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REGIONAL OFFICE FOR THE WESTERN PACIFIC

REPORT

INTERREGIONAL MEETING ON MALARIA CONTROL WITH EMPHASIS ON DRUG RESISTANCE

Manila, Philippines
21-24 October 1996

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REPORT

INTERREGIONAL MEETING ON MALARIA CONTROL WITH EMPHASIS ON DRUG RESISTANCE

Convened by the

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NOTE

The views expressed in this report are those of the participants in the Interregional Meeting on Malaria Control with Emphasis on Drug Resistance and do not necessarily reflect the policies of the World Health Organization.

This report has been prepared by the World Health Organization Regional Office for the Western Pacific for governments of Member States in the Region and for those who participated in the Interregional Meeting on Malaria Control with Emphasis on Drug Resistance which was held in Manila, Philippines on 21-24 October 1996.

CONTENTS

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SUMMARY

Countries of the Western Pacific and South-East Asia Regions share similar problems in malaria and its control, especially in border areas stretching from north-east India to southern China. In March 1993, representatives of these bordering countries met in Kunming, Yunnan Province, China to discuss and plan solutions to common problems associated with the epidemiology and control of malaria. This report of a meeting of malaria control programme managers and related staff from selected countries of the two Regions, convened in Manila in October 1996, provides a review of progress since the Kunming meeting. Practical guidance is given on strengthening of antimalarial drug policies related to artemisinin and its derivatives, the monitoring of drug efficacy and resistance, and guidance on developing information exchange on malaria control in border areas.

While drug resistance continues to pose serious problems to malaria control in bordering countries of the two Regions, reports show that the malaria situation is improving in some of these countries. The important lesson to the world community is that drug and insecticide resistance, altered vector behaviour and population movements are not intractable obstacles to malaria control if the right strategies are applied in the right way in the right place at the right time. Border meetings should involve not only administrators and policy-makers at the national level but also those operational staff and other field workers at the district and provincial levels who are actually dealing with specific problems in border areas.

Experience has shown that substantial drug pressure on world populations of *Plasmodium falciparum* invariably leads to the development of drug resistance. The current variability in the susceptibility of the parasite *in vitro* to artemisinin and its derivatives, and the ease with which resistance can be developed in animal models makes it almost certain that the current pattern of the use of these drugs will eventually lead to the development of resistance and loss of therapeutic efficacy. The best way of preventing the development of resistance is to reserve these drugs for the treatment of severe malaria. Artemisinin or artesunate combined with another drug also may help delay resistance. In addition, malaria control programmes which identify areas of multidrug resistance, should define vector control measures which can effectively reduce incidence of the disease, and apply them rapidly, vigorously and persistently.

This report also provides protocols for *in vivo* test for drug susceptibility of *P. falciparum* to artemisinin, artesunate and quinine, and *in vitro* field test for drug susceptibility of *P. falciparum* to artemisinin. The meeting also agreed on mechanisms for reporting malaria outbreaks in cross-border areas.

1. INTRODUCTION

Countries of the Western Pacific and South-East Asia Regions share similar problems in malaria and its control, especially in the border areas stretching from north-east India to southern China. These relate to population movements across country borders; the inadequacy of health services in these areas to prevent, diagnose and treat malaria disease, the improper use of antimalarial drugs; and the limited resources and operational difficulties in implementing malaria control activities in these areas. As a consequence, the two Regions face the most serious problems of multidrug resistance seen in the world today and these problems are spreading.

In November 1993, representatives of these bordering countries met in Kunming, Yunnan Province, China to discuss and plan solutions to common problems associated with the epidemiology and control of malaria in these border areas. This meeting identified collaborative control activities, training and operational research needs in the two Regions and defined achievable targets in malaria control in the affected areas. Particular attention was paid to the development and implementation of antimalarial drug policies, to malaria control in areas with high population mobility, and to the exchange of information.

This report of a meeting of malaria control programme managers and related staff from selected countries of the two Regions, WHO staff and partners in malaria control provides a review of progress since the Kunming meeting and provides practical guidance on the strengthening of antimalarial drug policies related to artemisinin and its derivatives, the monitoring of drug efficacy and resistance, and border malaria and information exchange.

1.1 Objectives

The specific objectives of the meeting were:

(1) Drug Policies: Artemisinin and its derivatives

(i) to strengthen antimalarial drug policies on the use of artemisinin and its derivatives with respect to:

registration;

quality control;

use in public and private sectors;

management of severe and complicated malaria; and

prevention of development of resistance.

(ii) to improve the monitoring of adverse reactions to artemisinin and its derivatives with particular emphasis on potential neurotoxic effects and its use during pregnancy.

(2) Monitoring of therapeutic efficacy and drug resistance

(i) to determine the role and scope of monitoring treatment failures as an early warning system for changes in antimalarial drug efficacy;

(ii) to develop a protocol for therapeutic efficacy of antimalarial drugs for use in areas of low and moderate transmission malaria, with particular reference to artemisinin and its derivatives and quinine

(iii) to propose norms for *in vivo* testing of susceptibility of *Plasmodium falciparum* to artemisinin, artesunate and quinine; and

(iv) to determine the essential elements and application of an *in vitro* test for the susceptibility of *P. falciparum* to artemisinin and its derivatives.

(3) Border malaria and information exchange

(i) to determine the essential elements for information exchange on border malaria with special emphasis on epidemic preparedness and importation of malaria; and

(ii) to agree upon a format of reporting and a mechanism of information exchange.

1.2 Organization

The meeting was held at the World Health Organization Regional Office for the Western Pacific (WPRO) in Manila, Philippines from 21 to 24 October 1996. It was partly funded by the Swedish International Development Administration (SIDA) and the Japan Voluntary Contribution (JVC). The detailed programme is given in Annex 1.

The Chairman of the meeting was Dr Leonard Ortega, Philippines, the Co-Chairman Dr Shiv Lal, India, and the Rapporteurs Dr Hasan bin Abdul Rahman, Malaysia and Dr Thomas Suroso, Indonesia.

1.3 Participants and resource persons

The list of participants and resource persons is given in Annex 2.

1.4 Opening ceremony

The meeting was opened on behalf of Dr S. T. Han, Regional Director of the Western Pacific Region of the World Health Organization by Dr B. P. Kean, Director, Programme Management. In his address, Dr Kean pointed out that while drug resistance continues to pose serious problems to malaria control in bordering countries of the Western and South-East Asia Regions, reports show that the malaria situation is improving in some of these countries. The important lesson to the world community is that drug and insecticide resistance, altered vector behaviour and population movements are not intractable obstacles to malaria control if the right strategies are applied in the right way in the right place at the right time. Dr Kean stressed that this depends on improving the quality and exchange of information and ensuring that information is used for decision making and action at the local level.

Dr V.S. Orlov, Senior Regional Adviser (Malaria and Vector Biology Control) presented a message from Dr Uton Muchtar Rafei, Regional Director of the South-East Asia Region of WHO welcoming the initiatives taken to improve coordination between the two Regions for solving common problems in the control of malaria as well as other communicable diseases. He noted that management of multidrug resistant malaria was expensive and urged participants to focus their efforts on reducing the burden of malaria in poor and underserved communities by ensuring malaria control was affordable to those in need.

2. PROCEEDINGS

2.1 Malaria in border areas of the South-East Asia and Western Pacific Regions of WHO

The malaria situation has deteriorated alarmingly in the eastern areas of Kachin and Shan States of Myanmar bordering China, Thailand and the Lao People's Democratic Republic where transmission is intense, substantial populations movements occur and malaria accounts for up to 30% of hospital admissions. Over 85% of the cases are due to multiresistant falciparum malaria. The incidence of malaria is underestimated because of the limited utilization of government health services at the periphery where only chloroquine and sulfadoxine/pyrimethamine combinations are available. Mefloquine is the first-line treatment in some townships near the border of Thailand and is available in the private sector. In 1995, the cure rate of mefloquine was 79% in Pa-am near the Myanmar-Thailand border but data from other border areas is limited. Severe cases are referred to township or state hospitals where they are generally treated with parenteral quinine. Artemisinin and its derivatives are widely available through private sources. There are no vector control activities carried out in any of the Myanmar border areas. The epicentre of intense transmission of multiresistant falciparum malaria in eastern Myanmar is, however, threatening the situation in western and northern Thailand, China and the Lao People's Democratic Republic.

