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# Monitoring Antimalarial Drug Resistance,

Report of a WHO consultation

Geneva, Switzerland 3–5 December 2001

**World Health Organization**
Department of Communicable Disease,
Surveillance and Response

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**Roll Back Malaria**

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# EXECUTIVE SUMMARY

Based on the five-year field experience of using the 1996 WHO protocol for the assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission, many experts have suggested the need to modify and up-date the protocol. Several teams in Africa have already introduced modifications. In response to the rapid spread of drug resistance and the slow rate at which new antimalarial drugs are developed, it is recommended that treatment policy should focus on therapy with antimalarial drug combinations. The implementation of combination therapy will likely require modification of the design of the therapeutic efficacy test. Furthermore, the WHO protocol for monitoring resistance of *Plasmodium falciparum* in low to moderate transmission areas and the *P. vivax* protocol are still in the draft stages.

It was therefore considered timely to convene a consultation on monitoring anti-malarial drug resistance. The consultation, which was jointly organized by Emerging Public Health Risks including Drug Resistance, Communicable Disease Surveillance and Response (CSR/EPH) and Roll Back Malaria (RBM), was held at WHO headquarters in Geneva from 3 to 5 December 2001.

The objectives of this consultation were:

- to review and update WHO protocols for assessing the therapeutic efficacy of antimalarial drugs for treatment of uncomplicated falciparum malaria in areas with intense transmission (WHO/MAL/96.1077) and in areas with low to moderate transmission;
- to review the draft guidelines for assessing the therapeutic efficacy of chloroquine for vivax malaria;
- to review the role of *in vitro* tests and current methods for detecting molecular markers in the surveillance of resistance to antimalarial drugs;
- to define the technical and operational elements needed for strengthening surveillance of drug resistance of both falciparum and vivax malaria at the country level.

The consultation was attended by scientists, physicians, researchers, epidemiologists and statisticians from Africa, the Americas, Asia and Europe (Annex 1). It included presentations based on working papers, plenary sessions and group discussions on three main topics: *P. falciparum* therapeutic efficacy test methods, *P. vivax* therapeutic efficacy test methods, and *in vitro* tests and molecular markers.

This report gives details of the various modifications and complementary information to be included, which was agreed by experts during the consultation. All these modifications will be taken into account in the new WHO protocol for monitoring the efficacy of antimalarial drugs for the treatment of *P. falciparum* and *P. vivax*.

The following are the main recommendations:

- The classification of therapeutic response should be modified in order to obtain a similar classification for both intense and low to moderate transmission areas.
- The analytical and statistical procedures should be revised.
- The quality assurance process to be used during the monitoring should be increased and better defined.
- The role and the choice of the sentinel site system should be clearly specified.
- The draft protocol for *P. vivax* should be simplified.
- Operational research for the development of new tools should be strengthened and the existing *in vitro* tests and molecular markers standardized.

# 1. BACKGROUND

## 1.1 Monitoring *Plasmodium falciparum* resistance to antimalarial drugs

The first standardized test systems for the assessment of *in vivo* drug response in *P. falciparum* were developed in 1965 shortly after the first reports of chloroquine resistance in this species. These test systems were subsequently revised in 1967 and remained largely unchanged until the WHO Scientific Group on the Chemotherapy of Malaria and Resistance to Antimalarials in 1972 modified them to their present form. The standardized tests were originally developed for chloroquine. In their performance, these tests follow set criteria for the administration of a standard treatment regimen of the appropriate drug, and daily parasitological blood examination for the stipulated period, i.e. 7 or 28 days for chloroquine. The performance of these tests in the field was constrained by the need for daily blood examination in the first week followed by twice-weekly blood examination, when follow-up was extended beyond 7 days. In addition, these tests were primarily conceived for the assessment of the parasitological response of *P. falciparum* in areas with low to moderate malaria transmission and took practically no note of the clinical response to the drugs and the immunity of the patient. Because of the lack of clinical information, which would in many situations be necessary to inform policy-makers, it was decided to introduce a simplified test system where the number of parasitological observations was reduced and complemented by standardized clinical observations.

A standardized protocol has been developed by the Centers for Diseases Control and Prevention, Atlanta (United States) 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 intense transmission. In the preparation of this protocol, due note has been taken of earlier work towards the same objective, as reflected in WHO document WHO/MAL/94.1070, *Antimalarial Drug Policies*. An advanced version of the protocol was reviewed and endorsed in August 1996, at the inter-country workshop on "Malaria Treatment and Resistance in Kenya, Zambia and Malawi" (Mangochi, Malawi). It is presented as the current WHO standard method for monitoring the therapeutic efficacy of antimalarial drugs for the treatment of children suffering from uncomplicated falciparum malaria in areas of intense transmission (WHO document WHO/MAL/96.1077, *Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission*). However, it is obvious that there are large areas in the South-East Asia, Western Pacific and East Mediterranean Regions, South and Central America, as well as in tropical Africa, where malaria transmission is of low intensity or shows large cyclical variations verging on epidemics. 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.

