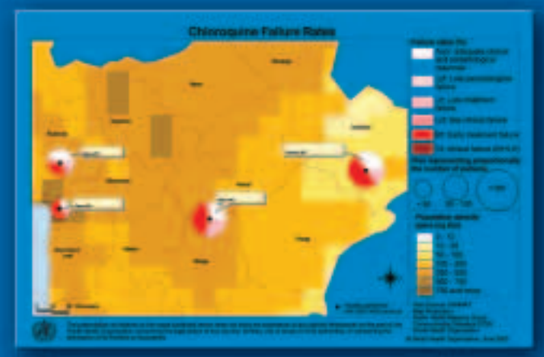
Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria



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ASSESSMENT AND MONITORING OF ANTIMALARIAL DRUG EFFICACY FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA

World Health Organization
Geneva

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1. Introduction

Antimalarial drug resistance has emerged as a leading threat to ongoing malaria control efforts. As resistance to one or more antimalarial drugs occurs more frequently, malaria control programmes and other concerned institutions need to be able to evaluate antimalarial drug efficacy in a way that provides timely, relevant, reliable, and understandable information.

Data derived from these evaluations are essential not only for maintaining confidence that current treatment recommendations are adequate in relation to malaria patients' needs, but also, should that not be the case, for generating convincing evidence that current treatment recommendations are in need of change. When such evaluations are conducted consistently over time and in a reasonable and representative selection of sites, programmes should be able to monitor drug efficacy in a way that will allow changes in treatment recommendations or policies to be made early enough to minimize the impact of a failing treatment regimen.

The primary goal of this protocol is to provide guidance in obtaining the ***minimum*** essential information about the clinical and parasitological response to antimalarial drugs among populations at greatest risk of severe morbidity or mortality due to malaria. The intended use of this protocol, therefore, is primarily as a tool for the collection of clinically relevant information for developing evidence-based antimalarial treatment policy. It is not intended for use as a more traditional biomedical research protocol and is not intended to replace well-designed clinical trials conducted under Good Clinical Practice (GCP) guidelines, as should be done when investigating new treatment lacking an adequate history of safe clinical use.

Considerable emphasis has been placed on maintaining as much simplicity and practicality as possible. Using this protocol, programmes lacking access to substantial financial resources or to state-of-the-art laboratory analysis — most often obtained through collaborative links with medical research institutions — should nonetheless be able to produce the information needed to ensure the best malaria treatment for the people living in their country. Programmes that do have adequate resources and expertise are encouraged to collect any additional information that they feel is relevant. However, they are also encouraged to ***at least*** collect this minimal data set in a way that is consistent with this protocol. Only through such standardization will it be possible to compare and interpret results over time and within or between regions.

2. BACKGROUND

2.1 Development of the WHO standardized in vivo test

The first well-documented reports of *Plasmodium falciparum* resistant to chloroquine were made between 1957 and 1960 in South-East Asia and South America (*1*, *2*). Because chloroquine had become the treatment of choice for malaria since its discovery in the 1930s, there was a clear need to be able to assess the extent of resistance to this drug in areas where malaria was being transmitted.

A standardized in vivo test system for assessing the response of *P. falciparum* to chloroquine was first developed in 1965 (*3*). This system was officially revised twice, first in 1967 and again in 1972 (*4*, *5*). The methodology that evolved from these revisions was fairly demanding, requiring daily blood examinations during the first week post-treatment followed by a prolonged period (28 days) of monitoring with weekly blood examinations. Because the outcome of primary interest was reappearance of parasites within the observation period, indicating treatment failure, patients were kept in a mosquito-free environment to prevent reinfection. Later modifications allowed for a choice between a shortened observation period of 7 days (the "WHO standard test") and the longer 28-day observation period (the "extended test"), depending on whether the possibility of reinfection could be excluded. The short observation period allowed the test to be conducted under typical field conditions and constraints.