Intensive control activities, including vector control, are carried out in border areas in Thailand. Malaria is well under control on the borders areas of Thailand with both the Lao People's Democratic Republic and Malaysia, and, with the closing of the border between Thailand and Cambodia, the situation is improving in these areas. Resistance to chloroquine and sulfadoxine/pyrimethamine is widespread in all border areas of Thailand and to mefloquine in areas bordering Myanmar and in Chanthaburi, Prachinaburi and Trat Provinces bordering Cambodia. Intensive transmission is only focal on the Thailand-Malaysian border.

In Cambodia, drug sensitivity studies conducted over the past decade indicate a high level of *P. falciparum* resistance, especially in the western provinces. In the north-west, chloroquine and sulfadoxine/pyrimethamine can no longer be used for treatment at the community level, and the choice of an affordable and acceptable alternative has been difficult. Mefloquine, as a single 20mg/kg dose, has been introduced in hospitals with reliable laboratory and management services and is the first-line drug in the central, northern and southern Provinces but this drug is also showing decreased sensitivity, especially in the western provinces bordering Thailand. A seven-day regimen of quinine/tetracycline is still effective and is the first line treatment in hospitals in the north-west and second-line treatment in central, northern and southern areas but compliance is difficult to obtain. An oral regimen of five-day artesunate plus a single dose of 20 mg/kg mefloquine is being evaluated and, if effective, could become the first-line treatment in the north-west in 1997. Chloroquine can still be used as the first-line drug at all levels in the north-east of

Cambodia but sulfadoxine/pyrimethamine is ineffective and mefloquine is an effective second-line drug in hospitals. Patients seek malaria treatment primarily in the extensive and unregulated private sector from practitioners and drug vendors who provide a large range of drugs, often inappropriate and with irrational regimens. Artemisinin and its derivatives are used throughout the country in the private sector, even in areas where chloroquine is still effective and vastly cheaper.

In central and southern Viet Nam, the levels of resistance to chloroquine and sulfadoxine/pyrimethamine are so high that both of these drugs are largely ineffective for the treatment of falciparum malaria. There are, however, some foci of relative susceptibility to chloroquine in the North. Incipient resistance to both quinine and mefloquine also occurs in the South. The main treatment of clinically diagnosed malaria in most provinces is oral artesunate or artemisinin given for five days. In the South confirmed falciparum malaria is treated with artemisinin or artesunate for three days with a single dose of 15mg/kg mefloquine. Severe malaria is treated with injectable artesunate or artemisinin suppositories. Quinine is being phased out in Viet Nam for severe malaria. The rapidly decreasing malaria morbidity and mortality in Viet Nam has been attributed in part to the large-scale deployment of artemisinin and artesunate, and to the targeting of vector control, especially impregnated mosquito nets to populations at high risk of malaria.

Chloroquine resistance in the Lao People's Democratic Republic is not yet as severe as in western Cambodia or southern or central Viet Nam. Chloroquine is the first-line drug and sulfadoxine/pyrimethamine the second line drug throughout the country. Quinine is used in hospitals to treat severe and complicated cases. Resistance to chloroquine and sulfadoxine/pyrimethamine is reported from border areas.

Yunnan, Guangxi and Hainan Provinces are the main malarious areas of China. Yunnan Province has borders with Myanmar, the Lao People's Democratic Republic and Viet Nam. Intensive surveillance and control activities are carried in these provinces. As a result, the number of cases and the incidence of malaria dropped by 13% and 16% respectively between 1992 and 1995 in Yunnan, where 15 249 cases and 34 deaths due to malaria were reported in 1995. Although success has been achieved in these border areas, further progress is being hindered by several factors. Chloroquine and sulfadoxine resistance occurs in all border areas and is spreading. Recent studies in south-east Yunnan Province bordering Viet Nam also show a decrease in sensitivity to piperaquine, pyronaridine and, importantly, artemisinin derivatives. Population movements have increased, especially in Yunnan Province but also in neighbouring parts of southern China, and the outreach of primary health care facilities has decreased. It is estimated that that over three-fold more cases of malaria are treated at the periphery and by private clinics, and 25% more in hospitals than currently reported.

Multidrug resistance is not a problem in other countries of the two Regions in western and middle south Asia, as well as Indonesia, Malaysia, the Philippines and south-west Pacific, where the levels of chloroquine resistance are variable. Resistance of *P. vivax* to chloroquine has now been reported from Indonesia (Irian Jaya Region), Myanmar, Papua New Guinea and Vanuatu. In some areas of Papua New Guinea and Irian Jaya, 20%-30% of patients infected with vivax malaria have recurrences of parasitaemia one to three weeks after a full treatment dose of chloroquine (25mg/kg). Cross-resistance to amodiaquine sometimes occurs.

2.2 Drug policy: issues related to artemisinin and its derivatives

Artemisinin and its derivatives are a group of rapidly acting antimalarial drugs, produced mainly in China and Viet Nam. They are effective against infections which are resistant to other

antimalarial drugs, but a 100% cure rate is not attained even with treatment courses as long as five to seven days. In studies in Myanmar and Thailand, parenteral treatment with artesunate and artemether have decreased the fatality rate of severe malaria when compared with parenteral quinine but such a difference has not been found in studies in Viet Nam and Africa, presumably because of better quinine susceptibility of the parasites in the latter areas. Artemisinin and its derivatives are safe, well tolerated and convenient to use for severe as well as for uncomplicated malaria. At prices that, in some areas, may be as low as US$ 1.00-US$ 2.00 per adult treatment dose, they have become popular among health professionals and populations in malaria endemic areas in South-East Asia. It is feared that their widespread and irrational use could lead to parasite resistance.

The extent of their availability is illustrated by the following data on sales of some of the formulations of these drugs. Sales of artesunate tablets by producers in China have increased steadily from 185 000 adult doses in 1991 to 2 545 000 adult doses in 1995. It is estimated that about 60% of this production is exported to countries such as Myanmar, Viet Nam and others. In Viet Nam, 1 060 000 adult doses of oral formulations of artemisinin and artesunate were produced by the major local manufacturers in 1994, and 630 000 adult doses were produced in 1995. The Vietnamese production is sold mainly on the domestic market. In Thailand, which does not produce these drugs, the annual consumption is estimated to be 50 000 treatments per year.

The indications and dosages of these drugs, were reviewed by WHO in 1995 (see *Management of uncomplicated malaria and the use of drugs for the protection of travellers,* WHO/MAL/96. 1075).

2.2.1 Registration

When the registration of a new drug or formulation is considered in all countries of the two Regions, a need must be documented, and safety, quality and efficacy guaranteed.

In Thailand, a certificate of free sale in the country of origin is normally required. If it is not available, a new drug has to undergo a clinical trial in Thailand before it can be registered. New formulations can be registered if they are superior to already registered formulations in terms of price, quality or other relevant factors. Most countries in the two Regions have similar registration schemes but the national capabilities for assessing safety, quality and efficacy are variable.

The implementation of national norms and policies is also variable and a large number of formulations, which have not been registered, can be bought in pharmacies and even in market-places in some countries, This may have advantages, as competition helps to keep prices low. However, in some areas, it becomes increasingly difficult for providers of care to use the wide range of formulations correctly.

2.2.2 Quality control

Quality control is an important issue in relation to artemisinin and its derivatives because most of the available formulations are not produced according to Good Manufacturing Practice (GMP) standards. The matter is further complicated by misleading advertising and black-market activities.

Most countries have some capacity to carry out quality control of drugs. This is normally linked to industry or to government. However, only few countries such as China, Malaysia,

Viet Nam and Thailand, can, at present, carry out quality control of artemisinin and its derivatives. The main problem is the absence of updated technologies and pharmacopoeial norms. Even where the technologies are available and norms have been established nationally for the main formulations of artemisinin and its derivatives, quality control may not be applied sufficiently in relation to the variety of formulations available in different areas of the country.

WHO has established collaboration for quality control with University Sains Malaysia, Penang, Malaysia, which is now accepting samples of any antimalarial drug from any country. This work is carried out with limited financial support. It is obviously impossible for this institution alone to cope with the demand for this service in the long run, and there is an urgent need for the establishment of additional international reference centres, as well as for strengthening national capacities.

It is particularly important that more effort is made to bring the existing productions up to full GMP level, and that the current practices of quality control are replaced by systems of quality assurance.

2.2.3 The role of artemisinin and its derivatives in severe malaria

It is a priority for all malaria control programmes to ensure early and effective treatment of severe malaria to prevent death. The early, effective treatment of uncomplicated malaria is essential for preventing severe malaria.

While quinine is still considered the drug of choice for severe malaria in those countries that do not have a problem of multidrug resistance, artemisinin and its derivatives are increasingly being used for this purpose in the countries from Myanmar in the west to Viet Nam in the east. Typically, artemether for intramuscular injection and/or artesunate for intramuscular or intravenous injection are available at hospitals, while the drug used for initial treatment before referral to hospital in many areas is intramuscular quinine.

