely rare in isolates (102–104). A single Ser108Asn mutation is not correlated with resistance to cycloguanil. The accumulation of several pyrimethamine-resistance type mutations gradually reduces sensitivity to cycloguanil.

Dihydropteroate synthase (*dhps*)

The mechanism of resistance to sulfadoxine is also associated with point mutations. Five sites of point mutation, on the Ser436Ala/Phe, Ala437Gly, Lys540Glu, Ala581Gly and Ala613Thr/Ser codons of the *dhps* gene, have been reported. It is unclear which is the key mutation, as a mutation at codon 108 is in *dhfr*, and mutations at 436, 437 and 540 may confer some degree of resistance. A higher level of resistance requires multiple mutations. The 581 and 613 mutations are rare or absent in Africa but could lead to a high level of resistance to sulfadoxine–pyrimethamine. A correlation between in vitro test results and the existence of mutations has not been completely established in isolates, as the protocol of the in vitro test for sulfadoxine is not standardized and the presence of folic acid in the medium–blood mixture considerably modifies the IC50 value (79, 105, 106).

P. falciparum chloroquine-resistance transporter (*pfcrt*)

The *pfcrt* gene is situated on chromosome 7 and codes for a transport protein in the vacuolar membrane (67). Experimental studies with clones and transfected parasites have shown that the *pfcrt* gene plays a major role in determining the phenotype of chloroquine resistance, when lysine has been replaced at codon 76 by threonine. This mutation is generally not isolated but is associated, depending on the geographical setting, with mutations at

other codons, Cys72Ser, Met74Ile, Asn75Glu, Ala220Ser, Gln271Glu, Asn326Ser, Ile356Thr and Arg371Ile, the role of which is not well defined. Clinical studies have confirmed that the Lys76Thr mutation is present in all isolates of *P. falciparum* after treatment failure with chloroquine (107, 108); however, these studies also showed that this mutation can be present in chloroquine-sensitive isolates, suggesting that other mutations in the *pfcrt* gene or other genes may be involved (109–121).

P. falciparum multidrug-resistance gene 1 (*pfmdr1*)

The *cg2* gene was initially suggested to be the key gene for chloroquine resistance, but its association with chloroquine resistance is probably artefactual and related to the fact that it is situated on chromosome 7 close to the *pfcrt* gene (122). The *pfmdr1* gene, situated on chromosome 5 and coding for the P-glycoprotein homologue 1, has also generated keen interest, because of two observations. In some mammalian cancer cells, overexpression of *mdr* genes encoding P-glycoprotein is directly correlated with an increased efflux of anti-cancer drugs from drug-resistant cells. Drug efflux can be inhibited by several pharmacological entities that are not anti-cancer agents, so that drug-resistant cancer cells are killed by the drug against which they are resistant. A similar phenomenon has been observed in vitro with chloroquine-resistant *P. falciparum* exposed to resistance modulators (e.g. verapamil modulates drug resistance in both cancer cells and malaria parasites). The Asn86Tyr mutation has been associated with chloroquine resistance, but studies conducted in vivo and in vitro in parallel have produced discordant results (123–132).

Several other compensatory mutations have also been described: Tyr184-Phe, Ser1034Cys, Asn1042Asp and Asp1246Tyr. Linkage disequilibrium between the Lys76Thr mutation on the *pfcrt* gene and the Asn86Tyr mutation on the *pfmdr1* gene has been observed in studies in Burkina Faso, Nigeria and Sudan, providing a supplementary argument for the hypothesis that polymorphisms have to converge before emergence of a phenotype that is strongly chloroquine-resistant (70, 133, 134). All highly chloroquine-resistant isolates have at least the Lys76Thr and Ala220Ser mutations in the *pfcrt* gene, generally associated with the Asn86Tyr mutation in the *pfmdr1* gene (135). An additional mechanism of chloroquine resistance suggested for *pfmdr1* is gene amplification, which was observed in some chloroquine-resistant reference clones. In laboratory-adapted clones selected for drug resistance in vitro, gene amplification might play a role in determining the drug resistance phenotype, although amplification does not appear be the main mechanism of chloroquine resistance in field isolates.

The *pfmdr1* gene has also been implicated in resistance to amino alcohols and artemisinin. Transfection studies have raised the possibility that the Asn86Tyr, Ser1034Cys, Asn1042Asp and Asp1246Tyr mutations are associated with quinine resistance as well as increased sensitivity to mefloquine, halofantrine, lumefantrine and artemisinin derivatives. Field studies have yielded contradictory results (44, 136–140). During a clinical trial in Zanzibar (United Republic of Tanzania), artemether–lumefantrine appeared to select for the wild-type *pfmdr1* Asn86 allele, which could be a marker for lumefantrine resistance (141). It remains unclear whether the *pfmdr1* gene is involved in mefloquine resistance through mutations or through amplification. Studies conducted in Thailand showed that increasing numbers of copies of the gene are responsible not only for resistance to mefloquine but also for reduced sensitivity to artemisinin (142, 143). The existence of the Asn86Tyr mutation is a negative marker for the presence of gene amplification.

Cytochrome *b*

When atovaquone, a hydroxynaphtoquinone, is administrated as monotherapy, resistant parasites are rapidly selected. In order to delay the emergence and spread of atovaquone resistance, the synergistic combination atovaquone–proguanil has been developed (144). Molecular analysis of recrudescent isolates demonstrated that atovaquone resistance was linked to a single mutation at the cytochrome *b* gene codon (Tyr26Asn or Tyr268Ser), inducing an approximately 10 000-fold increase in the atovaquone IC50 (145, 146). This mutation appeared to provide a sufficient explanation of the treatment failures observed with the atovaquone–proguanil combination; however, several treatment failures have occurred in patients infected with parasites that have none of these mutations (147–149). These cases call into question the isolated role of the mutation at codon 276 in atovaquone resistance.

4.2.2 Indications and limits

Like the in vitro tests, molecular markers of resistance might provide an early warning system in geographical or temporal monitoring for guiding treatment efficacy studies (83, 84, 87, 150–160). Molecular markers are useful for monitoring the prevalence of mutations after a drug has been withdrawn or when a drug combination is used (161). They can replace in vitro tests for antifolates, which present several technical difficulties (use of special medium, poor solubility in water). They are particularly useful for providing direct evidence that a treatment or prophylaxis failure is a result of selection of resistant parasite populations (162).

Polymerase chain reaction (PCR) methods of varying sensitivity are used in different laboratories (163, 164). Mixed infections are common in many endemic areas, and resistant parasite populations might be masked by sensitive populations if the method used is not adequate, possibly leading to a discordant result between therapeutic efficacy and molecular markers.

The identification of molecular markers has raised the hope of a predictive test of treatment efficacy, and several models have been proposed. To be useful as a public health measure, these markers should reliably predict the clinical and parasitological outcome. At present, the molecular markers do not give an accurate prediction in all epidemiological settings.

In antimalarial chemotherapy, sulfadoxine is always combined with pyrimethamine. The antimalarial activity of this combination is based on the specific inhibition of two successive enzymes in the biosynthesis of folic acid, with a subsequent synergistic action. Several mutations in the *dhfr* and *dhps* genes are necessary to induce treatment failure with the sulfadoxine–pyrimethamine combination. In one study conducted in Africa, triple mutations at codons 108, 51 and 59 of the *dhfr* gene and double mutations at codons 437 and 540 of the *dhps* gene correlated with treatment outcome (165). In population monitoring, the presence of mutations at codon 59 of the *dhfr* gene and codon 540 of the *dhps* gene (which is thought to play a more important role than codon 437) is strongly predictive of the quintuple mutation (codons 51, 58, 108 on the gene and 437 and 540 on the *dhps* gene) and treatment failure (166, 167). A prevalence of *dhfr* mutant Arg59 parasites and of wild-type parasites Cys59 parasites is also strongly correlated to clinical outcome (168). Treatment failure can, however, occur in the presence of fewer than five mutations on the *dhfr* and *dhps* genes, and, conversely, immune adults can eliminate parasites carrying the quintuple mutation after sulfadoxine–pyrimethamine treatment (89, 169–174). The relationship between the parasite genotype and therapeutic response to sulfadoxine–pyrimethamine is influenced by parasitic, pharmacokinetic and human factors. When a parasite has wild-type *dhfr* without mutation, the risk of failure is almost nil, regardless of the *dhps* alleles. In contrast, the more mutations in the *dhfr* gene the higher the risk of failure, in particular when a superadded mutation is present in the *dhps* gene or immunity is lacking (101). Cumulative mutations in the *dhfr* gene increase parasite clearance time and the risk of gametocyte carriage. As a result, even though the sulfadoxine–pyrimethamine combination remains effective, one or two mutations can increase the transmission of malaria potentially capable of increasing the spread of resistance (175).

The correlation between the presence of mutation at codon 76 of the *pfcrt* gene and treatment outcome is also strongly influenced by the immune status of the patient. The absence of mutation was strongly predictive of treatment success, while the presence of mutation was associated with failure in only one third of immune people in Mali (107). The authors proposed two age-adjusted indices for predicting treatment outcome from the prevalence of the key *pfcrt* mutation: the genotype resistance index and the genotype failure index. The genotype resistance index is defined as the ratio of the prevalence of mutant Thr76 *pfcrt* and parasitological and clinical failure. The genotype failure index is defined as the ratio of the prevalence of mutant Thr76 *pfcrt* and clinical failure. In Mali, the two indexes (values between 2 and 3) were shown to be stable both geographically and temporally, indicating that the prevalence of the molecular marker was two to three times higher than treatment failure (176). On the basis of the prevalence of mutation in samples from a cohort study, the genotype resistance index and the genotype failure index would allow prediction of the treatment efficacy of chloroquine. These indexes nevertheless have two drawbacks: they must be validated in other epidemiological contexts, and they cannot be used in areas where the prevalence of chloroquine resistance is already high, as the prevalence of mutation has reached a threshold that no longer allows prediction of treatment efficacy. Another index, based on the ratio of the prevalence of mutant Thr76 to that of wild-type Lys76 *pfcrt* codon, has been used in Uganda. This index is correlated with the prevalence of early treatment failure and chloroquine use in a community (177).

4.2 Genetic markers of recrudescence and reinfection

4.2.1 Applications

When monitoring is extended beyond 14 days and as most antimalarial drugs have no action on the effects of *P. falciparum* on the liver stages, techniques are required to distinguish cases of new infection from recrudescence, especially in areas of high transmission.

PCR has greatly facilitated analysis of the genetic diversity of *P. falciparum*. Three genes are commonly used as markers because of their considerable polymorphism: merozoite surface proteins 1 and 2 (*msp-1* and *msp-2*) and glutamate-rich protein (*glurp*) (178). Microsatellites and circumsporozoite protein have also been used (179, 180). The probability that an isolate is identical in two randomly selected specimens decreases significantly as the

number and polymorphism of the genetic markers used increases. The use of two or more genetic markers increases the detection of diversity and raises the confidence interval for the comparison. An analysis based on a single gene should be avoided with conventional techniques, as it might provide grounds for concluding that two specimens are different but not that they are identical (181, 182).

4.2.2 Limits

Sensitivity

PCR methods have different detection sensitivities, depending on the template preparation and primers used. Accordingly, failure of a PCR method to reveal a particular genotype does not necessarily signify that it is absent from the specimen.

Sequestration and asynchronism

P. falciparum infections can be asynchronous. Several paroxysms usually occur during the initial phase of an infection, which reflect the different growth dynamics of different parasite broods. A single specimen taken on day 0 does not allow collection of these different broods, and taking two successive specimens 24 h apart has been suggested as a way of addressing this shortcoming.

Transmission

An isolate can contain several broods of parasites, which can have different resistance and growth characteristics. Two genetically similar or different populations issuing from the same population can be detected in blood several days apart, leading to different interpretations of molecular tests. Conversely, a patient might be inoculated several days apart by the same mosquito or by another one carrying the same isolate. Such cases are relatively rare but demonstrate the complexity of interpretation of molecular tests.

DNA origin

The most important confounding factor is the presence of gametocytes at the time of recrudescence. Conventional techniques cannot distinguish gametocyte DNA from that of asexual forms present in the blood. Acute malaria therefore tends to be overclassified as recrudescence.

The ideal situation would be to avoid reinoculation, to co-administer drugs active against hepatic stages and gametocytes and to obtain a sufficient

number of specimens. Under these conditions, it might be possible to describe the parasite population dynamics, although a small degree of uncertainty will remain. Unless specimen collection protocols, DNA extraction, PCR amplification techniques for molecular markers and interpretation are standardized, it is difficult to compare the results obtained by different laboratories (178). Nevertheless, discordant molecular results from different laboratories are probably rarer than with in vitro assays for drug sensitivity.

5. MONITORING THE THERAPEUTIC EFFICACY OF ANTIMALARIAL DRUGS

After introducing the new therapeutic efficacy test in 1996, WHO funded clinical studies in three to four sites in 23 African countries and in five South-East Asian countries. Bilateral aid agencies also funded studies in several countries. Since 1997, approximately 2000 studies have been carried out with funding from a wide range of sources. Nevertheless, recent information on antimalarial drugs is not readily available to people delegated to set up a national policy or to agencies in charge of nationwide funding to combat malaria. Establishment of a regularly updated database and publication of detailed analyses of the available data are therefore of capital importance.

5.1 WHO database

A global database was set up by WHO in response to the challenge posed by the emergence of resistance to antimalarial drugs. A continually updated database is a valuable source of information for those working on malaria and specifically on drug resistance. It contains all the current, pertinent literature in one accessible location and allows the reader to analyse and filter the information as required.

5.1.1 Sources

The data in the database originate from three main sources.

Published data are an important source of information, obtained by searching for articles published in journals through a variety of databases in various languages. Chief among them are MEDLINE, PubMed and the Latin American and Caribbean Center on Health Sciences Information. Since 2004, the Institute for Scientific Information Web of Science database has also been consulted. Other articles have been found by systematic analysis of the reference lists of the selected articles.

Unpublished data are derived from reports drafted by ministries of health, national control programmes or nongovernmental organizations, consultant reports, theses and papers or posters presented at national or international conferences.

Regular monitoring data are raw data from surveillance studies conducted according to the WHO standard protocol. The results are incorporated in standardized Excel or EpiInfo files and sent from countries to WHO for validation and comments.

5.1.2 Database description

The WHO database contains the following information for each study: year and month during which the study was conducted, name of the country and site, drug and dose used, WHO protocol used and any modifications introduced to the standard protocol, quality control checks, percentage of patients lost to follow-up or excluded, number of patients entered in the analysis, percentage of clinical failures and adequate clinical responses for studies conducted in areas of high transmission and percentage of clinical and parasitological failures and adequate clinical and parasitological responses for studies conducted in areas of low-to-moderate transmission, and type of study reference (publication, report, thesis, presentation, raw data), with, in some cases, Internet hyperlinks to expand use of the database as a primary source of information. The rationale for expressing results either as clinical failures or as clinical and parasitological failures is that, up to 2004, the treatment objective in areas of high transmission was clinical cure whereas that in areas of low-to-moderate transmission was radical cure.

All studies conducted since 1996 with a follow-up period of at least 14 days and with drug doses in accordance with WHO recommendations have been incorporated into the database. This document covers the results of studies conducted with either the 1996 or the 2001 WHO protocol that included the minimum number of patients recommended in the protocols. Twelve studies based on asymptomatic people and six with unsupervised treatment were not taken into consideration. When data were available for different age groups in studies carried out in areas of high transmission, only the results for children under 5 years of age were included.

Studies conducted according to the 1973 protocol in African countries with high malaria transmission were not taken into consideration for several reasons. The S-RI-RII-RIII classification[1] defined in the 1973 WHO protocol is not comparable with that in the new protocols for areas of high trans-

[1] S or S/RI: in the extended test, the parasites are S if no asexual parasites are found by day 6 and parasites do not reappear by day 28. In the 7-day field test, the infection may be either S or resistant at RI (S/RI) level if no asexual parasites are found at day 6 and none are present on day 7. An S response and a RI response cannot be distinguished for the non-extended test since the difference between the 2 responses depends on the presence or absence of recrudescence between day 8 and day 28.

mission: the sum of RI-, RII- and RIII-type failures is not equivalent to early treatment failure plus late clinical failure. Moreover, the 1973 protocol underwent many adaptations and simplifications by various teams in Africa, with the result that various methods and various definitions of RI-, RII- and RIII-type failures have been used over time. It is thus difficult to compare the results. Nevertheless, several studies have shown that, when the protocol is used in areas of low-to-moderate transmission, the sum of early treatment failure, late clinical failure and late parasitological failure is equivalent to the sum of RI-, RII- and RIII-type failures (183–185). In order that an important repository of information is not lost, studies conducted with the 1973 WHO protocol in countries of low-to-moderate transmission are therefore included in the database.

5.2 Results

Between 1996 and 2004, 1691 studies were indexed and incorporated into the WHO database. For the purposes of analysis, 1418 studies were taken into consideration. Most of the studies were conducted with either the 1996 or the 2001 WHO protocol; 138 studies in Africa followed the 1973 protocol or developed their own inclusion criteria, classification and analytical methods, so that the results are not comparable with those of conventional studies.

5.2.1 Protocol modifications

One of the main problems in analysis of a database is standardization of the method and expression of results. Several changes to the WHO protocol (with varying degrees of justification) have been introduced in the field, including changes to the inclusion and exclusion criteria (age, history of fever, parasitaemia cut-off point) and the classification or analysis of data. Teams in Ethiopia and Namibia decided to adopt the protocol most suited to

RI: in the extended test, parasites are resistant at the RI level if asexual parasites disappear but return within 28 days, reinfection having been excluded.

In the 7-day field test, parasites are resistant at the RI level if asexual parasites disappear for at least 2 consecutive days but return and are present on day 7.

RII: the parasites are resistant at RII level if asexual parasitaemia does not clear but is reduced to 25% or less of the original pre-test level during the first 48 hours of treatment.

RIII : the parasites are resistant at RIII level if asexual parasitaemia is reduced by less than 75% during the first 48 hours or if it continues to rise.

their epidemiological profile (low-to-moderate transmission) rather than modify the inclusion criteria for countries of high malaria transmission.

Targeted age groups

In all regions, the methods used should give priority to effective treatment in young children (<5 years old) with clinical malaria. Even in populations with low levels of acquired immunity, young children often respond less favourably to antimalarial drugs than older children and adults (186, 187). In countries where transmission is low or where young children are much less exposed to the risk of infection than adults, preferential enrolment of children under 5 years of age has presented logistic problems owing to the considerable increase in the duration of enrolment. In practically all countries of Sahelian Africa, the maximum age at inclusion has been extended to 10 or 15 years or even to all age groups.

Fever or history of fever in areas of high transmission

The WHO Expert Committee on Malaria recommended in 2001 that, in regions of high transmission, patient enrolment should be based on the presence of fever. Similarly, determination of treatment outcome should be based solely on an objectively recorded temperature, history of fever alone not being considered an adequate indicator of clinical treatment failure. The Committee recognized that fever associated with malaria is not constant, that patients might have taken antipyretic drugs and that the absence of patent fever at the time of inclusion does not signify that the patient is not affected by malaria requiring treatment. The rationale for the requirement of fever at the time of inclusion was due regard for consistency of data and use of objective criteria to avoid including or classifying asymptomatic carriers as clinical failures. Many teams have preferred to use history of fever as an inclusion criterion. The enrolment of children with a history of fever could increase the number included by 40% (188). There are no studies which show that the outcome of the study was modified by the inclusion of people with a history of fever in the previous 24 h instead of proven fever. To maintain coherence, the criterion chosen (proven fever or history of fever in the previous 24 h) should be retained throughout the study. In principle, in sentinel sites situated in areas of high transmission, there should be no problem in enrolling a sufficient number of febrile children, and there should be no need to resort to including children with a history of fever. In the contrary event, local transmission should be re-evaluated and the protocol adapted accordingly. Nevertheless, there is no rationale for extending the history of fever to 48 or even 72 h.

In 2001, a temperature of ≥39.5 °C was no longer considered to be an exclusion criterion, and a new definition of severe malaria was given (189). The inclusion of children with temperatures ≥39.5 °C since 1997 has had no significant effect on the study results.

Parasitaemia

The limits of parasitaemia were changed in 2001 from those in the 1996 protocol, from 1000–30 000/µl to 1000–100 000/µl in regions of low transmission, and from 2000–100 000/µl to 2000–200 000/µl in regions of high transmission. These modifications are based on the WHO definition of hyperparasitaemia (189). The exclusion of mixed infections was generally respected, except in five studies.

The lower limit: of the minimum cut-off point has sometimes been lowered to 100/µl, which poses a significant risk of including asymptomatic subjects. South American countries have adopted a minimum parasitaemia limit of 250/µl because of difficulties of enrolment (190). Use of a low minimum parasitaemia cut-off point as an inclusion criterion is accompanied by two problems. In areas of high transmission, immune subjects are often asymptomatic carriers of low-grade parasitaemia, which can disappear spontaneously, thereby resulting in an underestimate of therapeutic failure. Secondly, microscopic reading of slides must be accurate in order to avoid early treatment failure classification errors, failure being based on a comparison of parasitaemia on day 0 and on day 2 or 3. In the Lao People's Democratic Republic, a high lower limit (5000/µl) has been adopted, which excludes a large number of patients from the study and thus potentially introduces selection bias (185).

After 1997, a maximum cut-off point of 250 000/µl was used in areas of high transmission, with no impact on the results. Raising the cut-off point allows a 25% increase in the number of patients who can be included under some conditions (14). Inclusion of patients with hyperparasitaemia (>5%) should nevertheless be avoided on the grounds of patient safety, especially when the treatment used is of uncertain efficacy or slow-acting. Furthermore, for some drugs, high parasitaemia is a risk factor for therapeutic failure (22, 191–193).

Previous intake of an antimalarial drug

In a considerable number of studies, people who had taken antimalarial drugs before consultation were not included, particularly if the total dose of treatment had been administered. The main reason for exclusion is patient

safety, as administration of the same or another treatment can induce side-effects caused by overdose or drug interactions. The other argument for excluding such patients is to avoid sequential treatment, which might modify the final treatment outcome. A history of use of antimalarial drugs or the presence of antimalarial drugs in the urine or blood is not an exclusion criterion in the WHO protocol. The protocol seeks to be pragmatic and is not designed for evaluating the efficacy of new developmental-phase drugs; that would require considerably more precise information.

In many places, previous treatment with antimalarial drugs is the rule rather than the exception, and exclusion of previously treated patients would mean that the sample was not representative of the target population (i.e. patients seeking consultation in health centres for treatment of uncomplicated malaria). Antimalarial drugs can be detected in the blood or urine of persons who deny self-medication, or, conversely, negative results can be found for persons who affirm that they have taken antimalarial treatment recently. These considerations suggest that histories of previous treatment are not reliable and that urine tests are more appropriate. If patients are included regardless of previous antimalarial treatment, information on prior drug use should be carefully collated and noted for each patient so that it can be used to stratify the results. Exclusion of patients with a history of self-medication or recent antimalarial treatment is a problem at sentinel sites at which the number of patients who satisfy the inclusion criteria is limited.

Duration of follow-up

The length of the follow-up period has given rise to heated debate. Many of the criticisms expressed are based on a misunderstanding of the basic objective of the 1996 WHO protocol. Confronted with an increasing number of alarming reports of chloroquine resistance, the 1996 protocol was designed to determine, in simple, practical studies, whether chloroquine and sulfadoxine–pyrimethamine were still effective in Africa. To do this, WHO adopted the "lot quality assurance sampling" method, which, it considered, would establish whether the prevalence of clinical failure was statistically close to or remote from the 25% cut-off point in a limited number of patients. This sophisticated statistical method was, however, poorly applied in the field. Retrospectively, it can be concluded that the method failed to determine and follow the exact prevalence of failures over time and proved to be unsuitable for use for drugs or drug combinations with low failure rates (190). In anticipation of a simplified test, the follow-up period was restricted to 14 days, especially since many national malaria

control programmes did not have access to the molecular biology laboratories that are crucial for confirming whether treatment failure is a result of recrudescence or reinfection.

The first draft versions of the protocol for areas of low-to-moderate transmission made immediate provision for a follow-up period of 28 days. WHO recommended not only a 28-day follow-up, while stressing limiting conditions in the field, but also suggested that monitoring should match the half-life of the drug(s) in all transmission areas. For drugs that had already been proven to be ineffective, extension beyond 28 days would permit recuperation of such a small number of late failures that the overall results would not be significantly modified, while the cost and risk of loss to follow-up would be increased (194).

The database allows a deeper analysis of the relation between duration of follow-up and outcome. The results at days 14 and 28 of tests for the efficacy of amodiaquine, amodiaquine+sulfadoxine–pyrimethamine, artemether–lumefantrine, artesunate+amodiaquine, artesunate+sulfadoxine–pyrimethamine and chloroquine+sulfadoxine–pyrimethamine indicate that the clinical failure rate and the total failure rate are highly correlated, as are the clinical and total failure rates between days 14 and 28 (Figures 1.1–1.4).

Figure 1.1
Correlation between clinical failure rate and total failure rate at day (D) 14

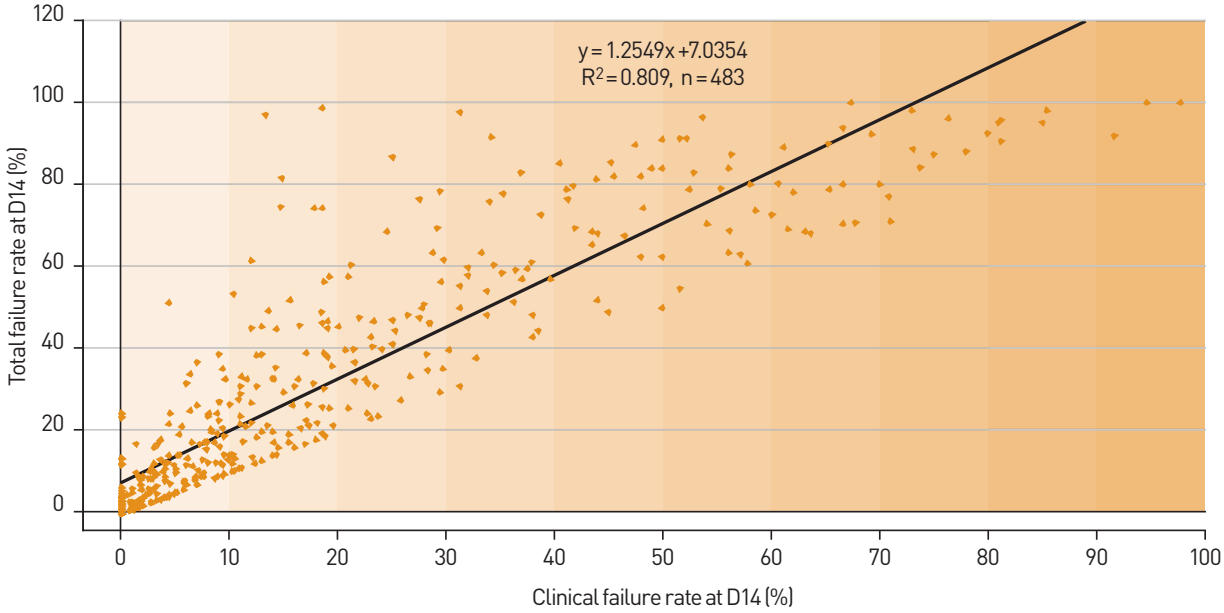


Figure 1.2
Correlation between clinical failure rate and total failure rate at day (D) 28

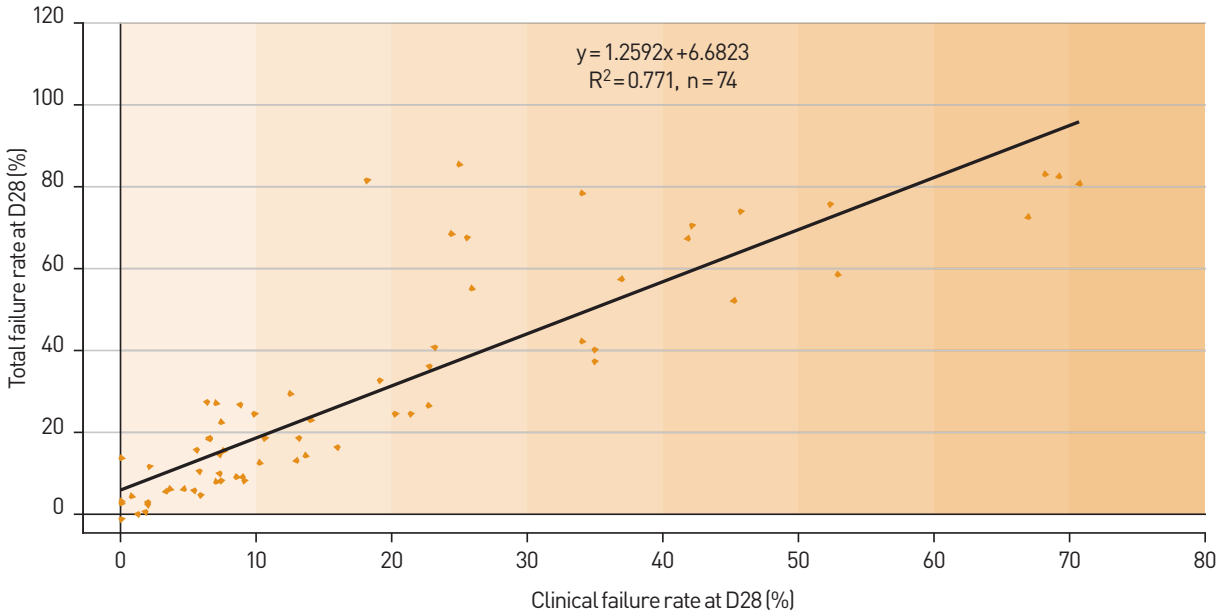


Figure 1.3
Correlation between clinical failure rate at days (D) 14 and 28

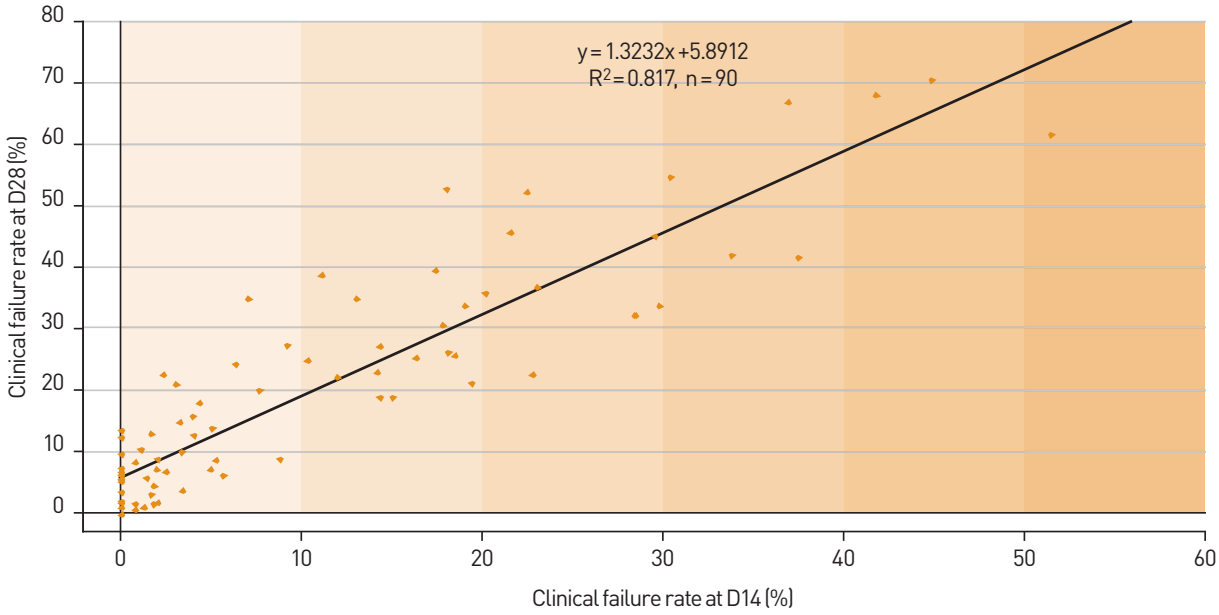
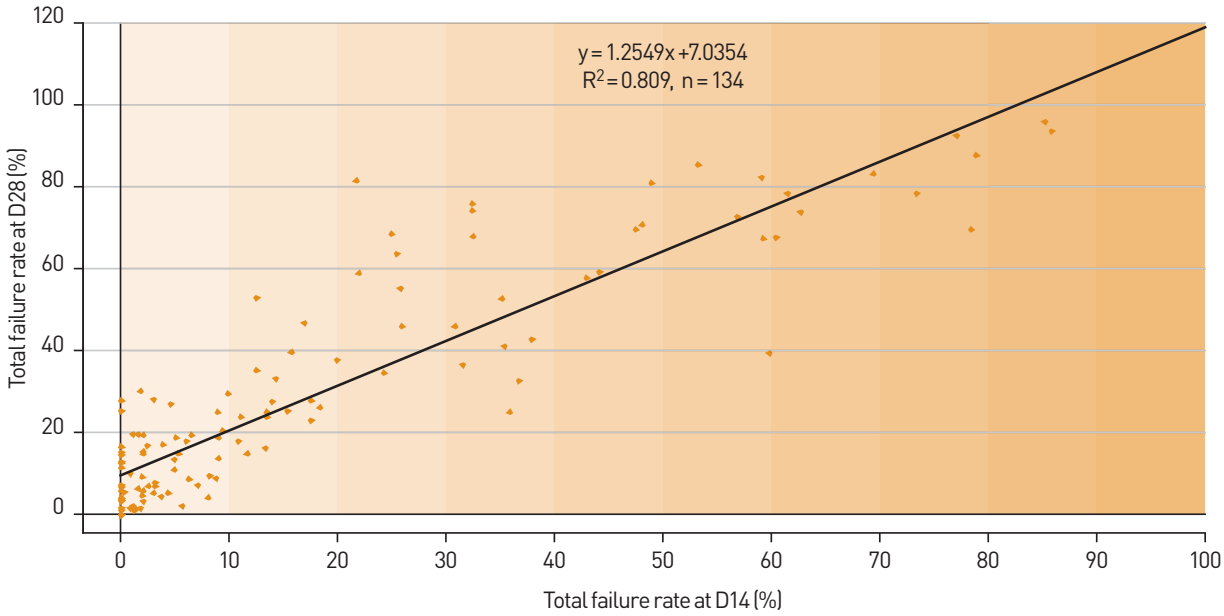


Figure 1.4
Correlation between total failure rate at days (D) 14 and 28



Treatment of parasitological failures

The main difference between the protocols for areas of high transmission and for areas of low-to-moderate transmission, apart from the inclusion criteria, is in the management of parasitological failures without clinical signs. The differences reflect regional programme priorities (treatment for clinical cure or for radical cure). In areas of low-to-moderate transmission, parasitological and clinical failures have the same weight, and in these regions rescue treatment can be provided for both asymptomatic parasitological failures and clinical failures. As underlying acquired immunity in people with lifetime exposure to intense malaria transmission allows for parasitological failures without concurrent clinical failure, rescue treatment is usually not given for asymptomatic parasitological failures but only for clinical failures. At the end of the follow-up period of a study, however, all patients with parasitaemia are given rescue treatment, regardless of their clinical status, for obvious ethical reasons. This interregional difference in decision-making is changing and will lead to a protocol update.