Therefore, a protocol was presented and reviewed at the "Interregional Meeting on Malaria Control with Emphasis on Drug Resistance", Manila, Philippines, October 1996, and at the expert meeting in Manaus Brazil, March 1998 (OPS/HCP/HCT/ 113/98, *Evaluación de la eficacia terapeutica de los medicamentos para el tratamiento del paludismo por* Plasmodium falciparum *sin complicaciones en las Americas*). During the "Informal Consultation on Monitoring Drug Resistance to Antimalarial Drugs in the Mekong Region", Phnom Penh, October 2000, several modifications were suggested and included in a draft protocol (March 2001) for adaptation to areas with low to moderate transmission rates, highlighting in particular the need for a comprehensible classification suitable both in areas of intense transmission and those with low to moderate transmission.

## 1.2 Monitoring *Plasmodium vivax* resistance to chloroquine

Resistance to chloroquine by *P. vivax* was first confirmed among Australians repatriated from Papua New Guinea. Reports of chloroquine-resistant *P. vivax* have since come from several regions in India, Indonesia and Myanmar, as well as from Brazil and Guatemala. It was also observed in travellers to Guyana (South America) repatriated to Canada. Other reports have documented uniform susceptibility to chloroquine in Azerbaijan, the Philippines and Thailand. Health policy decisions concerning chloroquine used for vivax malaria should be based upon evaluation of chloroquine-resistant *P. vivax* epidemiology using a standardized protocol.

The need for a standard procedure for diagnosis of chloroquine-resistant *P. vivax* was recognized at a meeting in Manaus, Brazil (March 1998) convened by the Pan American Health Authority (PAHO) to adapt the 1996 WHO protocol for monitoring the efficacy of antimalarial drugs for the treatment of falciparum malaria to a format relevant to the American setting. Experts from the American Region attending this meeting expressed their concern that therapeutic failure of *P. vivax* infections was already perceived as a problem of significant impact on public health, and that there was no standard protocol for assessing the problem. The PAHO expert group prepared a draft protocol for the assessment of chloroquine sensitivity of *P. vivax* in different parts of the world. As a follow-up to this meeting, meetings were held in São Luis (Brazil) in February 2000 and in Salvador (Brazil) in March 2001. The objectives were to discuss the use, viability and suggestions for improvement of the draft protocol for monitoring the response of *P. vivax* to chloroquine and to share the results of studies undertaken in the different regions using the draft *P. vivax* protocol. The draft protocol elaborated in Manaus was further modified, resulting in the present version which was discussed during this consultation.

# 2. OBJECTIVES OF THE CONSULTATION

- To review and update WHO protocols for the assessment of the therapeutic efficacy of antimalarial drugs for the treatment of uncomplicated falciparum malaria in areas with intense transmission (WHO/MAL/96.1077) and with low to moderate transmission.
- To review the draft guidelines for the assessment of therapeutic efficacy of chloroquine in vivax malaria.
- To review the role of *in vitro* tests and current methods for the detection of molecular markers in the surveillance of resistance to antimalarial drugs.
- To define the technical and operational issues of strengthening surveillance of drug resistance of both falciparum and vivax malaria at the country level.

# 3. *PLASMODIUM FALCIPARUM* THERAPEUTIC EFFICACY TEST METHODS

## 3.1 Introduction

The principal recommendation from the working group was to revise the current *in vivo* protocol in order to:

1. Develop a single, globally standardised protocol that outlines procedures for monitoring antimalarial drug efficacy against *P. falciparum* in endemic countries;
2. Recommend specific modifications that might be used to tailor these methods in accordance with differences in the local epidemiology of malaria, especially transmission intensity;
3. Modify the current *in vivo* response classification system to allow for a single system usable in all endemic areas;
4. Clarify areas of the current protocol that are currently ambiguous;
5. Provide ancillary notes to improve understanding of the test methodology.

The group focused on modifications that were relevant to the primary intent of the protocol, namely the monitoring of drug efficacy over time for strictly programmatic purposes. It was recognized that these methods would not, nor should be expected to, provide all possible scientific information necessary for understanding drug efficacy and resistance in a given environment. Rather, they are intended to ensure a minimal evidence base from which ministries of health can develop informed treatment guidelines and policies.

## 3.2 Specific recommendations

### 3.2.1 Inclusion and exclusion criteria

*Target age groups.* In all areas, the methods should emphasize treatment efficacy in young children (age < 5 years) with clinical malaria. The rationale behind this requirement is that, even in populations with low acquired immunity, younger children often have a less favourable response to antimalarial drugs than older children and adults. It was recognized, however, that in areas of low transmission, preferential enrolment of children < 5 years could pose logistical difficulties (e.g. greatly extending the period of enrolment). In such cases, or in environments where young children are at substantially lower risk of infection than adults (such as the occupational exposure seen in some South-East Asian countries), all ages can be enrolled. Nonetheless, wherever possible, it is recommended that a sufficient number of patients be enrolled to allow for stratification of results based on age (< 5 years and ≥ 5 years).

*Specific Drug Considerations.* Several drugs have specific exclusion criteria. For example, atovaquone-proguanil (Malarone®), artemether-lumefantrine (Co-artem®, Riamet®), and halofantrine have minimum age or weight cut-off points below which treatment is not recommended.

*Exclusion of children < 6 months.* Although this exclusion criterion will be retained, additional information is urgently needed to define differences regarding use, safety and efficacy in the very young child (< 6 months).