Despite irregular pharmacokinetics and generally low plasma levels after administration, artemisinin and artesunate suppositories have been used successfully for the treatment of severe malaria. There is no international consensus on dosage schedules but the national guidelines in Viet Nam recommend a mg/kg schedule identical to that of oral artemisinin or oral artesunate (see WHO/MAL/96. 1075). Suppositories of artemisinin and its derivatives are considered a promising approach to the treatment of severe malaria in circumstances where injections cannot be given safely.

In China, Thailand , and Viet Nam, the tendency is now to replace the use of quinine with artemisinin and its derivatives in the treatment of severe malaria. The reasons for this are as follows:

- quinine susceptibility is declining in several areas;
- parenteral formulations of artemether and artesunate are easier to apply than intramuscular quinine, especially in young children for whom the volumes of injection of quinine are small, and the therapeutic range is narrow;
- intramuscular quinine carries a risk of muscular necrosis, and has been associated with tetanus in Viet Nam. Artemisinin and artesunate suppositories are now available in Viet Nam; and

- it is possible that removing quinine from general use will ensure that its efficacy is preserved, so that it can be deployed again for the treatment of severe malaria, if resistance to artemisinin and its derivatives compromises their efficacy.

However, quinine is still considered preferable for treating pregnant women in the first trimester.

2.2.4 Use in private and public sectors

Based on an assessment of the epidemiological situations, including the extent of multidrug resistance and the characteristics of national malaria control programmes and health services, countries in the two Regions have taken four different approaches to the question of authorizing the use of artemisinin and its derivatives in public and private sectors. These are as follows:

- some countries do not have a problem of multidrug resistant malaria. They have no need to register artemisinin and its derivatives and these drugs are not available on the market (examples: Philippines, Bangladesh).

- some countries have registered artemisinin and its derivatives but, having strong malaria control programmes and well-regulated health services, they have restricted their use to specialized malaria services and to certain hospitals. In some cases it is under discussion whether the affected populations have sufficiently easy access to treatment with these drugs (example: Thailand).

- some countries have registered artemisinin and its derivatives but restricted their use to specialized malaria services and medical practice (example: Myanmar).

- some countries, realizing that some of the populations in endemic areas do not have access to either medical practitioners, or to public health services, allow these drugs to be traded by private and public pharmacies and market-sellers in villages (examples: China, Viet Nam).

In some areas and countries with severe multidrug resistance, artemisinin and its derivatives are available through the private sector at prices considerably above the typical world-market levels and above what is locally affordable. Such situations can lead to under-dosing and inappropriate combination treatments, ultimately resulting in treatment failure, risk of severe malaria, excessive costs to the patients and accelerated development of drug resistance. National programmes need to investigate such situations. If the prices are too high, corrective measures should be taken; in some instances, these may include more control, in others, increased liberalization.

2.2.5 Monitoring adverse reactions

Clinical trials including those with healthy volunteers, have demonstrated a transient decrease of reticulocyte counts and, at high dosage, a reduction of neutrophil counts in a limited number of cases following administration of artemisinin and its derivatives. A transient increase of plasma-levels of liver enzymes without any clinical correlate has also been observed in some trials. Mild febrile reactions soon after the start of treatment have been confirmed in several studies.

Animal experiments with injectable artemether and arteether have shown very severe neurotoxic effects, but only at daily dosages which were at least four times as high as those

conventionally applied for treatment, and only when treatment was prolonged or repeated several times In these studies, the audio-vestibular system was particularly affected. The results of these studies are disturbing, because it was not possible to detect milder warning signs at lower doses. In early dose-finding trials in China, careful neurological examination did not disclose any damage, but these investigations did not include audiometry or specialized examination of the audio-vestibular system. Likewise, there has been no evidence of neurotoxicity in more than 3000 cases treated in Thailand and followed up for 28 days or more.

A large number of Phase I-Phase III clinical trials as well as a rigorous system of post-marketing surveillance applied for two years in Thailand, have documented that artemisinin and its derivatives are safe at the dosage ranges which are currently applied for the treatment of malaria (see WHO/MAL/96.1075). Millions of patients have also been treated in China and Viet Nam, and many thousands in neighbouring countries. No deaths or serious adverse events which could be causally related to the treatment have been recorded. However, the post-marketing surveillance in Thailand where the drugs have been mainly used in combination with mefloquine has revealed a high frequency of side-effects which are similar to those documented after the administration of mefloquine alone. The possibility that the well-known adverse events of mefloquine could have 'masked' possible side effects of artemisinin and its derivatives should be considered.

Preclinical studies have consistently shown that artemisinin and its derivatives do not have mutagenic or teratogenic activity but all of these drugs caused foetal resorption in rats. Reports on the use of these drugs in pregnancy are limited but no abnormality was been detected in a small study of children born to mothers treated with artemisinin and its derivatives during the second and third trimester and followed for up to ten years. Malaria can be particularly hazardous during pregnancy. Thus, national and WHO guidelines recommend their use during pregnancy in areas of multidrug resistance if there is no other effective treatment available. They are contraindicated during the first trimester of pregnancy, but in a case of severe malaria, it may not be practically possible to ascertain whether a woman is pregnant before treatment is initiated. Their use should be based on consideration of the risks and benefits. For example, it may be justified with a case of severe malaria in an area with decreased quinine susceptibility of *P. falciparum*. As there is limited data, high priority should be given to the follow up of pregnancies during which artemisinin and its derivatives has been administered. This could be done in collaboration with Mother and Child Health programmes.

It can be concluded that artemisinin and its derivatives are safe as long as good quality formulations are used at conventional dosage. Nevertheless, repetitive treatment with these formulations should be treated with caution and, in areas where patients may be exposed to frequent treatments, specialized neurological examinations, including audiometry should be considered. In the future, it may become necessary to increase dosages, to try out new combinations or to apply repeat treatments in order to enhance efficacy. Such treatment schedules should undergo rigorous testing firstly in healthy volunteers, and with particular attention to specialized neurological examination, including audiometry.

2.2.6 Prevention of the development of resistance to artemisinin and its derivatives

Experience has shown that substantial drug pressure on wild populations of *P. falciparum* invariably leads to the development of drug resistance. The current variability in the susceptibility of the parasite *in vitro* to artemisinin and its derivatives, and the ease with which resistance can be developed in animal models makes it almost certain that the current pattern of use of these drugs will eventually lead to the development of resistance and an unacceptable low level of therapeutic efficacy. The best way of preventing the development of resistance is to reserve these drugs for

reserve these drugs for severe malaria. In such a situation the drug pressure would be slight and the efficacy of these rapidly acting drugs could probably be preserved for many years. However, until a novel, affordable, effective, safe and well tolerated drug for the treatment of uncomplicated malaria becomes available, the following measures may help in delaying the development of resistance:

- limit their use to areas only where there is a documented need;
- use as a combination treatment, for which mefloquine is currently the best 'partner'. Administration of three daily doses of artemisinin or artesunate with a single dose of mefloquine currently yields a cure rate of 100% even in multidrug resistant areas. However, this combination is relatively expensive, and it is possibly not the ideal solution in areas with intense transmission, where mefloquine with its long half-life could soon select in favour of mefloquine-resistant parasites;
- maintain strict compliance with treatment regimens;
- ensure strict follow-up of all treatments to ensure radical treatment of recrudescences. Experience has shown that this measure is least feasible in multidrug resistant areas with migrants or ethnic minorities; and
- experience shows that the development of resistance is generally less rapid in areas where transmission is effectively reduced by vector control. The application of effective vector control in multiresistant areas reduces the incidence of disease and, therefore, the need for treating with artemisinin and its derivatives.

2.3 Monitoring of therapeutic efficacy and parasite susceptibility to drugs

Monitoring therapeutic efficacy is the most important method for decision-making on treatment regimens and the development and review of national antimalarial drug policies. However, there is scope for monitoring drug susceptibility of the parasite to antimalarial drugs, both *in vivo* and *in vitro*, since a decrease in parasite susceptibility may provide early warning signs of the impending loss of therapeutic efficacy and the need to change local strategies for disease management. As artemisinin and its derivatives are used more and more widely, it becomes increasingly important to monitor resistance of malaria parasites to these drugs. Globally, an understanding of the dynamics of changes in the susceptibility of these drugs will be important for decision-making on their deployment in countries where they are not presently used. Table 1 provides a summary of the methods that can be used for monitoring therapeutic efficacy and parasite susceptibility of antimalarial drugs.