Systematic treatment has been administered before the end of follow-up to people with asymptomatic parasitological failure in studies carried out in areas of high transmission in Liberia and Sudan (195). This practice modifies the comparability of the results with those of other studies. The total number of clinical and parasitological failures remains unchanged; parasitological failure may remain asymptomatic or progress to clinical

failure, and it is rare to see complete eradication of parasites without additional treatment. Nevertheless, the proportion of clinical failure (indicator used by countries for policy change) is reduced, while the proportion of parasitological failure is increased.

Data analysis

Three types of analysis are principally used: "per protocol" analysis, life-table analysis and intent-to-treat analysis. Only the first two are recommended in the WHO protocol, the third being more suitable for randomized comparative clinical trials. A typical error in studies in which "per protocol" analysis is used is retention in the denominator of data on patients who were lost to follow-up or excluded from the study when calculating percentages. These patients should not be taken into consideration in the final analysis. This type of error is rectified before the data are incorporated into the WHO database. The Excel and EpiInfo programmes allow results to be analysed in both ways.

Reinfection and recrudescence

In studies with a follow-up period longer than 14 days in areas of high transmission and longer than 28 days in areas of low-to-moderate transmission, molecular tests are required to distinguish between reinfection and recrudescence. In areas of high transmission, PCR tests have sometimes been conducted from day 10 of follow-up, the earliest time that new infections have been detected. Although the indication for PCR is not open to debate, subsequent interpretation of results and data analysis can significantly modify the outcome of a study. According to the WHO protocol, cases of reinfection by *P. falciparum* or the appearance of *P. vivax* should lead to patient exclusion from the analysis. In both situations, particularly in the event of reinfection by *P. falciparum*, rescue treatment is administered. This treatment is unrelated to the infection for which the patient was enrolled in the study and could theoretically mask a true recrudescence when parasitaemia is not yet detectable, even by PCR. This should be considered as a protocol violation. A similar attitude should be adopted for cases in which PCR does not allow reinfection to be distinguished from recrudescence. Many teams do not exclude these patients (up to 50% of late failures in areas of high transmission), as the number of patients remaining for analysis would be markedly reduced. Cases of reinfection are often considered treatment successes, and cases of *P. vivax* infection appearing during the follow-up period are only mentioned. Failures in which PCR tests were not conducted or for which the result is indeterminate are sometimes

classified as treatment successes or as late failures. This kind of interpretation might be warranted in randomized clinical research studies in which the results are analysed in terms of intention to treat, but not within the framework of a surveillance protocol.

5.2.2 Analysis of available data for each drug

The problem of resistance affects all currently available drugs except for artemisinin derivatives, although the degree of resistance varies. The results of an analysis of the WHO database for antimalarial drug efficacy are summarized by country in Annex 5 and by monotherapy or combination therapy in Annex 6. These tables will be updated regularly on the Roll Back Malaria web site (www.who.int/malaria/resistance.html). The results are expressed for African countries (except South Africa and the area of moderate transmission in Sudan) as clinical failure (early treatment failure plus late clinical failure) after a follow-up of at least 14 days. Although results for day 28 and data on the prevalence of late parasitological failure are available in a limited number of studies, the vast majority of studies conducted in Africa adhered to the 1996 protocol, with follow-up for 14 days and a classification scheme that did not recognize late parasitological failure. In order to avoid losing information and to maintain coherence with the recommendations for policy change, data for the African countries are presented as clinical failures after a follow-up of 14 days. In fact, up to 2004, the main rationale in the African countries for changing national policy was the prevalence of clinical failure on day 14. For the other countries, including South Africa and the area of moderate transmission in Sudan, the results are expressed as total, clinical and parasitological failures (early treatment failure plus late clinical failure plus late parasitological failure) after a follow-up of 28 days. Most of the countries in areas of low-to-moderate transmission used a follow-up period of 28 days, although this was not systematic, in particular when the test drug showed a high failure rate on day 14. Follow-up was sometimes continued through to day 42 or day 63, depending on the drug used.

Chloroquine (Figures 2.1–2.3)

Since the first cases of resistance to chloroquine appeared in Thailand in 1957 and in Colombia and Venezuela in 1960, resistance has spread to all areas in which *P. falciparum* is endemic. Only the island of Hispaniola and the countries of Central America remained free of chloroquine resistance in early 2005. Chloroquine resistance in Africa occurred much later than in South America and South-East Asia. Molecular analysis has suggested that

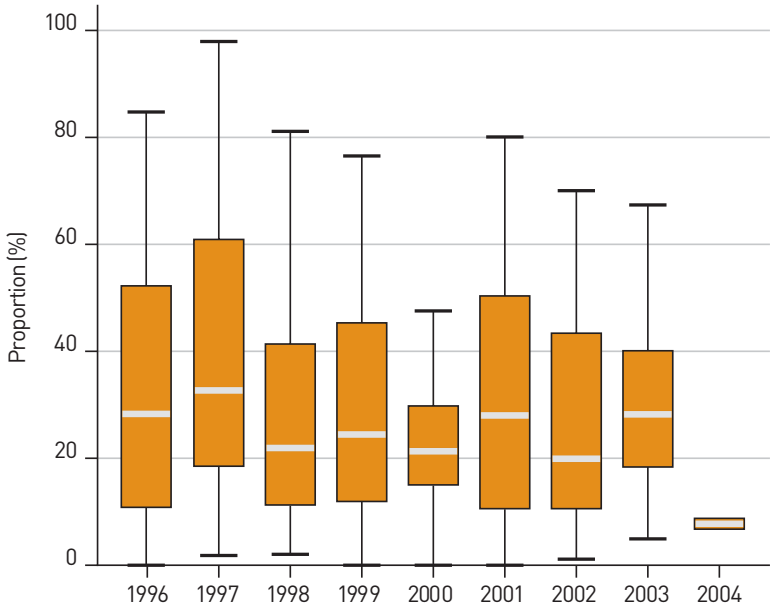
resistant mutants emerged independently at a limited number of sites in the world: two in South America, one in Asia, one in Papua New Guinea and one in the Philippines (99, 196–198). According to these studies, the advent of chloroquine resistance in Africa was not linked to the appearance of a new mutation but to the slow, gradual spread of chloroquine resistance from South-East Asia, with its final arrival in East Africa in 1978 (199).

Note. In all the box charts shown in the figures, the line within the box indicates the median value and the outline of the box represents the 25th and 75th percentiles. The whiskers correspond to the upper and lower adjacent values. These values correspond roughly to the complete range, but some outlines are omitted and are not displayed in the graphs. For years with three or two studies, the whiskers are omitted; for years with a single study, the value is shown with a single horizontal line. The box plots illustrate study results equally and do not account for varying sample size, reported protocol or study site location.

Since 1978, chloroquine resistance has spread in Africa both geographically and in intensity. In all African countries except Djibouti and Swaziland, at least one study has confirmed a treatment failure rate >25%. Increasing levels of chloroquine resistance or its stabilization at a high level have been confirmed in those countries that have maintained the same sentinel sites over time, both for treatment efficacy tests and for in vitro tests and molecular markers: Burkina Faso, Cameroon, Comoros, Eritrea, Guinea-Bissau, Madagascar, Mali, Mozambique, Senegal, Sudan, Uganda and Zambia (http://www.who.int/malaria/resistance.html).

Significant regression of chloroquine resistance has rarely been fully documented, except in Malawi after total withdrawal of chloroquine. Apparent regression is usually a result of a comparison of data from different study protocols (in particular different age groups) or to technical problems with in vitro tests (60, 200). A reduction in chloroquine resistance has nevertheless been described in some countries, usually on the basis of the results of in vitro tests or use of molecular markers. Chloroquine was abandoned as a first-line drug in Thailand in 1972 and replaced by sulfadoxine–pyrimethamine. In vitro tests conducted between 1984 and 1990 showed a significant fall in resistance (201, 202), and the results were partly confirmed by the successful treatment of clinical cases of *P. falciparum* infection diagnosed erroneously as caused by *P. vivax*, despite a long parasite clearance time (203).

Figure 2.1
Clinical failure after chloroquine treatment in Africa, by year (1996–2004)[a]

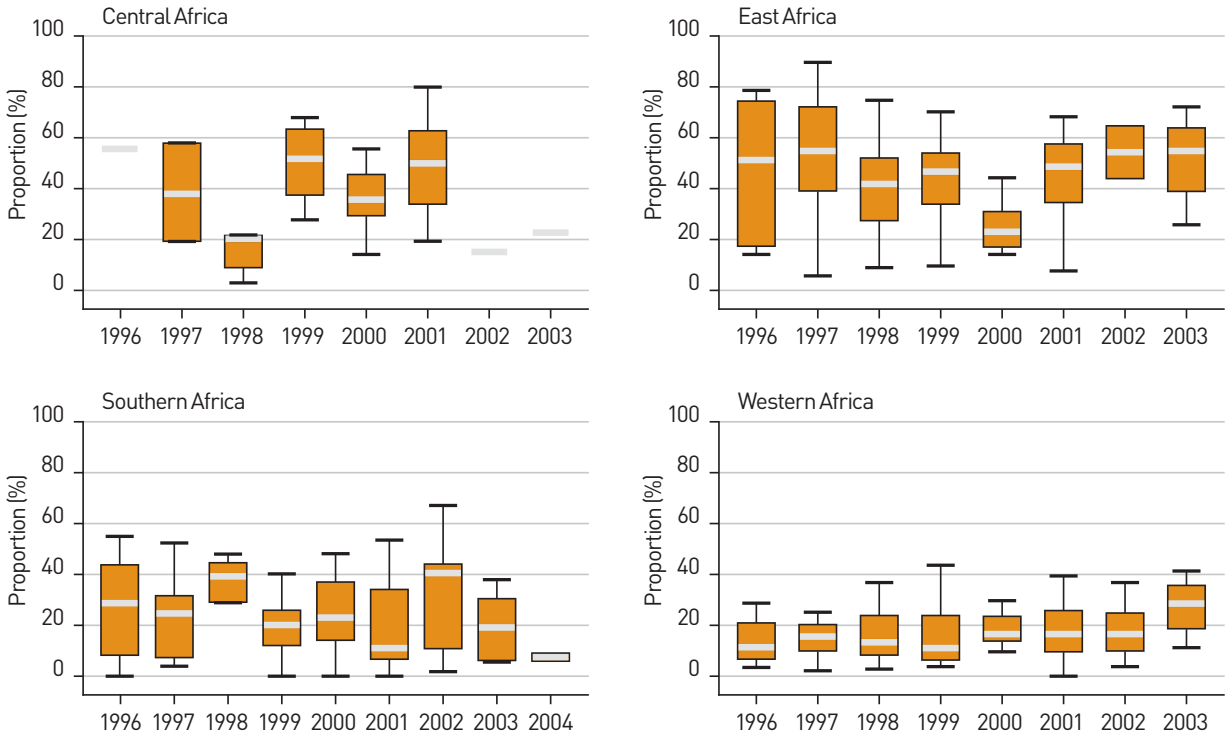


[a] Figure 2.1 shows that the clinical failure rate at day 14 of chloroquine in Africa remained steady throughout the study period. A median of 24% of all 405 studies considered experienced clinical failure with chloroquine. The values for individual years varied from a low of 21.4% in 2000 to a high of 32.5% in 1997, but the overall trend remained constant around the 24% median. The large numbers of studies of clinical failure of chloroquine in Africa allow annual analysis of the four subregions of Africa (see Annex 7).

Chloroquine resistance was reported to have regressed in a similar manner between 1981 and 1992 in Yunnan Province, China, where the mean IC50 values fell from 170 nmol/l to 110 nmol/l, respectively (204). Between 1981 and 1997 on the island of Hainan, the efficacy of chloroquine increased from 15.8% to 80.6%, in parallel with a fall in the IC50 by a ratio of 6. The number of patients was limited (20–30 per study), and the authors did not state to what extent the groups were comparable (58, 205).

In Viet Nam, two teams reported an increase in sensitivity to chloroquine in vitro in Binh Phuoc Province (206, 207). In light of these results, chloroquine was combined with artesunate (8 mg/kg over 3 days) for the treatment of two study populations of different ages and immunity levels in two provinces, Dac Lac and Binh Phuoc. Paradoxically, the prevalence of the *pfcrt* mutation varied from 60.6% to 95.8%, while chloroquine resistance in vitro ranged from 10.3% to 13.2%. Depending on the method used to obtain

Figure 2.2
Clinical failure after chloroquine treatment in Africa, by subregion and year (1996-2004)[a]

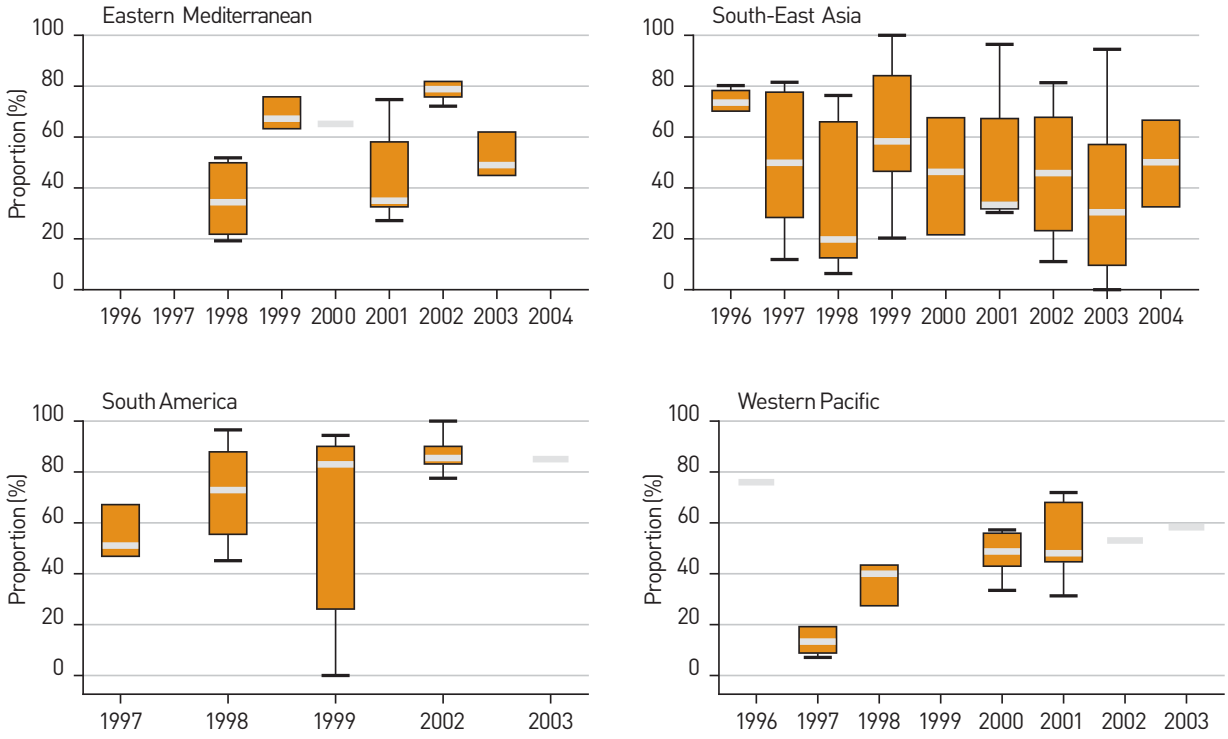


[a] Figure 2.2 shows that the stable estimates of clinical failure observed in Figure 2.1 mask substantial differences among the subregions. Many studies have been conducted in eastern, southern and western Africa (111, 105 and 156, respectively), but only 33 were conducted in Central Africa. Clinical failure was consistently highest in East Africa, with a median rate of 48.2%, followed by southern Africa with a rate of 22% and western Africa with a rate of 16.1%. Although there have been fewer studies, the estimate for Central Africa shows more variability, ranging from 14.2% (2002) to 51.6% (1999) with a median across all years of 38%.

the results, the treatment failure rate with the combination ranged from 3.6% to 48.5% with microscopic reading and from 25% to 45.5% with detection of parasites by PCR (208).

Malawi differs from these Asian countries by high levels of transmission, the almost exclusive prevalence of *P. falciparum* and, especially, by the fact that chloroquine disappeared from the market in 1993, the year in which the national health authorities changed their drug policy. In Blantyre, the prevalence of the mutant *pfcrt* gene at codon 76 fell from 85% in 1992 to 13% in 2000. In 2001, 55 asymptomatic adults were rendered completely parasite-free by treatment with chloroquine: no isolates were found to be chloroquine resistant in vitro and none were carriers of the mutant *pfcrt*

Figure 2.3
Total failure after chloroquine treatment in Africa, Asia and the Americas, by subregion and year (1996–2004)[a]

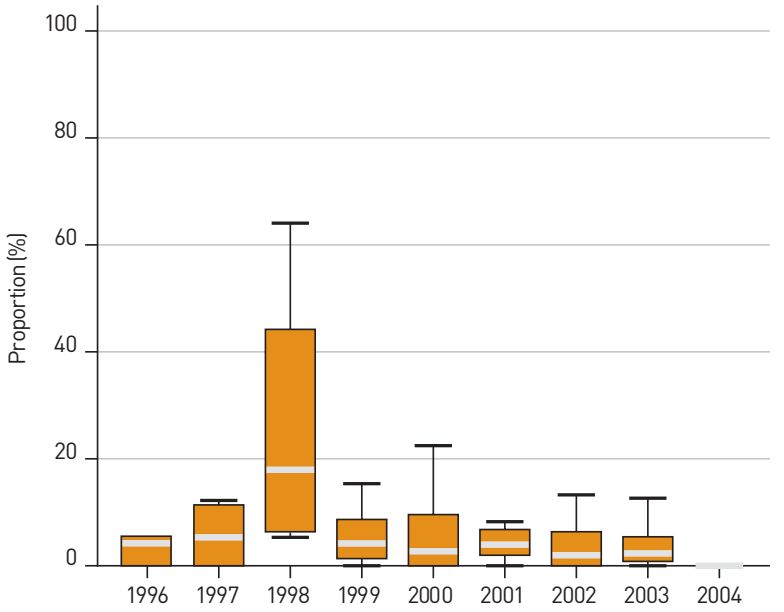


[a] Covering 151 studies, Figure 2.3 shows more variability in the total failure rate at day 28 in Asia and the Americas than in Africa. Despite a high median total failure rate in 1996 of 76.2% and a low of 33.2% in 2001, the overall trend remains constant around the median of 48.6% across the years.

gene (161). In Salima district, the prevalence of the Lys76Thr mutation in the *pfcrt* gene regressed from 17% to 2% between 1998 and 2000, and 90.6% of 53 asymptomatic children were successfully treated with chloroquine (57). The disappearance of parasites with the mutant *pfcrt* gene was confirmed in another study (209). Paradoxically, the prevalence of the Asn86Tyr mutation in the *pfmdr1* gene has not regressed similarly. The disappearance of parasites carrying the mutant *pfcrt* gene is thought to be linked to expansion of wild-type strains rather than to reversion of the Lys76Thr mutation (210).

These results are highly encouraging, but it is currently impossible to predict the probable outcome of wide-scale reintroduction of chloroquine in such a context and, in particular, how long it would take for chloroquine resistance to reappear in non-immune populations.

Figure 3
Clinical failure rates after amodiaquine treatment in Africa, by year (1996–2004)[a]

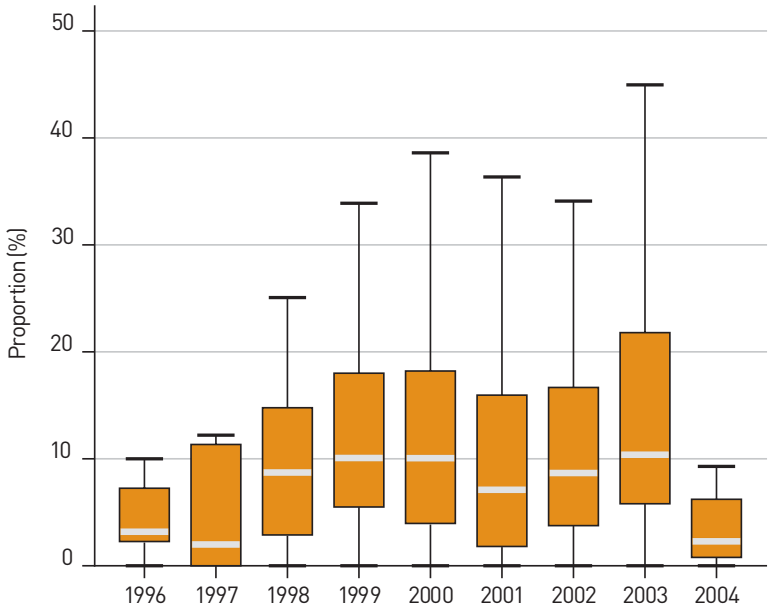


[a] Figure 3 indicates that the clinical failure rate at day 14 after amodiaquine therapy remains low in Africa. In these 90 studies, the clinical failure rate began at 4.4% in 1996 and stayed in the same range, to end at 1.8% in 2003, with an overall median of 3.3%.

Amodiaquine (Figure 3)

The efficacy of amodiaquine as a single-drug therapy at a dose of 25–30 mg/kg has been studied in about 20 African countries over the past 10 years. Despite cross-resistance between chloroquine and amodiaquine, which has been described many times, amodiaquine remains more effective than chloroquine in areas of chloroquine resistance (31, 211). Amodiaquine was accordingly chosen by several countries as the first-line drug in combination with either sulfadoxine–pyrimethamine or artesunate. Amodiaquine can, however, rapidly lose its efficacy if it is used intensively in areas where chloroquine resistance is widespread or where the level of chloroquine resistance is high, as in Rwanda. The 28-day clinical and parasitological failure rates at three sentinel sites ranged from 14.3% to 25.5% in 2001, and these rates had increased slightly to 19.7–27.9% at the same sites 1 year after adoption of the combination (212, 213). In 15 countries, at least one study has confirmed a clinical and parasitological failure rate ≥20%: Angola, Central African Republic, Democratic Republic of the Congo, Ethiopia, Gabon, Kenya, Liberia, Rwanda, Sierra Leone, Sudan, Uganda and United

Figure 4.1
Clinical failure rates after sulfadoxine–pyrimethamine treatment in Africa, by year (1996–2004)[a]



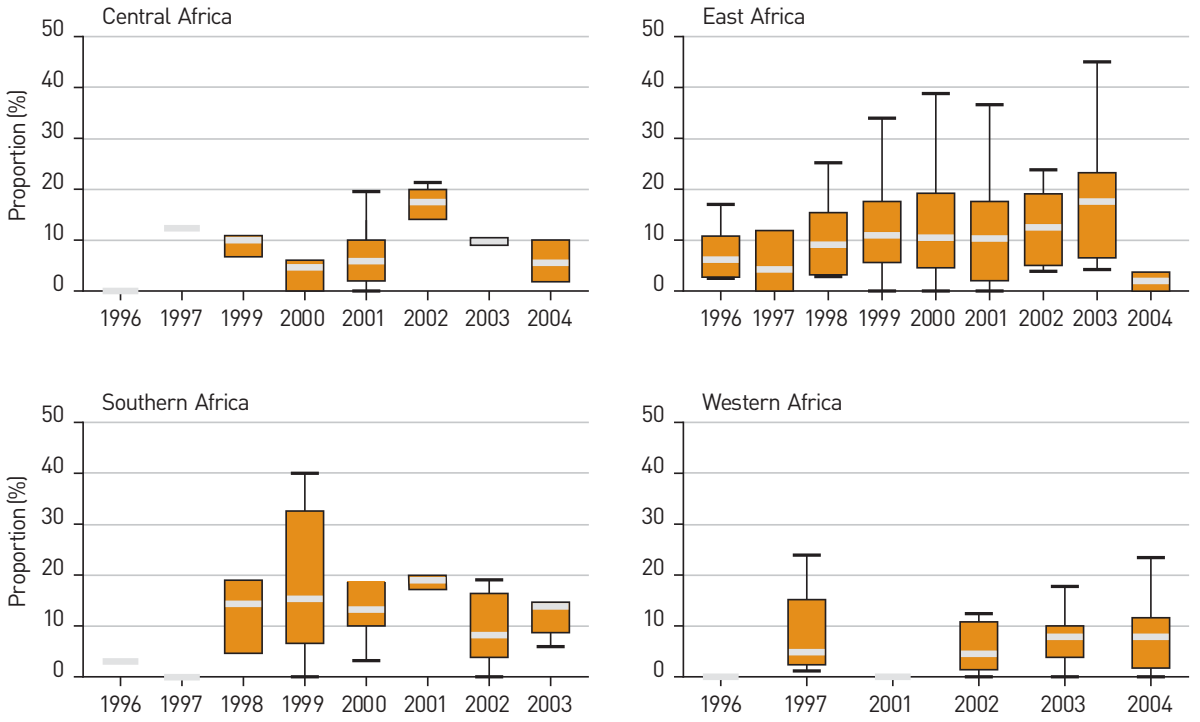
[a] The clinical failure rate at day 14 of sulfadoxine-pyrimethamine treatment remains low in Africa but is increasing. In the 234 studies considered, the clinical failure rate began at a low of 2.0% and rose over the period to a high of 10.4% in 2003. In addition to an increasing median, high clinical failure rates became more common, the 75th percentile increasing from 25% to 45% in 2003.

Republic of Tanzania in Africa; and Afghanistan, Colombia and Pakistan. In five countries, at least one study has confirmed a clinical and parasitological failure rate between 10% and 20% (Cameroon, Chad, Nigeria, Senegal and Somalia), and in three countries, the clinical and parasitological failure rates are <10%, although monitoring is generally for 14 days (Burkina Faso, Madagascar, Mozambique and Zanzibar).

Sulfadoxine–pyrimethamine (Figures 4.1–4.3)

Resistance to antifolate, unlike that to chloroquine, emerged very rapidly, after only 1–2 years' intensive use at the national level. The sulfadoxine–pyrimethamine combination has been used widely to treat chloroquine-resistant malaria. Monitoring of resistance, either by treatment efficacy tests or molecular markers, has confirmed that treatment efficacy in most endemic countries is <90% or the prevalence of triple mutants in the *dhfr* gene is high. Recent data on therapeutic efficacy are not available from

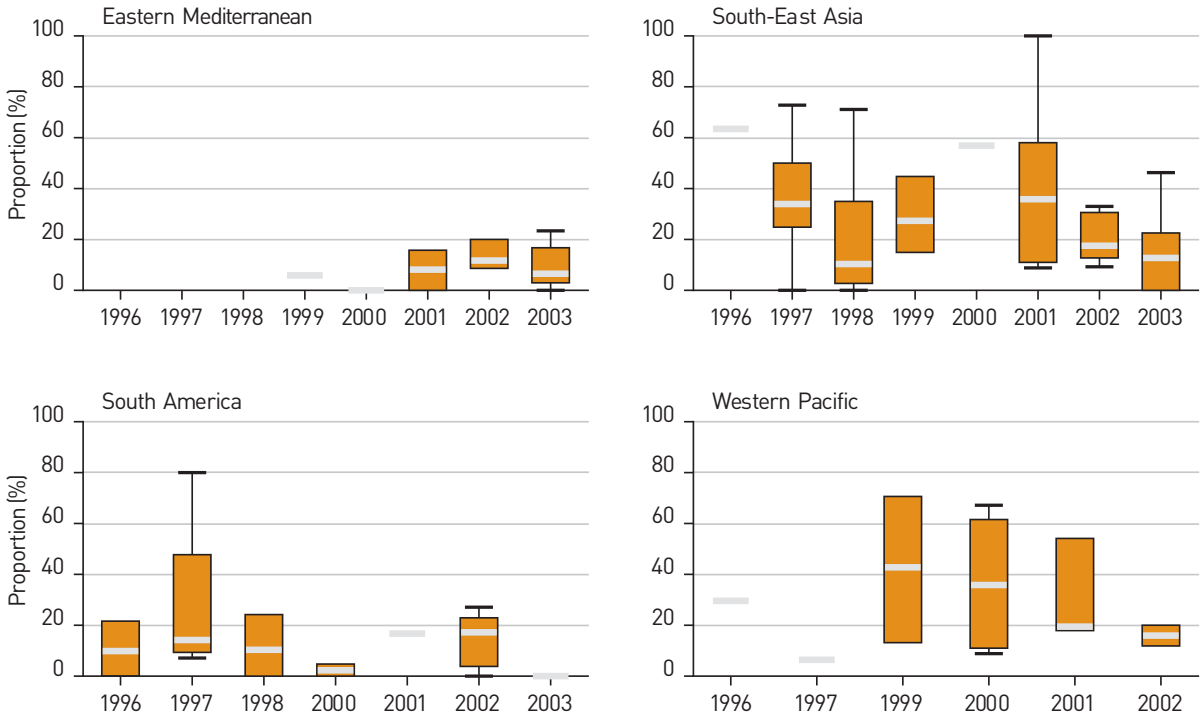
Figure 4.2
Clinical failure rates after sulfadoxine–pyrimethamine treatment in Africa, by subregion and year (1996–2004)[a]



[a] Across all subregions of Africa, the clinical failure rate of sulfadoxine-pyrimethamine is steady or rising. In 107 studies, East Africa showed a clear upwards trend. The clinical failure rate rose from 4.0% in 1997 to 17.2% in 2003. The 41 West African studies also showed a slight upwards trend, from 4.2% to 7.8%, but the trend was weaker. No discernible trends emerged for Central or southern Africa. Across all years, the 28 studies in Central Africa show variations around a median clinical failure rate of 8.6%, and the 58 studies in southern Africa show a similar median (8.9%).

Botswana, Central America, Djibouti, Guinea, Mauritania, Niger, Sao Tome and Principe, Saudi Arabia, Swaziland or Togo; however, in Mauritania, 12.6–18.6% of parasites are mutant in at least one of the key *dhfr* codons, and 15.8–22% of parasites are mutant at codon 437 of the *dhps* gene. No mutation was reported at codon 540. The treatment failure rate remains low in the Congo, in the Islamic Republic of Iran and in Mali. In the Congo, the clinical failure rate after 14 days is about 10%, but the prevalence of triple mutants is high (214). In the Islamic Republic of Iran, the clinical and parasitological failure rates do not exceed 6% after 28 days. Although the prevalence of double *dhfr* mutants is high (60–80%), that of triple mutants remains <15%. Mutations at codon 540 of the *dhps* gene are rare (151, 215).

Figure 4.3
Total failure rates of sulfadoxine-pyrimethamine treatment in Asia and the Americas (1996–2004)[a]



[a] Figure 4.3 shows the total failure rates at day 28 for sulfadoxine-pyrimethamine in Asia and the Americas. No trends emerge for these regions, but the data do indicate the relative failure rates across subregions. In 11 and 28 studies, the Eastern Mediterranean and Americas regions had the lowest total failure rates, of 8.7% and 12.2%, respectively. Total failure was more common in the 15 studies in the Western Pacific subregion, with a median of 18.7%, and the highest rate in the 50 studies in South-East Asia was 21.7%.

In Mali, the treatment efficacy rate remains higher than 90%, but the prevalence of triple *dhfr* mutants has reached 20% in some regions (26, 162). No or low therapeutic failure rates have been described in Madagascar and Yemen after 28 days of follow-up, and these results were confirmed by the absence or low prevalence of *dhfr* mutation at codon 108 (63, 87, 216).

In 1993, Malawi was one of the first countries in Africa to adopt the sulfadoxine–pyrimethamine combination as the first-line drug. Longitudinal monitoring with both treatment efficacy tests and molecular markers confirmed rapid development of resistance. After 7 years of use, the prevalence of quintuple *dhfr–dhps* mutants had reached 78%, and the currently published clinical and parasitological failure rates exceed the acceptable thresholds (150, 217, 218).

Studies of molecular markers and microsatellites suggest that spread of sulfadoxine–pyrimethamine resistance is not necessarily associated with the appearance of new resistant mutants and their propagation under drug pressure. Highly resistant strains might have a common ancestral origin, which propagated initially at the regional level, as has been described in South America, South-East Asia and southern Africa (99, 219–221). Moreover, it is thought that parasites highly resistant to sulfadoxine–pyrimethamine in Africa came originally from South-East Asia. These suggestions are alarming because 67% of isolates in Cambodia, Myanmar and Thailand carry the Leu164 mutation in the *dhfr* gene and because resistance to mefloquine and quinine has become common in South-East Asia.