In practice, individual researchers have made numerous variations and modifications. Unofficial modifications of the protocol began to show the usefulness of tracking clinical response to treatment, including calculating fever clearance times, initial symptom resolution, time to reappearance of parasites, and haematological response (*6–9*). Because of the growing emphasis on clinical response, these developments began to take into account such issues as the potential implications underlying acquired immunity and the appropriateness of inclusion of asymptomatic subjects. As a result, there was a movement towards including only symptomatic patients in the test and, in areas of intense transmission, focusing on the age group at greatest risk of severe morbidity and mortality (and least likely to have a well developed immune response to malaria), i.e. children less than 5 years of age. There was also a move away from the short, 7-day observation periods towards observation periods of sufficient length to allow observation of changes in clinical and haematological status (14 days or more).

These efforts culminated in a new standardized protocol jointly developed by the Centers for Disease Control and Prevention, Atlanta, USA, and WHO at an inter-country workshop – Malaria Treatment and Resistance in Kenya, Zambia and Malawi – in Mangochi, Malawi, in 1996 (*10*).

During the same period, a second protocol was developed specifically for areas of low

to moderate transmission, beginning with a meeting in Manila (Philippines) in 1996 and continuing at an expert meeting in Manaus (Brazil) in 1998 (*11*). Recommendations resulting from a meeting in Phnom Penh (Cambodia) in 2000 (*12*) achieved further refinement of the protocol.

Unfortunately, while these protocols responded to different programmatic priorities dictated by differences in the underlying epidemiology of malaria, the existence of two "official" protocols caused some confusion. Most importantly, while the two protocols used the same terms to classify treatment failures, the actual definitions of the terms differed between areas of intense transmission and areas of low to moderate transmission. Consequently, if the protocols were applied as recommended, results could not be compared between different areas of the world – or even within single countries with highly variable transmission rates.

The protocol presented here is an attempt to reconcile these protocols and produce a single, globally standardized protocol. Additionally, some methodological problems that became apparent while the 1996 protocol was in use have been corrected. Modifications to the 1996 protocol were discussed and agreed upon during an informal consultation in Geneva in 2001 (*13*).

The objectives of this consultation were:
• to review and update the existing WHO protocols for assessing the therapeutic efficacy of drugs for treatment of uncomplicated falciparum malaria in areas with intense transmission (*10*) and in areas with low to moderate transmission (which exist only in draft form);
• to review the draft guidelines for assessing the therapeutic efficacy of chloroquine against *P. vivax*;
• to review the potential role of in vitro tests and current molecular methods for detecting markers of resistance in the surveillance of resistance to antimalarial drugs; and
• to define the technical and operational elements needed for strengthening surveillance of drug resistance of both falciparum and vivax malaria at the country level.

2.2 Importance of in vivo tests to antimalarial treatment policy development

While treatment efficacy data represent only one element in a wide array of information needed to develop evidence-based policy for treatment of malaria, the results of in vivo tests conducted using the WHO standardized in vivo methodology are clearly critical. Throughout Africa, south America, and Asia, studies using the WHO in vivo methodology have provided valuable information on the efficacy of antimalarial drugs in current use as well as initial evaluations of potential alternative treatment regimens.

In South-East Asia, Thailand has seen the greatest change in treatment policies,

in part because of the rapidity with which resistance developed in that country. Since 1973, Thailand has changed first-line malaria treatment from chloroquine, to sulfadoxine–pyrimethamine (SP) plus mefloquine, to mefloquine alone, and finally to a combination of mefloquine and artesunate (*14*). Nearly all countries in South-East Asia have either recently changed first-line treatment recommendations for malaria and are using in vivo studies to monitor treatment efficacy prospectively, or are in the process of evaluating the efficacy of their current treatment recommendations as a first step towards potential change (*12*).

Chloroquine resistance was first reported in east Africa in 1978 and spread throughout the continent over the next 10 years. As resistance intensified, especially in east Africa, concern began to grow that treatment policies would need to eventually change in favour of more efficacious drugs.