If drug resistance is defined conventially as "the ability of a parasite strain to survive and/or multiply despite the administration and absorption of drugs in doses equal to or higher than those normally recommended but within the limits of tolerance of the subject", it is not possible, as yet, to define resistance to artemisinin and its derivatives since the tolerance limits have still to be defined. Furthermore the definition of resistance to these drugs is constrained by the fact that recrudescences are common after the standard recommended five-day regimen. The occurrence of recrudescences is thought to be related to the difficulty of maintaining effective blood levels to kill all sensitive parasites. This difficulty is only partially overcome by prolonged treatment since blood concentrations of the drugs and their active metabolites tend to decline during continuous treatment, possibly through a mechanism of enzyme induction.

2.3.1 Monitoring treatment failures

In settings where microscope diagnosis is available, treatment failures detected by the health services might provide an early warning of problems of treatment effectiveness which require further investigation. The proportion of cases that return with clinical symptoms less than 14 days after treatment in areas of intense transmission or within 28 days in other areas might be a suitable indicator. Unfortunately, there is limited experience with this approach. Such a system has been implemented in Papua New Guinea since 1993 where the proportion of malaria treatment failures has been officially added to the list of main health indicators. This experience has identified several problems including the following:

- underreporting and late reporting, e.g., patients dissatisfied with the treatment go elsewhere for further treatment and return only if this is also ineffective;
- the need for an efficient health information system with rapid feedback of data;
- the need for proper training and supervision of health personnel to recognize and report malaria treatment failures;
- poor coverage due to the lack of microscopy at the periphery;
- the preference of patients for a second treatment based on clinical diagnosis because of charges for microscopical diagnosis.

Monitoring of treatment failures by the health services may provide an early warning of problems of reduced drug susceptibility in areas with well developed health information systems. Success depends on collaboration between the general health services and national malaria control programmes.

2.3.2 Monitoring therapeutic efficacy and *in vivo* testing of drug susceptibility

The techniques for the assessment of therapeutic efficacy and of *in vivo* testing for drug susceptibility of parasites are closely related. Methodologies for assessment of therapeutic efficacy originated as *in vivo* tests into which a clinical component was incorporated. However, their use has two different purposes. Assessment of therapeutic efficacy aims at determining whether a treatment regimen of a single drug or drug combination works, i.e. cures the disease in a patient population in a given area. The purpose of *in vivo* testing for drug susceptibility is to determine the effect of a drug on a parasite population (constituting the infection of a human host). The drugs employed for both tests should be of a reliable and quality-controlled batch. WHO can provide collaboration in their procurement, if needed (see the table).

2.3.2.1 Monitoring therapeutic efficacy

A detailed protocol for assessing the therapeutic efficacy of antimalaria drug regimens used in countries of the South-East Asia and Western Pacific Regions was discussed at the meeting. A document will be issued from the WHO Malaria Unit in Geneva. It will be applicable to all drug regimens currently recommended for the treatment of uncomplicated malaria in the two Regions and is based on a protocol previously developed by WHO for use in children in tropical Africa.

The protocol proposes clinical examinations, temperature measurements, and parasite counts to be carried out on Days 0, 3, 7, 14, 21, and 28, with haemoglobin or haematocrit measurements on Days 0, 14 and 28 in areas where malaria-associated anaemia is important, such as Papua New Guinea. An additional evaluation could be carried out on Day 2 in areas where it is desirable to have comparative results with RIII levels measured in the past. If the patient returns with clinical deterioration on any other day between Day 0 and Day 28, he or she should be fully evaluated with a complete clinical examination, temperature measurement and assessment of parasitaemia. The clinical examination should include the assessment and recording of adverse events which may have been caused by the antimalarial drugs. Every effort should be made to ensure each patient is "followed up" the basic minimum of 14 days, but a 28-day test is more desirable in areas of low or moderate transmission. A 14-day test is more appropriate for areas with intense transmission.

The inclusion criteria are listed below:

- all age groups above six months (excluding pregnant women)
- monoinfection with *P. falciparum* or *P. vivax* in areas with a resistance problem
- *P. falciparum* parasite density of 1000/ul-10 000/µl (the upper limit can be set to 100 000/µl for artemisinin and its derivatives) or *P. vivax* parasite density of 1000/ul-10 000/µl
- absence of general danger signs or signs of severe and complicated malaria
- history of fever during the last 48 hours
- axillary temperature $< 39.5\ ^{\circ}C$
- absence of febrile conditions caused by diseases other than malaria
- ability to come for the stipulated follow-up visits, and easy access to the health facility at all times
- informed consent by the patient or by parent/guardian.

The following are the criteria for classification of therapeutic response:

Early treatment failure (ETF) if any of the following conditions occur:

- development of danger signs or severe malaria on Day 1, Day 2 or Day 3 in the presence of parasitaemia
- parasitaemia on Day 2 > Day 0 count
- parasitaemia on Day 3, 25% of Day 0 count

Late treatment failure (LTF) if the patient did not develop any ETF before, and one of the following conditions occur:

- development of danger signs or severe malaria after Day 3 in the presence of parasitaemia (same species as on Day 0)

- unscheduled return of the patient after three days because of clinical deterioration in the presence of parasitaemia (same species as on Day 0)

- persistent parasitaemia on any of scheduled return visits on Day 7, Day 14, Day 21 and Day 28 (same species as on Day 0)

Adequate clinical response (ACR) if the patient did not develop any of the conditions for ETF and LTF before, and parasitological clearance has been confirmed throughout the follow-up period.

Monitoring of therapeutic efficacy should be carried out through a system of well selected sentinel sites in order to obtain consistent longitudinal data and to document trends. At the initial stage, a national core group of experts should be established to coordinate all the activities, i. e. training, supervision, collection/analysis of data, and to forward recommendations to drug policy-makers. This core group should ensure good quality of diagnostic laboratories in the sentinel sites and provide continuous logistic support.

The minimal requirements to establish a sentinel site are the availability of trained clinical personnel and microscopists, with a laboratory for blood film examination. This can be at the periphery (community-based), or based at a health facility at district level. Hospitals of urban areas may not be suitable choices for sentinel site monitoring since the cases presenting at such facilities may have more complex clinical presentations, be more likely to have been referred because of previous drug failures and may be more difficult to follow up. Whenever possible the monitoring should be done at the periphery.

The following criteria should be considered in the selection of sentinel sites:

- distribution of malaria treatment failures reported by the health information system

- epidemiology of malaria, especially intensity and seasonality of transmission

- population mobility and migration (especially in border areas)

- the logistics for drug distribution

The sentinel sites should be selected to be representative of each major strata in which the country can be divided as regards to drug resistance. Empirically between three and six sentinel sites might be considered for each of the meaningful strata. When making such decisions, emphasis must be placed on the need for a "manageable" number of sites to ensure proper monitoring and supervision.

Monitoring can be carried out either by local personnel at the sentinel site or by a more specialized mobile team. The choice will vary with each country's situation, depending mainly on national resources and the availability of trained staff at the selected sentinel site. In Viet Nam, mobile teams at provincial or district level are assigned to carry out the tests. Due to the importance of the private sector in drug procurement and distribution in many countries, and the heterogeneity of drug resistance, drug utilization studies should be conducted, whenever feasible, in the areas selected for sentinel site monitoring.

2.3.2.2 *In vivo* testing of drug susceptibility

There is a particular need to monitor the drug susceptibility of *P. falciparum* to the oral formulations of artemisinin and its derivatives currently in widespread use, i.e., artemisinin and artesunate, and to quinine. A protocol for testing these drugs is given in Annex 3.

It is similar to that used for assessing therapeutic efficacy but differs in that:

- both patients and asymptomatic parasite carriers can be included, but the study report must state whether one or the other was used;
- only single-drug regimens are used and not drug combinations; and
- the intake of antimalarial drugs should be excluded by whatever means are available and appropriate in relation to the local pattern of drug use.

The meeting arrived at a consensus that drug susceptibility of artemisinin and artesunate should be assessed through analysis of the parasitological response during and after a seven-day treatment with artemisinin or artesunate. The choice of a seven-day regimen is based on the observation that a seven-day treatment is more efficacious than a five-day treatment, and the consideration that a treatment of longer duration although probably 'tolerable' and safe would never be practically applicable as a standard treatment of the disease.

The methodology is mainly of interest for monitoring of drug susceptibility of parasite populations at sentinel sites over a period of time. If results from these tests are to be compared, reproducibility of test conditions is essential. The follow up period must be at least 14 days, as a shorter follow up may be too insensitive to initial changes in susceptibility. Follow-up can be extended to 28 days if reinfection can be excluded.