*Fever vs. measured temperature elevation*

a) The measurement of fever is not always accurate and the correlation between axillary, rectal and aural elevations in temperature is unreliable. Nevertheless, fever, as defined in this protocol, is a useful working definition and excludes normal diurnal variation as a cause of an elevated temperature reading. Fever is defined as (i) axillary temperature ≥ 37.5 °C , (ii) rectal or tympanic temperature ≥ 38 °C. The protocol should specify the method used for measuring temperature.

b) In areas of intense transmission, enrolment of patients should be based on measured fever (an elevated body temperature indicating fever as per the definitions above). History of fever alone is not sufficient. This enrolment criterion does not suggest that parasitemic patients without a manifest fever do not have malaria requiring treatment. It only suggests that, for the purposes of this assessment, objective measures of fever are required for enrolment. Patients not meeting this admittedly restrictive definition of malaria still require treatment, albeit outside the context of the assessment.

c) In areas of intense transmission, determination of outcome should also be based on manifest, measured elevations in temperature only; history of fever alone will not be considered a sufficient indicator for determination of treatment failure.

d) In areas of low transmission, it is recommended that manifest fever also be used as an enrolment criterion. However, it is recognized that application of this requirement may pose logistical constraints in terms of enrolling sufficient numbers of patients in a reasonable time period. Therefore, in areas of low transmission only, where history of fever is deemed reliable, a measured elevation in temperature or a history of fever can be used as an adequate enrolment criterion.

e) In areas of low transmission, unlike most programmes in areas of intense transmission, the programmatic response to presence of parasitemia after treatment does not differ between patients with history of fever or with or without overt clinical symptoms (i.e. symptomatic and asymptomatic parasitemia are weighted equally and require rescue treatment as a treatment failure).

f) History of fever is defined as a history of fever within the previous 24 hours preceding enrolment only.

g) Patients will no longer be excluded on the basis of a measured temperature ≥ 39.5 °C, in keeping with the updated definitions of severe malaria in the

Transactions Supplement (2000) [Severe falciparum malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 94; supplement 1].

*Parasitemia*. Limits of parasite count for inclusion have been changed from 1 000–30 000/µl to 1 000–100 000/µl for low transmission area, and from 2 000–100 000/µl to 2 000–200 000/µl in areas of intense transmission. These changes are supported by the description of hyperparasitemia in the Transactions Supplement (2000) [Severe falciparum malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 94; supplement 1, S1/5 and S1/33].

### 3.2.2 Classification of response to treatment

A modified classification system of response to treatment has been developed (see Annex 3).

The definition of *Early Treatment Failure* for both intense and low to moderate areas should be modified to reflect the absolute requirement of a measured parasitemia on Day 2. In intense transmission areas, a blood slide at Day 2 is now mandatory and not only if fever is present. This criterion has been included for ethical purposes.

The definition of *Early Treatment Failure* for low to moderate areas should be added to reflect the absolute requirement of a measured increase in temperature on Day 3. The current wording specifically refers to the axillary temperature; this should be modified to allow for other methods of temperature determination and should specify the appropriate cut-off point for each method.

The definition of *Late Clinical Failure* remains for areas with intense transmission "Presence of parasitemia on any day from Day 4 to Day 14 and a measured axillary temperature ≥ 37.5 °C, without previously meeting any of the criteria of early treatment failure" and, for areas of low to moderate transmission, "Presence of parasitemia on any day from Day 4 to Day 28 and a measured axillary temperature ≥ 37.5 °C, without previously meeting any of the criteria of early treatment failure".

Insertion of a footnote is required for the definition of *Late Clinical Failure* in areas of low to moderate transmission. In assessments conducted in areas that have chosen to accept the addition of history of fever during the preceding 24 hours as an enrolment criterion, definitions of *Late Clinical Failure* should also include history of fever (e.g. "Presence of parasitemia and either measured elevation of temperature or history of fever during the preceding 24 hours from any day between Day 4 and Day 28, without previously meeting any of the criteria of early treatment failure."). Note. On Day 3 of the study, an important indicator of treatment failure is parasitemia plus measured elevation in temperature. Because of the frequency of fevers occurring on Day 2, allowing the use of history of fever on Day 3 will substantially and incorrectly increase the apparent early failure rate. Therefore, history of fever should not be used on Day 3 or before.

The definition of *Late Parasitologic Failure* for areas with intense transmission has been added: "Presence of parasitemia on Day 14 and a measured axillary temperature

of less than 37.5 °C, without previously meeting any of the criteria of early treatment failure or late clinical failure."

The definition of *Late Parasitologic Failure* for areas of low to moderate transmission has been added: "Presence of parasitemia on any day from Day 7 to Day 28 and a measured axillary temperature of less than 37.5 °C, without previously meeting any of the criteria of early treatment failure or late clinical failure."

The protocol should emphasize the need to use sound clinical judgement at all times to ensure patient safety and for determining the appropriateness of any individual patient's continuation in the assessment. This applies especially to patients identified as parasitemic but afebrile on Day 7 in areas of intense transmission. While the assessment calls for continued monitoring of these patients for an additional seven days, patients that are judged to be of particular concern (such as afebrile patients with high parasitemia) could and should be monitored more carefully, including admission to hospital for closer observation or reassessment on the following day. At any time, a patient that is deemed unfit to continue the assessment because of safety concerns may be withdrawn from the assessment and classified in the failure group.

The protocol should include the updated definition of severe malaria as outlined by WHO (2000). [Severe falciparum malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 94; supplement 1.]