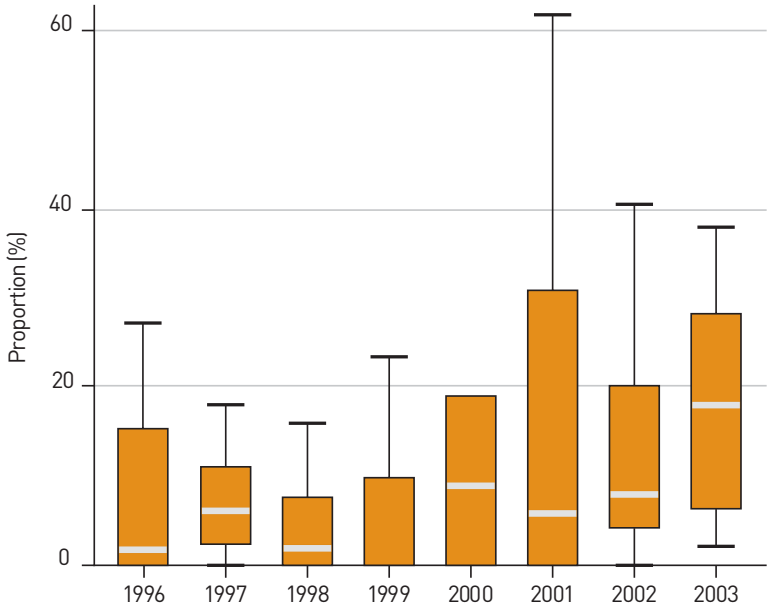
Resistance to sulfadoxine–pyrimethamine does not appear to evolve in the same way as that to chloroquine. Withdrawal of the combination in South-East Asia has not been followed by disappearance of the resistant mutant parasites. This may be a result of cross-resistance between sulfadoxine–pyrimethamine and antibiotics such as co-trimoxazole or the existence of compensatory mutations in resistant parasites (222). Furthermore, sulfadoxine–pyrimethamine is still circulating in large quantities in the informal sector, maintaining drug pressure on the regional parasite populations. After 2 years' use of impregnated mosquito nets in a village in the United Republic of Tanzania, however, the prevalence of wild-type strains had increased in comparison with that in a neighbouring village (223). Although this finding concurs with some mathematical models, additional field studies will be necessary to confirm regression of antifolate resistance (224).

Mefloquine (Figures 5.1–5.2)

Mefloquine resistance appeared at the Thai–Cambodian border barely a few years after introduction of the drug (225). This rapid onset was probably linked to two factors: the existence of strains with markedly reduced sensitivity to quinine and the long half-life of mefloquine. Other possible factors include common use of the low-dose, single-dose regimen (15 mg/kg) in this region and the availability of large quantities of the drug through illegal outlets. Mefloquine is used at doses of 15–25 mg/kg. The higher dose is more effective but is associated with more adverse effects, in particular vomiting, which may result in lower blood concentrations and hence treatment failure (226–228).

Much has been published on resistance in vitro and prophylactic failure in travellers (229, 230); however, some of the failures were successfully treated with a therapeutic dose of mefloquine, suggesting that the failures resulted from poor compliance or poor absorption (5, 231). Treatment failure in

Figure 5.1
Total failure rates after mefloquine treatment in Asia and the Americas, by year (1996–2004)[a]

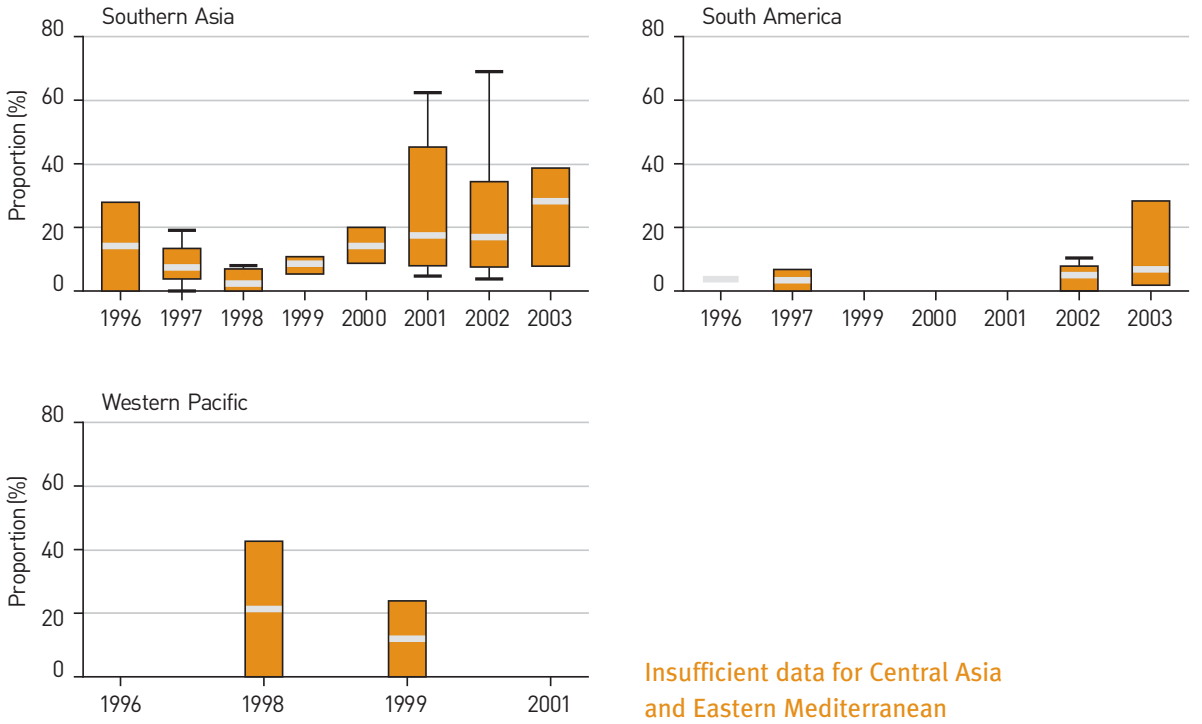


[a] Figure 5.1 indicates that the failure rates fluctuated widely in the 66 studies of total failure of mefloquine in Asia and the Americas. The rate of total failure was 1.7% in 1996, and the median was 18% in 2003. The overall median remained much lower, at 6%, but an increasing number of recent studies showed a high total failure rate to mefloquine.

travellers nevertheless remains an early warning of reduced sensitivity to mefloquine. In vitro data should be interpreted carefully, as the resistance thresholds for mefloquine vary according to the method used. Furthermore, plates pre-dosed with mefloquine have a short shelf-life (approximately 3–6 months), and rapid degradation of mefloquine results in an artificial rise in the IC50 value and overestimated levels of resistance.

Mefloquine resistance has become a major public health problem in South-East Asia. Through its monitoring system, Thailand has been able to observe a gradual decline in the efficacy of mefloquine at its sentinel sites. Even when the dose was increased from 15 mg/kg to 25 mg/kg, the rise in efficacy was only temporary, and the treatment failure rate is now >60% at some sites (54, 232). The situation is also of major concern in Cambodia, Myanmar and Viet Nam, where the treatment failure rate can be as high as 40% but where the standard dose remains 15 mg/kg (233, 234). A study in 1996–1997 showed an efficacy of <80% in Bangladesh, although this result should be confirmed, especially since more recent studies indicate that efficacy has been retained in Bhutan and India (235).

Figure 5.2
Total failure rates after mefloquine treatment in Asia and the Americas, by subregion and year (1996–2004)[a]



[a] The regional data in Figure 5.2 pertain mostly to the South-East Asia subregion. Of the 66 studies with mefloquine, 42 are from that subregion, 18 from the Americas Region and 6 from the Western Pacific subregion. The data for the South-East Asia subregion show a mixed pattern, the rate of total failure beginning at 13.6% in 1995 and ending at 18% in 2003, but with an overall median of 8.3%. The total failure rate in the Americas Region remained low, with a median of 6.4% in 2003 and a total median of 1.6%. Of the six studies in the Western Pacific subregion, four showed a total failure rate of 0% and the remaining two total failure rates of 12% and 21%.

In Africa, studies have shown that some strains of *P. falciparum* have reduced sensitivity to mefloquine in vitro but remain sensitive to chloroquine (236, 237). The fact that mefloquine has not been massively deployed in this region suggests innate parasite resistance rather than acquired resistance. A clinical failure rate of 10% was reported in a study in Malawi with the 15 mg/kg dose in 1998, and several children had blood concentrations of mefloquine >500 ng/ml on day 2 (238). Similar failure rates had been observed 10 years earlier (13). In South America, the efficacy of this drug exceeds 90%, except in Guyana, but the 15 mg/kg dose is still too frequently used in South America.

Isolated cases of treatment failure and sometimes early failure have been observed in Brazil, Cameroon, China, Indonesia, Nigeria, Senegal, Sudan,

United Republic of Tanzania and Zambia, but their public health impact remains limited. The results of these studies are shown in Table 2.

Quinine

Since adoption of artemisinin-based drug combinations as first-line treatment for uncomplicated cases of malaria, quinine has been reinstated as an option for second-line treatment. Many countries therefore have expressed interest in studying the level of parasite sensitivity to this drug; however, the standard protocol is not suitable for evaluating the efficacy of quinine. Quinine must be administered as three divided daily doses over 7 days. If treatment is prolonged beyond 3 days, most if not all patients show adverse effects, in particular tinnitus, temporary deafness or dizziness, and evaluation of the efficacy of quinine requires hospitalization of patients in order to ensure their compliance.

It is difficult to demonstrate resistance to quinine. Most failures during treatment with quinine occur in the third week of follow-up (253). There is no point in assaying plasma quinine concentration at the time of failure because of the very short half-life of the drug. A continuous concentration ≥6 µg/ml over at least 7 days is required to ensure cure (10). The commonly used in vitro resistance threshold of 500 nmol/l has not been validated correctly. If a 48-h parasite reduction percentage of 10^2–10^3 is assumed for quinine, the length of treatment should be adjusted to the parasite load (254). In the event of hyperparasitaemia, it may prove necessary to extend treatment beyond 7 days or to combine quinine with another antimalarial agent (255).

For all these reasons, it is difficult to estimate the extent of quinine resistance across the world. In addition, the study results are extremely diverse because of varying lengths and doses of quinine treatment, use alone and in combination and differences in the patient groups (adults, children, pregnant women) enrolled in the studies.

In Africa, the frequency of proven quinine resistance remains low. Most cases are not correctly documented or are due to insufficient doses of quinine (256-264). Many failures are a result of errors in the conversion of dose of quinine salts to quinine base (265). The clinical failure rates after treatment of acute uncomplicated malaria over 7 days with a follow-up of 14 days were 0% in Gabon, 4.8–5.5% in Equatorial Guinea and 6.3–9.3% in Sudan, where follow-up was for 28 days (266–268). In the Democratic Republic of the Congo, the clinical and parasitological efficacy of the quinine–clindamycin combination (3–5 days) was higher (94.1–96.9%) than that of quinine given as a single drug (86.1–90%) (269, 270). The efficacy of quinine remains high in

India and Malaysia, while it is considerably reduced in Bangladesh, at 79.6–81.6% (235, 271). In Thailand, the efficacy of quinine varied from 67% to 100%; when used in combination with doxycycline or clindamycin, the efficacy of quinine exceeded 97% (272), but the efficacy of the quinine–rifampicin combination was low because of pharmacokinetic interaction between the two drugs (273). The efficacy of quinine was also reduced in Venezuela (77.8–90.4%) and Viet Nam (81.2%) (274).

Further studies are needed to determine whether the standard protocol needs adjustment for evaluation of quinine. In a 28-day pilot study conducted by WHO in Tajikistan, the efficacy of quinine was 100%. In view of its relatively slow action, as confirmed by the 48-h parasite reduction percentage, use of the current criteria for early treatment failure might lead to an erroneous conclusion of early resistance, especially as a temporary rise in parasitaemia is often seen after initiation of treatment with quinine, whereas the course of the disease remains unaffected (H. Noedl unpublished data, 2004; 275–278).

Atovaquone–proguanil

Atovaquone is used in combination with proguanil for treatment and prophylaxis, but, in view of its exorbitant price, the combination is reserved for travellers from industrialized countries. Its efficacy was >95% in studies in endemic areas and in travellers, except in Kenya (93.1%) and Viet Nam (86%) (144, 279–283). In Kenya, two successive treatments with atovaquone–proguanil failed to cure a child (284). Its prophylactic efficacy is >96% against *P. falciparum* but, for unknown reasons, considerably lower against *P. vivax* (84%). A few treatment breakthroughs have been reported in travellers returning from Cameroon, Congo, Côte d'Ivoire (n = 3), Gambia, Kenya and United Republic of Tanzania (n = 2) and Mali and Nigeria (n = 2) (146–148, 285, 286). Of the 11 strains, 5 did not have a mutation in the cytochrome *b* gene, and assays for pharmacokinetics were conducted in only 4 cases.

Artemisinin and derivatives

Artemisinin and its derivatives act extremely rapidly, and most patients are clinically cured within 1–3 days. Short-course monotherapy for 3–5 days leads, however, to a high rate of recrudescence. Monotherapy with artemisinin derivatives is not recommended but, if necessary, should be for at least 7 days. A better option is to administer an artemisinin-based combination therapy.

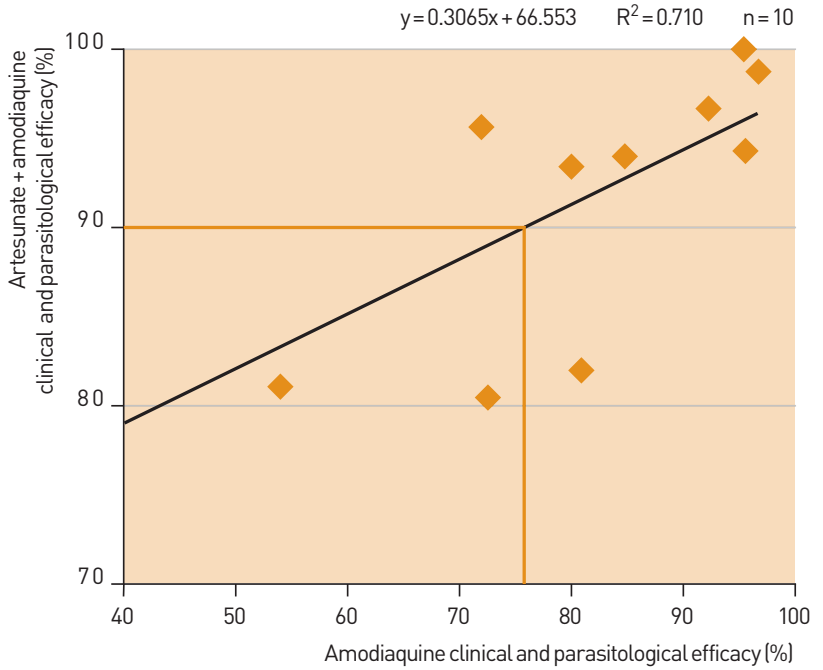
Resistance to artemisinin derivatives has been induced in murine models. In a new mechanism of action, recently described, artemisinin is thought to inhibit the *Plasmodium* sarcoendoplasmic reticulum Ca^{2+}–ATPase (PfATP6) (287, 288). In *P. falciparum* and *P. yoelii*, amplification of the *pfmdr1* gene (or its homologue) appears to be the common genetic mechanism of resistance (16, 289–292). Resistance to artemisinin derivatives is difficult to confirm, however, as the half-life of these drugs is short and the in vitro resistance threshold has not been defined (293, 294). Many cases of "resistance" were not properly documented and included failures after excessively short treatment, early deaths caused by cerebral malaria and absence of PCR data to eliminate reinfection (295, 296). Reduced immunity (resulting from human immunodeficiency virus (HIV) infection or splenectomy) and haemoglobin abnormalities can lead to excessively long delays in treatment response (297, 298).

Although resistance to artemisinin has not been formally confirmed, three types of evidence argue for enhanced monitoring. Monitoring over time, either recently or since the 1990s, has not provided in vitro evidence of an increased IC50 value for artemisinin derivatives in Cambodia, Cameroon or Thailand (37, 52, 56, 299). In Viet Nam, the IC50 for artemisinin remained stable between 1998 and 2001, while the IC90 and 99% inhibitory concentration (IC99) doubled and quadrupled, respectively (207). The sensitivity of *P. falciparum* to artesunate also fell significantly between 1988 and 1999 in China, where the IC50 tripled and the MIC doubled (300). A temporary rise in the IC50 for artemether was reported in 2002 in French Guyana (P. Esterre, unpublished data, 2004).

The second type of evidence comes from tests of treatment efficacy. In China, the efficacy of dihydroartemisinin given over 7 days at a dose of 12.8 mg/kg was 95.8–97.3% (301–303). In Thailand, artesunate used at a dose of 12 mg/kg over 7 days in pregnant women and at a dose of 13.2 mg/kg in other adults, had an efficacy of 90.5–100% (272). In Viet Nam, the efficacy of artesunate at a dose of 12 mg/kg over 5 days was 71–87.5% but increased to 93.1% at a dose of 16 mg/kg over 7 days (207, 234). Most of the failures were in late treatment; however, it has been shown that failures after artesunate treatment result not only from decreased sensitivity of strains to artesunate but also from relatively high pretreatment parasitaemia. The latter should dictate the appropriate treatment (304). In addition, as for quinine, it is not rare to witness a rise in parasitaemia after the start of treatment with artemisinin derivatives (305).

The third type of evidence comes from the observation of isolated cases. Four cases, two in India and two in Thailand, are suspected of being early failures (306–308). The precise immunological status and the presence or

Figure 6.1
Correlation between amodiaquine and artesunate+amodiaquine success rate at day 28[a]



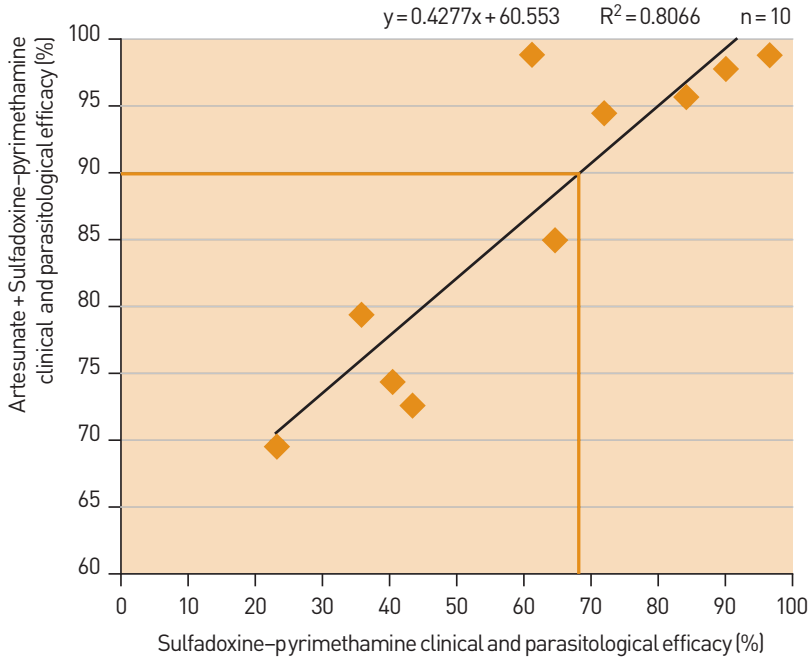
[a] Figures 6.1 and 6.2 show that the expected total failure rate for amodiaquine and sulfadoxine–pyrimethamine would need to reach 25% and 30%, respectively, in order for the efficacy rate of the combination with artesunate to fall below 90%.

absence of genetic disease were not established in all patients, and drug quality control is not mentioned. In India, an adult still had parasitaemia after 5-day parenteral treatment with artemether (total dose, 480 mg administered by intramuscular injection), and another adult reported recrudescence on day 14 after 7 days' treatment with artesunate at a dose of 13.3 mg/kg. In Thailand, two children aged 2 and 5 years had positive blood smears on day 7 after a dose of 12 mg/kg, and one had persistent parasitaemia throughout treatment.

Drug combinations

For all countries in which a change in first-line treatment is being considered, especially from a monotherapy, WHO recommends a combination therapy, if possible based on artemisinin. Five combinations are currently recommended: artesunate+amodiaquine, artesunate+sulfadoxine–pyrime-

Figure 6.2
Correlation between sulfadoxine–pyrimethamine and artesunate+sulfadoxine–pyrimethamine success rate at day 28[a]

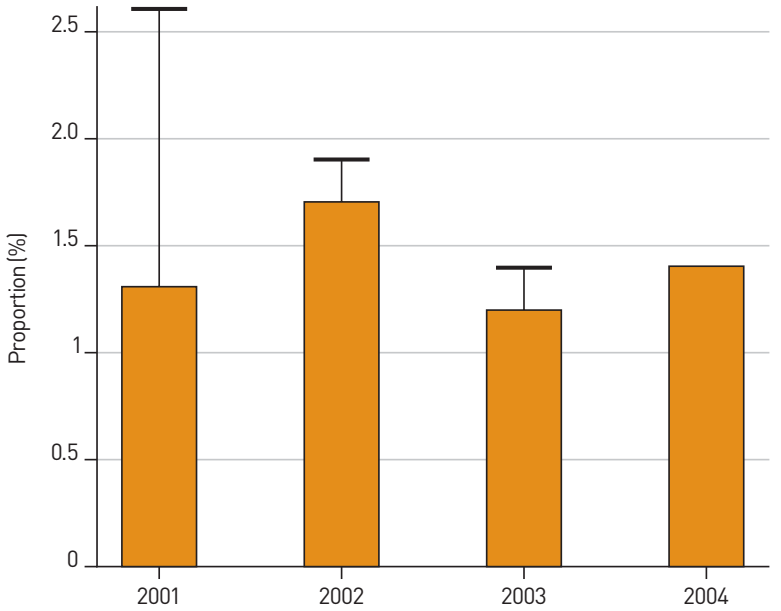


thamine, amodiaquine+sulfadoxine–pyrimethamine, artesunate+mefloquine and artemether–lumefantrine.

The rationale for use of drug combinations in the treatment of tuberculosis and HIV infection is well established. In malaria, the probability that a parasite that is resistant to two drugs with different modes of action will emerge is markedly reduced, from 1 in 10^9 to 1 in 10^{18}, when a combination is used (77). Artemisinin derivatives are particularly interesting constituents, as they are more active than the other antimalarial drugs, have a broad spectrum of activity on the parasite cycle and inhibit the production of gametocytes.

The total failure rate with amodiaquine or sulfadoxine–pyrimethamine used as monotherapy, below which these drugs are likely to remain adequately effective in combination with artesunate (>90%), has yet to be clearly established (309). Preliminary results from a limited number of studies suggest that, beyond a total failure rate of 20% for amodiaquine or sulfadoxine–pyrimethamine, the efficacy of the combination with artesunate does not exceed 90%. Analysis of 20 studies in the WHO database partly confirms this hypothesis (Figures 6.1 and 6.2).

Figure 7.1
Clinical failure rates after artesunate+amodiaquine treatment in Africa, by year (1996–2004)[a]

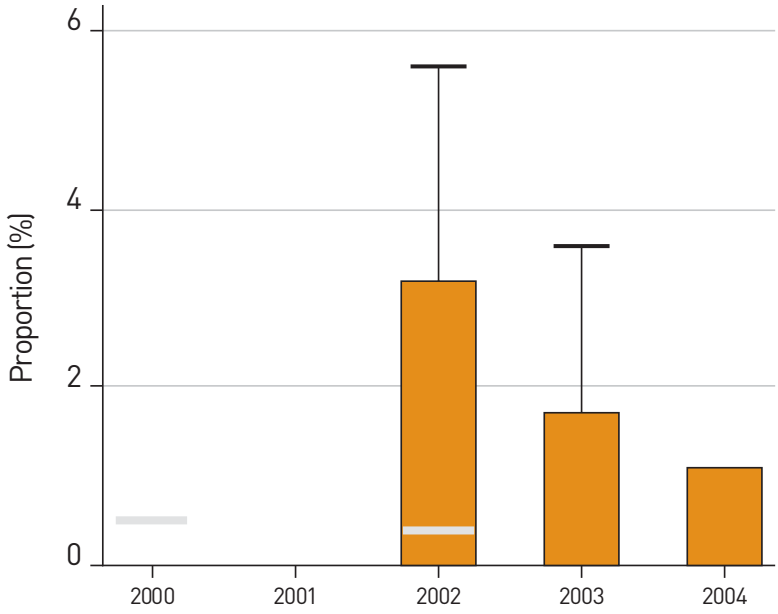


[a] Figure 7.1 summarizes the results of 30 studies of clinical failure at day 14 of artesunate+amodiaquine in Africa. The rate remained low, with a median of 0% for each year, and few studies showed clinical failure rates >1.5%. The few studies that demonstrated clinical failure were clustered in central and eastern Africa. Outside Africa, only five studies have addressed total clinical failure after artesunate+amodiaquine: four in Myanmar and one in Pakistan. The total failure rates in Myanmar reached a median of 4.0%, while the sole study in Pakistan showed a total failure rate of 18%.

One of the first outcomes of initial studies in Africa is that the combination of artesunate+chloroquine is of no practical use because of the already high prevalence of chloroquine resistance (309). These results have been confirmed in Pakistan and Viet Nam (M. Rowland, unpublished data, 2004; 208).

The highest rate of clinical and parasitological failure with the combination artesunate+amodiaquine, after 28-day monitoring and corrected for reinfection by PCR, varies substantially across the African continent: 1.2% in Angola, 3.5% in Senegal, 4% in Uganda, 6.8% in the Democratic Republic of the Congo, 9.2% in Zanzibar (United Republic of Tanzania), 7.2% in Sudan and 19.5% in Rwanda (Figure 7.1). Similar results have been recorded for the combination artesunate+sulfadoxine–pyrimethamine (Figure 7.2): 1.2% in Angola, 5% in South Africa, 5.5% in Ghana, 8.8% in Sudan, 16.5% in Zambia, 19.7% in the Democratic Republic of the Congo and 30.3% in Rwanda.

Figure 7.2
Clinical failure rates after artesunate+sulfadoxine–pyrimethamine treatment in Africa, by year (1996–2004)[a]

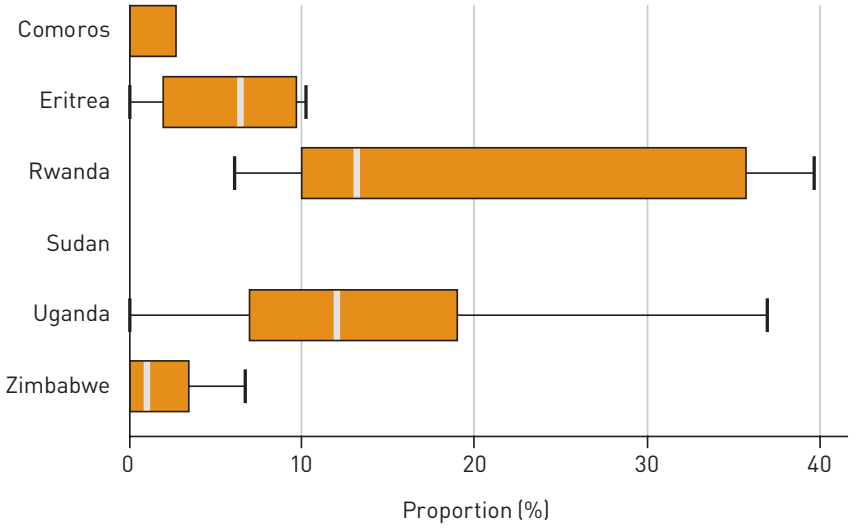


[a] Figure 7.2 summarizes the results of 24 studies of clinical failure at day 14 of artesunate+sulfadoxine-pyrimethamine in Africa. The clinical failure rate remained low, none of the medians exceeding 0.5%. Each subregion had a median clinical failure rate of 0%, except for a single study in West Africa which showed a rate of 0.75%. Only in studies in southern Africa did the clinical failure rate exceed 1.5%. Outside Africa, only 10 studies have addressed total clinical failure of artesunate+sulfadoxine-pyrimethamine: one in Indonesia, two in Myanmar, one in Pakistan, one in Sri Lanka and two in Viet Nam. The highest total failure rates were reported in Indonesia and Viet Nam, where the median value reached 4.3% and 33.2%, respectively.

Paradoxically, the combination artesunate+amodiaquine appears to be still adequately effective in Myanmar but not in Pakistan; this also applies to the combination artesunate+sulfadoxine–pyrimethamine, which remains effective in Ecuador, Indonesia, Pakistan, Peru and Sri Lanka but not in Viet Nam.

The combinations sulfadoxine–pyrimethamine+chloroquine and sulfadoxine–pyrimethamine+amodiaquine have been proposed as alternatives to combinations containing an artemisinin derivative (310, 311). The clinical failure rates with sulfadoxine–pyrimethamine+chloroquine (Figure 8) are very high after 14-day monitoring in Africa and can exceed 30% after 28-day monitoring both in Africa and in other endemic areas. Comoros, Ecuador, India, Tajikistan and Zimbabwe are the exceptions, but the failure rate with sulfadoxine–pyrimethamine does not exceed 20% on day 28 (day 14 for the Comoros and Zimbabwe), except in India.

Figure 8
Clinical failure rates after chloroquine+sulfadoxine–pyrimethamine treatment in Africa, by country (1996–2004)[a]

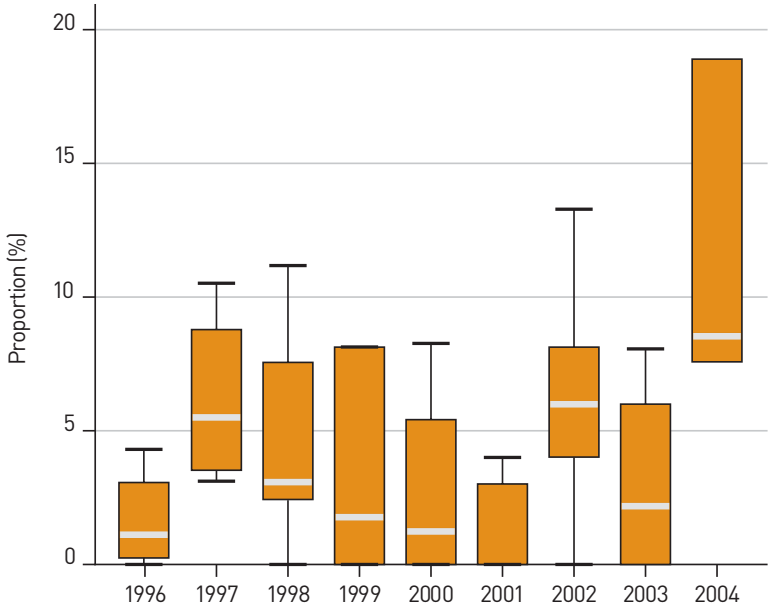


[a] In all, 55 studies of clinical failure with chloroquine+sulfadoxine-pyrimethamine were conducted in six countries between 1996 and 2004. Figure 8 presents the results of these studies by country. The clinical failure rate at day 14 varied substantially, from a low of 0% in the three studies in the Comoros to the highest median of 13.2% in Rwanda. Most of the results are for Uganda (15 studies) and Zimbabwe (25 studies), where the median clinical failure rates were 12% and 1.1%, respectively.

The combination sulfadoxine–pyrimethamine+amodiaquine is less effective than artesunate+amodiaquine, but the higher risk of failure with sulfadoxine–pyrimethamine+amodiaquine is counterbalanced by a lower risk of reinfection: the number of new treatments during 28 days' monitoring is therefore the same (312–314). The public health implications of this prophylactic effect should be studied in detail. Nevertheless, the 28-day clinical and parasitological failure rate (PCR-corrected) has reached high levels in Rwanda (28.2%) and Uganda (38%). Efficacy remains high in Cameroon (100%), Ghana (94.1%) and Senegal (98.8%) in Africa and in Afghanistan (97%), Colombia (89.2%) and Papua New Guinea (100%).

The combination artesunate+mefloquine (Figures 9.1 and 9.2) was introduced after increasing spread of resistance to mefloquine in Thailand. Not only did this combination prove to be highly effective when administered over 3 days (at a dose of 25 mg/kg mefloquine divided over 2 days and 12 mg/kg

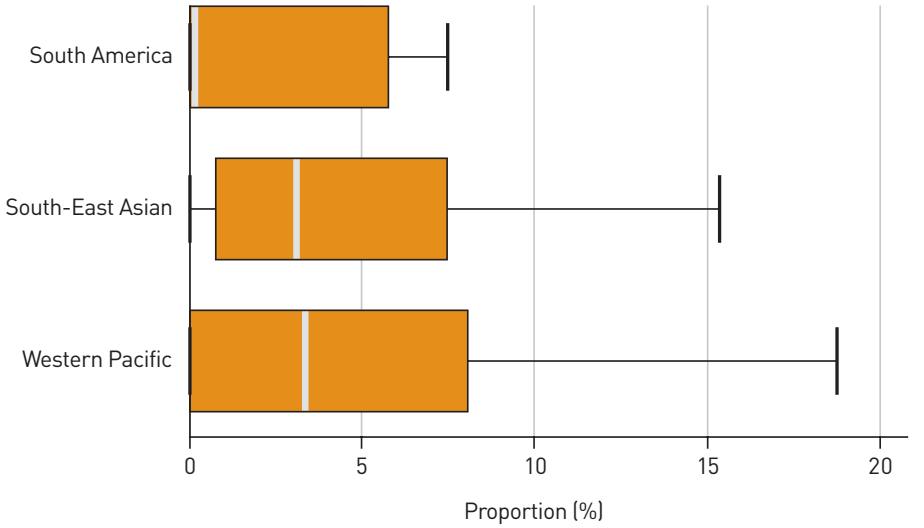
Figure 9.1
Total failure rates after artesunate+mefloquine treatment in Asia and the Americas, by year (1996–2004)[a]



[a] Artesunate+mefloquine has not been studied extensively in Africa: only five studies considered clinical failure. Outside of Africa, 69 studies have addressed the total failure rates of artesunate+mefloquine in Asia and the Americas. The results were highly variable, depending on the year, but a potentially increasing trend emerges. The total failure rates begin at a low of 1.2% in 1996, but the median for the three studies conducted in 2004 is 8.5%. The medians during the intervening years vary from 0% to 5%.

artesunate divided over 3 days) at the Thailand-Myanmar border, but, most importantly, in vitro studies showed a significant reduction in mefloquine IC50 values (315). These results were not, however, confirmed at the Thai–Cambodian border, where the treatment failure rate exceeded 21% on the Thai side and 10% on the Cambodian side. The difference in the results for the two regions of Thailand might be explained by use of two distinct treatment regimens, the total dose of 600 mg artesunate being divided over 2 or over 3 days. For the same dose of mefloquine, administration of artesunate over 2 or 3 days gives better results than administration in a single day (316–319). Although 2 days' treatment with artesunate combined with mefloquine might give good results, dividing the dose over 3 days is preferable and more effective (320, 321). Except in these two countries, the efficacy of the combination is rarely <90%, in particular in Africa, where it has been studied in Benin, Cameroon, Côte d'Ivoire and Senegal (322).