2.3.3 *In vitro* methods

In vitro tests are invaluable as a research tool to investigate a number of issues related to drug susceptibility of *P. falciparum*. Standard test kits are available for testing the susceptibility to chloroquine, amodiaquine, quinine, mefloquine and sulfadoxine/ pyrimethamine combinations. These kits are produced at cost by the Department of Health, Manila, Philippines. Standardized tests are also required for artemisinin and its derivatives since there is a great need for acquiring a better understanding of changing drug susceptibility patterns as these drugs are now used on a large scale. For example, it is important to know whether susceptibility decreases more rapidly in areas with intense transmission, whether it is more affected by higher or lower dosage schedules, and whether combination with mefloquine can really protect these drugs (in this context it is also important to monitor mefloquine susceptibility). Ideally, *in vitro* methods should be applied to representative samples in areas where artemisinin and its derivatives have never been applied, and where their use is envisaged, to obtain baseline levels. *In vitro* methods are also useful for investigating cross-resistance patterns (see the table).

It must be emphasized that *in vitro* monitoring should not be considered a routine programme activity. It should be applied with a definite purpose, with valid epidemiological sampling methods and good technique, respecting such factors as the shelf-life of the plates, and the need to exclude the presence of drugs in the patient's blood. It is technically demanding but a number of centres in the two Regions have the necessary capabilities, not only for carrying out *in vitro* tests but also for training new staff to do it.

A consensus was reached to apply one standard *in vitro* test with plates predosed with artemisinin. The choice of artemisinin as the reference drug is based on the observation that the derivatives cannot be preserved on predosed plates while, on the other hand, *in vitro* results with artemisinin correlate very well with results obtained with dihydroartemisinin, to which artesunate is hydrolyzed in aqueous solution.

The details of the *in vitro* test are described in Annex 4. It is a 24 hour incubation test, based on the WHO standard method for assessing inhibition of schizont maturation using the same materials as those contained in the WHO standard test-kits for other drugs. The concentration range for artemisinin corresponds to 3, 10, 30, 100, 300, 1000 and 3000 nmol per litre blood-medium mixture, the volume of blood-medium mixture being 50 microliter per well. It is foreseen that the production of artemisinin plates will be done at the test-kit production unit, Department of Health, Manila, Philippines. The plates will require refrigeration since they have a short shelf-life when kept at room-temperature. This is less than one week at room temperatures around 30°C. There may be a possibility of extending the shelf-life by sealing them under nitrogen but this requires validation. Under conditions where cold-chain transport is not feasible, it is proposed to supply a kit including non-dosed plates, artemisinin powder, standard quality artemisinin solvents and a description of the method of preparing stock and dosing solutions so that predosed plates can be prepared at the site of the test.

The IBM/PC software used for regression analysis of the data is the same as that used for other standard WHO *in vitro* tests. It will also be made available through the test kit production unit.

2.4 Border malaria and information exchange

Malaria in border areas continues to be a major problem for control programmes throughout both the South-East Asia and Western Pacific Regions. The features of malaria found in the South-East Asia hill zone generally have so many similarities that experiences in one country will be valid in many instances in another.

Most international borders are invisible and groups of people regularly cross back and forth without any sort of control. Many borders are in heavily forested regions where activities such as mining and logging attract large population groups with many coming from cities and towns. These workers may have no previous malaria exposure and so infections result in high rates of morbidity and mortality. Housing and sanitation conditions are poor, access to health facilities is often difficult, and all types of antimalarial drugs may be available through private channels leading to high rates of self-treatment. The combination of these factors with often high levels of malaria transmission provide optimum conditions for a high incidence of severe forms of malaria and the rapid development and spread of multidrug resistant parasites.

Malaria control programmes generally find it difficult to mount effective measures in border areas due to the difficult terrain, the highly mobile nature of the population, and security problems in many places. Some border areas are populated with minority ethnic groups whose contact and cooperation with governmental health services is limited. During the period of malaria eradication some countries maintained buffer areas along their borders where intensive control measures were applied as a barrier to the movement of malaria but in most cases, this approach has failed and has long been abandoned.

Even in areas where effective control activities are being carried out there is often little or no information about the malaria situation on opposite sides of the border. There are many

examples where workers, such as loggers and miners, are infected on one side of a border but return to the opposite side for treatment. In these cases an outbreak detected on one side is not reported to health officials on the opposite side because of the lack of a functional channel of communication. Even in places where control measures can be effectively applied, the impact may be significantly reduced by the lack of coordinated control measures on the other side of the border.

For some decades, the political and military problems in the south-east Asian peninsular were such that attempting collaboration for the control of border malaria appeared unlikely to succeed but this is now beginning to take place, albeit on a limited scale. For example, a number of provinces in Viet Nam and the Lao People's Democratic Republic with common borders have established bilateral cooperation which is encouraged by central authorities.

One mechanism for the exchange of information on malaria has been border meetings. These include border meetings for Brunei Darussalam and Indonesia, Malaysia and Singapore; regular meetings between Thailand and Malaysia; and the south-west Pacific malaria meeting involving Australia, Papua New Guinea, Solomon Islands, Vanuatu and Indonesia. Since August 1995, four border meetings were held in the South-East Asia Region, involving Bangladesh, Bhutan, India, Myanmar, and Nepal and two more will be held in the near future; the first involving India, Maldives and Sri Lanka and the second between Myanmar and Thailand. Regular meetings between Cambodia, the Lao People's Democratic Republic and Viet Nam are planned as part of the European Union's support for malaria control in these countries. Such meetings should not only aim to exchange information but should be used as a starting point to plan practical activities for collaboration between cross-border provinces. To achieve this aim they need to involve operational field staff from neighbouring provinces. The presence of operational staff would also establish personal contact and stimulate direct communication between local control programmes in border areas.

Indicators for assessing the malaria situation and its control have already been developed by the interregional meeting held in Kunming in 1993. These have been adapted by South-East Asia Regional Office as a set of forms to promote the exchange of information on border malaria. These forms are designed to provide a common framework for the exchange of epidemiological and operational information needed to coordinate border malaria control activities. When exchange of information across international borders is developed, local managers can be alerted more quickly to an abnormal development on the other side. It also provides an opportunity for joint critical assessments of local situations, exchange of experiences, possible joint action and mutual support in the face of critical situations. In many border areas, the status of drug resistance, and drug availability and utilization are often the most important issues to clarify. Control programme managers need valid and objective information on these issues to make rational decisions on the deployment of antimalarial drugs. The conclusions and recommendations contained in this Report should facilitate this initiative.

Rapid information exchange is imperative in epidemic and emergency situations. The minimum information requirements are given in Annex 5. This should be forwarded by the provincial or district malaria programme managers to central level and also to counterparts in neighbouring border provinces.

Progress in informatics technology, including mapping and geographical information systems, and the Internet has created a demand for improved information exchange. Such systems could have practical importance in improving rapid information exchange and action both internationally and nationally, at the central and provincial levels. Financial constraints have been

the main reason why such systems have not been widely established but increasingly, national malaria control programmes have access to computer technologies. Funding from the European Union could be instrumental in establishing such networks in Cambodia, the Lao People's Democratic Republic and Viet Nam.

3. CONCLUSIONS

3.1 Drug policy: issues related to artemisinin and its derivatives

- malaria control programmes which identify areas of multidrug resistance, should define vector control measures, which can effectively reduce incidence of the disease in those areas, and apply them rapidly, vigorously and persistently. Multidrug resistance is not the only criterion for application of vector control, but it is one of the most important factors to consider when resources are limited and priorities must be defined;

- WHO should ensure the establishment of international pharmacopoeial norms and standards for the formulations of artemisinin and its derivatives currently in operational use;

- more international reference centres for quality control of antimalarial drugs should be established in the two Regions;

- national malaria control programmes should include widespread and regular quality control of antimalarial drugs used in both public and private sectors as part of routine programme activities;

- priority should been given to the follow up of pregnancies during which artemisinin and its derivatives have been administrated since limited data exists for this indication.

3.2 Monitoring of therapeutic efficacy and drug resistance

- the protocol that includes both a clinical and parasitological evaluation for assessing therapeutic efficacy of antimalarial drug regimens should be adopted for the purpose formulating/modifying national drug policies;

- in view of the current widespread use of oral formulations of artemisinin and its derivatives and quinine, *in vivo* and *in vitro* studies on the effect of these drugs on *P. falciparum* should be urgently carried out to determine the changes to the patterns of susceptibility of parasites to these drugs;

- kits for testing the susceptibility of *P. falciparum* to artemisinin and its derivatives *in vitro*, should be produced and evaluated according to the guidelines given in this Report;

- national health information systems should be strengthened to allow the routine reporting of malaria treatment failures as an indicator of potential problems of drug resistance;

- a core group of expertise is required in national malaria control programmes to monitor the antimalarial drug resistance situation, to plan and implement monitoring activities, analyse data for decision making and to liaise with the general health services and those responsible for national antimalaria drug policies;

- a national network of sentinel sites representing different epidemiological situations should be established to monitor therapeutic efficacy and drug susceptibility using standard protocols; and

- a mechanism to monitor drug utilization pattern both in the government and private sector in the catchment of the sentinel sites used for drug resistance monitoring should be established.