A more detailed explanation of the difference between "treatment failure", "withdrawal", and "loss to follow-up" is required. For example, movement of a patient from the study site to a place outside the reach of active follow-up should be classified as "loss to follow-up" and not withdrawal. In all cases, reasons for withdrawal and loss to follow-up should be recorded and reported in detail. See also section on analytic plan below, regarding the analytic handling of withdrawals and patients loss to follow-up.

### 3.2.3 Analytic and statistical issues (including sample size calculations)

There should be a statement that the fundamental design of this protocol is intended to provide essential information for monitoring therapeutic efficacy of a range of antimalarial drugs against uncomplicated falciparum malaria as needed for programmatic purposes. Additions to the protocol that do not change its fundamental design could be made, such as measuring blood levels of the drugs, extending the period of follow up and testing for molecular markers. Certain other types of therapeutic trials, however, such as evaluation of new antimalarial drugs or comparative evaluation of two therapies usually require a different type of design not addressed by this protocol.

The recommendations for appropriate methods for calculating sample size represent a substantial departure from the most recent WHO protocol. The Lot Quality Assurance method (LQAS) has been used as a way to minimize the amount of field work required, but the patients included in the studies should have been chosen randomly.

This was rarely the case in the trials carried out since 1996. Also, although LQAS is used to determine sample size, it is far too often forgotten by the time the analysis is done (i.e. the study is analysed as if traditional sample size calculations were done and the interpretation is often incorrect). It has been agreed that LQAS is a valuable option if used properly and has the advantage of stopping the study after the 16$^{th}$ patient. Nevertheless, the group recommended the use of classical statistical methods for determining sample size, based on an expected proportion of treatment failures, desired confidence level (95%) and precision (5% or 10%). The table on minimal sample size for a prevalence study in the draft protocol can be simplified by including only the row corresponding to precision of 0.05 and 0.1. In the case of an expected failure rate lower than 15% and in order to be representative, a minimum of 50 patients should be included.

The preferred method for analysing data derived from these assessments is the life-table method. This method allows for inclusion of data from patients that are withdrawn or loss to follow-up without requiring that assumptions be made about ultimately unknown outcomes. This provides the essential benefits of intent-to-treat analysis with fewer of the intent-to-treat method's drawbacks. Recommendation of this method as the preferred method, however, has greater implications for explanation and training. Although life-table analysis can be accomplished by hand, access to computer assistance will greatly increase ease of analysis and reduce calculation errors.

It is recognized that adoption of the life-table method is a significant change from previously used methods (almost uniformly, the "per protocol" method), creating problems with comparing new data with historical data. It might also be necessary or desirable for preliminary analysis to be conducted by people unfamiliar with or untrained in life-table methodology (and by people without access to computer support). Therefore, it is also recommended that a traditional "per protocol" method be used in parallel (the "per protocol" method removes all unevaluable patients [i.e. those withdrawn or loss to follow-up] from the denominator). It is highly recommended that results from both types of analyses be reported.

Detailed instructions on how to analyse data by hand using both methods should be included in the final protocol document. However, computer-based applications will be developed to provide assistance in all aspects of data management and analysis.

### 3.2.4 Recommended duration of assessment

*Areas of intense transmission.* The recommended minimum length of follow-up is 14 days. Studies of longer duration in areas of intense transmission must be accompanied by molecular assessment (PCR) to assist in distinguishing recrudescence from re-infection. Several participants emphasized the need for a longer period of follow-up depending on the drug used. Based on several trials and experiences, it is obvious that a 14-day follow-up underestimates the true rate of failures. The most suitable duration of follow-up for chloroquine, amodiaquine, sulfadoxine-pyrimethamine, mefloquine and artemether-lumefantrine should be 28, 28, 42, 63 and 42 days respectively.

*Areas of low to moderate transmission.* The recommended length of follow-up for assessments conducted in areas of low to moderate transmission is 28 days. However, in some circumstances, assessments of shorter duration (minimum of 14 days) can still provide useful results and may be utilized. Molecular assessment to assist in distinguishing recrudescence from re-infection is recommended, but not strictly essential, for studies of more than 14 days duration.

### 3.2.5 Drug regimens not covered by this protocol

This protocol is not designed to assess drug regimens given over periods longer than 3 days, such as quinine for 7 days, combinations of quinine and tetracycline or doxycycline given over 7 days, or artemisinin derivatives given for 5 to 7 days.

### 3.2.6 Quality assurance

All aspects of the assessment should be subject to quality assurance procedures. Recommendations regarding quality assurance of four particularly important aspects (microscopy, temperatures, data management, drugs) are described below.

*Training.* Quality assurance begins with proper training and supervision of personnel, and attention to methods and data. Sufficient time and resources should be allocated to training prior to initiating the assessment. Many staff can be trained adequately with minimal time investment and appropriate ongoing supervision. Because the validity of the assessment and the safety of the patients are dependent upon them, the competence of key study personnel should be ensured, including microscopists and principal medical staff.

*Haematologic assessment.* It is an optional activity. Should it be used by a specific programme, haematologic status assessment ought to be subject to quality control procedures appropriate to the specific method used.