Figure 9.2
Total failure rates after artesunate+mefloquine treatment in Asia and the Americas, by subregion (1996–2004)[a]



[a] The total failure rate remains lowest in South America (median, 0%), whereas higher rates are observed in the South-East Asia and Western Pacific subregions, at 3.1% and 3.4%, respectively.

The combination artemether–lumefantrine was marketed a few years ago in a single tablet, which is an advantage in terms of treatment compliance as compared with blister-pack combinations. The outcome of the first clinical trials in Asia, in which four doses were divided over 2 days, was a high rate of recrudescence, unlike in Africa where the strains are probably more sensitive (323–328). The dosage was therefore increased to six doses divided over 3 days; this six-dose treatment should be made common policy, irrespective of strain sensitivity, in order to ensure optimal efficacy and avoid selection of resistant strains (183, 232, 329–333). The pharmacokinetics of lumefantrine varies considerably among individuals, and its absorption is enhanced by concomitant intake of fatty foods (334). Studies of the pharmacokinetics and pharmacodynamics of lumefantrine indicate that the main determinant of the efficacy of the artemether–lumefantrine combination is the area under the curve for the plasma concentration of lumefantrine or its surrogate, the plasma concentration of lumefantrine on day 7.

A lumefantrine plasma concentration >500 ng/ml on day 7 is associated with a treatment success rate >90% (335). There is thus every reason to give the six-dose treatment, as it increases the plasma concentration of lume-

fantrine (336). The efficacy of the 6-dose treatment exceeds 95%, except in Cambodia where several studies have confirmed high failure rates (17–30%). Investigations are under way to determine the contributions of resistance and interindividual variations in pharmacokinetics to these failures.

5.2.3 Specific groups

Adults

Since 1996, the WHO protocol does not recommend enrolment of adults in areas of high transmission (337). Table 3 gives a non-exhaustive list of studies of therapeutic efficacy in children aged <5 years and >5 years. The results confirm that, even in areas of low-to-moderate transmission, the therapeutic response in adults and in older children is better than that in young children.

People with HIV infection

HIV infection is associated with an increased frequency of malarial episodes and higher parasitaemia (347, 348) As immunity is a major factor in the therapeutic response, the immune suppression induced by HIV can compromise the outcome of antimalarial treatment. In a study of intermittent preventive treatment, sulfadoxine–pyrimethamine had to be given in monthly doses for the first two trimesters of pregnancy to women co-infected with HIV but at only two doses to seronegative women to achieve the same result (349). Treatment with chloroquine is less effective in children aged <5 years who are co-infected by the virus (350). The efficacy of the combinations artemether–lumefantrine and sulfadoxine–pyrimethamine is reduced in adults co-infected with HIV and with a CD4 count <300/µl (J.-P. Van Geertruyden, unpublished data, 2004), and the parasite clearance time after treatment with artemisinin is increased in seropositive patients (351). Chronic infections are an exclusion criterion for routine monitoring of therapeutic efficacy; however, because of the high prevalence of HIV positivity in areas in which malaria is endemic, clinical research is needed for this particular group. Therapeutic efficacy tests should be performed in this patient population, alone or in comparison with an HIV-negative control group. The risk that resistance to antimalarial drugs will extend with the AIDS epidemic must be considered seriously.

Pregnant women

During a first or second pregnancy, protective immunity against malaria tends to diminish, without disappearing completely. Together with changes

in pharmacokinetics (apparent increase in the volume of distribution, with a resulting reduction in plasma drug concentration), this fall in immunity is responsible for a higher treatment failure rate than in other women of the same age (4). Like *Plasmodium*–HIV co-infection, pregnancy is an exclusion criterion, but clinical research on this group is important, as studies on therapeutic efficacy are necessary for selecting and implementing intermittent preventive treatment within a national policy. Such studies are also important for determining tolerance and adverse effects in pregnant women (249, 352–363).

Patients with severe malaria

Severe, complicated malaria is a major exclusion criterion, and the appearance of signs of gravity after day o is considered to be treatment failure. Such patients cannot take drugs orally, but the efficacy of drugs administered by other routes has been evaluated in several studies. The results of these studies are difficult to interpret, as a considerable proportion of the patients may die at a time when their parasitaemia has completely disappeared (364–366).

6. IMPACT OF THE RESULTS ON POLICY CHANGE

Decision-markers need recommendations to help them decide on changes in treatment policy and to establish a strategy for implementing the new policy. The principal objective of antimalarial treatment is to reduce morbidity and, especially, mortality. Morbidity and mortality rates specifically for malaria should be the indicator used to determine whether a national treatment policy is satisfactory. Unfortunately, most deaths occur at home, and reliable data on specific morbidity and mortality are not accessible outside epidemiological studies. As a consequence, clinical and parasitological failure rates have been widely used as the sole guide for policy change.

6.1 Criteria for change

The cut-off points for changing treatment policy, which are sometimes based on parasitological or economic parameters, are often determined arbitrarily. Economic analysis has shown, for instance, that the critical cut-off failure rate for changing from chloroquine to sulfadoxine–pyrimethamine is situated between 18% and 23%, depending on the intensity of transmission. Other studies have suggested that policy should be changed when the early failure rate reaches 14–31%, when the rate of clinical failure reaches an unacceptable cut-off of 15–25%, when the median duration of clinical response is <14 days, or when haematological recovery is not as good as with an effective antimalarial treatment (367–370). At an international workshop in Malawi in 1996, the consensus was that an early clinical failure rate >5% indicates a significant problem and that a clinical failure rate >15–20% is an indication for policy change.

These suggested cut-off points are indicative, and decision-markers at the national level should feel free to initiate change at any time. In the past, many countries were too slow or too cautious in deciding on policy change, despite a high failure rate with chloroquine reported in the field.

6.2 Changes in criteria for change

At a workshop on antimalarial drug policies in Africa, held in Harare, Zimbabwe, in May 1999, a scale of dynamic change was established on the

basis of clinical failure rates on day 14. This scale gives reference points for actions to be undertaken or for change, in reference to the cost and half-life of alternative treatments. A schematic plan has been proposed, which provides a good "rule of thumb" for initiating debate on malaria treatment policies in a country (371). Four successive periods leading to a change in drug policy are defined on the basis of clinical failure rates: 0–4%, "grace"; 5–14%, "alert"; 15–24%, "action"; and ≥25%, "change". It should be noted that this system was drawn up primarily for regions of high transmission.

During an informal WHO consultation on the goal of antimalarial treatment policy in Africa, held in Harare, Zimbabwe, on 14 and 15 August 2003, the experts concluded that persistent asymptomatic parasitaemia after treatment is associated with an increased risk of clinical episodes, anaemia and gametocyte carriage. They agreed that parasitological response should be an additional indicator for interpretation of the therapeutic efficacy test. For data obtained with the standard WHO protocol, the cut-off point for policy change in areas of high transmission is now: adequate clinical and parasitological response <75% (total failure, ≥25%) and adequate clinical response <85% (clinical failure, ≥15%).

The drafting committee for malaria treatment guidelines suggested in 2005 that, with the introduction of more effective combination therapies, the efficacy of an antimalarial treatment should reach 95% and that a policy change should be seriously considered if the efficacy is below 90% on day 28.

6.3 Examples of countries that have changed their policies

Since 1996, 28 African and 25 countries in other regions have changed their policies, mainly on the basis of the results of therapeutic efficacy tests. Table 4 lists these countries.

While it might be clear from surveillance at sentinel sites that a first-line treatment is ineffective, the choice of a replacement drug or drug combination at consensus meetings has sometimes proved difficult. According to the 2001 WHO recommendations, an endemic country in which drug resistance to monotherapy is observed should change to a combination therapy, if possible based on artemisinin. The currently recommended options are artemether–lumefantrine, artesunate+amodiaquine, artesunate+ sulfadoxine–pyrimethamine, amodiaquine+sulfadoxine–pyrimethamine and artesunate+

mefloquine, although the last combination is at present recommended only for areas of low-to-moderate transmission) (372).

The efficacy of first- and second-line drugs is generally evaluated more frequently than that of the combination therapies that might serve as replacements. Countries usually base their choice on existing data for the new combinations, but such data are often not available. Countries must then either use data provided by neighbouring countries that are members of the same network or choose a drug combination on the basis of recommendations, the availability of the components, the efficacy of one of the drug component in previous tests for therapeutic efficacy or extrapolation of results from in vitro assays for drug sensitivity or molecular markers. Some countries prefer to postpone their choice while awaiting the results of studies on the various options available.

7. UPDATE OF THE THERAPEUTIC EFFICACY TEST, 2005

7.1 Recommendation of the drafting committee for malaria treatment guidelines

The conclusions and recommendations of the drafting committee for malaria treatment guidelines, which met several times in Geneva, Switzerland, in 2004 and 2005, stress that the objective of antimalarial treatment, even in areas of high transmission, is radical cure of the disease. An effective treatment prevents progression towards a severe form of the disease and the morbidity associated with treatment failure. Reduction of transmission and prevention of resistance are also crucial factors that should be taken into consideration. The consequences of drug resistance include delayed therapeutic response, a socioeconomic burden and increased mortality (373). From a clinical point of view, the persistence of parasites resulting from treatment failure can lead to anaemia, gametocyte carriage and the risk of recurrent clinical sign and symptoms. These three harmful consequences of treatment failure are taken into consideration in the modified WHO protocol.

7.1.1 Anaemia

Severe anaemia is one of the principal causes of malaria-related mortality (374). Several studies have shown that ineffective treatment with chloroquine leads to failure to improve anaemia present before treatment or aggravation of anaemia in areas of chloroquine resistance (368, 375, 376). Exacerbation of anaemia is not limited to clinical failure. Despite alleviation of clinical signs and symptoms such as attenuation of fever, the persistence of asymptomatic parasitaemia prevents remission from anaemia. The haematological response (i.e. improvement of anaemia or a post-treatment increase in erythrocyte volume fraction) was proposed as one of the criteria for evaluating therapeutic response (367). The most marked haematological effects are observed in early treatment failure. It is therefore apparent that reducing parasitaemia is not sufficient: only a radical cure of parasitaemia enables a significant increase in haemoglobin concentration.

7.1.2 Gametocyte carriage

One manifestation associated with treatment failure is an increased capacity for production of gametocytes, resulting in increased transmission

of resistant parasites (377). The extent of gametocytogenesis and of gametocyte infectivity varies, depending on the drugs to which the parasites are resistant (373, 378–383). Artemisinin derivatives, given as monotherapy or in combination with other drugs, not only diminish gametocyte carriage but also lower infectivity in mosquitoes, without preventing it completely, in particular if the combination contains an antimalarial drug against which the parasites are resistant (384–386).

7.1.3 Clinical malaria

Few clinical studies in Africa have included follow-up of asymptomatic children long enough to allow determination of the proportion of asymptomatic carriers who progress to clinical malaria. In the United Republic of Tanzania, 66% of children with parasitological failure on day 7 after treatment with sulfadoxine–pyrimethamine developed clinical signs within the follow-up period of 28 days (387). This proportion is slightly higher than that recorded during monitoring of an asymptomatic cohort in Uganda (50%) (388). These results suggest that patients who respond with late parasitological failure should be treated with drugs or drug combinations that are more effective than those administered on day 0, in order to avoid delayed manifestation of clinical signs and symptoms.

7.2 Modification of the protocol

7.2.1 Scope

The proposed modifications will affect the protocol essentially for areas of high transmission, in particular with regard to classification, management of late parasitological failure and the duration of follow-up. The inclusion criteria will remain distinct for areas of high transmission and areas of low-to-moderate transmission:

- age, 6–59 months;[2]
- infection only with *P. falciparum*;
- parasitaemia, 2000–200 000 asexual forms per µl;[3]

[2] In so far as possible, the study should include only children under 5 years of age; however, when enrolment proves difficult, in particular in areas of low-to-moderate transmission, all age groups may be enrolled.

[3] In areas of low-to-moderate transmission, the cut-off point is set between 1000 and 100 000 asexual forms per µl.

- axillary temperature, ≥37.5 °C;[4]
- ability to attend stipulated follow-up visits;
- easy access to a health facility; and
- informed consent of a parent or guardian.

7.2.2 Classification

A single classification scheme will henceforth be used (Annex 8). Late parasitological failure in areas of high transmission should no longer be monitored until the onset of clinical signs and symptoms, but should be treated immediately with a rescue antimalarial drug.

7.2.3 Duration of follow-up

Although a follow-up period of 42 days is optimal, a 28-day follow-up would be adequate to detect most cases of late treatment failure for drugs with elimination half-lives of <7 days (amodiaquine, quinine, halofantrine, atovaquone–proguanil, sulfadoxine–pyrimethamine). For drugs with longer elimination half-lives (lumefantrine, mefloquine, piperaquine), a follow-up period of at least 42 days is necessary (253). Most failures in clinical studies with artemisinin-based combinations occur after day 21. The drafting committee for malaria treatment guidelines agreed that the minimum duration should be at least 28 days, regardless of the level of transmission. Studies in areas of high transmission lasting more than 14 days and studies in areas of low-to-moderate transmission lasting more than 28 days should be complemented by molecular tests in order to distinguish cases of recrudescence from reinfection.

7.3 Future challenges

Countries that conduct therapeutic efficacy tests with a follow-up of at least 28 days will be confronted by additional operational constraints, as the increase in the length of follow-up and use of PCR tests will inevitably increase the cost of studies. When constraints caused by limited budgets or limited numbers of qualified personnel are difficult to overcome, the available resources should be channelled into more complete and regular studies at fewer sites in order to obtain better information.

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[4] When enrolment proves difficult, in particular in areas of low-to-moderate transmission, proven fever or a history of fever within the previous 24 h are acceptable alternatives.

Increased use of molecular markers and in vitro tests for surveillance of drug resistance will require the analysis of more samples. Molecular and in vitro tests are still considered research tools, and the number of laboratories in developing countries with the necessary technical capacity and expertise is limited. It will be too costly to establish and equip laboratories with expertise in molecular biology, cell culture and pharmacological assays for malaria control programmes. Plans to develop such laboratories at each sentinel site are inappropriate, at least in the immediate future. Finger-prick capillary blood samples collected on filter papers can be readily transported, and PCR should be performed at national or regional reference centres. In vitro tests pose additional logistic problems, as samples must be processed within 48 h. This short delay is likely to limit use of this assay to sites that are relatively close to reference centres. New in vitro assays based on enyzme-linked immunosorbent assays should be validated as soon as possible under field conditions. The role of reference centres should not be limited to the technical aspects of laboratory tools, such as logistic support for laboratory activities, ensuring data comparability by following the standard protocol and quality control, they should also assist in collecting regional or national information related to antimalarial drug resistance.

This type of centre already exists in some endemic countries, but they require more assistance in terms of training and resources. The exchange of results, standardization of methods and quality control will be essential for ensuring data continuity and quality.

8. REFERENCES

1. Bruce-Chwatt LJ et al. *Chemotherapy of malaria*, revised 2nd ed. Geneva, World Health Organization, 1986.

2. Trenholme MG et al. Host failure in treatment of malaria with sulfalene and pyrimethamine. *Annals of Internal Medicine*, 1975, 82:219–223.

3. Bayly AM, Macreadie IG. Folic acid antagonism of sulfa drug treatments. *Trends in Parasitology*, 2002,18:49–50.

4. White NJ. Why is it that antimalarial drug treatments do not always work? *Annals of Tropical Medicine and Parasitology*, 1998, 92:449–458.

5. Ringwald P et al. Failure of anti-malarial prophylaxis with mefloquine in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1990, 84:348–349.

6. Herzog C et al. Possible role of drug malabsorption in recrudescence of falciparum malaria. *Lancet*, 1982, 20:1157–1158.

7. Simpson JA, Aarons L, White NJ. How can we do pharmacokinetic studies in the tropics? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:347–351.

8. Aubouy A et al. Combination of drug level measurement and parasite genotyping data for improved assessment of amodiaquine and sulfadoxine–pyrimethamine efficacies in treating *Plasmodium falciparum* malaria in Gabonese children. *Antimicrobial Agents and Chemotherapy*, 2003, 47:231–237.

9. Ringwald P et al. Chimioresistance de *P. falciparum* en milieu urbain à Yaoundé, Cameroun. Part 1: Surveillance *in vitro* et *in vivo* de la resistance de *Plasmodium falciparum* à la chloroquine entre 1994 et 1999 à Yaoundé, Cameroun [Chemoresistance of *P. falciparum* in urban areas of Yaounde, Cameroon. Part 1: Surveillance of in vitro and in vivo resistance of *Plasmodium falciparum* to chloroquine from 1994 to 1999 in Yaounde, Cameroon]. *Tropical Medicine and International Health*, 2000, 5:612–619.

10. Pukrittayakamee S et al. Quinine pharmacokinetic–pharmacodynamic relationships in uncomplicated falciparum malaria. *Antimicrobial Agents and Chemotherapy*, 2003, 47:3458–3463.

11. Dua VK et al. Chloroquine and desethylchloroquine concentrations in blood cells and plasma from Indian patients infected with sensitive or resistant *Plasmodium falciparum*. *Annals of Tropical Medicine and Parasitology*, 2000, 94:565–570.

12. Maitland K et al. Plasma chloroquine concentrations in young and older malaria patients treated with chloroquine. *Acta Tropica*, 1997, 66:155–161.

13. Slutsker LM et al. Mefloquine therapy for *Plasmodium falciparum* malaria in children under 5 years of age in Malawi: in vivo/in vitro efficacy and correlation of drug concentration with parasitological outcome. *Bulletin of the World Health Organization*, 1990; 68:53–59.

14. Talisuna AO, Bloland P, D'Alessandro U. History, dynamics, and public health importance of malaria parasite resistance. *Clinical Microbiology Reviews*, 2004, 17:235–254.

15. Wernsdorfer WH, Noedl H. Molecular markers for drug resistance in malaria: use in treatment, diagnosis and epidemiology. *Current Opinion in Infectious Diseases*, 2003, 16:553–558.

16. White NJ. Antimalarial drug resistance. *Journal of Clinical Investigation*, 2004, 113:1084–1092.

17. Wongsrichanalai C et al. Epidemiology of drug-resistant malaria. *Lancet Infectious Diseases*, 2002, 2:209–218.

18. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. *Pharmacological Reviews*, 2005, 57:117–145.

19. Mharakurwa S. *Plasmodium falciparum* transmission rate and selection for drug resistance: a vexed association or a key to successful control? *International Journal of Parasitology*, 2004, 34:1483–1487.

20. *Chemotherapy of malaria and resistance to antimalarials. Report of a WHO Scientific Group*. Geneva, World Health Organization, 1973 (WHO Technical Report Series, No. 529).

21. Bloland PB et al. Chloroquine in Africa: critical assessment and recommendations for monitoring and evaluating chloroquine therapy efficacy in sub-Saharan Africa. *Tropical Medicine and International Health*, 1998, 3:543–552.

22. ter Kuile FO et al. Predictors of mefloquine treatment failure: a prospective study of 1590 patients with uncomplicated falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1995 89:660–664.

23. *Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission*. Geneva, World Health Organization, 1996 (unpublished document WHO/MAL/96.1077).

24. *Evaluación de la eficacia terapeutica de los medicamentos para el tratamiento del paludismo por* Plasmodium falciparum *sin complicaciones en las Americas [Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated* Plasmodium falciparum *malaria in the Americas]*. Washington, Organización Mundial de la Salud, 1998 (unpublished document OPS/HCP/HCT/113/98).

25. *Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria*. Geneva, World Health Organization, 2003 (unpublished document WHO/HTM/RBM/2003.50).

26. Djimde AA et al. Molecular diagnosis of resistance to antimalarial drugs during epidemics and in war zones. *Journal of Infectious Diseases*, 2004, 15;190:853–855.

27. East African Network for Monitoring Antimalarial Treatment (EANMAT). The efficacy of antimalarial monotherapies, sulphadoxine–pyrimethamine and amodiaquine in East Africa: implications for sub-regional policy. *Tropical Medicine and International Health*, 2003, 8:860–867.

28. East African Network for Monitoring Antimalarial Treatment (EANMAT). Monitoring antimalarial drug resistance within national malaria control programmes: the EANMAT experience. *Tropical Medicine and International Health*, 2001, 6:891–898.

29. Tinto H et al. Etude de la sensibilité in vitro de 232 isolats de *Plasmodium falciparum* aux antipaludéens au Burkina Faso (Afrique de l'Ouest) [In vitro susceptibility of Burkina faso (West Africa) 232 isolates of *Plasmodium falciparum* to antimalarials]. *Bulletin de la Société de Pathologie Exotique*, 2001, 94:188–191.

30. Ringwald P, Bickii J, Basco LK. In vitro activity of dihydroartemisinin against clinical isolates of *Plasmodium falciparum* in Yaounde, Cameroon. *American Journal of Tropical Medicine and Hygiene*, 1999, 61:187–192.

31. Ringwald P, Bickii J, Basco LK. Amodiaquine as the first-line treatment of malaria in Yaounde, Cameroon: presumptive evidence from activity *in vitro* and cross-resistance patterns. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1998, 92:212–213.

32. Pradines B et al. In vitro susceptibility of Gabonese wild isolates of *Plasmodium falciparum* to artemether, and comparison with chloroquine, quinine, halofantrine and amodiaquine. *Parasitology*, 1998, 117:541–545.

33. Randrianarivelojosia M et al. In vitro sensitivity of *Plasmodium falciparum* to amodiaquine compared with other major antimalarials in Madagascar. *Parasitologia*, 2002, 44:141–147.

34. Brockman A et al. *Plasmodium falciparum* antimalarial drug susceptibility on the north-western border of Thailand during five years of extensive use of artesunate–mefloquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, 94:537–544.

35. Wernsdorfer WH et al. Inverse correlation of sensitivity in vitro of *Plasmodium falciparum* to chloroquine and mefloquine in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, 88:443–444.

36. Simon F et al. Reduced sensitivity of *Plasmodium falciparum* to mefloquine in West Africa. *Lancet*, 1988, i:467–468.

37. Basco LK, Ringwald P. In vitro activities of piperaquine and other 4-aminoquinolines against clinical isolates of *Plasmodium falciparum* in Cameroon. *Antimicrobial Agents and Chemotherapy*, 2003, 47:1391–1394.

38. Basco LK, Bickii J, Ringwald P. In vitro activity of lumefantrine (benflumetol) against clinical isolates of *Plasmodium falciparum* in Yaounde, Cameroon. *Antimicrobial Agents and Chemotherapy*, 1998, 42:2347–2351.

39. Pradines B et al. In vitro susceptibility of African isolates of *Plasmodium falciparum* from Gabon to pyronaridine. *American Journal of Tropical Medicine and Hygiene*, 1999, 60:105–108.

40. Pradines B, In vitro activity of pyronaridine and amodiaquine against African isolates (Senegal) of *Plasmodium falciparum i*n comparison with standard antimalarial agents. *Journal of Antimicrobial Chemotherapy*, 1998, 42:333–339.

41. Wernsdorfer WH et al. Activity of benflumetol and its enantiomers in fresh isolates of *Plasmodium falciparum* from East Africa. *Acta Tropica*, 1998, 70:9–15.

42. Lopes D et al. Molecular characterisation of drug-resistant *Plasmodium falciparum* from Thailand. *Malaria Journal*, 2002, 1:12.

43. Pettinelli F et al. Short report: High prevalence of multidrug-resistant *Plasmodium falciparum* malaria in the French territory of Mayotte. *American Journal of Tropical Medicine and Hygiene*, 2004, 70:635–637.

44. Zalis MG et al. Characterization of *Plasmodium falciparum* isolated from the Amazon region of Brazil: evidence for quinine resistance. *American Journal of Tropical Medicine and Hygiene*, 1998, 58:630–637.

45. Yang HL et al. Sensitivity in vitro of *Plasmodium falciparum* to chloroquine, pyronaridine, artesunate and piperaquine in south Yunnan. *Chinese Journal of Parasitology and Parasitic Diseases*, 1992, 10(3):198–200.

46. Mberu EK et al. The changing in vitro susceptibility pattern to pyrimethamine/ sulfadoxine in *Plasmodium falciparum* field isolates from Kilifi, Kenya. *American Journal of Tropical Medicine and Hygiene*, 2000, 62:396–401.

47. Ouedraogo JB et al. In vitro sensitivity of *Plasmodium falciparum* to halofantrine compared with chloroquine, quinine and mefloquine in the region of Bobo-Dioulasso, Burkina Faso (West Africa). *Tropical Medicine and International Health*, 1998, 3:381–384.

48. Schwenke A et al. Declining chloroquine resistance of *Plasmodium falciparum* in Lambarene, Gabon from 1992 to 1998. *Wiener Klinische Wochenschrift*, 2001, 113:63–64.

49. Reynes JM et al. In vitro responses of *Plasmodium falciparum* isolates to five antimalaria drugs in French Guiana during 1994 and 1995. *Memorias do Instituto Oswaldo Cruz*, 1997, 92:251–252.

50. Yang H et al. Longitudinal surveillance of sensitivity of *Plasmodium falciparum* to pyronaridine in South Yunnan. *Chinese Journal of Parasitology and Parasitic Diseases*, 1998, 16:81–83.

51. Yang H et al. Changes in susceptibility of *Plasmodium falciparum* to artesunate in vitro in Yunnan Province, China. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:226–228.

52. Lim P et al. In vitro monitoring of *Plasmodium falciparum* susceptibility to artesunate, mefloquine, quinine and chloroquine in Cambodia: 2001–2002. *Acta Tropica*, 2005, 93:31–40.

53. Wongsrichanalai C et al. In vitro susceptibility of *Plasmodium falciparum* isolates from Myanmar to antimalarial drugs. *American Journal of Tropical Medicine and Hygiene*, 2001, 65:450–455.

54. Rojanawatsirivet C et al. Declining mefloquine sensitivity of *Plasmodium falciparum* along the Thai–Myanmar border. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2004, 35:560–565.

55. Pilz JB et al. In vitro sensitivity of *Plasmodium falciparum* to lumefantrine in north-western Thailand. *Wiener Klinische Wochenschrift*, 2004;116 (Suppl. 4): 41–46.

56. Woitsch B et al. Comparative study of the in vitro sensitivity of *Plasmodium falciparum* to artemisinin in two border areas of Thailand. *Wiener Klinische Wochenschrift*, 2004, 116 (Suppl. 4):35–40.

57. Mita T et al. Recovery of chloroquine sensitivity and low prevalence of the *Plasmodium falciparum* chloroquine resistance transporter gene mutation K76T following the discontinuance of chloroquine use in Malawi. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:413–415.

58. Liu D et al. Changes in chloroquine resistance of *Plasmodium falciparum* in Hainan province. *Chinese Journal of Parasitology and Parasitic Diseases*, 1999, 17(1):32–34.

59. Pradines B et al. In vitro activities of benflumetol against 158 Senegalese isolates of *Plasmodium falciparum* in comparison with those of standard antimalarial drugs. *Antimicrobial Agents and Chemotherapy*, 1999, 43:418–420.

60. Borrmann S. Reassessment of the resistance of *Plasmodium falciparum* to chloroquine in Gabon: implications for the validity of tests in vitro vs. in vivo. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:660–663.

61. Moreno A. Use of the DELI-microtest to determine the drug sensitivity of *Plasmodium falciparum* in Burkina Faso. *Annals of Tropical Medicine and Parasitology*, 2001, 95:309–312.

62. Randrianarivelojosia M et al. Susceptibility of *Plasmodium falciparum* to the drugs used to treat severe malaria (quinine) and to prevent malaria (mefloquine, cycloguanil) in Comoros Union and Madagascar. *South African Medical Journal*, 2004, 94:47–51.

63. Rason MA et al. Monitoring the drug-sensitivity of *Plasmodium falciparum* in coastal towns in Madagascar by use of *in vitro* chemosensitivity and mutation detection tests. *Parasite*, 2002, 9:247–253.

64. Yang HL et al. In vitro sensitivity of *Plasmodium falciparum* to eight antimalarials in China–Myanmar and China–Lao PDR border areas. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1997, 28:460–464.

65. Hatabu T et al. In vitro susceptibility of *Plasmodium falciparum* isolates to chloroquine and mefloquine in southeastern Mindanao Island, the Philippines. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2003, 34:546–551.

66. Noedl H et al. Drug-resistant malaria in Bangladesh: an in vitro assessment. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:140–142.

67. Fidock DA et al. Mutations in the *P. falciparum* digestive vacuole transmembrane protein *PFCRT* and evidence for their role in chloroquine resistance. *Molecular Cell*, 2000, 6:861–671.

68. Foote SJ, Galatis D, Cowman AF. Amino acids in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum* involved in cycloguanil resistance differ from those involved in pyrimethamine resistance. *Proceedings of the National Academy of Sciences of the United States of America*, 1990, 87:3014–3017.

69. Peterson DS, Milhous WK, Wellems TE. Molecular basis of differential resistance to cycloguanil and pyrimethamine in *Plasmodium falciparum* malaria. *Proceedings of the National Academy of Sciences of the United States of America*, 1990, 87:3018–3022.

70. Tinto H et al. Relationship between the *Pfcrt* T76 and the *Pfmdr*-1 Y86 mutations in *Plasmodium falciparum* and in vitro/in vivo chloroquine resistance in Burkina Faso, West Africa. *Infection, Genetics and Evolution*, 2003, 3:287–292.

71. Basco LK, Ringwald P. Molecular epidemiology of malaria in Yaounde, Cameroon. VI. Sequence variations in the *Plasmodium falciparum* dihydrofolate reductase–thymidylate synthase gene and *in vitro* resistance to pyrimethamine and cycloguanil. *American Journal of Tropical Medicine and Hygiene*, 2000, 62:271–276.

72. Basco LK. Molecular epidemiology of malaria in Cameroon. XII. In vitro drug assays and molecular surveillance of chloroquine and proguanil resistance. *American Journal of Tropical Medicine and Hygiene*, 2002, 67:383–387.

73. Reeder JC et al. Point mutations in the dihydrofolate reductase and dihydropteroate synthetase genes and in vitro susceptibility to pyrimethamine and cycloguanil of *Plasmodium falciparum* isolates from Papua New Guinea. *American Journal of Tropical Medicine and Hygiene*, 1996, 55:209–213.

74. Ndounga M, Basco LK, Ringwald P. Variabilité de l'activité *in vitro* du proguanil et du cycloguanil sur les stades érythrocytaires de *Plasmodium falciparum* en fonction des conditions de culture [Variability of in vitro activity of proguanil and cycloguanil on erythrocyte stages of *Plasmodium falciparum* as a function of culture conditions]. *Bulletin de la Société de Pathologie Exotique*, 1999, 92:313–316.

75. Nzila-Mounda A et al. Kenyan *Plasmodium falciparum* field isolates: correlation between pyrimethamine and chlorcycloguanil activity in vitro and point mutations in the dihydrofolate reductase domain. *Antimicrobial Agents and Chemotherapy*, 1998, 42:164– 169.

76. Khalil I et al. Dihydrofolate reductase and dihydropteroate synthase genotypes associated with in vitro resistance of *Plasmodium falciparum* to pyrimethamine, trimethoprim, sulfadoxine, and sulfamethoxazole. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:586–589.

77. Yeung S et al. Antimalarial drug resistance, artemisinin based combination therapy, and the contribution of modeling to elucidating policy choices. *American Journal of Tropical Medicine and Hygiene*, 2004, 71(Suppl. 2): 179–186.

78. Basco LK et al. Molecular epidemiology of malaria in Cameroon. IX. Characteristics of recrudescent and persistent *Plasmodium falciparum* infections after chloroquine or amodiaquine treatment in children. *American Journal of Tropical Medicine and Hygiene*, 2002, 66:117–123.

79. Ndounga M, Basco LK, Ringwald P. Evaluation of a new sulfadoxine sensitivity assay *in vitro* for field isolates of *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:55–57.

80. Hankins EG, Warhurst DC, Sibley CH. Novel alleles of the *Plasmodium falciparum dhfr* highly resistant to pyrimethamine and chlorcycloguanil, but not WR99210. *Molecular and Biochemical Parasitology*, 2001, 117:91–102.

81. Krudsood S et al. Artesunate–dapsone–proguanil treatment of falciparum malaria: genotypic determinants of therapeutic response. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2005, 99:142–149.

82. van den Broek IV et al. Drug resistance in *Plasmodium falciparum* from the Chittagong Hill Tracts, Bangladesh. *Tropical Medicine and International Health*, 2004, 9:680–687.

83. Vasconcelos KF et al. Mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthase of isolates from the Amazon region of Brazil. *Memorias do Instituto Oswaldo Cruz*, 2000, 95:721–728.

84. Biswas S et al. Prevalence of point mutations in the dihydrofolate reductase and dihydropteroate synthetase genes of *Plasmodium falciparum* isolates from India and Thailand: a molecular epidemiologic study. *Tropical Medicine and International Health*, 2000, 5:737–743.

85. Basco LK et al. Point mutations in the dihydrofolate reductase– thymidylate synthase gene and pyrimethamine and cycloguanil resistance in *Plasmodium falciparum*. *Molecular and Biochemical Parasitology*, 1995, 69:135–138.

86. Kim N et al. Polymorphisme et chimiorésistance de *Plasmodium falciparum* au Cambodge [Chemoresistance and polymorphism in falciparum malaria in Cambodia]. *Médecine et Maladies Infectieuses*, 2004, 34:S61–S64.