3.3 Border malaria and information exchange

- bilateral meetings should be supported as one mechanism for the exchange of information about malaria and other important diseases affecting populations in border areas;

- border meetings should involve not only administrators and policy-makers at the national level but also those operational staff and other field workers at the district and provincial levels who are actually dealing with the specific problems in the border areas;

- a directory should be compiled that contains the names, addresses, phone and FAX (and e-mail) numbers of both malaria control staff and health staff in border areas. This will greatly facilitate the direct exchange of information such as reports on outbreaks, information on the opening of new settlement areas, and major population movements that may affect the malaria situation. This directory may ideally contain simple maps showing border districts, major tows and administrative centres;

- a special form should be adopted as the standard mechanism for the exchange of information on outbreaks or epidemics in border areas. This form should be sent directly to the malaria control officer or responsible health official on the opposite side of the border as the first step in mounting a coordinated response to the outbreak or epidemic; and

- interregional cooperation should be strengthened to enable border countries to develop common strategies and approaches for the control of multiresistant falciparum malaria. The Biregional meetings on the Control of Communicable Diseases provide a mechanism for such initiatives.

TABLE. MONITORING OF THERAPEUTIC EFFICACY AND DRUG SUSCEPTIBILITY

Type of test	Routine treatment failure	Therapeutic Efficacy	Drug Susceptibility *in vivo*	*In vitro* tests
Purpose	Alerts programme managers to changes in therapeutic efficacy	Determines therapeutic efficacy of standard treatment i.e. does the drug cure the patient	Determines drug susceptibility of the parasite i.e. does the drug kill the parasite in the patient	Determines drug susceptibility of the parasite i.e. does the drug kill the parasite
Source of information	Routine reporting from health information	Study using standardised protocol	Study using standardized protocol for artemisinin and derivatives, and quinine	Research Study (non routine)
Programme component	General health services	General health services/malaria control programme	Malaria control programme/research institutions	Malaria control programme/ research institution
Target group	Patients with uncomplicated malaria	Patients with uncomplicated malaria	Asymptomatic and symptomatic patients with uncomplicated malaria.	Parasite isolates
Indicator	Proportion of patients returning within 14-28 days of treatment with clinical symptoms	Early and late treatment failure, adequate clinical response during a minimum of 14 days follow up	Parasitaemia on Days 2, 7, 14 and 28.	EC_{50}, EC_{90}, MIC
Outcomes	Early warning system	Provides relevant information for drug policy formulation/revision	Provides information on changes of drug susceptibility that may precede changes in therapeutic efficacy	Baseline data on drug susceptibility. Cross-resistance patterns. Changes in drug sensitivity with time
Specific exclusion/inclusion	-	Includes multiple drug treatment. No exclusion if patient previously treated	Exclusion if previous treatment. Only single drug treatment	-
Advantages/ constraints	Potentially large coverage and early warning. Major effort required to incorporate into health information system. Poor coverage.	High sensitivity. Reinfection may confuse interpretation of late clinical failures after day 14.	High sensitivity. Reinfection may confuse interpretation of recrudescences after day 14	Limited number of institutions with technical capability.
Requirements	Efficient health information system. Laboratory diagnosis. Health services at periphery.	Trained clinical and laboratory staff at sentinel sites or mobile teams. Follow up of patients essential	Trained laboratory staff at site or mobile teams. Follow-up of patients essential	Well trained technical staff. Laboratory support.

ANNEX 1

DETAILED PROGRAMME OF THE INTERREGIONAL MEETING ON MALARIA CONTROL WITH EMPHASIS ON DRUG RESISTANCE 21-24 October 1996

Chairman: Dr Leonard Ortega, Philippines
Co-Chairman: Dr Shiv Lal, India

Monday, 21 October 1996

0800 - Registration

0900 - Opening ceremony
Regional Director's opening address
Self introductions
Designation of Chairman, Co-Chairman and Rapporteurs
Administrative announcements
Group photograph

0930 - Coffee break

1000 - Objectives and content of meeting

1015 - Malaria suveillance and control with special reference to interregional and border issues in the Regions

1115 - Country presentations (Cambodia and Thailand)

1200 - Lunch break

1330 - Continuation of country presentations (Lao People's Democratic Republic, Myanmar, China and Viet Nam)

1445 - Coffee break

1500 - Continuation of country presentations (India, Bangladesh, Malaysia and Indonesia)

1600 - Informal get-together (hosted by Regional Director)

Tuesday, 22 October 1996

0800 - Continuation of country presentations (Philippines, Solomon Islands, Papua New Guinea and Vanuatu)

0900 - **Antimalarial drugs**

Artemisinin derivatives and other new drugs
(Professor Looareesuwan, Thailand)

Surveillance of side effects to artesunate and artemether
(Ms Hutangkabodee, Thailand)

0930 - Coffee break

1000 - Quality control. Drugs and *in vitro* test kits
(Dr Trigg)

Drug policies and distribution. The role of the private sector
(Dr Denis, Cambodia)

1100 **Drug resistance and surveillance**

In vitro methods and their role in the surveillance of drug response of *P. falciparum* (Professor Wernsdorfer)

1120 *In vitro* moniroting of the susceptability of *P. falciparum* to artemisinin and its derivatives (Dr Tang Lin Hua, China. Discussants: Dr Nguyen Duy Sy, Dr Jeeraphat Sirichaisinthop, Professor Wernsdorfer)

1200 Lunch break

1400 *In vivo* monitoring of the susceptability of *P. falciparum* to artemisinin and its derivatives
- Viet Nam (Dr Schapira and Dr Nguyen Duy Sy)
- China (Dr Li Guoqiao)
- Discussant: Dr Bjorkman

1445 Protocol for assessment of therapeutic efficacy of antimalarial drugs
(Dr Bosman and Dr Trigg)

1530 Coffee break

Wednesday, 23 October 1996

0800 - **Border malaria and information exchange**

Malaria surveillance and control in Yunnan Province and other border areas of China (Dr Tang, China)

Design and implementation of information systems: TDR related activities
(Mr Wayling)

Operational research priorities
(Dr Orlov)

0930 - Coffee break

1000 - Discussion on border malaria and information exchange in SEAR and WPR countries

1200 - Lunch break

1330 - Group discussions: Disease management and drug policies, drug resistance surveillance, malaria control in border areas and information exchange

1430 - Coffee break

1500 - Continuation of group discussions

Thursday, 24 October 1996

0800 - Group discussions and reports

0930 - Coffee break

1000 - Group drafting of recommendations

1100 - Plenary session: Presentation of Group 1

1200 - Lunch break

1300 - Plenary session: Presentation of Groups 2 and 3

1445 - Coffee break

1500 - Finalization of recommendations and closure of meeting

ANNEX 2

LIST OF PARTICIPANTS, TEMPORARY ADVISERS, REPRESENTATIVES/OBSERVERS AND SECRETARIAT

1. PARTICIPANTS

CAMBODIA	Dr Yi Poravuth Medical Officer National Malaria Centre Phnom Penh
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ANNEX 3

IN VIVO TESTS FOR ASSESSMENT OF THE SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO ARTEMISININ AND ITS DERIVATIVES AND QUININE

The WHO 7 and 28 day *in vivo* tests have only been standardized for chloroquine. They have proven useful for assessing the response of parasite strains to such antimalarial drugs as sulfadoxine-pyrimethamine and mefloquine, but they are not immediately applicable to very short-acting drugs, which need to be given in prolonged regimens.

Monitoring of therapeutic efficacy is the most important method for decision-making regarding antimalaria drug policies. However, monitoring drug susceptibility of parasite strains is still important. It may, for example, provide early warning signs about impending loss of therapeutic efficacy, and this could lead to a change in the local control strategy. Parasite susceptibility may be determined by *in vivo* and *in vitro* techniques. However, to define *in vitro* threshold levels for resistance, it is necessary to correlate *in vivo* and *in vitro* test results obtained on the same subjects, after first having defined which *in vivo* results should be interpreted as resistant and which as sensitive. One important purpose of *in vivo* testing as proposed here is to provide a basis for such correlations.