*Quality control of microscopy*

a) Quality control of microscopy involves ensuring that i) quality Giemsa stain is used, ii) staining procedures adhere to recognized methods, iii) the equipment is of adequate quality and in good repair, and iv) the microscopic results are reliable. The current protocol adequately describes the staining procedures, but should be expanded to better describe the other issues. In particular, methods for assuring the quality of microscopic results should be provided.

b) It is recommended that microscopic results be assessed with the following procedure that emphasizes reproducibility in outcome over reproducibility in exact parasite counts. The rationale for this system is that wide variation in parasite counts can be found even between two highly experienced microscopists, especially at higher parasite densities. For the purposes of this assessment, those discrepant results that lead to a change in outcome classification are emphasized over discrepant results for individual blood slides that may make no difference in

assigning an ultimate classification. In an ideal situation, two different qualified microscopists should read separately all the slides. If this is not feasible, a 10% random selection of enrolled patients or a minimum of 10 randomly selected patients (whichever is greater) should be selected for re-checking. The second microscopist who is blinded to the patient number, day of follow-up, original results and patient outcome should re-examine all microscope slides from these patients. The second microscopist should provide results as if reading the slide for the first time (i.e. determine if negative or positive and if positive, provide a parasite count using standard procedures). After all slides have been reviewed, the patient-specific new data should be reassembled by patient and the results should be used to assign an outcome using the recommended classification system. The new outcome should be compared to the original outcome and discrepancies noted. If more than 10% of the sub-sample (i.e. 1 patient for a sub-sample of 10 patients) have discordant outcomes, then all study results should be reviewed.

*Quality control for measured temperatures.* Since outcome classifications are dependent on measured temperature (especially in areas of high transmission), both thermometers and temperature-taking technique should be reviewed and their quality ensured. The quality of technique should be ensured through proper training and supervision. Additionally, the current protocol already recommends that measured temperatures of less than 36.0 °C should be repeated. Thermometers should be tested prior to the assessment in a water bath of known temperature (i.e. the temperature of the water bath should be measured using a reliable thermometer, preferably of laboratory quality, before testing the study thermometers). If logistically possible, this procedure should be repeated during and at the end of the assessment.

*Quality control of data management.* Data management in this instance refers to all aspects of data collection, entry, management and analysis. All case report forms should be reviewed by the study supervisor on a regular basis during the assessment, preferably daily, for completeness and accuracy. Data should be computerized using double entry or a random sample of 10% of computerized records should be selected and compared to hard-copy case report forms for confirmation of consistency.

*Drug quality*. Not all drugs of public health importance are produced under good manufacturing practice (GMP). Even pharmaceutical companies following GMP standards might produce drugs of poor quality. Full analysis of drug quality using United States pharmacopoeia or British pharmacopoeia standards is prohibitively expensive and difficult to arrange for many programs. Some drugs of public health importance do not have established pharmacopoeia standards. To the extent possible, programs should obtain test drugs from WHO or other internationally recognized sources of quality drugs. For drugs that cannot be obtained through such sources, samples of test drugs should be submitted to the WHO collaborating centres or regional reference laboratories on quality of drugs for analysis.

### 3.2.7 Sentinel site surveillance system

Control programmes should establish sentinel site surveillance to monitor antimalarial drug efficacy. Although no definitive scientific advice can be given regarding the number of sites needed, experience suggests that between four and eight sites achieve a balance between representativeness and practicality. Programmes should increase or decrease this range as needed to account for geographic size, population distribution and density, differing malaria epidemiology or ecology and other factors deemed important to the program. When making such decisions, emphasis must be placed on the need for a "manageable" number of sites to ensure proper monitoring and supervision.

Again, based on experience rather than definitive science, it is recommended that assessments be conducted not less frequently than once every 24 months. For comparability, assessments should be conducted during the same time of year. Most programmes conducting sentinel site surveillance of therapy efficacy find it easiest to alternate test sites (e.g. four sites tested per year with each site being assessed every other year).

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 (national malaria control programme, ministry of health, universities, institutes of research, national reference laboratory) should be established to coordinate all the activities, i.e. training, supervision, collection and analysis of data, and to forward recommendations to the drug policy-makers. This core group should ensure a good quality of laboratory diagnostic skill in the sentinel sites and provide continuous logistic support.

The minimal requirements to establishing a sentinel site are the availability of trained and motivated 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 in urban settings 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 characteristics should be considered in the selection of sentinel sites:

- population density;
- accessibility to and feasibility of supervision;
- epidemiology of malaria, especially intensity and seasonality of transmission;
- population mobility and migration (especially in border areas);
- distribution of malaria treatment failures reported by health information system.

The sentinel sites should be selected to be representative of each major epidemiological strata into which the country can be divided.

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 the country's situation

depending mainly on national resources and availability of trained staff at selected sentinel sites.

Due to the importance of the private sector in drug procurement and distribution in many countries, and the heterogeneity of drug resistance, drug utilization and drug quality studies should be conducted, whenever feasible, in the areas selected for sentinel site monitoring.

It is likely that results between sites will not be uniform: some sites may identify a substantial deterioration in treatment efficacy while other sites continue to record an acceptable response to the same drug. The programme should consider how to respond to this eventuality: can specific treatment guidelines be targeted to affected areas without changing national policy or guidelines? How many sites need to show unacceptable treatment failures before national policy or treatment guidelines are altered? Once a site demonstrates a high level of treatment failure with the existing first-line drug and national policy or treatment guidelines are not altered, is there a need to continue to evaluate that drug or site relationship in the future? At what level of treatment failure would this occur?