87. Wang et al. Resistance to antifolates in *Plasmodium falciparum* monitored by sequence analysis of dihydropteroate synthetase and dihydrofolate reductase alleles in a large number of field samples of diverse origins. *Molecular and Biochemical Parasitology*, 1997, 89:161–177.

88. Zhang ZX, Yang YM, Lui H. Gene point mutation in the dihydrofolate reductase–thymidylate synthase gene of *Plasmodium falciparum*. *Chinese Journal of Parasitology and Parasitic Diseases*, 2001, 19:87–89.

89. Kublin JG et al. Molecular assays for surveillance of antifolate-resistant malaria. *Lancet*, 1998, 351:1629–1630.

90. Masimirembwa et al. Molecular epidemiology of *Plasmodium falciparum* antifolate resistance in Vietnam: genotyping for resistance variants of dihydropteroate synthase and dihydrofolate reductase. *International Journal of Antimicrobial Agents*, 1999, 12:203–211.

91. Plowe CV et al. Mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine–sulfadoxine use and resistance. *Journal of Infectious Diseases*, 1997, 176:1590–1596.

92. Ahmed A et al. *Plasmodium falciparum* isolates in India exhibit a progressive increase in mutations associated with sulfadoxine–pyrimethamine resistance. *Antimicrobial Agents and Chemotherapy*, 2004, 48:879–889.

93. Cox-Singh J et al. Short report. Differences in dihydrofolate reductase but not dihydropteroate synthase alleles in *Plasmodium falciparum* isolates from geographically distinct areas in Malaysia. *American Journal of Tropical Medicine and Hygiene*, 2001, 64:28–31.

94. Ochong E et al. Molecular monitoring of the Leu-164 mutation of dihydrofolate reductase in a highly sulfadoxine/pyrimethamine-resistant area in Africa. *Malaria Journal*, 2003, 2:46.

95. Wichmann O et al. Molecular surveillance of the antifolate-resistant mutation I164L in imported African isolates of *Plasmodium falciparum* in Europe: sentinel data from TropNetEurop. *Malaria Journal*, 2003, 2:17.

96. Hastings MD et al. Highly pyrimethamine-resistant alleles of dihydrofolate reductase in isolates of *Plasmodium falciparum* from Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:674–676.

97. Mookherjee S et al. Identification and analysis of dihydrofolate reductase alleles from *Plasmodium falciparum* present at low frequency in polyclonal patient samples. *American Journal of Tropical Medicine and Hygiene*, 1999, 61:131–140.

98. Farnert A et al. Polyclonal *Plasmodium falciparum* malaria in travelers and selection of antifolate mutations after proguanil prophylaxis. *American Journal of Tropical Medicine and Hygiene*, 2002, 66:487–491.

99. Cortese JF et al. Origin and dissemination of *Plasmodium falciparum* drug-resistance mutations in South America. *Journal of Infectious Diseases*, 2002, 186:999–1006.

100. Bates et al. Rare, highly pyrimethamine-resistant alleles of the *Plasmodium falciparum* dihydrofolate reductase gene from 5 African sites. *Journal of Infectious Diseases*, 2004, 190:1783–1792.

101. Sibley CH et al. Pyrimethamine–sulfadoxine resistance in *Plasmodium falciparum*: what next? *Trends in Parasitology*, 2001, 17:582–588.

102. Syafruddin D et al. Frequency distribution of antimalarial drug-resistant alleles among isolates of *Plasmodium falciparum* in Purworejo district, Central Java Province, Indonesia. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:614–620.

103. Biswas S. *Plasmodium falciparum* dihydrofolate reductase Val-16 and Thr-108 mutation associated with in vivo resistance to antifolate drug: a case study. *Indian Journal of Malariology*, 2001, 38:76–83.

104. Zindrou S et al. *Plasmodium falciparum*: mutation pattern in the dihydrofolate reductase–thymidylate synthase genes of Vietnamese isolates, a novel mutation, and coexistence of two clones in a Thai patient. *Experimental Parasitology*, 1996, 84:56–64.

105. Mberu EK et al. *Plasmodium falciparum*: in vitro activity of sulfadoxine and dapsone in field isolates from Kenya: point mutations in dihydropteroate synthase may not be the only determinants in sulfa resistance. *Experimental Parasitology*, 2002, 101:90–96.

106. Wang P et al. Sulfadoxine resistance in the human malaria parasite *Plasmodium falciparum* is determined by mutations in dihydropteroate synthetase and an additional factor associated with folate utilization. *Molecular Microbiology*, 1997, 23:979–986.

107. Djimde A et al. A molecular marker for chloroquine-resistant falciparum malaria. *New England Journal of Medicine*, 2001, 344:257–263.

108. Basco LK et al. Molecular epidemiology of malaria in Cameroon. XIV. *Plasmodium falciparum* chloroquine resistance transporter (*PFCRT*) gene sequences of isolates before and after chloroquine treatment. *American Journal of Tropical Medicine and Hygiene*, 2002, 67:392–395.

109. Basco LK. Molecular epidemiology of malaria in Cameroon. XIII. Analysis of *pfcrt* mutations and in vitro chloroquine resistance. *American Journal of Tropical Medicine and Hygiene*, 2002, 67:388–391.

110. Durrand V et al. Variations in the sequence and expression of the *Plasmodium falciparum* chloroquine resistance transporter (*Pfcrt*) and their relationship to chloroquine resistance in vitro. *Molecular and Biochemical Parasitology*, 2004, 136:273–285.

111. Thomas SM et al. In vitro chloroquine susceptibility and PCR analysis of *pfcrt* and *pfmdr1* polymorphisms in *Plasmodium falciparum* isolates from Senegal. *American Journal of Tropical Medicine and Hygiene*, 2002, 66:474–480.

112. Casey JG et al. Molecular analysis of *Plasmodium falciparum* from drug treatment failure patients in Papua New Guinea. *American Journal of Tropical Medicine and Hygiene*, 2004, 70:251–255.

113. Chen N et al. Levels of chloroquine resistance in *Plasmodium falciparum* are determined by loci other than *pfcrt* and *pfmdr1*. *Journal of Infectious Diseases*, 2002, 185:405–407.

114. Happi TC et al. Point mutations in the *pfcrt* and *pfmdr-1* genes of *Plasmodium falciparum* and clinical response to chloroquine, among malaria patients from Nigeria. *Annals of Tropical Medicine and Parasitology*, 2003, 97:439–451.

115. Vathsala PG et al. Widespread occurrence of the *Plasmodium falciparum* chloroquine resistance transporter (*Pfcrt*) gene haplotype SVMNT in *P. falciparum* malaria in India. *American Journal of Tropical Medicine and Hygiene*, 2004, 70:256–259.

116. Berens N et al. *Plasmodium falciparum*: correlation of in vivo resistance to chloroquine and antifolates with genetic polymorphisms in isolates from the south of Lao PDR. *Tropical Medicine and International Health*, 200 3, 8:775–782.

117. Schneider AG et al. A point mutation in codon 76 of *pfcrt* of *P. falciparum* is positively selected for by chloroquine treatment in Tanzania. *Infection, Genetics and Evolution*, 2002, 1:183–189.

118. Maguire JD et al. The T76 mutation in the *pfcrt* gene of *Plasmodium falciparum* and clinical chloroquine resistance phenotypes in Papua, Indonesia. *Annals of Tropical Medicine and Parasitology*, 2001, 95:559–572.

119. Basco LK, Ringwald P. Analysis of the key *pfcrt* point mutation and in vitro and in vivo response to chloroquine in Yaounde, Cameroon. *Journal of Infectious Diseases*, 2001, 183:1828–1831.

120. Major AG et al. Prevalence of the K76T mutation in the putative *Plasmodium falciparum* chloroquine resistance transporter (*pfcrt*) gene and its relation to chloroquine resistance in Mozambique. *Journal of Infectious Diseases*, 2001, 183:1413–1416.

121. Ngo T et al. Analysis of *pfcrt*, *pfmdr1*, *dhfr*, and *dhps* mutations and drug sensitivities in *Plasmodium falciparum* isolates from patients in Vietnam before and after treatment with artemisinin. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:350–356.

122. Su X et al. Complex polymorphisms in an approximately 330 kDa protein are linked to chloroquine-resistant *P. falciparum* in Southeast Asia and Africa. *Cell*, 1997, 91:593–603.

123. Gomez-Saladin E et al. *Plasmodium falciparum mdr1* mutations and in vivo chloroquine resistance in Indonesia. *American Journal of Tropical Medicine and Hygiene*, 1999, 61:240–244.

124. Nagesha HS et al. Mutations in the *pfmdr1*, *dhfr* and *dhps* genes of *Plasmodium falciparum* are associated with in vivo drug resistance in West Papua, Indonesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:43–49.

125. Basco LK, Ringwald P. *pfmdr1* gene mutation and clinical response to chloroquine in Yaoundé, Cameroon. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1997, 91:210–211.

126. Basco LK, Ringwald P. Molecular epidemiology of malaria in Yaounde, Cameroon. III. Analysis of chloroquine resistance and point mutations in the multidrug resistance 1 (*pfmdr 1*) gene of *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene*, 1998, 59:577–581.

127. Bhattacharya PR, Pillai CR. Strong association, but incomplete correlation, between chloroquine resistance and allelic variation in the *pfmdr-1* gene of *Plasmodium falciparum* isolates from India. *Annals of Tropical Medicine and Parasitology*, 1999, 93:679–684.

128. Bhattacharya PR, Biswas S, Kabilan L. Alleles of the *Plasmodium falciparum Pfmdr1* gene appear not to be associated with chloroquine resistance in India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1997, 91:454–455.

129. McCutcheon KR et al. Two mutations in the multidrug-resistance gene homologue of *Plasmodium falciparum*, *pfmdr1*, are not useful predictors of in vivo or in vitro chloroquine resistance in southern Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93:300–302.

130. von Seidlein L et al. Polymorphism of the *Pfmdr1* gene and chloroquine resistance in *Plasmodium falciparum* in The Gambia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1997, 91:450–453.

131. Grobusch MP et al. *Plasmodium falciparum*: in vitro chloroquine susceptibility and allele-specific PCR detection of *Pfmdr1* Asn86Tyr polymorphism in Lambarene, Gabon. *Parasitology*, 1998, 116:211–217.

132. Flueck TP et al. Correlation of in vivo-resistance to chloroquine and allelic polymorphisms in *Plasmodium falciparum* isolates from Uganda. *Medicine and International Health*, 2000, 5:174–178.

133. Adagut IS, Warhurst DC. *Plasmodium falciparum*: linkage disequilibrium between loci in chromosomes 7 and 5 and chloroquine selective pressure in northern Nigeria. *Parasitology*, 2001, 123:219–224.

134. Babiker HA et al. High-level chloroquine resistance in Sudanese isolates of *Plasmodium falciparum* is associated with mutations in the chloroquine resistance transporter gene *pfcrt* and the multidrug resistance gene *pfmdr1*. *Journal of Infectious Diseases*, 2001, 183:1535–1538.

135. Howard EM, Zhang H, Roepe PD. A novel transporter, *Pfcrt*, confers antimalarial drug resistance. *Journal of Membrane Biology*, 2002, 190:1–8.

136. Reed MB et al. *Pgh1* modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. *Nature*, 2000, 403:906–909.

137. Duraisingh MT et al. The tyrosine-86 allele of the *pfmdr1* gene of *Plasmodium falciparum* is associated with increased sensitivity to the anti-malarials mefloquine and artemisinin. *Molecular and Biochemical Parasitology*, 2000, 108:13–23.

138. Duraisingh MT et al. Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the *pfmdr1* gene of *Plasmodium falciparum*. *Molecular Microbiology*, 2000, 36:955–961.

139. Basco LK, Ringwald P. Molecular epidemiology of malaria in Cameroon. X. Evaluation of *PFMDR1* mutations as genetic markers for resistance to amino alcohols and artemisinin derivatives. *American Journal of Tropical Medicine and Hygiene*, 2002, 66:667–671.

140. Pillai DR et al. Lack of prediction of mefloquine and mefloquine–artesunate treatment outcome by mutations in the *Plasmodium falciparum* multidrug resistance 1 (*pfmdr1*) gene for *P. falciparum* malaria in Peru. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:107–110.

141. Sisowath C et al. In vivo selection of *Plasmodium falciparum pfmdr1* 86N coding alleles by artemether–lumefantrine (Coartem©). *Journal of Infectious Diseases*, 2005, 191:1014–1017.

142. Price RN et al. The *pfmdr1* gene is associated with a multidrug-resistant phenotype in *Plasmodium falciparum* from the western border of Thailand. *Antimicrobial Agents and Chemotherapy*, 1999, 43:2943–2949.

143. Price RN et al. Mefloquine resistance in *Plasmodium falciparum* and increased *pfmdr1* gene copy number. *Lancet*, 2004, 364:438–447.

144. Looareesuwan S et al. Malarone™ (atovaquone and proguanil hydrochloride): a review of its clinical development for treatment of malaria. Malarone Clinical Trials Study Group. *American Journal of Tropical Medicine and Hygiene*, 1999, 60:533–541.

145. Gil JP et al. Detection of atovaquone and Malarone resistance conferring mutations in *Plasmodium falciparum* cytochrome *b* gene (*cytb*). *Molecular and Cellular Probes*, 2003, 17:85–89.

146. Fivelman QL et al. Malarone treatment failure and in vitro confirmation of resistance of *Plasmodium falciparum* isolate from Lagos, Nigeria. *Malaria Journal*, 2002, 1:1.

147. Färnert A et al. Evidence of *Plasmodium falciparum* malaria resistant to atovaquone and proguanil hydrochloride: case reports. *British Medical Journal*, 2003, 326:628–629.

148. Wichmann O et al. Malarone treatment failure not associated with previously described mutations in the cytochrome *b* gene. *Malaria Journal*, 2004, 3:14.

149. Wichmann O et al. Screening for mutations related to atovaquone/proguanil resistance in treatment failures and other imported isolates of *Plasmodium falciparum* in Europe. *Journal of Infectious Diseases*, 2004, 190:1541–1546.

150. Bwijo B et al. High prevalence of quintuple mutant *dhps*/*dhfr* genes in *Plasmodium falciparum* infections seven years after introduction of sulfadoxine and pyrimethamine as first line treatment in Malawi. *Acta Tropica*, 2003, 85:363–373.

151. Jafari S et al. Molecular survey of *Plasmodium falciparum* resistance in southeastern Iran. *Annals of Tropical Medicine and Parasitology*, 2003, 97:119–124.

152. Eberl KJ et al. Prevalence of polymorphisms in the dihydrofolate reductase and dihydropteroate synthetase genes of *Plasmodium falciparum* isolates from southern Mauritania. *Tropical Medicine and International Health*, 2001, 6:756–760.

153. Basco LK, Ringwald P. Molecular epidemiology of malaria in Yaounde, Cameroon IV. Evolution of pyrimethamine resistance between 1994 and 1998. *American Journal of Tropical Medicine and Hygiene*, 1999, 61:802–806.

154. Basco LK et al. Molecular epidemiology of malaria in Cameroon. XI. Geographic distribution of *Plasmodium falciparum* isolates with dihydrofolate reductase gene mutations in southern and central Cameroon. *American Journal of Tropical Medicine and Hygiene*, 2002, 67:378–382.

155. Basco LK. Molecular epidemiology of malaria in Cameroon. XVI. Longitudinal surveillance of in vitro pyrimethamine resistance in *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:174–178.

156. Alifrangis M et al. Prediction of *Plasmodium falciparum* resistance to sulfadoxine/pyrimethamine in vivo by mutations in the dihydrofolate reductase and dihydropteroate synthetase genes: a comparative study between sites of differing endemicity. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:601–606.

157. Mbaisi A et al. Drug susceptibility and genetic evaluation of *Plasmodium falciparum* isolates obtained in four distinct geographical regions of Kenya. *Antimicrobial Agents and Chemotherapy*, 2004, 48:3598–3601.

158. Kyosiimire-Lugemwa J, et al. The Lys-76–Thr mutation in *PfCRT* and chloroquine resistance in *Plasmodium falciparum* isolates from Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:91–95.

159. Jelinek T et al. Diagnostic value of molecular markers in chloroquine-resistant falciparum malaria in southern Mauritania. *American Journal of Tropical Medicine and Hygiene*, 2002, 67:449–453.

160. Labbe AC et al. Molecular markers for chloroquine-resistant *Plasmodium falciparum* malaria in Thailand and Laos. *Annals of Tropical Medicine and Parasitology*, 2001, 95:781–788.

161. Kublin JG et al. Reemergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. *Journal of Infectious Diseases*, 2003, 187:1870–1875.

162. Doumbo OK et al. Rapid selection of *Plasmodium falciparum* dihydrofolate reductase mutants by pyrimethamine prophylaxis. *Journal of Infectious Diseases*, 2000, 182:993–996.

163. Ranford-Cartwright LC et al. Critical comparison of molecular genotyping methods for detection of drug-resistant *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:568–572.

164. Durand R et al. Rapid detection of a molecular marker for chloroquine-resistant falciparum malaria. *Antimicrobial Agents and Chemotherapy*, 2002, 46:2684–2686.

165. Kublin et al. Molecular markers for failure of sulfadoxine–pyrimethamine and chlorproguanil–dapsone treatment of *Plasmodium falciparum* malaria. *Journal of Infectious Diseases*, 2002, 185:380–388.

166. Talisuna AO et al. Two mutations in dihydrofolate reductase combined with one in the dihydropteroate synthase gene predict sulphadoxine–pyrimethamine parasitological failure in Ugandan children with uncomplicated falciparum malaria. *Infection, Genetics and Evolution*, 2004, 4:321–327.

167. Dorsey G et al. Principal role of dihydropteroate synthase mutations in mediating resistance to sulfadoxine–pyrimethamine in single-drug and combination therapy of uncomplicated malaria in Uganda. *American Journal of Tropical Medicine and Hygiene*, 2004, 71:758–763.

168. Talisuna AO et al. Population-based validation of dihydrofolate reductase gene mutations for the prediction of sulfadoxine–pyrimethamine resistance in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:338–342.

169. Staedke SG et al. Relationship between age, molecular markers, and response to sulphadoxine–pyrimethamine treatment in Kampala, Uganda. *Tropical Medicine and International Health*, 2004, 9:624–629.

170. Eriksen J et al. Patterns of resistance and *DHFR*/*DHPS* genotypes of *Plasmodium falciparum* in rural Tanzania prior to the adoption of sulfadoxine–pyrimethamine as first-line treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2004, 98:347–353.

171. Khalil I et al. Pyrimethamine/sulfadoxine combination in the treatment of uncomplicated falciparum malaria: relation between dihydropteroate synthase/

dihydrofolate reductase genotypes, sulfadoxine plasma levels, and treatment outcome. *American Journal of Tropical Medicine and Hygiene*, 2002, 67:225–229.

172. Basco LK, Tahar R, Ringwald P. Molecular basis of in vivo resistance to sulfadoxine–pyrimethamine in African adult patients infected with *Plasmodium falciparum* malaria parasites. *Antimicrobial Agents and Chemotherapy*, 1998, 42:1811–1814.

173. Basco LK et al. Sequence variations in the genes encoding dihydropteroate synthase and dihydrofolate reductase and clinical response to sulfadoxine–pyrimethamine in patients with acute uncomplicated falciparum malaria. *Journal of Infectious Diseases*, 2000, 182:624–628.

174. Aubouy A et al. *DHFR* and *DHPS* genotypes of *Plasmodium falciparum* isolates from Gabon correlate with in vitro activity of pyrimethamine and cycloguanil, but not with sulfadoxine–pyrimethamine treatment efficacy. *Journal of Antimicrobial and Chemotherapy*, 2003, 52:43–49.

175. Mendez F et al. Determinants of treatment response to sulfadoxine–pyrimethamine and subsequent transmission potential in falciparum malaria. *American Journal of Epidemiology*, 2002, 156:230–238.

176. Djimde A et al. Application of a molecular marker for surveillance of chloroquine-resistant falciparum malaria. *Lancet*, 2001, 358:890–891.

177. Talisuna AO et al. Role of the *pfcrt* codon 76 mutation as a molecular marker for population-based surveillance of chloroquine (CQ)-resistant *Plasmodium falciparum* malaria in Ugandan sentinel sites with high CQ resistance. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:551–556.

178. Snounou G, Beck HP. The use of PCR genotyping in the assessment of recrudescence or reinfection after antimalarial drug treatment. *Parasitology Today*, 1998, 14:462–467.

179. Leclerc MC et al. Genetic diversity and population structure of *Plasmodium falciparum* isolates from Dakar, Senegal, investigated from microsatellite and antigen determinant loci. *Microbes and Infections*, 2002, 4:685–692.

180. Basco LK, Ringwald P. Molecular epidemiology of malaria in Yaounde, Cameroon. VII. Analysis of recrudescence and reinfection in patients with uncomplicated falciparum malaria. *American Journal of Tropical Medicine and Hygiene*, 2000, 63:215–221.

181. Cattamanchi A et al. Distinguishing recrudescence from reinfection in a longitudinal antimalarial drug efficacy study: comparison of results based on genotyping of *msp-1*, *msp-2*, and *glurp*. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:133–139.

182. Farnert A et al. Genotyping of *Plasmodium falciparum* infections by PCR: a comparative multicentre study. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:225–232.

183. Mayxay M et al. Randomized comparison of chloroquine plus sulfadoxine–pyrimethamine versus artesunate plus mefloquine versus artemether–lumefantrine in the treatment of uncomplicated falciparum malaria in the Lao People's Democratic Republic. *Clinical Infectious Diseases*, 2004, 39: 1139–1147.

184. Mayxay M et al. A randomized comparison of oral chloroquine and sulfadoxine– pyrimethamine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Laos. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:343–344.

185. Mayxay M et al. Chloroquine versus sulfadoxine–pyrimethamine for treatment of *Plasmodium falciparum* malaria in Savannakhet Province, Lao People's Democratic Republic: an assessment of national antimalarial drug recommendations. *Clinical Infectious Diseases*, 2003, 37:1021–1028.

186. Djimde AA et al. Clearance of drug-resistant parasites as a model for protective immunity in *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:558–563.

187. Mawili-Mboumba DP et al. Antibody responses to *Plasmodium falciparum* merozoite surface protein 1 and efficacy of amodiaquine in Gabonese children with *P. falciparum* malaria. *Journal of Infectious Diseases*, 2003, 187:1137–1141.

188. Basco L, Same-Ekobo A, Ringwald P. Le nouveau test de chimiosensibilité *in vivo* pour le paludisme: test d'efficacité thérapeutique. *Bulletin de Liaison de l'OCEAC*, 1999, 32:14–20.

189. World Health Organization. Severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, 94(Suppl. 1): S1-S90.

190. Ruebush TK II et al. Practical aspects of in vivo antimalarial drug efficacy testing in the Americas. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:391–397.

191. Ndounga M, Basco LK. Rapid clearance of *Plasmodium falciparum* hyperparasitaemia after oral amodiaquine treatment in patients with uncomplicated malaria. *Acta Tropica*, 2003, 88:27–32.

192. Sowunmi A et al. *Plasmodium falciparum* hyperparasitaemia in children. Risk factors, treatment outcomes, and gametocytaemia following treatment. *Parasite*, 2004, 11:317–323.

193. Hess FI et al. Risk factors of chloroquine resistance in *Plasmodium falciparum* malaria. *Acta Tropica*, 1996, 61:293–306.

194. Ringwald P. Monitoring antimalarial drug efficacy. *Clinical Infectious Diseases*, 2004, 38:1192–1193.

195. Checchi F et al. High *Plasmodium falciparum* resistance to chloroquine and sulfadoxine–pyrimethamine in Harper, Liberia: results in vivo and analysis of point mutations. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:664–669.

196. Mehlotra RK et al. Evolution of a unique *Plasmodium falciparum* chloroquine-resistance phenotype in association with *pfcrt* polymorphism in Papua New Guinea and South America. *Proceedings of the National Academy of Sciences of the United States of America*, 2001, 98:12689–12694.

197. Wootton JC et al. Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*. *Nature*, 2002, 418:320–323.

198. Chen N et al. *pfcrt* Allelic types with two novel amino acid mutations in chloroquine-resistant *Plasmodium falciparum* isolates from the Philippines. *Antimicrobial Agents and Chemotherapy*, 2003, 47:3500–3505.

199. Wellems TE, Plowe CV. Chloroquine-resistant malaria. *Journal of Infectious Diseases*, 2001, 184:770–776.

200. Louis FJ, Ringwald P, Chambon R. Surveillance épidémiologique de la sensibilité de *Plasmodium falciparum* à la chloroquine au Cameroun: nécessité d'une adaptation des politiques de santé publique [Epidemiological surveillance of *Plasmodium falciparum* sensitivity to chloroquine in Cameroon: necessity of public health policy adaptation]. *Médecine Tropicale*, 1995, 55 (4 Suppl.):77–79.

201. Thaithong S et al. Evidence of increased chloroquine sensitivity in Thai isolates of *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1988, 82:37–38.

202. Wongsrichanalai C et al. *In vitro* sensitivity of *Plasmodium falciparum* isolates in Thailand to quinine and chloroquine, 1984–1990. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1992, 23:533–536.

203. Kain KC et al. In vivo response of falciparum malaria to chloroquine in southern Thailand. *Journal of Infectious Diseases*, 1994, 170:258–259.

204. Yang HL et al. Longitudinal surveillance of chloroquine resistance of *Plasmodium falciparum* after cessation of medication in south Yunnan. *Chinese Journal of Parasitology and Parasitic Diseases*, 1994, 12:31–33.

205. Liu DQ et al. Changes in the resistance of *Plasmodium falciparum* to chloroquine in Hainan, China. *Bulletin of the World Health Organization*, 1995, 73:483–486.

206. Thanh NV et al. Assessment of susceptibility of *Plasmodium falciparum* to chloroquine, quinine, mefloquine, sulfadoxine–pyrimethamine and artemisinin in southern Viet Nam. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:513–517.

207. Huong MN et al. Resistance of *Plasmodium falciparum* to antimalarial drugs in a highly endemic area of southern Viet Nam: a study in vivo and in vitro. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:325–329.

208. Nguyen MH et al. Treatment of uncomplicated falciparum malaria in southern Vietnam: can chloroquine or sulfadoxine–pyrimethamine be reintroduced in combination with artesunate? *Clinical Infectious Diseases*, 2003, 37:1461–1466.

209. Wilson PE et al. Prevalence of *pfcrt* mutations in Congolese and Malawian *Plasmodium falciparum* isolates as determined by a new Taqman assay. *Acta Tropica*, 2005, 93:97–106.

210. Mita T et al. Expansion of wild type allele rather than back mutation in *pfcrt* explains the recent recovery of chloroquine sensitivity of *Plasmodium falciparum* in Malawi. *Molecular and Biochemical Parasitology*, 2004, 135:159–163.

211. Olliaro P et al. Systematic review of amodiaquine treatment in uncomplicated malaria. *Lancet*, 1996, 348:1196–1201.

212. Rwagacondo CE et al. Efficacy of amodiaquine alone and combined with sulfadoxine–pyrimethamine and of sulfadoxine–pyrimethamine combined with artesunate. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:743–747.

213. Rwagacondo CE et al. Is amodiaquine failing in Rwanda? Efficacy of amodiaquine alone and combined with artesunate in children with uncomplicated malaria. *Tropical Medicine and International Health*, 2004, 9:1091–1098.

214. Nsimba B et al. Efficacy of sulfadoxine/pyrimethamine in the treatment of uncomplicated *Plasmodium falciparum* malaria in Republic of Congo. *American Journal of Tropical Medicine and Hygiene*, 2004, 70:133–138.

215. Zakeri S et al. High prevalence of double *Plasmodium falciparum dhfr* mutations at codons 108 and 59 in the Sistan–Baluchistan province, Iran. *Journal of Infectious Diseases*, 2003, 187:1828– 1829.

216. Randrianarivelojosia M et al. Current absence of pyrimethamine resistance of *Plasmodium falciparum* in Madagascar. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:557–559.

217. Plowe CV et al. Sustained clinical efficacy of sulfadoxine–pyrimethamine for uncomplicated falciparum malaria in Malawi after 10 years as first line treatment: five year prospective study. *British Medical Journal*, 2004, 328:545.

218. Ringwald P. Sulfadoxine–pyrimethamine for uncomplicated falciparum malaria: treatment failure and resistance in Malawi remain subject for debate. *British Medical Journal*, 2004, 328:1259–1260.

219. Roper C et al. Antifolate antimalarial resistance in southeast Africa: a population-based analysis. *Lancet*, 2003, 361:1174–1181.

220. Nair S et al. A selective sweep driven by pyrimethamine treatment in Southeast Asian malaria parasites. *Molecular Biology and Evolution*, 2003, 20:1526–1536.

221. Roper C et al. Intercontinental spread of pyrimethamine-resistant malaria. *Science*, 2004, 305:1124.

222. Laufer MK, Plowe CV. Withdrawing antimalarial drugs: impact on parasite resistance and implications for malaria treatment policies. *Drug Resistance Updates*, 2004, 7:279–288.

223. Alifrangis M et al. Increasing prevalence of wildtypes in the dihydrofolate reductase gene of *Plasmodium falciparum* in an area with high levels of sulfadoxine/pyrimethamine resistance after introduction of treated bed nets. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:238–243.

224. Talisuna AO et al. Intensity of transmission and spread of gene mutations linked to chloroquine and sulphadoxine–pyrimethamine resistance in falciparum malaria. *International Journal of Parasitology*, 2003, 33:1051–1058.

225. Boudreau EF et al. Type II mefloquine resistance in Thailand. *Lancet*, 1982, ii:1335.

226. ter Kuile FO et al. Mefloquine treatment of acute falciparum malaria: a prospective study of non-serious adverse effects in 3673 patients. *Bulletin of the World Health Organization*, 1995, 73:631–642.

227. Smithuis FM et al. Comparison of two mefloquine regimens for treatment of *Plasmodium falciparum* malaria on the northeastern Thai–Cambodian border. *Antimicrobial Agents and Chemotherapy*, 1993, 37:1977–1981.

228. Simpson JA et al. Mefloquine pharmacokinetic–pharmacodynamic models: implications for dosing and resistance. *Antimicrobial Agents and Chemotherapy*, 2000, 44:3414–3424.

229. Schlagenhauf P. Mefloquine for malaria chemoprophylaxis 1992–1998: a review. *Journal of Travel Medicine*, 1999, 6:122–133.

230. Mockenhaupt FP. Mefloquine resistance in *Plasmodium falciparum*. *Parasitology Today*, 1995, 11:248–253.

231. Lobel HO et al. Monitoring for mefloquine-resistant *Plasmodium falciparum* in Africa: implications for travelers' health. *American Journal of Tropical Medicine and Hygiene*, 1998, 59:129–132.

232. Rojanawatsirivej C et al. Monitoring the therapeutic efficacy of antimalarials against uncomplicated falciparum malaria in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2003, 34:536–541.

233. Smithuis F et al. Comparison of chloroquine, sulfadoxine/pyrimethamine, mefloquine and mefloquine–artesunate for the treatment of falciparum malaria in Kachin State, north Myanmar. *Tropical Medicine and International Health*, 2004, 9:1184–1190.

234. Trung TN et al. Treatment of falciparum malaria in Vietnamese children: the need for combination therapy and optimized dosage regimens. *Annals of Tropical Pediatrics*, 2001, 21:307–312.

235. Rahman MR et al. A randomized controlled trial on the efficacy of alternative treatment regimens for uncomplicated falciparum malaria in a multidrug-resistant falciparum area of Bangladesh—narrowing the options for the national malaria control programme? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:661–667.

236. Oduola AM et al. Innate resistance to new antimalarial drugs in *Plasmodium falciparum* from Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1992, 86:123–126.

237. Oduola AM et al. Reduced in vitro susceptibility to mefloquine in West African isolates of *Plasmodium falciparum*. *Lancet*, 1987, ii:1304–1305.

238. MacArthur J et al. Efficacy of mefloquine and sulfadoxine– pyrimethamine for the treatment of uncomplicated *Plasmodium falciparum* infection in Machinga District, Malawi, 1998. *American Journal of Tropical Medicine and Hygiene*, 2001, 65:679–684.

239. Jiang JB et al. Antimalarial activity of mefloquine and qinghaosu. *Lancet*, 1982, ii:285–288.

240. Hoffman SL et al. RII and RIII type resistance of *Plasmodium falciparum* to combination of mefloquine and sulfadoxine/ pyrimethamine in Indonesia. *Lancet*, 1985, ii:1039–1040.

241. Noronha E et al. Resistência à mefloquina do tipo RIII em crianças com malária falciparum em Manaus, AM, Brasil [RIII mefloquine resistance in children with falciparum malaria in Manaus, AM, Brazil]. *Revista da Sociedade Brasileira de Medicina Tropical*, 2000, 33:201–205.

242. Brasseur P et al. Multi-drug resistant falciparum malaria in Cameroon in 1987–1988. II. Mefloquine resistance confirmed in vivo and in vitro and its correlation with quinine resistance. *American Journal of Tropical Medicine and Hygiene*, 1992, 46:8–14.

243. Salako LA et al. Sensitivity of *Plasmodium falciparum* to mefloquine–sulphadoxine–pyrimethamine (Fansimef®) in vivo and to mefloquine alone in vitro in Nigeria. *Annals of Tropical Medicine and Parasitology*, 1988, 82, 325–330.

244. Sowunmi A et al. The relationship between the response of *Plasmodium falciparum* malaria to mefloquine in African children and its sensitivity *in vitro*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1992, 86:368–371.

245. Fadeke Aderounmu A et al. Comparison of the susceptibility of falciparum malaria to mefloquine–sulphadoxine–pyrimethamine and chloroquine in Nigeria. *African Journal of Medicine and Medical Science*, 1988, 17:195–200.

246. Okoyeh et al. Responses of multidrug-resistant *Plasmodium falciparum* parasites to mefloquine in Nigerian children. *Tropical Medicine and International Health*, 1997, 2:319–324.