Although the problem of failing chloroquine efficacy was fully appreciated by medical researchers in Africa, this appreciation was slow to develop among those responsible for formulating national malaria treatment policy (*15*). Doubtless, there were many reasons for this slow change in attitude, but it was clear that decision-makers, who did not necessarily have biomedical research, or even medical, backgrounds, either did not fully understand the implications of parasitological failure or were otherwise unconvinced by the data. This situation was further complicated by the fact that, owing to acquired immunity and the antipyretic effect of chloroquine, patients treated with chloroquine appeared to get better even when parasites remained or returned.

As the in vivo protocol evolved to focus more on clinical outcomes, increased emphasis was also placed on identifying how best to communicate efficacy data to policy-makers in ways that were understandable, accessible, and convincing. Although South Africa changed first-line treatment from chloroquine to SP in one province in 1988, primarily on the basis of in vitro data, Malawi was the first country in Africa to make the same change on a national level. The impetus for this change was a series of in vivo studies that clearly showed high rates of chloroquine treatment failure, the temporary nature of the initial favourable clinical response to chloroquine treatment, and the inadequate haematological response to chlorquine, compared with that to SP, among anaemic children (*16*).

Since then, more African countries have recognized the need for similar policy changes. The advent and success of multi-country subregional networks — such as the East African Network for Monitoring Antimalarial Treatment (EANMAT) — have greatly increased countries' experience with and capacity to conduct reliable in vivo studies. Similar networks have developed elsewhere in Africa, as well as in South-East Asia and south America. As a result, in vivo efficacy data have become more influential in the development of national malaria treatment policy (*17*).

3. Overview of study design

The fundamental design of this protocol is intended to evaluate the therapeutic efficacy of a range of antimalarial drugs used for treating uncomplicated falciparum malaria, providing the minimum information essential for programmatic decision-making. Studies that follow this basic design, when conducted periodically in a number of appropriately selected sentinel sites, can also form the basis of a surveillance system capable of monitoring drug efficacy changes over time.

The design is a simple, one-arm, prospective evaluation of the clinical and parasitological response to directly observed treatment for uncomplicated malaria. Additions to the protocol that do not change its fundamental design or intended purpose can be made and – when technically and logistically feasible, such as when measuring blood levels of the drugs, extending the period of follow-up, and testing for molecular markers to help distinguish reinfection from recrudescence – are even encouraged.

Programmes will probably wish to evaluate more than one drug. For example, it is common for programmes to evaluate both the current first-line treatment as well as one or more potential replacement treatments. However, the protocol is ***not*** designed for the evaluation of new or experimental drugs; such studies, as well as comparative clinical trials, usually require design, ethical, and statistical considerations that are beyond the scope of this protocol. Furthermore, this protocol is not designed to assess drug regimens administered over periods longer than 3 days, such as quinine (given for 7 days), combinations of quinine and tetracycline or doxycycline (given over 7 days), or artemisinin monotherapy (given for 5–7 days).

NOTE: Major changes to the 1996 protocol and the rationale behind those changes are presented throughout in highlighted text boxes such as this.

4. STUDY POPULATION

4.1 Target age group

In all areas, regardless of the intensity of malaria transmission, the evaluation of antimalarials for uncomplicated malaria should emphasize treatment efficacy in children < 5 years with clinically apparent malaria. The rationale for this requirement is that, even in populations with little acquired immunity (as occurs in areas of low or highly seasonal malaria transmission), younger children often have a less favourable therapeutic response to antimalarial drugs than do older children and adults. Obviously, in areas of low malaria transmission, exclusive enrolment of children < 5 years is likely to pose logistic difficulties because of the relative infrequency of malaria infection in this age group. In such cases, or in environments where young children are at substantially lower risk of infection than adults, such as occurs with occupational exposure in some South-East Asian countries, patients of 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).