### 3.2.8 Transmission intensity (see unresolved issues)

Recommendations of methods to use for estimating the intensity of transmission in areas where this is not already known or otherwise in question should be provided. In all cases, these methods produce a general guideline for applying this assessment in characterizing sentinel sites. They are not intended to be used for official risk stratification and should not be considered as definitive. In the absence of a reliable surveillance system which could provide data on the actual incidence of malaria in the sentinel sites, potential methods include:

- Rapid community surveys based on spleen rate, parasite prevalence among children under 1 year, parasite prevalence distribution or prevalence of true clinical cases across all age groups.
- Review of existing hospital or health information system data to characterize pattern of outpatient department attendance, severe morbidity attributed to malaria or malaria-attributed mortality rates.
- Existing entomological data.

### 3.2.9 Unresolved issues

It was recognized that further consultations will be required to provide recommendations regarding the following issues:

*Early stopping rule.* Would it be possible to devise a system of interim analysis that would allow for halting the assessment before the entire sample size has completed the entire follow-up period if failure rates are above a certain level? With LQAS

method, the study could be stopped if the observed number of treatment failures is greater than 5 among the 16 first randomized patients (6/16 = 37.5%).

*Overall study validity.* At what level of loss to follow-up should the study be considered invalid? Should this level differ depending on the duration of follow-up (realizing that loss to follow-up is greater for longer duration studies)? The number of losses has been arbitrarily limited to < 10% for a 14-day follow-up period in the former protocol.

*Transmission intensity.* Additional information needs to be gathered in order to better guide users in assessing malaria transmission intensity. The group discussed offering a range of options, perhaps prioritized. In any case, sufficient detail on methods to be used and appropriate cut-off points (with references) needs to be provided.

# 4. *PLASMODIUM VIVAX* THERAPEUTIC EFFICACY TEST METHODS

## 4.1 Introduction

A WHO protocol for the assessment of the therapeutic efficacy of chloroquine for vivax malaria exists already as a working draft. The objective of the working group was to critically review the draft protocol and suggest guidelines to monitor *P. vivax* sensitivity to chloroquine or chloroquine in combination with other drugs (specifically primaquine). This was done also with due consideration to the modifications introduced during the present consultation in the protocol for assessing drug efficacy in *P. falciparum* malaria.

## 4.2 Specific recommendations

### 4.2.1 Inclusion and exclusion criteria

Compared with the draft protocol the adopted inclusion and exclusion criteria were the following:

*Inclusion criteria*

- Patients age > 6 months
- Positive for *P. vivax* monoinfection with parasite density above 250/µl (the lower limit was 1 000/µl in the previous protocol)
- History of fever during 48 hours prior to time of recruitment
- Ability and willingness to participate based on information given to parent or guardian and access to health facility
- Informed consent
- Axillary temperature ≥ 39.5 °C is no longer considered as a criterion for exclusion

*Exclusion criteria*

- Presence of clinical condition requiring hospitalization
- Presence of severe malnutrition
- Pregnancy
- Significant concomitant febrile illness which would interfere with follow-up
- Chronic infectious diseases other than malaria (e.g. tuberculosis)
- G6PD deficient when primaquine is assessed
- Known allergy and/or intolerance to drug(s) being tested

### 4.2.2 Classification of response to treatment

The distinction between early and late treatment failure has been abandoned. The group opted for a single definition of treatment failure, i.e. no distinction between early and late treatment failure. Subjects presenting a *P. falciparum* infection after clearance of *P. vivax* parasites should be treated accordingly and withdrawn. These should not be considered as *P. vivax* treatment failures.

The definition of treatment failure includes the following:

- Clinical deterioration due to *P. vivax* illness requiring hospitalization in presence of parasitemia.
- Presence of parasitemia and axillary temperature ≥ 37.5 °C any time between Day 3 and Day 28.
- Presence of parasitemia on any day between Day 7 and Day 28, irrespective of clinical conditions.

### 4.2.3 Follow-up procedures

| Days | 0 | 1 | 2 | 3 | 7 | 14 | 21 | 28 | Other day |
|---|---|---|---|---|---|---|---|---|---|
| Clinical examination and temperature | X | X | X | X | X | X | X | X | X |
| Treatment with chloroquine | X | X | X | | | | | | |
| Parasitemia | X | | X | X | X | X | X | X | X |
| Haemoglobin | X | | | | | | | X | |
| CQ + metabolite blood levels | X | | | | | | | | reappearance |
| Molecular markers | X | | | | | | | | reappearance |

Measurements of chloroquine and desethylchloroquine levels and determination of molecular markers is optional as only few laboratories will be able to do it. Measurement of chloroquine blood level may help in distinguishing between recrudescence and re-infection. Parasitemia in presence of chloroquine and desethylchloroquine blood levels at 100 ng/ml or more is considered as treatment failure. However, the rationale behind this threshold has been questioned and needs to be validated.

### 4.2.4 Analytic and statistical issues (including sample size calculations) (see *P. falciparum* group)

### 4.2.5 Quality assurance (see *P. falciparum* group)

### 4.2.6 Sentinel site surveillance system (see *P. falciparum* group)

### 4.2.7 Guidance on use of data

The trial profile should be presented in a standard format. Results of the tests should be presented to policy-makers in verbal as well as written form. Data should be used by national authority and for subsequent regional and international comparison. Exchange of information among countries in the same geographic region should be promoted.