247. Ededinachi ENU et al. Efficacy and tolerability of a low-dose mefloquine–sulfadoxine–pyrimethamine combination compared with chloroquine in the treatment of acute malaria infection in a population with multiple drug-resistant *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene*, 1999, 61:114–119.

248. Hatin I et al. Susceptibility of *Plasmodium falciparum* strains to mefloquine in an urban area in Senegal. *Bulletin of the World Health Organization*, 1982, 70:363–367.

249. Adam I et al. Mefloquine in the treatment of falciparum malaria during pregnancy in eastern Sudan. *Saudi Medical Journal*, 2004, 25:1400–1402.

250. Bygbjerg IC et al. Mefloquine resistance of falciparum malaria from Tanzania enhanced by treatment. *Lancet*, 1983, i:774–775.

251. Kofi Ekue JM et al. A double-blind comparative clinical trial of mefloquine and chloroquine in symptomatic falciparum malaria. *Bulletin of the World Health Organization*, 1983, 61:713–718.

252. Gay F et al. Mefloquine failure in child contracting falciparum malaria in West Africa. *Lancet*, 1990, 335:120–121.

253. Stepniewska K et al. In vivo assessment of drug efficacy against *Plasmodium falciparum* malaria: duration of follow-up. *Antimicrobial Agents and Chemotherapy*, 2004, 48:4271–4280.

254. White N. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. *Antimicrobial Agents and Chemotherapy*, 1997, 41:1413–1422.

255. Edwards G, Krishna S. Pharmacokinetic and pharmacodynamic issues in the treatment of parasitic infections. *European Journal of Clinical Microbiology and Infectious Diseases*, 2004, 23:233–242.

256. Molinier S et al. Paludisme à *Plasmodium falciparum*: résistance de type R1 à la quinine en Afrique de l'Est [*Plasmodium falciparum* malaria: type R1 quinine resistance in East Africa]. *Presse Medicale*, 1994, 23:1494.

257. Carles M et al. Résistance de type R1 de *Plasmodium falciparum* à la quinine au retour de Côte d'Ivoire [*Plasmodium falciparum* type R1 resistance to quinine after returning from Ivory Coast]. *Presse Medicale*, 1996, 25:1303.

258. Garavelli PL. Malaria da *Plasmodium falciparum* recidivante dopo terapia con chinino [Recurrent malaria caused by *Plasmodium falciparum* after therapy with quinine]. *Recenti Progressi in Medicina*, 1998, 89:406.

259. Biseru B. Quinine-resistant *Plasmodium falciparum* in Zambia. *Central African Journal of Medicine*, 1988, 34:17–18.

260. Turner GDS. Kenyan chloroquin, Fansidar and quinine resistant *P. falciparum*. *Central African Journal of Medicine*, 1984, 30:136–137.

261. Palmieri F et al. Genetic confirmation of quinine-resistant *Plasmodium falciparum* malaria followed by postmalaria neurological syndrome in a traveler from Mozambique. *Journal of Clinical Microbiology*, 2004, 42:5424–5426.

262. Warhurst DC, Hall AP, Tjokrosonto S. RI quinine–Fansidar resistant falciparum malaria from Malawi. *Lancet*, 1985, ii:330.

263. Rolfe M. Multiple drug resistant *Plasmodium falciparum* malaria in a pregnant indigenous Zambian woman. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1988, 82:554–557.

264. Longuet C et al. Paludisme à *Plasmodium falciparum*: résistance de type R1 à la quinine en Afrique de l'ouest [*Plasmodium falciparum* malaria: type R1 resistance to quinine in west Africa]. *Presse Médicale*, 1998, 27:522.

265. Delacollette C. Quinine base and quinine salt: a persistent confusion. *Annales de la Société belges de Médecine Tropicale*, 1990, 70:317–318.

266. Brandts CH et al. Effect of paracetamol on parasite clearance time in *Plasmodium falciparum* malaria. *Lancet*, 1997, 350:704–709.

267. Roche J et al. A. Surveillance of in vivo resistance of *Plasmodium falciparum* to antimalarial drugs from 1992 to 1999 in Malabo (Equatorial Guinea). *American Journal of Tropical Medicine and Hygiene*, 2003, 68:598–601.

268. Adam I et al. Efficacies of chloroquine, sulfadoxine–pyrimethamine and quinine in the treatment of uncomplicated, *Plasmodium falciparum* malaria in eastern Sudan. *Annals of Tropical Medicine and Parasitology*, 2004, 98:661–666.

269. Ngimbi NP, Tona Lutete, Ngoma Z. L'association de la clindamycine à la chloroquine ou à la quinine dans le traitement du paludisme à Kinshasa (Zaire). Résultats préliminaires. *Médecine d'Afrique Noire*, 1998, 45:270–272.

270. Mueller MS et al. Randomized controlled trial of a traditional preparation of *Artemisia annua* L. (annual wormwood) in the treatment of malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2004, 98:318–321.

271. Mohanty AK et al. Randomized control trial of quinine and artesunate in complicated malaria. *Indian Journal of Pediatrics*, 2004, 71:291–295.

272. Pukrittayakamee S et al. Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria. *Antimicrobial Agents and Chemotherapy*, 2004, 48:1329–1334.

273. Pukrittayakamee S et al. Adverse effect of rifampin on quinine efficacy in uncomplicated falciparum malaria. *Antimicrobial Agents and Chemotherapy*, 2003, 47:1509–1513.

274. de Vries PJ, Combinations of artemisinin and quinine for uncomplicated falciparum malaria: efficacy and pharmacodynamics. *Antimicrobial Agents and Chemotherapy*, 2000, 44:1302–1308.

275. Gachot B et al. Possible prognostic significance of a brief rise in parasitaemia following quinine treatment of severe *Plasmodium falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1996, 90:388–390.

276. Demar M, Carme B. *Plasmodium falciparum* in vivo resistance to quinine: description of two RIII responses in French Guiana. *American Journal of Tropical Medicine and Hygiene*, 2004, 70:125–127.

277. Uchiyama H et al. Quinine-resistant severe falciparum malaria effectively treated with atovaquone and proguanil hydrochloride combination therapy. *Internal Medicine*, 2004, 43:624–647.

278. Jelinek T et al. Quinine resistant falciparum malaria acquired in east Africa. *Tropical Medicine and Parasitology*, 1995, 46:38–40.

279. Marra F, Salzman JR, Ensom MHH. Atovaquone–proguanil for prophylaxis and treatment of malaria. *Annals of Pharmacotherapy*, 2003, 37:1266–1275.

280. McKeage K, Scott LJ. Atovaquone/proguanil. A review of its use for the prophylaxis of *Plasmodium falciparum* malaria. *Drugs*, 2003, 63:597–623.

281. Giao PT et al. Atovaquone–proguanil for recrudescent *Plasmodium falciparum* in Vietnam. *Annals of Tropical Medicine and Parasitology*, 2003, 97:575–580.

282. Ling J et al. Randomised, placebo-controlled trial of atovaquone/ proguanil for the prevention of *Plasmodium falciparum* or *Plasmodium vivax* malaria among migrants to Papua, Indonesia. *Clinical Infectious Diseases*, 2002, 35:825–833.

283. Povinelli L et al. *Plasmodium vivax* malaria in spite of atovaquone/ proguanil (Malarone) prophylaxis. *Journal of Travel Medicine*, 2003, 10:353–355.

284. Anabwani G et al. Combination atovaquone and proguanil hydrochloride vs. halofantrine for treatment of acute *Plasmodium falciparum* malaria in children. *Pediatric Infectious Disease Journal*, 1999, 18:456–461.

285. David KP et al. Atovaquone/proguanil resistance in Africa: a case report. *Scandinavian Journal of Infectious Diseases*, 2003, 35:897–898.

286. Schwartz E, Bujanover S, Kain KC. Genetic confirmation of atovaquone–proguanil-resistant *Plasmodium falciparum* malaria acquired by a nonimmune traveler to East Africa. *Clinical Infectious Diseases*, 2003, 37:450–451.

287. Eckstein Ludwig U et al. Artemisinins target the SERCA of *Plasmodium falciparum*. *Nature*, 2003, 424:957–961.

288. Krishna S, Uhlemann AC, Haynes RK. Artemisinins: mechanisms of action and potential for resistance. *Drug Resistance Updates*, 2004, 7:233–244.

289. Ferrer-Rodriguez I et al. *Plasmodium yoelii*: identification and partial characterization of an *MDR1* gene in an artemisinin resistant line. *Journal of Parasitology*, 2004, 90:152–160.

290. Xiao SH et al. Selection and reversal of *Plasmodium berghei* resistance in the mouse model following repeated high doses of artemether. *Parasitology Research*, 2004, 92:215–219.

291. Chawira AN, Warhurst DC, Peters W. Qinghaosu resistance in rodent malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1986, 80:477–480.

292. Walker DJ et al. Mechanisms of artemisinin resistance in the rodent malaria pathogen *Plasmodium yoelii*. *Antimicrobial Agents and Chemotherapy*, 2000, 44:344–347.

293. Randrianarivelojosia M et al. Madagascan isolates of *Plasmodium falciparum* showing low sensitivity to artemether in vitro. *Annals of Tropical Medicine and Parasitology*, 2001, 95:237–243.

294. Gay F et al. In vitro resistance of *Plasmodium falciparum* to qinghaosu derivatives in West Africa. *Lancet*, 1994, 343:850–851.

295. Jena RK et al. Failure of artemisinin in cerebral malaria. *Journal of the Association of Physicians of India*, 1997, 45:671–672.

296. Sahr F et al. Apparent drug failure following artesunate treatment of *Plasmodium falciparum* malaria in Freetown, Sierra Leone: four case reports. *Annals of Tropical Medicine and Parasitology*, 2001, 95:445–449.

297. Thu LTA et al. Delayed parasite clearance in a splenectomized patient with falciparum malaria who was treated with artemisinine derivatives. *Clinical Infectious Diseases*, 1997, 25:923–925.

298. Yuthavong Y et al. Decreased sensitivity to artesunate and chloroquine of *Plasmodium falciparum* infecting hemoglobin H and/or hemoglobin Constant Spring erythrocytes. *Journal of Clinical Investigation*, 1989, 83:502–505.

299. Wongsrichanalai C et al. *In vitro* sensitivity of *Plasmodium falciparum* to artesunate in Thailand. *Bulletin of the World Health Organization*, 1999, 77:392–398.

300. Henglin Y et al. Changes in susceptibility of *Plasmodium falciparum* to artesunate in vitro in Yunnan province, China. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:226–228.

301. Li GQ et al. Dose findings of dihydroartemisinin in treatment of falciparum malaria. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1999, 30:17–19.

302. Xu C, Ding Y, Qi Z. Efficacy of dihydroartemisinin in treatment of 37 malaria cases. *Chinese Journal of Internal Medicine*, 1997, 36:187–189.

303. Liu DQ et al. Study on treatment of multi-drug resistant falciparum malaria by using a combination of dihydroartemisinin and pyronaridine. *Chinese Journal of Parasitology and Parasitic Diseases*, 2002, 20:193–196.

304. Ittarat W et al. Recrudescence in artesunate-treated patients with falciparum malaria is dependent on parasite burden not on parasite factors. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:147–152.

305. Silachamroon U et al. Frequency of early rising parasitemia in falciparum malaria treated with artemisinine derivatives. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2001, 32:50–56.

306. Gogtay NJ et al. Probable resistance to parenteral artemether in *Plasmodium falciparum*: case reports from Mumbai (Bombay), India. *Annals of Tropical Medicine and Parasitology*, 2000, 94:519–520.

307. Luxemburger C et al. Two patients with falciparum malaria and poor *in vivo* responses to artesunate. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1998, 92:668–669.

308. Singh RK. The response of a case of multidrug-resistant, *Plasmodium falciparum* malaria to an unusual combination of antimalarial drugs. *Annals of Tropical Medicine and Parasitology*, 2002, 96:417–418.

309. Adjuik M et al. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet*, 2004, 363:9–17.

310. Gasasira AF et al. Comparative efficacy of aminoquinoline–antifolate combinations for the treatment of uncomplicated falciparum malaria in Kampala, Uganda. *American Journal of Tropical Medicine and Hygiene*, 2003, 68:127–132.

311. Talisuna AO et al. Efficacy of sulphadoxine–pyrimethamine alone or combined with amodiaquine or chloroquine for the treatment of uncomplicated falciparum malaria in Ugandan children. *Tropical Medicine and International Health*, 2004, 9:222–229.

312. Staedke SG et al. Amodiaquine, sulfadoxine/pyrimethamine, and combination therapy for treatment of uncomplicated falciparum malaria in Kampala, Uganda: a randomised trial. *Lancet*, 2001, 358:368–374.

313. Staedke SG et al. Combination treatments for uncomplicated falciparum malaria in Kampala, Uganda: randomized clinical trial. *Lancet*, 2004, 364:1950–1957.

314. Dorsey G et al. Sulfadoxine/pyrimethamine alone or with amodiaquine or artesunate for treatment of uncomplicated malaria: a longitudinal randomised trial. *Lancet*, 2002, 360:2031–2038.

315. Nosten F et al. Effects of artesunate–mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet*, 2000, 356:297–302.

316. Nosten F et al. Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate–mefloquine combination. *Journal of Infectious Diseases*, 1994, 170:971–977.

317. Looareesuwan S et al. Comparative clinical trial of artesunate followed by mefloquine in the treatment of acute uncomplicated falciparum malaria: two- and three-day regimens. *American Journal of Tropical Medicine and Hygiene*, 1996, 54:210–213.

318. Looareesuwan S et al. Treatment of acute uncomplicated falciparum malaria with a short course of artesunate followed by mefloquine. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1993, 24:230–234.

319. Looareesuwan S et al. Randomized trial of mefloquine alone and artesunate followed by mefloquine for the treatment of acute uncomplicated falciparum malaria. *Annals of Tropical Medicine and Parasitology*, 1994, 88:131–136.

320. Wilairatana P et al. A clinical trial of combination of artesunate and mefloquine in the treatment of acute uncomplicated falciparum malaria: a short and practical regimen. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1998, 29:696–701.

321. Nosten F et al. Malaria misconceptions. *Lancet*, 2005, 365:653.

322. Massougbodji A et al. Randomized, double-blind study on the efficacy and safety of a practical three-day regimen with artesunate and mefloquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:655–659.

323. Kshirsagar NA et al. A randomized, double-blind, parallel-group, comparative safety, and efficacy trial of oral co-artemether versus oral chloroquine in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in adults in India. *American Journal of Tropical Medicine and Hygiene*, 2000, 62:402–408.

324. Looareesuwan S et al. A randomized, double-blind, comparative trial of a new oral combination of artemether and benflumetol (CGP 56697) with mefloquine in the treatment of acute *Plasmodium falciparum* malaria in Thailand. *American Journal of Tropical Medicine and Hygiene*, 1999, 60:238–243.

325. van Vugt M et al. Randomized comparison of artemether–benflumetol and artesunate–mefloquine in treatment of multidrug-resistant falciparum malaria. *Antimicrobial Agents and Chemotherapy*, 1998, 42:135–139.

326. Hatz C et al. Efficacy and safety of CGP 56697 (artemether and benflumetol) compared with chloroquine to treat acute falciparum malaria in Tanzanian children aged 1–5 years. *Tropical Medicine and International Health*, 1998, 3:498–504.

327. von Seidlein L et al. A randomized controlled trial of artemether/ benflumetol, a new antimalarial and pyrimethamine/sulfadoxine in the treatment of uncomplicated falciparum malaria in African children. *American Journal of Tropical Medicine and Hygiene*, 1998, 58:638–644.

328. Sun ZW et al. Clinical comparative trial of co-artemether and benflumetol (two formulations) in the treatment of falciparum malaria. *Chinese Journal of Parasitology and Parasitic Diseases*, 2000, 18:159–161.

329. Stohrer JM et al. Therapeutic efficacy of artemether–lumefantrine and artesunate–mefloquine for treatment of uncomplicated *Plasmodium falciparum* malaria in Luang Namtha Province, Lao People's Democratic Republic. *Tropical Medicine and International Health*, 2004, 9:1175–1183.

330. Vugt MV et al. Efficacy of six doses of artemether–lumefantrine (benflumetol) in multidrug-resistant *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene*, 1999, 60:936–942.

331. van Vugt M et al. Artemether–lumefantrine for the treatment of multidrug-resistant falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, 94:545–548.

332. Krudsood S et al. Comparative clinical trial of two-fixed combinations dihydroartemisinin–napthoquine–trimethoprim (DNP) and artemether–lumefantrine (Coartem/Riamet) in the treatment of acute uncomplicated falciparum malaria in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2003, 34:316–321.

333. Ndayiragije A et al. Efficacité de combinaisons thérapeutiques avec des dérivés de l'artemisinine dans le traitement de l'accès palustre non-compliqué au Burundi [Efficacy of therapeutic combinations with artemisinin derivatives in the treatment of non complicated malaria in Burundi]. *Tropical Medicine and International Health*, 2004, 9:673–679.

334. Ezzet F, Mull R, Karbwang J. Population pharmacokinetics and therapeutic response of CGP 56697 (artemether+benflumetol) in malaria patients. *British Journal of Clinical Pharmacology*, 1998, 46:553–561.

335. White NJ, van Vugt M, Ezzet F. Clinical pharmacokinetics and pharmacodynamics and pharmacodynamics of artemether–lumefantrine. *Clinical Pharmacokinetics*, 1999, 37:105–125.

336. Ezzet F et al. Pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute falciparum malaria. *Antimicrobial Agents and Chemotherapy*, 2000, 44:697–704.

337. White NJ. The assessment of antimalarial drug efficacy. *Trends in Parasitology*, 2002, 18:458–464.

338. Kassi RR et al. Efficacité thérapeutique de la chloroquine dans le traitement du paludisme simple dû à *Plasmodium falciparum* chez les enfants du dispensaire de San Pedro en Côte d'Ivoire [Efficacy of chloroquine in treatment of non-complicated malaria with *Plasmodium falciparum* in children at the San Pedro dispensary in Cote d'Ivoire]. *Bulletin de la Société de Pathologie Exotique*, 2004, 97:129–130.

339. Sutherland CJ et al. The addition of artesunate to chloroquine for treatment of *Plasmodium falciparum* malaria in Gambian children delays, but does not prevent treatment failure. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:19–25.

340. Plowe CV et al. Chloroquine treatment of uncomplicated *Plasmodium falciparum* malaria in Mali: parasitologic resistance versus therapeutic efficacy. *American Journal of Tropical Medicine and Hygiene*, 2001, 64:242–246.

341. Parola P et al. Chloroquine sensitivity of *Plasmodium falciparum* at the Gamkalley Clinic and the Nigerian armed forces PMI (Niamey, Niger). *Bulletin de la Société de Pathologie Exotique*, 1999, 92:317–319.

342. van den Broek IV et al. Chloroquine, sulfadoxine–pyrimethamine and amodiaquine efficacy for the treatment of uncomplicated *Plasmodium falciparum* malaria in Upper Nile, south Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:229–235.

343. Kamya MR et al. The comparative efficacy of chloroquine and sulfadoxine–pyrimethamine for the treatment of uncomplicated falciparum malaria in Kampala, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:50–55.

344. Rahman M et al. Efficacy of combined chloroquine and sulfadoxine–pyrimethamine in uncomplicated *Plasmodium falciparum* malaria in Bangladesh. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2004, 98:438–441.

345. Tjitra E et al. Therapy of uncomplicated falciparum malaria: a randomized trial comparing artesunate plus sulfadoxine–pyrimethamine versus sulfadoxine–pyrimethamine alone in Irian Jaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*, 2001, 65:309–317.

346. Schwobel B et al. Therapeutic efficacy of chloroquine plus sulphadoxine/pyrimethamine compared with monotherapy with either chloroquine or sulphadoxine/pyrimethamine in uncomplicated *Plasmodium falciparum* malaria in Laos. *Tropical Medicine and International Health*, 2003, 8:19–24.

347. Whitworth J et al. Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. *Lancet*, 2000, 356:1051–1056.

348. Francesconi P et al. HIV, malaria parasites, and acute febrile episodes in Ugandan adults: a case–control study. *AIDS*, 2001, 15:2445–2450.

349. Parise ME et al. Efficacy of sulfadoxine–pyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection. *American Journal of Tropical Medicine and Hygiene*, 1998, 59:813–822.

350. Kamya MR, Kigonya CN, McFarland W. HIV infection may adversely affect clinical response to chloroquine therapy for uncomplicated malaria in children. *AIDS*, 2001, 15:1187–1188.

351. Birku Y et al. Delayed clearance of *Plasmodium falciparum* in patients with human immunodeficiency virus co-infection treated with artemisinin. *Ethiopian Medical Journal*, 2002, 40(Suppl. 1):17–26.

352. Sowunmi A. Randomised trial of artemether versus artemether and mefloquine for the treatment chloroquine/sulfadoxine–pyrimethamine-resistant falciparum malaria during pregnancy. *Journal of Obstetrics and Gynaecology*, 1998, 18:322–327.

353. Sowunmi A. Efficacy of chloroquine plus chlorpheniramine in chloroquine-resistant falciparum malaria during pregnancy in Nigerian women: a preliminary study. *Journal of Obstetrics and Gynaecology*, 1998, 18:524–527.

354. McGready R et al. Randomized comparison of quinine–clindamycin versus artesunate in the treatment of falciparum malaria in pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:651–656.

355. McGready R et al. Artesunate–atovaquone–proguanil rescue treatment of multidrug-resistant *Plasmodium falciparum* malaria in pregnancy: a preliminary report. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:592–594.

356. McGready R et al. Artemisinin antimalarials in pregnancy: a prospective treatment study of 539 episodes of multidrug-resistant *Plasmodium falciparum*. *Clinical Infectious Diseases*, 2001, 33:2009–2016.

357. McGready R et al. The effects of quinine and chloroquine antimalarial treatments in the first trimester of pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:180–184.

358. Bounyasong S. Randomized trial of artesunate and mefloquine in comparison with quinine sulfate to treat *P. falciparum* malaria pregnant women. *Journal of Medical Association of Thailand*, 2001, 84:1289–1299.

359. McGready R et al. Artemisinin derivatives in the treatment of falciparum malaria in pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1998, 92:430–433.

360. Rukaria-Kaumbutho RM, Ojwang SB, Oyieke JB. Resistance to chloroquine therapy in pregnant women with malaria parasitemia. *International Journal of Gynecology and Obstetrics*, 1996, 53:235–241.

361. McGready R et al. Randomized comparison of mefloquine–artesunate versus quinine in the treatment of multidrug-resistant falciparum malaria in pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, 94:689–693.

362. McGready R et al. Quinine and mefloquine in the treatment of multidrug-resistant *Plasmodium falciparum* malaria in pregnancy. *Annals of Tropical Medicine and Parasitology*, 1998, 92:643–653.

363. Deen JL et al. The safety of the combination artesunate and pyrimethamine–sulfadoxine given during pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2001, 95:424–428.

364. Moyou-Somo R et al. Clinical trial of beta-arteether versus quinine for the treatment of cerebral malaria in children in Yaounde, Cameroon. *American Journal of Tropical Medicine and Hygiene*, 2001, 64:229–232.

365. Faiz MA et al. A randomized controlled trial comparing artemether and quinine in the treatment of cerebral malaria in Bangladesh. *Indian Journal of Malariology*, 2001, 38:9–18.

366. Birku Y, Makonnen E, Bjorkman A. Comparison of rectal artemisinin with intravenous quinine in the treatment of severe malaria in Ethiopia. *East African Medical Journal*, 1999, 76:154–159.

367. Schapira A, Beales PF, Halloran ME. Malaria: living with drug resistance. *Parasitology Today*, 1993, 9:168–174.

368. Bloland PB et al. Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Journal of Infectious Diseases*, 1993, 167:932–937.

369. Sudre P et al. Treatment of chloroquine-resistant malaria in African children: a cost–effectiveness analysis. *International Journal of Epidemiology*, 1992, 21:146–154.

370. Goodman CA, Coleman PG, Mills AJ. Changing the first line drug for malaria treatment — cost–effectiveness analysis with highly uncertain inter-temporal trade-offs. *Health Economics*, 2001, 10:731–749.

371. Kitua AY. Antimalarial drug policy: making systematic change. *Lancet*, 1999, 354(Suppl. IV):32.

372. *Antimalarial drug combination therapy. Report of a WHO technical consultation.* Geneva, World Health Organization, 2001 (unpublished document WHO/CDS/RBM/2001.35).

373. Hogh B et al. The differing impact of chloroquine and pyrimethamine/sulfadoxine upon the infectivity of malaria species to the mosquito vector. *American Journal of Tropical Medicine and Hygiene*, 1998, 58:176–182.

374. Bojang KA et al. Predictors of mortality in Gambian children with severe malaria anaemia. *Annals of Tropical Paediatrics*, 1997, 17:355–359.

375. Zucker JR. Childhood mortality during and after hospitalization in western Kenya: effect of malaria treatment regimens. *American Journal of Tropical Medicine and Hygiene*, 1996, 55:655–660.

376. Price RN et al. Factors contributing to anemia after uncomplicated falciparum malaria. *American Journal of Tropical Medicine and Hygiene*, 2001, 65:614–622.

377. Sutherland CJ et al. Gambian children successfully treated with chloroquine can harbor and transmit *Plasmodium falciparum* gametocytes carrying resistance genes. *American Journal of Tropical Medicine and Hygiene*, 2002, 67:578–585.

378. Price R et al. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. *American Journal of Tropical Medicine and Hygiene*, 1999, 60:1019–1023.

379. Sokhna CS, Trape JF, Robert V. Gametocytaemia in Senegalese children with uncomplicated falciparum malaria treated with chloroquine, amodiaquine or sulfadoxine+pyrimethamine. *Parasite*, 2001, 8:243–250.

380. Sowunmi A, Fateye BA. *Plasmodium falciparum* gametocytaemia in Nigerian children: before, during and after treatment with antimalarial drugs. *Tropical Medicine and International Health*, 2003, 8:783–792.

381. Sowunmi A et al. Risk factor for gametocyte carriage in uncomplicated falciparum malaria in children. *Parasitology*, 2004, 129:255–262.

382. Bousema JT et al. Treatment failure of pyrimethamine–sulphadoxine and induction of *Plasmodium falciparum* gametocytaemia in children in western Kenya. *Tropical Medicine and International Health*, 2003, 8:427–430.

383. Puta C, Manyando C. Enhanced gametocyte production in Fansidar-treated *Plasmodium falciparum* malaria patients: implications for malaria transmission control programmes. *Tropical Medicine and International Health*, 1997, 2:227–229.

384. Drakeley CJ et al. Addition of artesunate to chloroquine for treatment of *Plasmodium falciparum* malaria in Gambian children causes a significant but short-lived reduction in infectiousness for mosquitoes. *Tropical Medicine and International Health*, 2004, 9:53–61.

385. Hallett RL et al. Combination therapy counteracts the enhanced transmission of drug-resistant malaria parasites to mosquitoes. *Antimicrobial Agents and Chemotherapy*, 2004, 48:3940–3943.

386. Price RN et al. Effects of artemisinin derivatives on malaria transmissibility. *Lancet*, 1996, 347:1654–1658.

387. Mutabingwa T et al. Chlorproguanil–dapsone for treatment of drug-resistant falciparum malaria in Tanzania. *Lancet*, 2001, 358:1218–1223.

388. Njama-Meya D, Kamya MR, Dorsey G. Asymptomatic parasitaemia as a risk factor for symptomatic malaria in a cohort of Ugandan children. *Tropical Medicine and International Health*, 2004, 9:862–868.

List of annexes and tables

ANNEX 1

CLASSIFICATION OF TREATMENT OUTCOMES ACCORDING TO WHO PROTOCOL, 1996

Three categories of therapeutic response are recognized: *early treatment failure, late treatment failure* and *adequate clinical response*, which are defined as described below.

Early treatment failure

A therapeutic response is classified as early treatment failure if the patient develops one of the following conditions during the first 3 days of follow-up:

- danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia;
- axillary temperature ≥37.5 °C on day 2 with parasitaemia greater than that on day 0;
- axillary temperature ≥37.5 °C on day 3 in the presence of parasitaemia; or
- parasitaemia on day 3 ≥25% of count on day 0.

Late treatment failure

A therapeutic response is classified as late treatment failure if the patient develops one of the following conditions in the presence of parasitaemia on any day between days 4 and 14 of follow-up, without previously meeting any of the criteria of early treatment failure:

- danger signs or severe malaria; or
- axillary temperature ≥37.5 °C.

Adequate clinical response

The response to treatment is classified as an adequate clinical response if the patient shows one of the following conditions during follow-up to day 14, without previously meeting any of the criteria of early or late treatment failure:

- absence of parasitaemia on day 14, irrespective of axillary temperature; or
- axillary temperature ‹37.5 °C, irrespective of the presence of parasitaemia.

ANNEX 2

CLASSIFICATION OF TREATMENT OUTCOMES ACCORDING TO WHO PROTOCOL, 2001

Early treatment failure

INTENSE TRANSMISSION AREA

- Development of danger signs or severe malaria on day 1, day 2 or day 3, in the presence of parasitaemia
- Parasitaemia on day 2 higher than day 0 count irrespective of axillary temperature
- Parasitaemia on day 3 with axillary temperature ≥37.5 °C
- Parasitaemia on day 3 ≥25% of count on day 0

LOW-TO-MODERATE TRANSMISSION AREA

- Development of danger signs or severe malaria on day 1, day 2 or day 3, in the presence of parasitaemia
- Parasitaemia on day 2 higher than day 0 count irrespective of axillary temperature
- Parasitaemia on day 3 with axillary temperature ≥37.5 °C
- Parasitaemia on day 3 ≥25% of count on day 0

Late treatment failure

Late clinical failure

- Development of danger signs or severe malaria after day 3 in the presence of parasitaemia, without previously meeting any of the criteria of early treatment failure
- Presence of parasitaemia and axillary temperature ≥37.5 °C on any day from day 4 to day 14, without previously meeting any of the criteria of early treatment failure

Late parasitological failure

- Presence of parasitaemia on day 14 and axillary temperature ‹37.5 °C, without previously meeting any of the criteria of early treatment failure or late clinical failure

Late clinical failure

- Development of danger signs or severe malaria after day 3 in the presence of parasitaemia, without previously meeting any of the criteria of early treatment failure
- Presence of parasitaemia and axillary temperature ≥37.5 °C (or history of fever) on any day from day 4 to day 28, without previously meeting any of the criteria of early treatment failure

Late parasitological failure

- Presence of parasitaemia on any day from day 7 to day 28 and axillary temperature ‹37.5 °C, without previously meeting any of the criteria of early treatment failure or late clinical failure

Adequate clinical and parasitological response

- Absence of parasitaemia on day 14 irrespective of axillary temperature without previously meeting any of the criteria of early treatment failure or late clinical failure or late parasitological failure

- Absence of parasitaemia on day 28 irrespective of axillary temperature without previously meeting any of the criteria of early treatment failure or late clinical failure or late parasitological failure

ANNEX 3

PRINCIPAL MODIFICATIONS TO WHO PROTOCOL 1996 FOR AREAS OF HIGH TRANSMISSION OF MALARIA

Follow-up

Taking a thick blood film on day 2 is now systematic, because the criteria for early treatment failure have changed.

Inclusion criteria

The parasitaemia cut-off points have changed: 2000-200 000 per µl instead of 2000–100 000 per µl.

Fever above 39.5 °C is no longer an exclusion criterion.

Samples

Calculation of the sample size required by estimating prevalence (P) is preferred to the lot quality assurance sampling method. The selected confidence interval is 95% with a precision (d) of 5% or 10%. If the prevalence of expected treatment failure is unknown, P should be chosen as 0.50. If the prevalence of the expected failure rate is low, a minimum of 50 patients must be enrolled in order for the sample to be representative.

	Calculated sample size, 95% confidence interval									
	Prevalence (*P*)									
Precision (d)	**0.05**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**	**0.35**	**0.4**	**0.45**	**0.5**
5%	73	138	196	246	288	323	350	369	380	384
10%	50	50	50	61	72	81	87	92	95	96

Classification

Early treatment failure:

- danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia;
- parasitaemia on day 2 higher than on day 0, *irrespective of axillary temperature;*
- parasitaemia on day 3 with axillary temperature ≥37.5 °C; or
- parasitaemia on day 3 ≥25% of count on day 0.

Late clinical failure:

- danger signs or severe malaria in the presence of parasitaemia on any day after day 4 and before day 14, without the patient previously meeting any of the criteria of early treatment failure; or
- axillary temperature ≥37.5 °C in the presence of parasitaemia on any day between day 4 and day 14 without the patient previously meeting any of the criteria of early treatment failure.

Late parasitological failure:

- presence of parasitaemia on day 14 with temperature ‹ 37.5 °C, without the patient previously meeting any of the criteria of early treatment failure or late clinical failure.

Adequate clinical and parasitological response:

- absence of parasitaemia on day 14, irrespective of axillary temperature, without the patient meeting any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

ANNEX 4

SUBREGIONAL NETWORKS FOR MONITORING THE EFFICACY OF ANTIMALARIAL DRUGS

AFRICA

- East African Network for Monitoring Antimalarial Treatment: Burundi, Kenya, Rwanda, Uganda, United Republic of Tanzania
- Horn of Africa Network for Monitoring Antimalarial Treatment: Djibouti, Eritrea, Ethiopia, Somalia, Sudan, Yemen
- Réseau d'Afrique Centrale pour le Traitement Antipaludique: Angola, Cameroon, Central African Republic, Chad, Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon
- Réseau d'Afrique de l'Ouest pour le Traitement Antipaludique I: Cape Verde, Gambia, Guinea, Guinea-Bissau, Mauritania, Senegal
- Réseau d'Afrique de l'Ouest pour le Traitement Antipaludique II: Benin, Burkina Faso, Côte d'Ivoire, Ghana, Mali, Niger, Nigeria, Sierra Leone, Togo
- Réseau d'Etude de la Résistance aux antipaludiques dans la sous région Océan Indien: Comoros, Madagascar, Mayotte (French Overseas Territory)

SOUTH-EAST ASIA

- Mekong network: Cambodia, China, Lao People's Democratic Republic, Myanmar, Thailand, Viet Nam

SOUTH AMERICA

- Red Amazónica para la Vigilancia de la Resistencia a las Drogas Antimaláricas: Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru, Suriname, Venezuela

ANNEX 5

SUMMARY OF RESULTS FOR THE EFFICACY OF ANTIMALARIAL DRUGS, BY COUNTRY (1996–2004), EXPRESSED AS TREATMENT FAILURE

Latest update: February 2005

Notes:

Median, range and percentiles are based on percentage clinical failure with at least 14-day follow up for countries in Africa south of the Sahara. For all other areas, including South Africa and moderate/low transmission areas of Sudan, percentage total failure is used.