4.2 Inclusion criteria

Patients should be selected on the basis of the following criteria:

- Aged between 6 and 59 months (but see comments on target age group in section 4.1 above).
- Absence of severe malnutrition (defined as a child whose weight-for-height is below –3 standard deviation or less than 70% of the median of the NCHS/WHO normalized reference values, or who has symmetrical oedema involving at least the feet) (*18*).
- A slide-confirmed infection with *P. falciparum* only (i.e. no mixed infections).
- Initial parasite density. The range of initial parasite densities appropriate for inclusion differs by level of malaria transmission intensity:
  – for areas of low to moderate malaria transmission, the acceptable range is between 1000 and 100 000 asexual parasites/μl;
  – for areas of high transmission, the acceptable range is between 2000 and 200 000 asexual parasites/μl.

NOTE: These recommended parasite densities reflect a change from the previous protocol. The changes are supported by the currently accepted definition of hyperparasitaemia (*19*).

- Absence of general danger signs among children < 5 years (see Box 1) or other signs of severe and complicated falciparum malaria according to current WHO definitions (see Annex 1).

Box 1: GENERAL DANGER SIGNS OF SEVERE ILLNESS

- Inability to drink or breastfeed
- Vomiting everything
- Recent history of convulsions
- Lethargy or unconsciousness
- Inability to sit or stand up

• Measured axillary temperature ≥ 37.5 °C or rectal/tympanic temperature ≥ 38.0 °C.

NOTE: Patients will no longer be excluded on the basis of a measured temperature > 39.5 °C, in keeping with the current definitions of severe malaria (*18*). See Box 2 for a discussion of the use of measured fever vs history of fever.

• Ability to attend the stipulated follow-up visits, and easy access to the health facility.
• Informed consent provided by patient or parent/guardian (see example, Annex 2).
• Absence of history of hypersensitivity reactions to any of the drugs being evaluated. A history of adverse reactions to antimalarials or other drugs is vital medical information that should be marked with a red pen or a highlighter on the patient record form. While such reactions to chloroquine, quinine, and mefloquine are fairly rare and relatively mild, reactions to drugs containing sulfonamides may be life-threatening. Life-threatening hypersensitivity reactions to artemisinins have been described, but appear to be very rare. In the case of a history of allergic reactions to drugs, the precise nature of which cannot be determined, it is advisable to exclude the patient from tests involving the suspect drug(s) and to give the recommended alternative treatment.

4.3 Exclusion criteria

In general, exclusion criteria are the opposites of inclusion criteria. However, some specific drugs have unique exclusion criteria that must be taken into account (although these are subject to change, as data accumulate). For example, for atovaquone–proguanil, artemether–lumefantrine, and halofantrine there are minimum age or weight cut-off points below which treatment is not recommended. Additionally, for studies allowing the inclusion of patients > 5 years, other exclusion criteria may be required, such as pregnancy or lactation or the existence of underlying chronic severe illness (e.g. cardiac, renal, hepatic diseases, HIV/AIDS).

A history of previous antimalarial drug use or the presence of antimalarial drugs in the urine or blood is not an exclusion criterion. Because, in many settings, prior anti-malarial treatment is the rule rather than the exception, exclusion of previously treated patients would not yield a representative sample of the target population (i.e. patients attending health facilities for the treatment of uncomplicated malaria). In many settings it has also been shown that antimalarial drugs can be found in the blood or urine of patients who deny previous use of antimalarial drugs, suggesting that a history of previous treatment is unreliable. Nonetheless, information on previous drug use should be carefully collected and recorded for each patient and can be used to stratify the results. This is particularly important for long-acting antimalarial drugs, such as SP and mefloquine.

A more reliable approach to determining previous drug use is to screen urine for antimalarial drugs. While this may give a more objective indication of current drug use in the population, it is not a mandatory component of this protocol. A number of urine tests are described in Annex 3.

Box 2: Use of measured elevation in body temperature *vs.* history of fever

The requirement to use only objectively measured elevations in body temperature (i.e. "measured fever") as opposed to allowing the use of a history of fever has been very controversial. The following is a description of some of the concerns and issues involved with definitions of fever and is intended to provide a rationale for current recommendations.