### 4.2.8 Possible rescue treatments

There is no standard alternative therapy established for *P. vivax* treatment failure. The working group proposed re-treatment with chloroquine, as breakthrough parasitemia could be due to problems of absorption, relapse, new infection, or to residual low levels of chloroquine. However, during the plenary session this has been challenged. Many countries have this policy although it is not ideal. It was agreed that in these cases re-treatment with chloroquine combined with primaquine as from Day 0 would be a better solution. In these cases the problem of G6PD deficiency should be taken into account and tested. Quinine can be administered according to local conditions. Amodiaquine is a possible rescue treatment, although there is little information on its efficacy against *P. vivax*.

# 5. *IN VITRO* TESTS AND MOLECULAR MARKERS

Therapeutic efficacy test results remain the basis for antimalarial drug policy decision. However, tools such as *in vitro* tests or molecular markers can help to clarify or complete the overall picture. The objectives of the working group were to update methods based on accumulated experience and recent advances in understanding drug action and resistance and to provide information regarding the use or potential use of supporting technologies such as *in vitro* and molecular methods.

## 5.1 *In vitro* tests

### 5.1.1 Introduction

Considering their technical difficulties and their cost, *in vitro* tests should be carried out only by centres with adequate resources and expertise. *In vitro* tests can be used:

1. to assess patterns of cross-resistance between different drugs;
2. to assess the baseline susceptibility to drugs to be introduced;
3. to temporally and geographically monitor parasite susceptibility to drugs.

*Patterns of cross-resistance.* The patterns of cross-resistance may vary between different regions and occur mainly among drugs of the same chemical family (quinine–mefloquine), but also among drugs belonging to different chemical families (mefloquine–artemisinin). These data can be important when changing the first-line drug, when deciding on second-line treatment and on rescue treatment during therapeutic efficacy tests or when combining two or more different drugs.

*Assessment of baseline susceptibility to drugs to be introduced.* Ideally, *in vitro* test results should be available before introducing a new treatment (monotherapy or combination therapy). Such tests should be performed on an adequate sample size (between 50 and 100 per sentinel site) and cover all sentinel sites in a given country. In case *in vitro* test results are indeterminate, their correct interpretation may require the genetic analysis of the isolates in order to identify molecular markers for resistance.

*Temporal and geographical monitoring of parasite susceptibility. In vitro* tests may provide an early warning of impending resistance before it becomes clinically apparent and may help to target therapeutic efficacy studies. *In vitro* tests can also be useful in monitoring changes in susceptibility to a drug that has been withdrawn. In situations where a drug combination is used and where it is not possible to carry out therapeutic efficacy tests for each component of the combination, *in vitro* tests can monitor susceptibility to each drug of the combination.

### 5.1.2 Recommendations

*In vitro* testing can be used for the surveillance of drug resistance at country or regional level and should not be used for individual diagnosis. A link between malaria control and research programmes should be established to facilitate such a system. It is recommended that *in vitro* tests should be carried out by central reference laboratories located in endemic countries. There is the need for standardizing the methodology, especially the cultivation procedures and the reporting of results. Threshold levels defining resistance must be validated by clinical trials in non-immune patients specifically for each type of *in vitro* test. A quality control system should be set up and should include reference clones.

## 5.2 Drug resistance molecular markers

Molecular markers have the potential for predicting therapeutic efficacy on a broad scale and models for their implementation have already been proposed. The collection, storage and transport of samples for molecular analysis are much easier than for the *in vitro* tests and this is a major advantage. However, molecular markers of resistance are available for only a few drugs and thus far only valid for *P. falciparum* (sulfadoxine, pyrimethamine, cycloguanil and chloroquine), while for other drugs they are not yet determined. This is why the working group recommends the collection of anonymous blood samples on filter paper from patients participating in the therapeutic efficacy tests. The analysis of these samples may help in validating new molecular markers for other drugs. Ethical issues of collecting blood samples for future use was considered by the working group. The group felt that these issues could be dealt with by unlinking blood samples to patient identifiers and then conducting anonymous testing. Regardless, the collection of blood samples for future molecular analysis should be mentioned and well explained during the informed consent process.

Molecular analysis of blood samples should be carried out in centres with adequate resources and expertise. Several studies have already been carried out and results published. Such centres already exist in endemic countries and need more support in terms of training and resources. Data sharing and method standardization among groups should be promoted and possibly a global network set up.

In a way similar to *in vitro* tests, molecular studies of resistance markers could also provide an early warning system or can target therapeutic efficacy studies. They can also be useful in monitoring the prevalence of molecular markers in places where a drug has been withdrawn or where a drug combination is in use.

The models for implementing molecular surveillance require validation in different epidemiological settings. To date, such a system has been evaluated only in Mali. Such a system would require close collaboration between malaria control programmes and research groups involved in the molecular analysis. The final aim would be to investigate how to use markers to help inform policy decisions by improving the understanding of how the presence of specific markers relates to clinical outcomes.

## 5.3 Operational research

The working group has identified several research subjects that are considered as priorities.

### 5.3.1 Genotyping of *P. falciparum.*

Presently, MSP1, MSP2 and GLURP are well established as genotyping markers for distinguishing between re-infection and recrudescence in treatment failures. There is a need to standardize the definition of recrudescence and re-infection based on molecular analysis. Considering the possible complexity of the parasite population in a single individual, it is proposed to investigate the usefulness of analysing blood samples collected at Day 0 and at Day 1. This would require a modification of the follow-up schedule. Other research subjects identified as important are the assessment of mixed species infection on the clinical and parasitological outcome of the test and the relationship between presence of molecular markers of resistance and anaemia.