If drug resistance is defined as conventionally, *the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject,* then it is not possible to define resistance to artemisinin and derivatives, for the limits of tolerance of these drugs have not been defined yet.

The recommended standard duration of monotherapy with artemisinin at a maintenance dose of 10 mg/kg per day or artesunate at 2 mg/kg per day is 5 days, but higher efficacy may be attained with a 7 day regimen. In a trial on 50 subjects, with 28 days of follow-up, where reinfection was excluded, treatment with artesunate for 5 days cured 80%, while a 7 days treatment cured 96%. There is no good data on the efficacy of even longer artemisinin or artesunate regimens, but because of compliance problems, it is unlikely that regimens lasting for more than 7 days would ever be used routinely. Animal experiments have raised concerns about the possibility of neuro-toxicity related to high, prolonged or repeated dosing, but the lowest total doses per kg body weight associated with such toxicity have been several times higher than the total doses of the current 3, 5 and 7 day regimens for uncomplicated malaria. It is therefore proposed that the standard duration of treatment for *in vivo* assessment of sensitivity to artemisinin and its derivatives should be 7 days.

In contrast to the artemisinin drugs, quinine has unpleasant minor side effects, and a maintenance dose of 24 mg base per kg body per day is probably close to the limit of tolerance. The normally recommended regimens include quinine for up to 7 days in combination with tetracycline or doxycycline. A treatment duration of 7 days for a standard *in* vivo test for sensitivity to quinine is therefore a logical choice.

Annex 3

Standard treatment regimens for *in vivo* tests

1. Artemisinin tablets

   20 mg per kg (single dose) on Day 0, and

   10mg/kg (single dose) daily on Days 1 to 6.

2. Artesunate tablets

   4 mg per kg (single dose) on Day 0, and

   2 mg per kg (single dose) daily on Days 1 to 6.

3. Quinine tablets

   8 mg base per kg 3 times daily for 7 days. (8 mg base corresponds to 10 mg quinine hydrochloride, dihydrochloride or sulfate).

It is of paramount importance that the quality of the medicaments is guaranteed. The treatment must be fully supervised: all doses must be taken in front of competent staff; in case of vomiting within half an hour after intake, the dose must be repeated and the occurrence recorded.

Sampling and inclusion criteria

The methodology is of interest for longitudinal monitoring by examining samples of patients at sentinel sites. Consecutive, systematic or random sampling schemes can be applied. Sample size should be determined on the basis of the expected proportion of unusual findings.

The inclusion criteria for *in vivo* studies are as follows:

- Patients who are not severely ill or asymptomatic parasite carriers can be included, but the study report must state whether the subjects studied belong to one or the other of these two categories.

- Asexual parasite density must be at least 1000/µl, and mixed infection excluded.

- By whatever means are available, intake of antimalarials during the latest month should be excluded, as their intake several weeks before might influence the response in a drug test. This criterion may be difficult to apply. As a minimum, intake during latest week should be excluded by careful questioning. Where chloroquine or sulfadoxine-pyrimethamine are commonly used, urine tests should be applied.

Follow-up

The follow-up period should optimally be 28 days, but follow-up for 14 days is also expected to yield valuable results. With 14 or 28 days follow-up, reinfection should preferably be excluded, but if that is not possible, data indicating the intensity of transmission should be provided with the study results. Complete compliance with follow-up is important, as defaulting may confound the interpretation of the results. The occurrence of treatment failures leading to interruption of the *in vivo* test must be included in the study report.Slides should be taken for thick and thin films just before start of treatment (D0), on D2 (48 hours after start of treatment), D3 (72 hours after start)

and on D7, D14, D21 and D28. Also at any time the patient has fever after D3. Attention must be paid to the preparation of the thick film. Each oil immersion field must have about 20 white blood cells. Exactly 100 fields of the thick film are examined (approximately 0.25 µl). Parasites should be counted against white blood cells, and parasite density calculated on the assumption of 8000 leukocytes/µl.

It has recently been reported that a positive *Para*Sight™-F test on day 14 may be a good predictor of parasitological recrudescence on day 28. This result needs further corroboration but represents a potentially important advance.

If necessary, additional slide-checks can be included. *In vivo* testing may be combined with assessment of therapeutic efficacy, provided that the inclusion criteria for both methodologies are respected, and it is relevant to assess therapeutic efficacy of a 7 day regimen. It is also possible to conduct a study of therapeutic efficacy and include the patients which satisfy *in vivo* test entry criteria in the analysis for susceptibility.

Analysis and interpretation

The main outcome variables are:

- Proportion of cases positive (i.e. patent asexual parasites of *P.falciparum*) on D2
- Proportion of cases positive on D3
- Proportion of cases positive on any day from D4 to D7
- Proportion of cases positive on any day from D8 to D14
- Potentially: Positive *Para*Sight™-F test or similar test on D14
- Proportion of cases positive on any day from D15 to D28
- Parasite clearance time

These proportions can be compared statistically between different materials. Standard software such as EPI Info may be suitable for such comparisons, when the full set of patient data is entered as records, and will also allow some control of potential confounding factors, such as age and initial parasite density.

Resistance can be said to be present, if the slide is positive on D7 or, if reinfection can be excluded, on any day after D7. Recrudescences during the third and fourth week after treatment will be indicative of early signs of resistance. Further studies are needed, before it can be stated, whether cases classified as resistant by this test are generally caused by parasites with low drug susceptibility or whether host factors play an important role. However, a significant change over time in the outcome variables, if test conditions are constant, can plausibly be ascribed to a change in parasite susceptibility.

A positive slide on D2 or D3 suggests an unusually long parasite clearance time for artemisinin and its derivatives. Therefore an increase of the proportion of positive slides on D2 or D3 or of the parasite clearance time is potentially useful for monitoring changes in drug susceptibility, but this requires further validation.

ANNEX 4

IN VITRO FIELD TEST FOR ASSESSING THE SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO ARTEMISININ

1. Introduction

In view of the increasing therapeutic use of artemisinin and its derivatives it is desirable to have a standard method for assessing the in vitro sensitivity of *Plasmodium falciparum* to this group of drugs. This need is accentuated by the fact that recrudescence after treatment with these drugs may occur in infections with parasites which are basically sensitive and where the reason for the recrudescence may have to be sought in host factors such as individual pharmacokinetic or metabolic features.

Several test systems for measuring the sensitivity of *Plasmodium falciparum* to artemisinin and its derivatives have been developed in the past, most of them applicable to continuous culture systems. The adaptation of the WHO standard sensitivity test system to artemisinin, including validation, dates from 1991. The method consists of measuring the concentration-dependent inhibition of schizont maturation (SMIT) under the influence of artemisinin.

Attempts were also made to adapt the SMIT system to artemether and dihydro-artemisinin, but it was observed that the bio-activity of artemether on the plate decreases fast, due to rapid absorption into the plastic material of the plate. With dihydro-artemisinin the shelf-life of the predosed plates is too short. However, comparative determination of the sensitivity to artemisinin and artemether, or artemisinin and dihydro-artemisinin, in paired isolates of *P. falciparum*, using freshly prepared plates, has shown a close correlation of the quantitative response.

2. Test Plates

2.1 Lay-out of plates

As the dose-response line of artemisinin is relatively flat and only 7 drug-dosed wells are available for each test line of the standard 8 x 12 microtitre plate (Falcon 3070) the customary two-fold progression will produce too narrow a concentration range. Therefore a geometric progression of about 3 has been chosen. A concentration range equivalent to 3 - 3000 nmol/l blood-medium-mixture (BMM) has proven to be appropriate. The specific doses per well and the corresponding concentrations per litre BMM are given in the following Table:

Well	Dose in pmol	Concentration in nmol/l BMM
A	0	0
B	0.15	3
C	0.50	10
D	1.50	30
E	5.00	100

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F	15.00	300
G	50.00	1000
H	150.00	3000

2.2 Routine production of plates

At the central production facility, Manila, Philippines, the plates are produced according to a standard protocol which provides also for appropriate quality control.

2.3 Storage and transport of test plates

Freighting to users is to be done under cold-chain conditions (4°C). At destination it will be advantageous to resume storage at -20°C if the plates will not be used within three months. Otherwise storage at 4°C will be adequate. Before setting up the tests, the plates should be allowed to acquire room temperature while they are still sealed in the plastic bag in order to avoid (a) the condensation of water which could interfere with sterility and (b) cold shock of the parasites.

The test plate contains 12 test lines. These will be rarely used up in one test round. The plate can be used safely, without loss of activity, for one further round if the rounds follow each other without interruption or if the plate is stored at 4°C between the rounds. However, once incubated, the plate should be discarded when not used up within one month.