AFRICA	study years	number of studies	median	range low	range high	percentile 25th	percentile 75th
Angola							
Chloroquine	2002	6	41.8	8.2	54.1	14.8	52.1
Sulfadoxine–pyrimethamine	2002–2003	8	5.7	0.0	28.2	2.7	8.8
Amodiaquine	2002–2003	2	8.7	3.9	13.4	3.9	13.4
Artesunate+amodiaquine	2003	1	0.0				
Artesunate+sulfadoxine–pyrimethamine	2003	1	1.2				
Benin							
Chloroquine	1998–2002	14	19.4	3.4	47.6	14.1	23.2
Sulfadoxine–pyrimethamine	2002	5	9.5	1.6	17.2	4.8	16.8
Botswana							
Chloroquine	1997–2000	6	24.4	20.7	44.0	20.7	44.0
Burkina Faso							
Chloroquine	1996–2003	24	12.0	5.3	35.5	10.0	21.7
Sulfadoxine–pyrimethamine	1998–2003	9	0.8	0.0	6.3	0.0	4.3
Amodiaquine	1996	1	4.4				
Burundi							
Chloroquine	2001	4	69.2	52.4	73.7	58.9	73.4
Sulfadoxine–pyrimethamine	2001	4	30.8	10.9	52.8	20.2	42.4
Artemether+lumefantrine	2001	2	0.0	0.0	0.0	0.0	0.0
Artesunate+amodiaquine	2001	2	1.3	0.0	2.6	0.0	2.6
Cameroon							
Chloroquine	1994–2001	12	33.0	2.0	66.6	15.9	58.2
Sulfadoxine–pyrimethamine	1997–2003	8	9.0	0.0	14.1	6.7	11.0
Amodiaquine	1997–2003	9	1.6	0.0	5.3	0.0	3.2
Amodiaquine+sulfadoxine–pyrimethamine	2001–2003	4	0.0	0.0	0.0	0.0	0.0
Central African Republic							
Chloroquine	1997–1998	5	20.8	19.0	57.1	19.3	39.6

	number of studies			range		percentile	
	study years		median	low	high	25th	75th
Chad							
Chloroquine	1999–2003	3	21.5	14.2	67.4	14.2	67.4
Sulfadoxine–pyrimethamine	2002–2003	2	11.7	4.0	19.4	4.0	19.4
Amodiaquine	2002–2003	2	3.4	1.9	4.9	1.9	4.9
Comoros							
Chloroquine	1997–2001	9	57.1	31.2	75.0	42.4	67.3
Sulfadoxine–pyrimethamine	2004	2	1.5	0.0	3.0	0.0	3.0
Chloroquine+sulfadoxine–pyrimethamine	2003	3	0.0	0.0	2.6	0.0	2.6
Artemether+lumefantrine	2004	3	0.0	0.0	1.8	0.0	1.8
Artesunate+amodiaquine	2003	3	0.0	0.0	0.0	0.0	0.0
Artesunate+sulfadoxine–pyrimethamine	2003	3	0.0	0.0	3.6	0.0	3.6
Congo							
Chloroquine	1999–2001	2	44.0	38.0	50.0	38.0	50.0
Sulfadoxine–pyrimethamine	1999–2002	3	0.0	0.0	9.5	0.0	9.5
Côte d'Ivoire							
Chloroquine	1997–2002	26	16.4	1.8	43.1	11.4	19.3
Sulfadoxine–pyrimethamine	1999	2	14.8	5.9	23.6	5.9	23.6
Democratic Republic of the Congo							
Chloroquine	2000–2001	7	48.0	29.4	80.0	34.0	50.0
Sulfadoxine–pyrimethamine	2000–2004	12	9.3	0.0	30.2	4.4	18.3
Amodiaquine+sulfadoxine–pyrimethamine	2002–2004	5	1.7	0.0	6.0	0.7	4.4
Artesunate+amodiaquine	2003–2004	3	0.0	0.0	1.4	0.0	1.4
Artesunate+sulfadoxine–pyrimethamine	2002–2004	6	0.0	0.0	5.6	0.0	3.4
Equatorial Guinea							
Chloroquine	1996–1999	2	48.9	42.1	55.6	42.1	55.6
Sulfadoxine–pyrimethamine	1996–1999	2	5.0	0.0	10.0	0.0	10.0
Eritrea							
Chloroquine	1997–2001	29	42.8	12.6	66.6	28.6	47.3
Sulfadoxine–pyrimethamine	2001–2002	6	3.1	0.0	15.4	0.0	10.3
Chloroquine+sulfadoxine–pyrimethamine	2002–2003	4	6.5	0.0	10.2	1.9	9.7
Artesunate+amodiaquine	2002–2003	3	0.0	0.0	1.4	0.0	1.4
Ethiopia							
Chloroquine	1996–1998	18	70.0	5.0	97.8	55.8	85.2
Sulfadoxine–pyrimethamine	1997–2003	17	10.3	0.0	44.9	2.0	26.1
Amodiaquine	1998	7	18.9	6.2	66.7	6.5	45.8
Artemether+lumefantrine	2003	4	0.0	0.0	0.0	0.0	0.0
Gabon							
Chloroquine	2001	2	57.1	52.2	62.0	52.2	62.0
Sulfadoxine–pyrimethamine	2000	1	4.4				
Amodiaquine	1997–2002	5	12.5	3.2	14.0	7.9	14.0
Artemether+lumefantrine	2001–2002	2	0.8	0.0	1.6	0.0	1.6
Artesunate+amodiaquine	2001–2002	2	0.9	0.0	1.7	0.0	1.7

	number of studies			range		percentile	
	study years		median	low	high	25th	75th
Gambia							
Chloroquine	1998–2003	4	12.2	2.9	28.2	6.1	21.6
Artesunate+chloroquine	2000	1	3.2				
Ghana							
Chloroquine	1998–2003	9	23.2	9.0	31.3	15.8	29.7
Sulfadoxine–pyrimethamine	1998–2003	3	3.0	0.0	5.2	0.0	5.2
Amodiaquine+sulfadoxine–pyrimethamine	2002	1	1.4				
Artemether+lumefantrine	2003	1	0.0				
Artesunate+amodiaquine	2003	1	0.0				
Artesunate+sulfadoxine–pyrimethamine	2002	1	0.8				
Guinea							
Chloroquine	1996–2001	8	15.6	7.7	28.3	9.9	22.6
Guinea-Bissau							
Chloroquine	2001	3	6.8	5.4	10.9	5.4	10.9
Kenya							
Chloroquine	1996–1999	7	65.8	15.2	84.8	31.7	80.4
Sulfadoxine–pyrimethamine	1996–2003	27	8.4	0.0	51.6	3.4	17.9
Amodiaquine	1996–2003	24	2.4	0.0	23.1	0.0	8.3
Amodiaquine+sulfadoxine–pyrimethamine	2003	2	2.0	1.6	2.4	1.6	2.4
Liberia							
Chloroquine	1999	2	25.9	22.5	29.2	22.5	29.2
Amodiaquine	2001	1	7.4				
Madagascar							
Chloroquine	1996–2004	13	9.5	0.0	25.6	6.9	17.1
Sulfadoxine–pyrimethamine	2003	1	0.0				
Amodiaquine	2004	1	0.0				
Malawi							
Sulfadoxine–pyrimethamine	1998–2002	15	18.6	2.8	40.0	16.6	33.3
Mefloquine	1998	1	10.2				
Mali							
Chloroquine	1996–2003	19	11.0	2.0	24.3	4.2	13.0
Sulfadoxine–pyrimethamine	2001–2003	3	0.6	0.0	2.0	0.0	2.0
Mauritania							
Chloroquine	1998	2	24.0	11.6	36.4	11.6	36.4
Mozambique							
Chloroquine	1998–2001	20	35.9	13.0	53.0	22.1	42.9
Sulfadoxine–pyrimethamine	1998–2002	10	5.4	0.2	17.3	2.7	13.7
Amodiaquine	2001	1	8.4				
Amodiaquine+sulfadoxine–pyrimethamine	2001	1	0.0				
Artesunate+amodiaquine	2001	1	0.0				
Artesunate+sulfadoxine–pyrimethamine	2001	1	0.0				

	number of studies			range		percentile	
	study years		median	low	high	25th	75th
Namibia							
Chloroquine	1997–2003	9	19.0	4.0	66.7	6.5	35.1
Sulfadoxine–pyrimethamine	1997–2003	5	8.8	0.0	22.8	0.0	18.6
Niger							
Chloroquine	1998–2001	2	19.2	17.4	20.9	17.4	20.9
Nigeria							
Chloroquine	1998–2003	11	25.8	2.0	53.7	13.6	38.7
Sulfadoxine–pyrimethamine	2001–2003	7	9.3	5.7	43.5	7.7	40.5
Amodiaquine	2001–2002	2	1.5	0.0	2.9	0.0	2.9
Rwanda							
Chloroquine	1997–2000	6	52.4	18.5	60.6	33.2	59.2
Sulfadoxine–pyrimethamine	2000	3	35.1	11.6	35.7	11.6	35.7
Amodiaquine	2001–2002	6	0.0	0.0	2.3	0.0	2.0
Chloroquine+sulfadoxine–pyrimethamine	2000	6	13.2	6.1	39.7	8.1	37.7
Amodiaquine+sulfadoxine–pyrimethamine	2001	3	0.0	0.0	0.0	0.0	0.0
Artesunate+amodiaquine	2002	3	0.0	0.0	1.6	0.0	1.6
Artesunate+sulfadoxine–pyrimethamine	2001	3	0.0	0.0	0.0	0.0	0.0
Senegal							
Chloroquine	1996–2002	19	12.9	2.7	30.7	10.1	16.6
Sulfadoxine–pyrimethamine	2001–2002	7	3.3	1.7	10.2	2.0	5.8
Amodiaquine	2001–2002	3	2.8	2.0	5.1	2.0	5.1
Amodiaquine+sulfadoxine–pyrimethamine	2001–2003	4	0.0				
Artemether+lumefantrine	2003	1	0.0				
Artesunate+amodiaquine	2002	2	0.0	0.0	0.0	0.0	0.0
Artesunate+mefloquine	2002	2	0.0	0.0	0.0	0.0	0.0
Sierra Leone							
Chloroquine	1998–2003	7	34.5	26.3	58.5	32.0	51.5
Sulfadoxine–pyrimethamine	2001–2003	5	11.2	7.8	23.4	9.1	17.7
Amodiaquine	2002–2003	5	1.8	0.0	7.6	0.0	5.8
Somalia							
Chloroquine	1997–2003	5	51.0	27.5	78.0	30.4	74.0
Sulfadoxine–pyrimethamine	1997–2003	3	4.0	2.0	5.9	2.0	5.9
South Africa							
Chloroquine	1997	4	53.8	40.0	62.5	44.2	60.8
Sulfadoxine–pyrimethamine	1997–2002	6	7.3	3.6	87.8	3.7	55.7
Artemether+lumefantrine	2002	1	0.0				
Artesunate+sulfadoxine–pyrimethamine	2004	1	5.0				

	number of studies			range		percentile	
	study years		median	low	high	25th	75th
Sudan							
high transmission area							
Chloroquine	2001–2003	5	53.1	16.6	60.7	32.4	59.4
Sulfadoxine–pyrimethamine	2001–2002	3	6.0	0.0	12.0	0.0	12.0
Amodiaquine	2001	2	6.5	6.0	7.0	6.0	7.0
Artesunate+amodiaquine	2003	2	0.4	0.0	0.8	0.0	0.8
Artesunate+sulfadoxine–pyrimethamine	2003	2	1.7	0.8	2.5	0.8	2.5
moderate-to-low transmission area							
Chloroquine	1996–2003	24	47.6	0.0	76.9	33.8	57.4
Sulfadoxine–pyrimethamine	1996–2003	7	4.2	0.0	11.7	2.0	8.1
Mefloquine	1999	1	2.5				
Chloroquine+sulfadoxine–pyrimethamine	2003	2	10.2	5.9	14.4	5.9	14.4
Swaziland							
Chloroquine	2000	1	12.5				
Togo							
Chloroquine	1998–2001	6	6.1	0.0	28.8	1.6	23.7
Uganda							
Chloroquine	1996–2001	18	29.3	7.5	81.2	16.4	58.7
Sulfadoxine–pyrimethamine	1996–2002	25	11.4	0.0	25.0	5.0	16.8
Amodiaquine	1999–2002	5	8.8	0.0	14.5	1.6	12.3
Chloroquine+sulfadoxine–pyrimethamine	1996–2003	15	12.0	0.0	37.0	7.0	19.0
Amodiaquine+sulfadoxine–pyrimethamine	1999–2003	12	1.6	0.0	13.0	0.5	3.5
Artesunate+amodiaquine	2002–2003	5	1.0	0.0	4.0	0.5	3.7
Artesunate+sulfadoxine–pyrimethamine	2000	1	0.5				
United Republic of Tanzania							
mainland							
Chloroquine	1997–1999	8	43.0	27.6	71.0	36.6	53.5
Sulfadoxine–pyrimethamine	1997–2002	15	10.5	1.4	33.8	5.6	16.9
Amodiaquine	1999–2002	12	3.7	0.0	10.8	1.6	6.9
Amodiaquine+sulfadoxine–pyrimethamine	1999	1	3.4				
Zanzibar							
Chloroquine	2001	2	60.5	60.2	60.8	60.2	60.8
Sulfadoxine–pyrimethamine	2001	2	8.9	4.7	13.1	4.7	13.1
Amodiaquine	2001	2	5.6	4.7	6.5	4.7	6.5
Artemether+lumefantrine	2002	2	1.0	0.0	2.0	0.0	2.0
Artesunate+amodiaquine	2002	2	1.9	1.8	1.9	1.8	1.9
Zambia							
Chloroquine	1996–2002	22	31.9	6.6	54.0	24.6	46.3
Sulfadoxine–pyrimethamine	1996–2003	17	7.9	0.0	17.9	3.3	14.2
Artemether+lumefantrine	2003	3	0.0	0.0	0.0	0.0	0.0
Artesunate+sulfadoxine–pyrimethamine	2002–2003	5	0.0	0.0	1.7	0.0	0.9
Zimbabwe							
Chloroquine	1999–2003	28	10.8	0.0	42.3	5.0	19.9
Sulfadoxine–pyrimethamine	1999	2	10.0	0.0	20.0	0.0	20.0
Chloroquine+sulfadoxine–pyrimethamine	2001–2004	25	1.1	0.0	8.6	0.0	3.9

ASIA	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Afghanistan							
Chloroquine	1999–2002	4	67.7	60.0	89.5	61.7	80.8
Sulfadoxine–pyrimethamine	2002–2003	3	8.7	4.0	22.7	4.0	22.7
Amodiaquine	2004	1	37.7				
Amodiaquine+sulfadoxine–pyrimethamine	2003–2004	2	2.0	1.0	3.0	1.0	3.0
Bangladesh							
Chloroquine	1996–1999	3	63.6	50.0	77.2	50.0	77.2
Mefloquine	1996	1	27.2				
Chloroquine+sulfadoxine–pyrimethamine	1996–2003	7	30.7	12.9	37.2	24.0	33.0
Artemether+lumefantrine	2002	1	0.8				
Artesunate+mefloquine	2002	1	0.9				
Bhutan							
Chloroquine	1997	4	78.1	64.7	80.7	70.8	80.0
Sulfadoxine–pyrimethamine	1998	1	34.8				
Mefloquine	1999	1	9.7				
Artesunate combinations	2000–2003	8	4.9	1.1	12.0	2.2	8.7
Cambodia							
Artemether+lumefantrine	2001–2004	3	26.9	13.5	30.0	13.5	30.0
Artesunate+mefloquine	2001–2004	12	3.7	0.0	14.3	1.1	8.1
China							
Chloroquine	1997–1999	2	29.6	18.4	40.7	18.4	40.7
India							
Chloroquine	1996–2004	25	34.0	0.0	95.9	23.6	65.4
Sulfadoxine–pyrimethamine	1999–2003	12	17.9	0.0	68.2	3.0	45.4
Mefloquine	1996–2001	3	4.5	0.0	7.8	0.0	7.8
Chloroquine+sulfadoxine–pyrimethamine		1	6.5				
Artesunate+mefloquine	2001	2	6.4	1.9	10.9	1.9	10.9
Indonesia							
Chloroquine	1995–2003	18	69.5	11.1	100.0	49.5	78.3
Sulfadoxine–pyrimethamine	1996–2003	12	17.8	0.0	82.9	12.0	43.0
Chloroquine+sulfadoxine–pyrimethamine	1999–2003	2	22.2	6.2	38.2	6.2	38.2
Artesunate+sulfadoxine–pyrimethamine	1999	1	4.3				
Iran (Islamic Republic of)							
Chloroquine	2000–2002	4	72.5	61.0	75.0	66.4	74.2
Sulfadoxine–pyrimethamine	1999–2001	3	0.0	0.0	5.7	0.0	5.7
Lao People's Democratic Republic							
Chloroquine	1998–2002	5	44.8	31.3	52.8	36.7	49.5
Sulfadoxine–pyrimethamine	2001–2002	3	18.0	17.9	18.7	17.9	18.7
Mefloquine	2001	1	0.0				
Chloroquine+sulfadoxine–pyrimethamine	2001	2	12.3	7.8	16.7	7.8	16.7
Artemether+lumefantrine	2001–2003	2	4.7	3.1	6.3	3.1	6.3
Artesunate+mefloquine	2001–2003	2	0.0	0.0	0.0	0.0	0.0

	number of studies			range		percentile	
	study years		median	low	high	25th	75th
Malaysia							
Chloroquine	2003	1	45.2				
Sulfadoxine-pyrimethamine	1996	1	29.4				
Mefloquine	1996	1	0.0				
Chloroquine+sulfadoxine–pyrimethamine	1999–2003	4	47.6	31.3	62.5	37.5	57.0
Myanmar							
Chloroquine	1997–2002	18	24.7	6.0	76.0	12.5	34.7
Sulfadoxine–pyrimethamine	1997–2002	18	27.8	0.0	100.0	7.9	37.7
Mefloquine	1997–2002	18	6.0	0.0	44.4	0.0	16.4
Artemether+lumefantrine	2003	3	2.0	0.0	2.0	0.0	2.0
Artesunate+amodiaquine	2003	4	4.0	3.0	7.0	3.5	5.5
Artesunate+sulfadoxine–pyrimethamine	2003	2	0.0	0.0	0.0	0.0	0.0
Artesunate+mefloquine	1996–2003	10	1.5	0.0	8.0	0.0	5.1
Nepal							
Sulfadoxine–pyrimethamine	1997–2003	7	22.0	0.0	88.2	0.0	72.7
Pakistan							
Chloroquine	2001–2002	13	28.9	18.2	79.0	25.9	66.6
Sulfadoxine–pyrimethamine	2001–2002	4	13.0	8.7	18.7	9.8	16.9
Amodiaquine	2002	1	83.3				
Artesunate+amodiaquine	2002	1	18.0				
Artesunate+chloroquine	2002	1	28.8				
Artesunate+sulfadoxine–pyrimethamine	2002	1	0.0				
Papua New Guinea							
Chloroquine+sulfadoxine–pyrimethamine	1998–2003	4	0.0	0.0	27.0	0.0	13.5
Amodiaquine+sulfadoxine–pyrimethamine	1998	1	0.0				
Philippines							
Chloroquine	1996–2000	9	42.1	16.4	76.2	32.1	52.0
Sulfadoxine–pyrimethamine	1999–2001	7	42.6	8.5	66.7	12.5	60.6
Chloroquine+sulfadoxine–pyrimethamine	2001–2002	3	18.4	11.1	29.6	11.1	29.6
Saudi Arabia							
Chloroquine	1997–1998	2	15.4	12.4	18.4	12.4	18.4
Solomon Islands							
Chloroquine	1997–2001	5	27.8	10.7	66.7	12.2	49.8
Sri Lanka							
Chloroquine	2002–2003	2	31.8	10.0	53.5	10.0	53.5
Artesunate+sulfadoxine–pyrimethamine	1999	1	0.0				
Tajikistan							
Chloroquine	2002	1	56.0				
Sulfadoxine–pyrimethamine	2002	1	16.0				
Chloroquine+sulfadoxine–pyrimethamine	2003	1	2.1				

	number of studies			range		percentile	
	study years		median	low	high	25th	75th
Thailand							
Mefloquine	1995–2003	19	13.8	2.0	68.4	7.5	28.0
Artemether+lumefantrine	1996–2002	6	2.6	0.0	3.9	0.5	3.5
Artesunate+mefloquine	1995–2003	34	3.6	0.0	21.4	1.2	8.1
Timor-Leste							
Chloroquine	2000	1	66.7				
Sulfadoxine–pyrimethamine	2001	1	10.0				
Vanuatu							
Chloroquine+sulfadoxine–pyrimethamine	2001	1	16.0				
Viet Nam							
Chloroquine	1997–2001	4	52.3	6.2	71.9	27.0	64.3
Sulfadoxine–pyrimethamine	1997–2002	4	16.6	12.2	70.6	13.0	41.9
Mefloquine	1998–1999	4	11.7	0.0	42.3	0.0	32.8
Artemether+lumefantrine	2001	1	2.2				
Artesunate+chloroquine		2	37.4	28.0	46.8	28.0	46.8
Artesunate+sulfadoxine–pyrimethamine		2	33.2	8.3	58.1	8.3	58.1
Artesunate+mefloquine	1998–2000	2	5.6	0.0	11.1	0.0	11.1
Yemen							
Chloroquine	1998–2003	9	42.4	9.0	57.0	23.3	44.9
Sulfadoxine–pyrimethamine	2003	1	0.0				

THE AMERICAS	study years	number of studies	median	range low	range high	percentile 25th	percentile 75th
Bolivia							
Sulfadoxine–pyrimethamine	2002	1	18.7				
Mefloquine	2001	2	0.0	0.0	0.0	0.0	0.0
Artesunate+mefloquine	2001	3	0.0	0.0	0.0	0.0	0.0
Brazil							
Mefloquine	1996–2002	6	5.2	0.0	9.7	0.5	7.9
Colombia							
Chloroquine	1997–1998	5	66.6	44.5	96.6	47.3	83.7
Sulfadoxine–pyrimethamine	1997–2002	12	10.8	0.0	26.5	1.9	15.8
Amodiaquine	1997–2002	7	11.5	0.0	50.0	3.2	27.3
Mefloquine	2002–2003	3	2.2	0.0	6.4	0.0	6.4
Chloroquine+sulfadoxine–pyrimethamine	2002	2	17.4	12.1	22.6	12.1	22.6
Amodiaquine+sulfadoxine–pyrimethamine	2001–2003	4	2.3	0.0	10.8	1.1	6.6
Ecuador							
Chloroquine	1998–2003	4	85.4	83.3	94.4	84.2	90.1
Sulfadoxine–pyrimethamine	2001–2003	3	4.0	0.0	17.1	0.0	17.1
Chloroquine+sulfadoxine-pyrimethamine	2003	1	0.0				
Artesunate+sulfadoxine–pyrimethamine	2003	2	0.0	0.0	0.0	0.0	0.0
French Guiana							
Mefloquine	1996	1	3.4				
Guyana							
Chloroquine	1998	1	55.6				
Mefloquine	2003	1	28.1				
Artesunate+mefloquine	2003	1	7.5				
Peru							
Chloroquine	1998–2002	6	86.4	75.6	90.0	78.3	89.8
Sulfadoxine–pyrimethamine	1998–2002	9	11.8	0.0	80.0	1.7	65.2
Mefloquine	1999–2000	4	0.0	0.0	0.0	0.0	0.0
Artesunate+sulfadoxine–pyrimethamine	2000	1	1.1				
Artesunate+mefloquine	2000	1	0.0				
Suriname							
Mefloquine	2002	1	7.3				
Artemether+lumefantrine	2003	2	2.0	1.9	2.0	1.9	2.0
Artesunate+mefloquine	2002–2003	2	4.1	2.4	5.8	2.4	5.8
Venezuela							
Chloroquine	1997–2002	5	48.6	0.0	100.0	13.1	88.6
Sulfadoxine–pyrimethamine	1997–1999	3	20.0	0.0	23.0	0.0	23.0
Chloroquine	1997–1998	5	66.6	44.5	96.6	47.3	83.7

ANNEX 6

SUMMARY OF RESULTS FOR THE EFFICACY OF ANTIMALARIAL DRUGS, BY TYPE OF THERAPY (1996–2004), EXPRESSED AS TREATMENT FAILURE

Latest update: February 2005

Notes: Median, range and percentiles are based on percentage clinical failure with at least 14-day follow up for countries in Africa south of the Sahara. For all other areas, including South Africa and moderate or low transmission areas of Sudan, percentage total failure is used.

CHLOROQUINE monotherapy	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Africa							
Angola	2002	6	41.8	8.2	54.1	14.8	52.1
Benin	1998–2002	14	19.4	3.4	47.6	14.1	23.2
Botswana	1997–2000	6	24.4	20.7	44.0	20.7	44.0
Burkina Faso	1996–2003	24	12.0	5.3	35.5	10.0	21.7
Burundi	2001	4	69.2	52.4	73.7	58.9	73.4
Cameroon	1994–2001	12	33.0	2.0	66.6	15.9	58.2
Central African Republic	1997–1998	5	20.8	19.0	57.1	19.3	39.6
Chad	1999–2003	3	21.5	14.2	67.4	14.2	67.4
Comoros	1997–2001	9	57.1	31.2	75.0	42.4	67.3
Congo	1999–2001	2	44.0	38.0	50.0	38.0	50.0
Côte d'Ivoire	1997–2002	26	16.4	1.8	43.1	11.4	19.3
Democratic Republic of the Congo	2000–2001	7	48.0	29.4	80.0	34.0	50.0
Equatorial Guinea	1996–1999	2	48.9	42.1	55.6	42.1	55.6
Eritrea	1997–2001	29	42.8	12.6	66.6	28.6	47.3
Ethiopia	1996–1998	18	70.0	5.0	97.8	55.8	85.2
Gabon	2001	2	57.1	52.2	62.0	52.2	62.0
Gambia	1998–2003	4	12.2	2.9	28.2	6.1	21.6
Ghana	1998–2003	9	23.2	9.0	31.3	15.8	29.7
Guinea	1996–2001	8	15.6	7.7	28.3	9.9	22.6
Guinea-Bissau	2001	3	6.8	5.4	10.9	5.4	10.9
Kenya	1996–1999	7	65.8	15.2	84.8	31.7	80.4
Liberia	1999	2	25.9	22.5	29.2	22.5	29.2
Madagascar	1996–2004	13	9.5	0.0	25.6	6.9	17.1
Mali	1996–2003	19	11.0	2.0	24.3	4.2	13.0
Mauritania	1998	2	24.0	11.6	36.4	11.6	36.4
Mozambique	1998–2001	20	35.9	13.0	53.0	22.1	42.9
Namibia	1997–2003	9	19.0	4.0	66.7	6.5	35.1
Niger	1998–2001	2	19.2	17.4	20.9	17.4	20.9
Nigeria	1998–2003	11	25.8	2.0	53.7	13.6	38.7
Rwanda	1997–2000	6	52.4	18.5	60.6	33.2	59.2

CHLOROQUINE monotherapy	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Senegal	1996–2002	19	12.9	2.7	30.7	10.1	16.6
Sierra Leone	1998–2003	7	34.5	26.3	58.5	32.0	51.5
Somalia	1997–2003	5	51.0	27.5	78.0	30.4	74.0
South Africa	1997	4	53.8	40.0	62.5	44.2	60.8
Sudan							
high transmission area	1996–2003	5	53.1	16.6	60.7	32.4	59.4
moderate-to-low transmission area	1996–2003	24	47.6	0.0	80.3	33.8	65.6
Swaziland	2000	1	12.5				
Togo	1998–2001	6	6.1	0.0	28.8	1.6	23.7
Uganda	1996–2001	18	29.3	7.5	81.2	16.4	58.7
United Republic of Tanzania							
mainland	1997–2001	8	43.0	27.6	71.0	36.6	60.5
Zanzibar	1997–2001	2	60.5	27.6	71.0	36.6	60.5
Zambia	1996–2002	22	31.9	6.6	54.0	24.6	46.3
Zimbabwe	1999–2003	28	10.8	0.0	42.3	5.0	19.9
Total: Africa	**1994–2004**	**433**	**24.0**	**0.0**	**97.8**	**12.6**	**43.9**

Asia

Afghanistan	1999–2002	4	67.7	60.0	89.5	61.7	80.8
Bangladesh	1996–1999	3	63.6	50.0	77.2	50.0	77.2
Bhutan	1997	4	78.1	64.7	80.7	70.8	80.0
China	1997–1999	2	29.6	18.4	40.7	18.4	40.7
India	1996–2004	25	34.0	0.0	95.9	23.6	65.4
Indonesia	1995–2003	18	69.5	11.1	100.0	49.5	78.3
Iran (Islamic Republic of)	2000–2002	4	72.5	61.0	75.0	66.4	74.2
Lao People's Democratic Republic	1998–2002	5	44.8	31.3	52.8	36.7	49.5
Malaysia	2003	1	45.2				
Myanmar	1997–2002	18	24.7	6.0	76.0	12.5	34.7
Pakistan	2001–2002	13	28.9	18.2	79.0	25.9	66.6
Philippines	1996–2000	9	42.1	16.4	76.2	32.1	52.0
Saudi Arabia	1997–1998	2	15.4	12.4	18.4	12.4	18.4
Solomon Islands	1997–2001	5	27.8	10.7	66.7	12.2	49.8
Sri Lanka	2002–2003	2	31.8	10.0	53.5	10.0	53.5
Tajikistan	2002	1	56.0				
Timor-Leste	2000	1	66.7				
Viet Nam	1997–2001	4	52.3	6.2	71.9	27.0	64.3
Yemen	1998–2003	9	42.4	9.0	57.0	23.3	44.9
Total: Asia	**1995–2004**	**130**	**44.1**	**0.0**	**100.0**	**26.3**	**66.7**

CHLOROQUINE monotherapy	number of studies / study years		median	range low	range high	percentile 25th	percentile 75th
The Americas							
Colombia	1997–1998	5	66.6	44.5	96.6	47.3	83.7
Ecuador	1998–2003	4	85.4	83.3	94.4	84.2	90.1
Guyana	1998	1	55.6				
Peru	1998–2002	6	86.4	75.6	90.0	78.3	89.8
Venezuela	1997–2002	5	48.6	0.0	100.0	13.1	88.6
Total: The Americas	**1997–2003**	**21**	**81.0**	**0.0**	**100.0**	**52.8**	**88.8**

SULFADOXINE-PYRIMETHAMINE monotherapy	number of studies / study years		median	range low	range high	percentile 25th	percentile 75th
Africa							
Angola	2002–2003	8	5.7	0.0	28.2	2.7	8.8
Benin	2002	5	9.5	1.6	17.2	4.8	16.8
Burkina Faso	1998–2003	9	0.8	0.0	6.3	0.0	4.3
Burundi	2001	4	30.8	10.9	52.8	20.2	42.4
Cameroon	1997–2003	8	9.0	0.0	14.1	6.7	11.0
Chad	2002–2003	2	11.7	4.0	19.4	4.0	19.4
Comoros	2004	2	1.5	0.0	3.0	0.0	3.0
Congo	1999–2002	3	0.0	0.0	9.5	0.0	9.5
Côte d'Ivoire	1999	2	14.8	5.9	23.6	5.9	23.6
Democratic Republic of the Congo	2000–2004	12	9.3	0.0	30.2	4.4	18.3
Equatorial Guinea	1996–1999	2	5.0	0.0	10.0	0.0	10.0
Eritrea	2001–2002	6	3.1	0.0	15.4	0.0	10.3
Ethiopia	1997–2003	17	10.3	0.0	44.9	2.0	26.1
Gabon	2000	1	4.4				
Ghana	1998–2003	3	3.0	0.0	5.2	0.0	5.2
Kenya	1996–2003	27	8.4	0.0	51.6	3.4	17.9
Madagascar	2003	1	0.0				
Malawi	1998–2002	15	18.6	2.8	40.0	16.6	33.3
Mali	2001–2003	3	0.6	0.0	2.0	0.0	2.0
Mozambique	1998–2002	10	5.4	0.2	17.3	2.7	13.7
Namibia	1997–2003	5	8.8	0.0	22.8	0.0	18.6
Nigeria	2001–2003	7	9.3	5.7	43.5	7.7	40.5
Rwanda	2000	3	35.1	11.6	35.7	11.6	35.7
Senegal	2001–2002	7	3.3	1.7	10.2	2.0	5.8
Sierra Leone	2001–2003	5	11.2	7.8	23.4	9.1	17.7
Somalia	1997–2003	3	4.0	2.0	5.9	2.0	5.9

SULFADOXINE-PYRIMETHAMINE monotherapy	study years	number of studies	median	range low	range high	percentile 25th	percentile 75th
South Africa	1997–2002	6	7.3	3.6	87.8	3.7	55.7
Sudan high transmission area	1996–2003	3	6.0	0.0	12.0	0.0	12.0
moderate-to-low transmission area	1996–2003	7	4.2	0.0	22.0	1.0	9.9
Uganda	1996–2002	25	11.4	0.0	25.0	5.0	16.8
United Republic of Tanzania							
mainland	1997–2002	15	10.5	1.4	33.8	5.2	15.9
Zanzibar	1997–2002	2	8.9	1.4	33.8	5.2	15.9
Zambia	1996–2003	17	7.9	0.0	17.9	3.3	14.2
Zimbabwe	1999	2	10.0	0.0	20.0	0.0	20.0
Total: Africa	**1996–2004**	**247**	**8.6**	**0.0**	**52.8**	**3.3**	**16.6**