### 5.3.2 Other important research issues

- The impact of resistance on anaemia, childhood mortality, gametocyte rate and malaria transmission.
- Identification of early parasitological and clinical indicators suitable for predicting treatment failure and the relationship between specific drug pharmacokinetic and pharmacodynamics and therapeutic outcome.
- Rapid tests to detect counterfeit drugs at peripheral level (a test for chloroquine, sulfadoxine-pyrimethamine and artesunate exists already).
- Rapid and less expensive methods for detecting G6PD deficiency.
- Simpler and less expensive methods to estimate haemoglobin levels.
- More specific rapid tests to detect significant levels of drugs in blood and urine.
- *In vitro* tests and genetic markers for resistance to chloroquine and pyrimethamine for *P. vivax.*
- Drug efficacy in pregnant women and HIV-positive individuals.
- Assessment of available drugs for alternative treatment of *P. vivax* chloroquine failures.

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# Annex 2. Agenda

## Monday 3 December 2001

| | | |
|---|---|---|
| 08:30 | Register - Files and badges will be available outside room M205 | |
| *Chair:* | | *T. Mutabingwa* |
| *Rapporteurs:* | | *P. Bloland and U. D'Alessandro* |
| 09:00 | Welcome and opening of the meeting | *D. Alnwick/G. Rodier* |
| 09:10 | Review of current WHO protocols for monitoring therapeutic efficacy of antimalarials | *P. Ringwald* |
| 09:20 | Experience with monitoring of therapeutic efficacy in African countries using WHO protocol | *T. Sukwa* |
| 09.40 | Operational problems with the current WHO protocol (choice of sentinels sites, teams, training, quality control of drugs, quality control of the data, analysis of the data) | *T. Mutabingwa* |
| 10:00 | Technical problems with the current WHO protocol (inclusion/exclusion criteria, classification criteria, urinary test, haemoglobin, adaptation according to transmission area or drugs) | *P. Bloland* |
| 10:30 | Coffee break | |
| 11: 00 | Discussion on WHO efficacy protocols | |
| 11:50 | Role of *in vitro* testing in monitoring drug resistance | *J. Le Bras* |
| | Discussion | |
| 12:30 | Lunch | |
| 14:00 | Molecular markers and monitoring drug resistance | |
| | Correlation of molecular markers and clinical data: *dhfr* and *dhps* | *C. Plowe* |
| | Other drugs (aminoquinolines, aminoalcohol, artemisinin) | *D. Warhurst* |
| | Use of PCR genotyping in therapeutic efficacy tests | *G. Snounou* |
| | Discussion | |
| 15.30 | Coffee break | |
| 16.00 | Comparison of WHO protocol with other protocols used in clinical research | *P. Olliaro* |
| 16:20 | Epidemiological basis for the choice of sentinel sites in different epidemiological setting | *U. D'Alessandro* |
| 16.50 | Statistical methods in therapeutic efficacy test (sampling, analytical plan) | *J. Simpson* |
| | Discussion on the last three presentations | |

## Tuesday 4 December 2001

*Chair:* *W. Wernsdorfer*
*Rapporteurs:* *P. Bloland and U. D'Alessandro*

09:00 Experience with monitoring the efficacy of chloroquine in the treatment of *P. vivax*
- Azerbaijan — *P. Ringwald*
- Peru — *P. Ringwald/T. Ruebush*
- Indonesia — *E. Tjitra*

09:30 Operational and technical problems for monitoring the efficacy of chloroquine for the treatment of vivax malaria — *D. Fryauff*

Discussion on *P. vivax* protocol

10.30 Coffee break

11:00 Discussion groups

Presentation of the objectives — *P. Ringwald*

Participants split in 3 groups (*P. falciparum* protocol, *P. vivax* protocol, other tools – *in vitro* and molecular markers)

12:30 Lunch

14:00 Discussion groups

15:30 Coffee break

16:00-18:00 Discussion groups

## Wednesday 5 December 2001

*Chair:* *W. Watkins*
*Rapporteurs:* *P. Bloland and U. D'Alessandro*

09:00 Presentation of the conclusions by rapporteurs of each group

Discussion

10:30 Coffee break

11.00 Presentation of the conclusions by rapporteurs of each group

Discussion

12:30 Lunch

14:30 Presentation of summary of conclusions and recommendations — *Rapporteurs*

15:30 Conclusion of the meeting and closing remarks — *Chairman*

# Annex 3. Classification of response to treatment

## Early Treatment Failure (ETF)

### INTENSE TRANSMISSION AREA

**ETF**

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

### LOW TO MODERATE TRANSMISSION AREA

**ETF**

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

## Late Treatment Failure (LTF)

**Late Clinical Failure**

- Development of danger signs or severe malaria after Day 3 in the presence of parasitemia, without previously meeting any of the criteria of early treatment failure
- Presence of parasitemia 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 parasitemia 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 parasitemia, without previously meeting any of the criteria of early treatment failure
- Presence of parasitemia 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 parasitemia 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 (ACPR)

**ACPR**

- Absence of parasitemia 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.

**ACPR**

- Absence of parasitemia 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.