3. Performance and analysis of the test

Pre-evaluation of subjects, preparation of growth medium and performance of the micro-test follow the instructions in Section 4 of WHO document MAP/87.2 (Corr. 1 incl.) Revision 1. This document can be requested from CTD/INF.DOC, WHO, CH-1211 Geneva 27, Switzerland, or the WHO Regional Office for the Western Pacific, P.O. Box 2932, 1000 Manila, Philippines.

The counting procedure for the SMIT with artemisinin is the same as used for chloroquine, quinine, mefloquine and amodiaquine. This is described in Sub-Section 5.2 of WHO document MAP/87.2 (Corr.1 incl.) Revision 1.

The quantitative analysis of grouped test data can be done according to the log-concentration/response probit procedure of Litchfield & Wilcoxon (*J. Pharm. Exp. Ther, 96*: 99-113, 1949). A computer adaptation of this method for processing sensitivity test data has been developed (Wernsdorfer & Wernsdorfer, *Mitt.Osterr.Ges.Trop.Med.Par,* 17: 221-228, 1995). The appropriate software will be available, on request, through the WHO Regional Office for the Western Pacific.

4. Preparation of artemisinin-dosed test plates in the field

In some circumstances it will be difficult to guarantee cold chain conditions for the transport of artemisinin-dosed plates between central producer and the site of use. This constraint may be overcome by production of the plates at or near the site of use.

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4.1 Equipment and material required

The only large piece of equipment required is an incubator which will also be required for sensitivity testing. All other material can be assembled in a kit including the following items:

	No. required
Pelletier bulb (for volumes < 50 ml)	1
Graduated pipette, 1 ml, sterile	10
Graduated pipette, 10 ml, sterile	20
Disposable syringe, 10 ml	10
Millipore filter, disposable, 0.22 µm	10
Adjustable volume pipette, 10-100 µl	1
Linoleic acid, purissimum (SIGMA L-1626) bottle, 50 ml	1
Polyoxyethylene sorbitan mono-oleate (SIGMA P-1780), bottle, 50 ml	1
Ethanol, analytic grade, bottle, 50 ml	1
Sterile bidist. water, bottle, 500 ml	2
Sterilin spray, spray-bottle, 250 ml	1
Sterilin vial, 25 ml, sterile	80
Falcon 3070 microtitre plate, sterile	20
Falcon 3071 plate covers, sterile	20
Sealstrip for Falcon 3070 plate, sterile	25
Sterilin vial, 25 ml, containing 42 mg artemisinin	5
Eppendorf tips (100 µl), sterile, dispensing box of 100	1

This will be available through the WHO Regional Office for the Western Pacific, P.O. Box 2932, 1000 Manila, Philippines.

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4.2 Preparation of stock solutions and dosing solutions

Well equipped laboratories can produce test plates from a stock solutions prepared as follows:

4.2.1 Stock solutions

4.2.1.1 Artemisinin stock solution

The stock solution should contain artemisinin at a concentration of 10^{-2} mol/l. It is prepared as follows:

(1) Add 5 ml ethanol, 5 ml linoleic acid, and 5 ml polyoxyethylene sorbitan mono-oleate to the vial containing 42 mg artemisinin;

(2) Dissolve by vigorous shaking until the solution is completely clear.

(3) Pass solution by syringe through millipore filter into sterile sterilin vial. When kept at 4°C this stock solution can be used for 1 month.

4.2.1.2 Solubilizer stock solution

(1) Add 5 ml ethanol to 4 ml linoleic acid and 5 ml polyoxyethylene sorbitan mono-oleate and shake until mixture is homogenous;

(2) Pass solution by syringe through millipore filter into sterile vial. When kept at 4°C this stock solution will remain stable for one year.

4.2.2 Dosing solutions

The indicated quantities of dosing solutions suffice for the production of a batch of up to 10 plates, using hand-dosing and a dispensing volume of 25 µl. The preparation of the dosing solutions is carried out under sterile conditions throughout. When water is mentioned as a diluent this denotes distilled, sterile water. When the adjustable pipette is used it must be armed with a sterile tip.

4.2.2.1 Preparation of dosing solution for control well (A)

Set fixed-volume pipette to 25 µl. Fill 2.5 ml water into sterile sterilin vial and add 25 µl of solubilizer stock solution. Mix thoroughly. This yields the intermediate dilution (ID).

Fill 7.5 ml water into sterile sterilin vial and add 25 µl of ID. Mix thoroughly. This is the dosing solution for well A (DSA).

4.2.2.2 Preparation of dosing solutions for wells B - H (drug wells)

Set adjustable pipette to 50 µl. Fill 0.95 ml water into sterile sterilin vial (with 1 ml pipette) and add 50 µl of artemisinin stock solution. Mix thoroughly. This is the intermediate stock solution (ISS).

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Set adjustable pipette to 73 µl. Fill 6 ml water sterile sterilin vial and add 73 µl of ISS. Mix thoroughly. This will be the dosing solution for well H (DSH). The other dosing solutions are obtained as follows, using sterile, appropriately marked, sterilin vials as required:

Add 2 ml of DSH to 4.0 ml water. Mix thoroughly. This yields dosing solution G (DSG);

Add 2 ml of DSG to 4.7 ml water. Mix thoroughly. This yields dosing solution F (DSF);

Add 2 ml of DSF to 4.0 ml water. Mix thoroughly. This yields dosing solution E (DSE);

Add 2 ml of DSE to 4.7 ml water. Mix thoroughly. This yields dosing solution D (DSD);

Add 2 ml of DSD to 4.0 ml water. Mix thoroughly. This yields dosing solution C (DSC);

Add 2 ml of DSC to 4.7 ml water. Mix thoroughly. This yields dosing solution B (DSB);

4.3 Preparation of test plates

The adjustable pipette is set at 25µl. The required number of microlitre plates is unwrapped and serially dosed with DSA for wells A, DSB for wells B, and so forth, using a dosing volume of 25µl throughout. (Attention: do not touch the interior wall of the holding vials with the non-sterile part of the pipette). When dosing has been completed, ending with wells H, the plates are placed into the pre-treated incubator for drying. (NB: for pre-treatment the interior of the incubator is sprayed with the sterilin spray. A 5-second spray suffices for a 150 1 cabinet. This treatment should be applied at least 2 hours before loading. The cabinet should be kept closed until 10 minutes before loading, when it should be slightly opened in order to let remaining fumes escape before placing the plates for drying). The plates are dried overnight. When dry, they should be closed with the sterile plastic seal strips and put back into their original plastic wrappings, closing those with a sealing tape. If not immediately used the plates should be stored at 4°C for short term storage or -20°C for long-term storage.

4.4 Preparing test plates without the kit

Well equipped laboratories could produce test plates without the kit using the reagents and procedures mentioned under 4.1 - 4.3. The stock solution should be prepared from pure artemisinin that should contain at least 97% artemisinin and not more than 1% dihydroartemisinin. The substance should, therefore, originate from a quality-controlled source. The molecular weight of artemisinin is 283.34.

ANNEX 5

REPORT OF MALARIA OUTBREAKS/EPIDEMICS IN CROSS BORDER AREAS

DATE: _____

COUNTRY: _____ PROVINCE: _____ DISTRICT: _____

NAME AND LOCATION OF UNIT REPORTING: _____

NAME OF OFFICER IN CHARGE: _____

PHONE: (___)_____ FAX: (___)_____

NUMBER OF LOCALITIES _____ TOTAL POPULATION INVOLVED _____

NAMES OF LOCALITIES INVOLVED:

_____

_____

_____

DATE REPORT RECEIVED __/__/__

DATE OF INVESTIGATION: __/__/__

DATE OF ONSET OF OUTBREAK: __/__/__

NUMBER OF CASES REPORTED:

MICROSCOPICALLY CONFIRMED: _____

CLINICALLY DIAGNOSED: _____

NUMBER OF DEATHS: _____

RESULTS OF INVESTIGATIONS:

SLIDES EXAMINED _____

SLIDES POSITIVE TOTAL _____ PF ___ PV ___ PM ___ MIX___

NUMBER OF INDIGENOUS CASES _____

NUMBER OF IMPORTED CASES _____

SOURCE OF IMPORTED CASES _____

_____

DESCRIBE AGE, SEX, OCCUPATION OF CASES _____

_____

_____

CONTROL MEASURES:

HOUSES SPRAYED _____ DATE: ___/___/_____

BED NETS DISTRIBUTED _____ DATE: ___/___/_____

POPULATION COVERED BY MDA _____ DATE: ___/___/_____

OTHER MEASURES (SPECIFY) _____