Asia

Afghanistan	2002–2003	3	8.7	4.0	22.7	4.0	22.7
Bhutan	1998	1	34.8				
India	1999–2003	12	17.9	0.0	68.2	3.0	45.4
Indonesia	1996–2003	12	17.8	0.0	82.9	12.0	43.0
Iran (Islamic Republic of)	1999–2001	3	0.0	0.0	5.7	0.0	5.7
Lao People's Democratic Republic	2001–2002	3	18.0	17.9	18.7	17.9	18.7
Malaysia	1996	1	29.4				
Myanmar	1997–2002	18	27.8	0.0	100.0	7.9	37.7
Nepal	1997–2003	7	22.0	0.0	88.2	0.0	72.7
Pakistan	2001–2002	4	13.0	8.7	18.7	9.8	16.9
Philippines	1999–2001	7	42.6	8.5	66.7	12.5	60.6
Tajikistan	2002	1	16.0				
Timor-Leste	2001	1	10.0				
Viet Nam	1997–2002	4	16.6	12.2	70.6	13.0	41.9
Yemen	2003	1	0.0				
Total: Asia	**1996–2003**	**78**	**18.4**	**0.0**	**100.0**	**8.5**	**40.8**

The Americas

Bolivia	2002	1	18.7				
Colombia	1997–2002	12	10.8	0.0	26.5	1.9	15.8
Ecuador	2001–2003	3	4.0	0.0	17.1	0.0	17.1
Peru	1998–2002	9	11.8	0.0	80.0	1.7	65.2
Venezuela	1997–1999	3	20.0	0.0	23.0	0.0	23.0
Total: The Americas	**1997–2003**	**28**	**12.2**	**0.0**	**80.0**	**1.7**	**19.4**

AMODIAQUINE monotherapy	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Africa							
Angola	2002–2003	2	8.7	3.9	13.4	3.9	13.4
Burkina Faso	1996	1	4.4				
Cameroon	1997–2003	9	1.6	0.0	5.3	0.0	3.2
Chad	2002–2003	2	3.4	1.9	4.9	1.9	4.9
Ethiopia	1998	7	18.9	6.2	66.7	6.5	45.8
Gabon	1997–2002	5	12.5	3.2	14.0	7.9	14.0
Kenya	1996–2003	24	2.4	0.0	23.1	0.0	8.3
Liberia	2001	1	7.4				
Madagascar	2004	1	0.0				
Mozambique	2001	1	8.4				
Nigeria	2001–2002	2	1.5	0.0	2.9	0.0	2.9
Rwanda	2001–2002	6	0.0	0.0	2.3	0.0	2.0
Senegal	2001–2002	3	2.8	2.0	5.1	2.0	5.1
Sierra Leone	2002–2003	5	1.8	0.0	7.6	0.0	5.8
Sudan							
high transmission area	2001	2	6.5	6.0	7.0	6.0	7.0
Uganda	1999–2002	5	8.8	0.0	14.5	1.6	12.3
United Republic of Tanzania							
mainland	1999–2002	12	3.7	0.0	10.8	1.6	7.9
Zanzibar	1999–2002	2	5.6	0.0	10.8	1.6	7.9
Total: Africa	**1996–2004**	**90**	**3.3**	**0.0**	**66.7**	**0.0**	**8.4**
Asia							
Afghanistan	2004	1	37.7				
Pakistan	2002	1	83.3				
Total: Asia	**2002–2004**	**2**	**60.5**	**37.7**	**83.3**	**37.7**	**83.3**
The Americas							
Colombia	1997–2002	7	11.5	0.0	50.0	3.2	27.3
Total: The Americas	**1997–2002**	**7**	**11.5**	**0.0**	**50.0**	**3.2**	**27.3**

MEFLOQUINE monotherapy	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Africa							
Malawi	1998	1	10.2				
Sudan moderate-/low-transmission area	1999	1	2.5				
Total: Africa	**1998–1999**	**2**	**2.5**	**2.5**	**2.5**	**2.5**	**2.5**
Asia							
Bangladesh	1996	1	27.2				
Bhutan	1999	1	9.7				
India	1996–2001	3	4.5	0.0	7.8	0.0	7.8
Lao People's Democratic Republic	2001	1	0.0				
Malaysia	1996	1	0.0				
Myanmar	1997–2002	18	6.0	0.0	44.4	0.0	16.4
Thailand	1995–2003	19	13.8	2.0	68.4	7.5	28.0
Viet Nam	1998–1999	4	11.7	0.0	42.3	0.0	32.8
Total: Asia	**1995–2003**	**48**	**8.0**	**0.0**	**68.4**	**2.7**	**18.5**
The Americas							
Bolivia	2001	2	0.0	0.0	0.0	0.0	0.0
Brazil	1996–2002	6	5.2	0.0	9.7	0.5	7.9
Colombia	2002–2003	3	2.2	0.0	6.4	0.0	6.4
French Guiana	1996	1	3.4				
Guyana	2003	1	28.1				
Peru	1999–2000	4	0.0	0.0	0.0	0.0	0.0
Suriname	2002	1	7.3				
Total: The Americas	**1996–2003**	**18**	**1.6**	**0.0**	**28.1**	**0.0**	**6.2**

CHLOROQUINE + SULFADOXINE-PYRIMETHAMINE combination therapy	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Africa							
Comoros	2003	3	0.0	0.0	2.6	0.0	2.6
Eritrea	2002–2003	4	6.5	0.0	10.2	1.9	9.7
Rwanda	2000	6	13.2	6.1	39.7	8.1	37.7
Sudan moderate-to-low transmission area	2003	2	10.2	5.9	14.4	5.9	14.4
Uganda	1996–2003	15	12.0	0.0	37.0	7.0	19.0
Zimbabwe	2001–2004	25	1.1	0.0	8.6	0.0	3.9
Total: Africa	**1996–2004**	**55**	**4.3**	**0.0**	**39.7**	**0.5**	**10.1**
Asia							
Bangladesh	1996–2003	7	30.7	12.9	37.2	24.0	33.0
India		1	6.5				
Indonesia	1999–2003	2	22.2	6.2	38.2	6.2	38.2
Lao People's Democratic Republic	2001	2	12.3	7.8	16.7	7.8	16.7
Malaysia	1999–2003	4	47.6	31.3	62.5	37.5	57.0
Papua New Guinea	1998–2003	4	0.0	0.0	27.0	0.0	13.5
Philippines	2001–2002	3	18.4	11.1	29.6	11.1	29.6
Tajikistan	2003	1	2.1				
Vanuatu	2001	1	16.0				
Total: Asia	**1996–2003**	**25**	**24.0**	**0.0**	**62.5**	**7.2**	**33.0**
The Americas							
Colombia	2002	2	17.4	12.1	22.6	12.1	22.6
Ecuador	2003	1	0.0				
Total: The Americas	**2002–2003**	**3**	**12.1**	**0.0**	**22.6**	**0.0**	**22.6**

AMODIAQUINE + SULFADOXINE-PYRIMETHAMINE combination therapy	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Africa							
Cameroon	2001–2003	4	0.0	0.0	0.0	0.0	0.0
Democratic Republic of the Congo	2002–2004	5	1.7	0.0	6.0	0.7	4.4
Ghana	2002	1	1.4				
Kenya	2003	2	2.0	1.6	2.4	1.6	2.4
Mozambique	2001	1	0.0				
Rwanda	2001	3	0.0	0.0	0.0	0.0	0.0
Senegal	2001–2003	4	0.0	0.0	0.0	0.0	0.0
Uganda	1999–2003	12	1.6	0.0	13.0	0.5	3.5
United Republic of Tanzania mainland	1999	1	3.4				
Total: Africa	**1999–2004**	**33**	**1.0**	**0.0**	**13.0**	**0.0**	**2.2**
Asia							
Afghanistan	2003–2004	2	2.0	1.0	3.0	1.0	3.0
Papua New Guinea	1998	1	0.0				
Total: Asia	**1998–2004**	**3**	**1.0**	**0.0**	**3.0**	**0.0**	**3.0**
The Americas							
Colombia	2001–2003	4	2.3	0.0	10.8	1.1	6.6
Total: The Americas	**2001–2003**	**4**	**2.3**	**0.0**	**10.8**	**1.1**	**6.6**

ARTEMETHER + LUMEFANTRINE combination therapy	study years	number of studies	median	range low	range high	percentile 25th	percentile 75th
Africa							
Burundi	2001	2	0.0	0.0	0.0	0.0	0.0
Comoros	2004	3	0.0	0.0	1.8	0.0	1.8
Ethiopia	2003	4	0.0	0.0	0.0	0.0	0.0
Gabon	2001-2002	2	0.8	0.0	1.6	0.0	1.6
Ghana	2003	1	0.0				
Senegal	2003	1	0.0				
South Africa	2002	1	0.0				
United Republic of Tanzania Zanzibar	2002	2	1.0	0.0	2.0	0.0	2.0
Zambia	2003	3	0.0	0.0	0.0	0.0	0.0
Total: Africa	**2001–2004**	**19**	**0.0**	**0.0**	**2.0**	**0.0**	**0.0**
Asia							
Bangladesh	2002	1	0.8				
Cambodia	2001–2004	3	26.9	13.5	30.0	13.5	30.0
Lao People's Democratic Republic	2001–2003	2	4.7	3.1	6.3	3.1	6.3
Myanmar	2003	3	2.0	0.0	2.0	0.0	2.0
Thailand	1996–2002	6	2.6	0.0	3.9	0.5	3.5
Viet Nam	2001	1	2.2				
Total: Asia	**1996–2004**	**16**	**2.6**	**0.0**	**30.0**	**1.5**	**5.1**
The Americas							
Suriname	2003	2	2.0	1.9	2.0	1.9	2.0
Total: The Americas	**2003**	**2**	**2.0**	**1.9**	**2.0**	**1.9**	**2.0**

ARTESUNATE + AMODIAQUINE combination therapy	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Africa							
Angola	2003	1	0.0				
Burundi	2001	2	1.3	0.0	2.6	0.0	2.6
Comoros	2003	3	0.0	0.0	0.0	0.0	0.0
Democratic Republic of the Congo	2003–2004	3	0.0	0.0	1.4	0.0	1.4
Eritrea	2002–2003	3	0.0	0.0	1.4	0.0	1.4
Gabon	2001–2002	2	0.9	0.0	1.7	0.0	1.7
Ghana	2003	1	0.0				
Mozambique	2001	1	0.0				
Rwanda	2002	3	0.0	0.0	1.6	0.0	1.6
Senegal	2002	2	0.0	0.0	0.0	0.0	0.0
Sudan high transmission area	2003	2	0.4	0.0	0.8	0.0	0.8
Uganda	2002–2003	5	1.0	0.0	4.0	0.5	3.7
United Republic of Tanzania Zanzibar	2002	2	1.9	1.8	1.9	1.8	1.9
Total: Africa	**2001–2004**	**30**	**0.0**	**0.0**	**4.0**	**0.0**	**1.5**
Asia							
Myanmar	2003	4	4.0	3.0	7.0	3.5	5.5
Pakistan	2002	1	18.0				
Total: Asia	**2002–2003**	**5**	**4.0**	**3.0**	**18.0**	**3.5**	**12.5**

ARTESUNATE + CHLOROQUINE combination therapy	study years	number of studies	median	range low	range high	percentile 25th	percentile 75th
Africa							
Gambia	2000	1	3.2				
Total: Africa	**2000**	**1**	**3.2**	**3.2**	**3.2**	**3.2**	**3.2**
Asia							
Pakistan	2002	1	28.8				
Viet Nam		2	37.4	28.0	46.8	28.0	46.8
Total: Asia	**2002**	**3**	**28.8**	**28.0**	**46.8**	**28.0**	**46.8**

ARTESUNATE + SULFADOXINE–PYRIMETHAMINE combination therapy	number of studies: study years		median	range: low	range: high	percentile: 25th	percentile: 75th
Africa							
Angola	2003	1	1.2				
Comoros	2003	3	0.0	0.0	3.6	0.0	3.6
Democratic Republic of the Congo	2002–2004	6	0.0	0.0	5.6	0.0	3.4
Ghana	2002	1	0.8				
Mozambique	2001	1	0.0				
Rwanda	2001	3	0.0	0.0	0.0	0.0	0.0
South Africa	2004	1	5.0				
Sudan high transmission area	2003	2	1.7	0.8	2.5	0.8	2.5
Uganda	2000	1	0.5				
Zambia	2002–2003	5	0.0	0.0	1.7	0.0	0.9
Total: Africa	**2000–2004**	**24**	**0.0**	**0.0**	**5.6**	**0.0**	**1.1**
Asia							
Indonesia	1999	1	4.3				
Myanmar	2003	2	0.0	0.0	0.0	0.0	0.0
Pakistan	2002	1	0.0				
Sri Lanka	1999	1	0.0				
Viet Nam		2	33.2	8.3	58.1	8.3	58.1
Total: Asia	**1999–2003**	**7**	**0.0**	**0.0**	**58.1**	**0.0**	**8.3**
The Americas							
Ecuador	2003	2	0.0	0.0	0.0	0.0	0.0
Peru	2000	1	1.1				
Total: The Americas	**2000–2003**	**3**	**0.0**	**0.0**	**1.1**	**0.0**	**1.1**

ARTESUNATE + MEFLOQUINE combination therapy	study years	number of studies	median	range low	range high	percentile 25th	percentile 75th
Africa							
Senegal	2002	2	0.0	0.0	0.0	0.0	0.0
Total: Africa	**2002**	**2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Asia							
Bangladesh	2002	1	0.9				
Cambodia	2001–2004	12	3.7	0.0	14.3	1.1	8.1
India	2001	2	6.4	1.9	10.9	1.9	10.9
Lao People's Democratic Republic	2001–2003	2	0.0	0.0	0.0	0.0	0.0
Myanmar	1996–2003	10	1.5	0.0	8.0	0.0	5.1
Thailand	1995–2003	34	3.6	0.0	21.4	1.2	8.1
Viet Nam	1998–2000	2	5.6	0.0	11.1	0.0	11.1
Total: Asia	**1995–2004**	**63**	**3.1**	**0.0**	**21.4**	**0.0**	**7.7**
The Americas							
Bolivia	2001	3	0.0	0.0	0.0	0.0	0.0
Guyana	2003	1	7.5				
Peru	2000	1	0.0				
Suriname	2002–2003	2	4.1	2.4	5.8	2.4	5.8
Total: The Americas	**2000–2003**	**7**	**0.0**	**0.0**	**7.5**	**0.0**	**5.8**
Asia							
Bhutan	2000–2003	8	4.9	1.1	12.0	2.2	8.7
Total: Asia	**2000–2003**	**8**	**4.9**	**1.1**	**12.0**	**2.2**	**8.7**

ANNEX 7

REGIONAL GROUPINGS OF COUNTRIES FOR WHICH RESULTS WERE AVAILABLE

Regional and subregional classification of countries and territories

The information from countries and territories considered to be malaria-endemic is presented from three broad global regions: Africa, Asia and the Americas, which are further divided into subregions. Groupings are based on geographical proximity and, secondarly, on existing WHO regional groupings.

Africa

Central Africa

Cameroon, Central African Republic, Chad, Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon

East Africa

Burundi, Comoros, Eritrea, Ethiopia, Kenya, Rwanda, Somalia, Sudan, Uganda, United Republic of Tanzania

Southern Africa

Angola, Botswana, Madagascar, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia, Zimbabwe

Western Africa

Benin, Burkina Faso, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, Togo

Americas

South America

Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, Venezuela

Asia

Central Asia

Tajikistan

Eastern Mediterranean
Afghanistan, Islamic Republic of Iran, Pakistan, Saudi Arabia, Yemen

South-East Asia

Bangladesh, Bhutan, India, Indonesia, Myanmar, Nepal, Sri Lanka, Thailand, Timor-Leste

Western Pacific

Cambodia, China, Lao People's Democratic Republic, Malaysia, Papua New Guinea, Philippines, Solomon Islands, Vanuatu, Viet Nam

ANNEX 8

CLASSIFICATION OF TREATMENT OUTCOMES ACCORDING TO WHO PROTOCOL, 2005

Early treatment failure:

- danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia;
- parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature;
- parasitaemia on day 3 with axillary temperature ≥37.5 °C;
- parasitaemia on day 3 ≥25% of count on day 0.

Late clinical failure:

- danger signs or severe malaria in the presence of parasitaemia on any day after day 4 and before day 28, without the patient previously meeting any of the criteria of early treatment failure;
- axillary temperature ≥37.5 °C in the presence of parasitaemia on any day between day 4 and day 28, without the patient previously meeting any of the criteria of early treatment failure.

Late parasitological failure:

- presence of parasitaemia between day 7 and day 28 with temperature ‹37.5 °C, without the patient previously meeting any of the criteria of early treatment failure or late clinical failure.

Adequate clinical and parasitological response:

- absence of parasitaemia on day 28, irrespective of axillary temperature, without the patient meeting any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

ANNEX 9

MALARIA TRANSMISSION AREAS AND REPORTED DRUG RESISTANCE, 2004

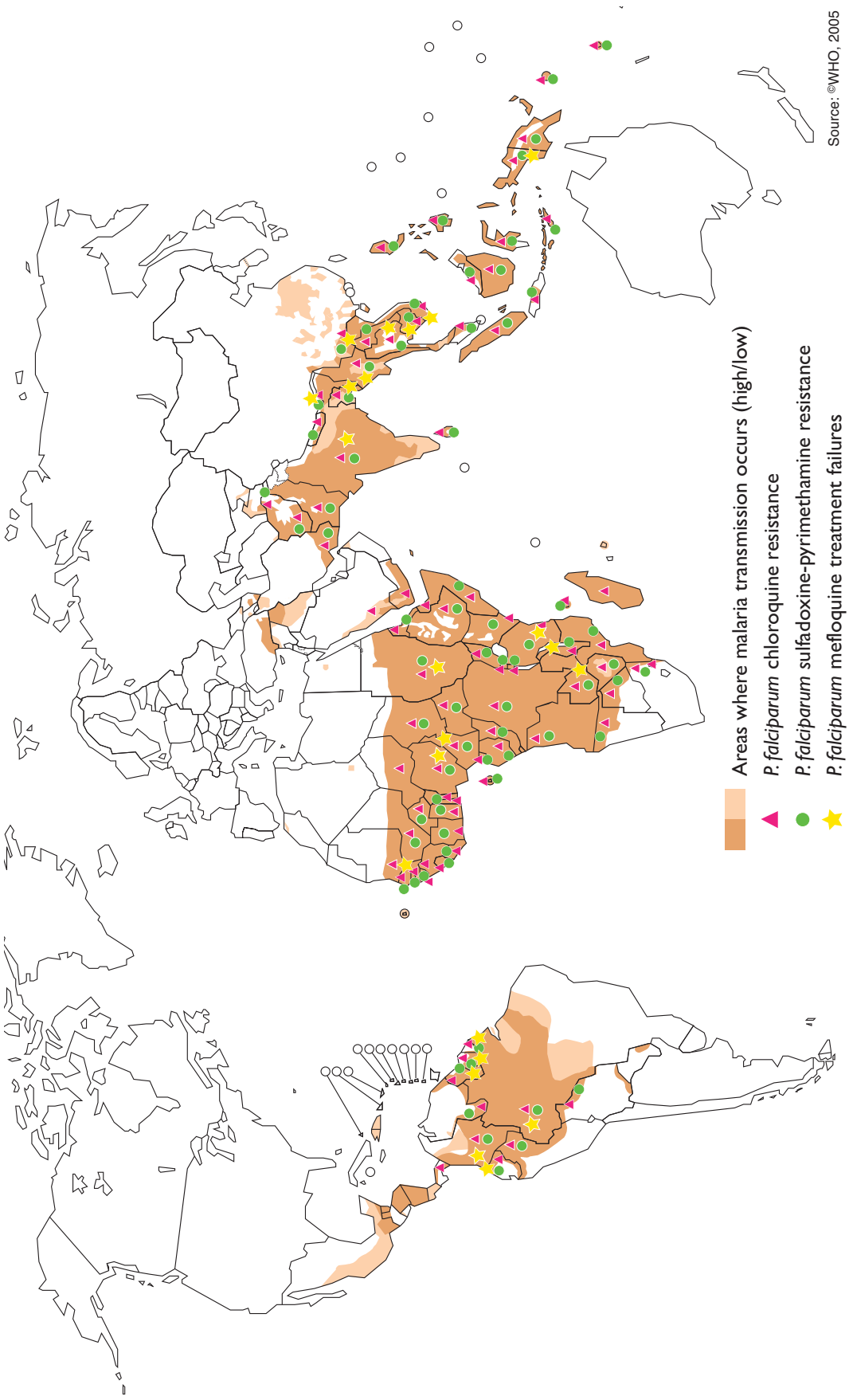

TABLE 1

TOOLS FOR MONITORING ANTIMALARIAL DRUG EFFICACY AND DRUG RESISTANCE

	Therapeutic efficacy test	In vitro sensitivity assay	Molecular markers
Definition	• Treatment of symptomatic *P. falciparum*-infected patients with a standard dose of an antimalarial drug and subsequent follow-up of parasitaemia and clinical signs and symptoms over a defined period (response of the host–parasite system to the drug)	• Cultivation of *P. falciparum* parasites in vitro with a range of antimalarial drug concentrations (response of the parasites to the drug)	• Detection of gene mutation(s) or amplification that modify drug-target (enzymes) or drug-transporter functions or affinities (genetic characterization of drug targets or transport)
Indications	• Gold standard for monitoring antimalarial drug efficacy and for guiding drug policy	• Detect reduced parasite response to antimalarial drug • Early warning system (adjunct to therapeutic efficacy test)	• Detect resistance-related mutations or amplification • Early warning system (adjunct to therapeutic efficacy test)
Advantages	• Easily interpretable results • Simple method with minimal training required (except microscopy) • Minimal equipment and supplies required • Relatively inexpensive to conduct (depending on local conditions) if integrated into the national malaria control programmes	• Avoids host confounding factors • Accurate for detecting true drug resistance • Provides quantitative results • Multiple tests can be performed with a single isolate, and several drugs can be assessed simultaneously • Experimental drugs can be tested (except pro-drugs) • In vitro resistance precedes in vivo resistance	• Avoids host confounding factors • Accurate for detecting true drug resistance • Samples on filter paper easily obtained, transported and stored • Multiple tests can be performed with a single filter paper and molecular targets of several drugs can be characterized • If known, targets of new and experimental drugs can be tested (e.g. atovaquone) • Mutations precedes in vivo resistance
Drawbacks	• Interference of immunity, previous drug intake, variation of drug absorption or metabolism • Misclassification of reinfection and recrudescence • Treatment failures do not reflect the level of true drug resistance • Difficult to conduct in areas of low transmission given the limited numbers of eligible patients • Overestimation of early treatment failures for slowly acting drugs • Numerous local adaptations and modifications result in poor ability to compare between sites • Long duration of patient monitoring may result in high patient loss to follow-up	• Correlation with therapeutic efficacy test not fully established • Presence of mixed population with different drug sensitivity phenotypes • Expensive equipment and supplies required • Training required • Numerous available methods but not always comparable • Lack of standardized in vitro protocol • Threshold of resistance not validated	• Correlation with therapeutic efficacy test not fully established • Presence of mixed population with mixed alleles • Expensive equipment and supplies required • Training required • Identified for a limited number of antimalarial drugs • Lack of standardized PCR protocol including sample collection and DNA extraction

TABLE 2

CASES OF TREATMENT FAILURE WITH MEFLOQUINE

Country	Year(s)	Treatment(s)	Dose of mefloquine	No. of failures/ no. included	Day (D) of failure	Comments	Reference
China	1980-1981	Mefloquine	1000 mg for an adult	4/51	D28 and D3 RII-RIII	No mefloquine dosage; no in vitro test	239
Indonesia (Irian Jaya)	1982	Mefloquine-sulfadoxine-pyrimethamine	15 mg/kg	2/36	RII and RIII	For one case: isolate sensitive in vitro; mefloquine concentration at D1 = 1904 ng/ml	240
Brazil	1997	Mefloquine	20 mg/kg	3/51	D3, D4 and D5	Three successive patients in same study; no mefloquine dosage; no in vitro test	241
Cameroon	1987	Mefloquine	25 mg/kg	6/57 asymptomatic children	Before D7	Follow-up until D7 only; mefloquine concentration ›500 ng/ml at D3 in all cases; IC50 = 18, 25, 46 and 79 nmol/l	242
Nigeria	1987	Mefloquine-sulfadoxine-pyrimethamine	5.6-12.5 mg/kg	1/33	D5	Mefloquine underdosed	243
Nigeria	1989-1990	Mefloquine	15 mg/kg 25 mg/kg	2/40 1/45	D31-D33 D32	MIC for two isolates = 41.8 and 67 nmol/l; reinfection not excluded; successfully treated with mefloquine at 25 mg/kg	244
Nigeria	unknown	Mefloquine-sulfadoxine-pyrimethamine	5.6-12.5 mg/kg	4/32	D14 (*n* = 1) and D21 (*n* = 3)	Mefloquine underdosed	245
Nigeria	unknown	Mefloquine	25 mg/kg	2/33	D16 and D28	No mefloquine dosage; no in vitro test; reinfection not excluded	246
Nigeria	1995-1996	Mefloquine-sulfadoxine-pyrimethamine	250 mg/20 kg	6/68	D7 (*n* = 4) and D14 (*n* = 2)	Spontaneous parasite clearance in 5/6 cases	247
Senegal	1988	Mefloquine	12.5 mg/kg	2/31	D5 and D14	Mefloquine concentration = 150, 180 ng/ml at D2; IC50 = 32 nmol/l in one case	248
Sudan	1998-2001	Mefloquine	25 mg/kg	1/40 pregnant women	D14	No mefloquine dosage; no in vitro test	249
United Republic of Tanzania	1982	Mefloquine	1500 mg/90 kg 2000 mg/90 kg	Case report of one adult	D30 D56	Peak mefloquine, 2850 ng/ml ; IC50 = 40 nmol/l Peak mefloquine, 2014 ng/ml ; IC50 = 80 nmol/l Successful treatment with mefloquine at 2000 mg and tetracycline at 2 g/day, 10 days	250
Zambia	?	Mefloquine	1000 mg	Case report of one adult	D19	Isolate sensitive in vitro; reinfection not excluded	251
Senegal, Morocco Côte d'Ivoire	1988	Mefloquine	65 mg/kg	Case report of one child	D21	Mefloquine concentration at D21 = 328 ng/ml	252

TABLE 3

COMPARISON OF THERAPEUTIC EFFICACY OF DIFFERENT ANTIMALARIAL DRUGS IN CHILDREN AGED UNDER AND OVER 5 YEARS

Country	Drug(s)	Age group 0.5–5 years		Age group >5 years		Ref.
		Clinical failure (%)	Total failure (%)			
Africa						
Côte d'Ivoire	Chloroquine	**22.6**	–	**9.8**	–	338
Gambia	Chloroquine	**15**	–	**6.8**	–	339
Gambia	Artesunate+chloroquine	**3.2**	–	**1**	–	339
Madagascar	Chloroquine	**8**	24	**3.2**	16.1	*
Mali (Mopti)	Chloroquine	–	30.3	–	14.1	340
Mali (Bandiagara)	Chloroquine	–	19.6	–	11.2	340
Niger	Chloroquine	**20.9**	25.6	**6.1**	14.3	341
Sudan	Chloroquine	**16.6**	20.8	**11.6**	13.5	342
Uganda	Chloroquine	**76.4**	96.1	**27.9**	44.2	343
Outside Africa						
Bangladesh	Quinine+sulfadoxine–pyrimethamine	–	16.6	–	6.9[a]	82
Bangladesh	Chloroquine+sulfadoxine–pyrimethamine	–	32.8[b]	–	25[a]	344
Indonesia	Sulfadoxine–pyrimethamine	–	16.1	–	0	345
Indonesia	Artesunate+sulfadoxine–pyrimethamine	–	5.1	–	0	345
Lao People's Democratic Republic	Chloroquine+sulfadoxine–pyrimethamine	–	37.1	–	12.5	346
Myanmar	Chloroquine	–	93.3	–	71.4	233
Myanmar	Sulfadoxine–pyrimethamine	–	76.5	–	69.2	233
Myanmar	Mefloquine	–	26.7	–	12.7	233
Myanmar	Artesunate+mefloquine	–	7.1	–	6.9	233
Viet Nam	Artesunate	–	36	–	11	234
Viet Nam	Mefloquine	–	37	–	0	234

[a] For age group >15 years

[b] For age group 0.5–15 years

* M. Randrianarivelojosia, unpublished data, 2004

TABLE 4

COUNTRIES THAT HAVE CHANGED THEIR NATIONAL MALARIA TREATMENT POLICY BETWEEN 1996 AND 2004 ON THE BASIS OF THE RESULTS OF THERAPEUTIC EFFICACY TESTS

Country	Previous drug policy	Year of change	New drug policy
African Region			
Benin	Chloroquine	2004	Artemether–lumefantrine
Botswana	Chloroquine	1998	Sulfadoxine–pyrimethamine
Burundi	Sulfadoxine–pyrimethamine	2003	Artesunate+amodiaquine
Cameroon	Chloroquine/amodiaquine	2004	Artesunate+amodiaquine
Comoros	Chloroquine	2003	Artemether–lumefantrine
Côte d'Ivoire	Chloroquine	2003	Amodiaquine/sulfadoxine–pyrimethamine
Democratic Republic of the Congo	Chloroquine	2001	Sulfadoxine–pyrimethamine
Equatorial Guinea	Chloroquine	2004	Artesunate+amodiaquine
Eritrea	Chloroquine	2002	Chloroquine+sulfadoxine–pyrimethamine
Ethiopia	Chloroquine	2004	Artemether–lumefantrine
Gabon	Chloroquine	2003	Artesunate+amodiaquine
Ghana	Chloroquine	2004	Artesunate+amodiaquine
Kenya	Sulfadoxine–pyrimethamine	2004	Artemether-lumefantrine
Liberia	Chloroquine	2004	Artesunate+amodiaquine
Madagascar	Chloroquine	2004	Artesunate+amodiaquine
Mali	Chloroquine	2004	Artemether–lumefantrine
Mozambique	Chloroquine	2003 2004	Amodiaquine+sulfadoxine-pyrimethamine Artesunate+amodiaquine
Namibia	Chloroquine	2004	Artemether–lumefantrine
Nigeria	Chloroquine	2004	Artemether–lumefantrine
Rwanda	Chloroquine	2001	Amodiaquine+sulfadoxine–pyrimethamine
Sao Tome and Principe	Chloroquine	2004	Artesunate+amodiaquine
Senegal	Chloroquine	2003	Amodiaquine+sulfadoxine–pyrimethamine
Sierra Leone	Chloroquine	2004	Artesunate+amodiaquine
South Africa	Sulfadoxine–pyrimethamine	2001	Artemether–lumefantrine/ artesunate+sulfadoxine–pyrimethamine

Country	Previous drug policy	Year of change	New drug policy
Uganda	Chloroquine+sulfadoxine-pyrimethamine	2004	Artemether–lumefantrine
United Republic of Tanzania			
mainland	Sulfadoxine-pyrimethamine	2004	Artemether–lumefantrine
Zanzibar	Chloroquine	2001	Artesunate+amodiaquine
Zambia	Chloroquine	2002	Artemether–lumefantrine
Zimbabwe	Chloroquine	2000	Chloroquine+sulfadoxine–pyrimethamine
Region of the Americas			
Bolivia	Quinine	2001	Artesunate+mefloquine
Colombia	Amodiaquine+sulfadoxine–pyrimethamine	2004	Artesunate+mefloquine (proposed)
Ecuador	Chloroquine	2004	Artesunate+sulfadoxine–pyrimethamine
Guyana	Mefloquine	2004	Artemether–lumefantrine
Peru	Sulfadoxine–pyrimethamine/ quinine+tetracycline	2001	Artesunate+sulfadoxine-pyrimethamine/ artesunate+mefloquine
Suriname	Quinine+doxycycline	2003	Artemether–lumefantrine
Venezuela	Chloroquine	2004	Artesunate+mefloquine
Eastern Mediterranean Region			
Afghanistan	Chloroquine	2004	Artesunate+sulfadoxine–pyrimethamine
Iran (Islamic Republic of)	Chloroquine	2004	Artesunate+sulfadoxine–pyrimethamine
Saudi Arabia	Chloroquine	2003	Sulfadoxine–pyrimethamine
Sudan	Chloroquine	2004	Artesunate+sulfadoxine-pyrimethamine
low-transmission area			
European Region			
Tajikistan	Chloroquine	2004	Artesunate+sulfadoxine–pyrimethamine

Country	Previous drug policy	Year of change	New drug policy
South-East Asia Region			
Bangladesh	Sulfadoxine–pyrimethamine	2004	Artemether–lumefantrine
Bhutan	Artesunate+doxycycline	2004	Artemether–lumefantrine
India	Chloroquine	2004 in some areas	Sulfadoxine–pyrimethamine/ artesunate+chloroquine
Indonesia	Chloroquine	2004	Artesunate+amodiaquine
Myanmar	Chloroquine	2002	Artesunate+mefloquine/ artemether–lumefantrine
Thailand	Mefloquine	1995 (until 2005)	Artesunate+mefloquine
Timor-Leste	Chloroquine	2002	Chloroquine/sulfadoxine–pyrimethamine
Western Pacific Region			
Cambodia	Mefloquine	2000	Artesunate+mefloquine
Lao People's Democratic Republic	Chloroquine	2001	Chloroquine+sulfadoxine–pyrimethamine (interim)
Philippines	Chloroquine	2002	Chloroquine+sulfadoxine–pyrimethamine
Papua New Guinea	Chloroquine	1997	Chloroquine/amodiaquine+ sulfadoxine-pyrimethamine
Solomon Islands	Chloroquine	2001	Chloroquine+sulfadoxine–pyrimethamine

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