The greatest challenge to the use of a history of fever in this context is its unreliability, especially among caregivers' reports of fever in young children. Additionally, in many situations, a caregiver will modify their response if they feel that the child will receive different treatment based on a positive or negative report of fever. For example, if participation in a study such as this is generally perceived as beneficial, the caregiver may misreport symptoms in an attempt to ensure the child's enrolment. Randomization in clinical trials reduces the chance for bias being introduced by the study team; similarly, the use of objective measures of illness, whenever possible, reduces potential bias introduced by subjective assessments made by either caregivers or study personnel.

Therefore, the global recommendation is that ***only objectively measured elevations in body temperature should be used*** for both enrolment and post-treatment evaluations. This recommendation ***does not*** imply that parasitaemic patients without manifest fever do not require treatment. It suggests only that, for the purposes of this assessment, objective measures of fever are required for enrolment. Patients not meeting this restrictive entry criterion still require treatment, albeit outside the context of the assessment.

Modification of this recommendation on the basis of transmission intensity may be necessary in some circumstances. In areas of low to moderate transmission, ***history of fever within the previous 24 hours*** can be used for enrolment and post-treatment evaluation. However, this modification should be used ***only*** when insistence on requiring an objectively measured increase in body temperature would cause great logistic and financial hardship due to difficulty in patient recruitment and protracted enrolment periods. This modification should ***not*** be used if sufficient numbers of patients with measured fever can be identified in a timely fashion.

The principal reason for allowing this flexibility in areas of low to moderate transmission and not in areas of high transmission is the difference in prevailing programmatic response to in vivo test results. In most areas of low to moderate transmission, the programmatic response to the presence of parasitaemia after treatment does not differ between patients with or without overt clinical symptoms (i.e. symptomatic and asymptomatic parasitaemia are weighted equally and require rescue treatment as a treatment failure). This is not typically the case in areas of intense transmission, where resolution of clinical symptoms is weighted more heavily in decision-making than is persistence of parasitaemia without overt symptoms. Accurate reflection of clinical indicators therefore becomes more important in areas of intense transmission.

5. Study methods and procedures

5.1 Overview

The study methods and procedures are broken down into multiple activity sections, including sample size calculations, screening evaluation, enrolment evaluation, informed consent procedures, treatment, patient follow-up evaluation, definition of study end-points, and determination of study outcomes. Additional sections will describe ethical considerations, laboratory techniques, data management, data analysis, reporting, study and data quality assurance, evaluation of study validity, recommendations for determining the level of malaria transmission intensity, and recommendations for establishing a sentinel site surveillance system for treatment efficacy.

5.2 Sample size considerations

NOTE: The current recommendations for appropriate methods of calculating sample size represent a substantial departure from the most recent WHO protocol.

The previous protocol recommended using the Lot Quality Assurance method (LQAS), primarily as a way to minimize the amount of fieldwork required. Cumulative experience in the context of in vivo assessments of antimalarial drug efficacy has suggested substantial methodological and analytical problems with this method in practice.

Proper use of the LQAS method requires patients to be randomly selected for enrolment, which is rarely, if ever, done in practice. Additionally, many programmes used this statistical method as a justification for enrolling small numbers of patients and conducting very short studies, but then dismissed or failed to understand the statistical implications inherent in this method (i.e. the study was often analysed as if traditional sample size calculations were done). This led to many reports with incorrect interpretation of study results.

The current recommendations therefore call for 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%). While this method results in sample sizes greater than would normally be the case for LQAS, the sample sizes are still well within the capacity of programmes. For example, in the case of a test drug with an expected failure rate lower than 15%, a minimum of 50 patients should be included in order to be representative (see also Annex 4).

5.3 Screening evaluation

A rapid screening procedure should be used in an outpatient setting to identify patients who may meet enrolment criteria. The exact procedures used, the clinical and laboratory evaluations performed, and the sequence in which they are done during screening may vary

from site to site. The typical screening data set includes age, sex, temperature, and body weight and height. If the local situation, available resources, and capacity of the outpatient unit permits, initial blood slide examination and haemoglobin concentration (or haematocrit[1]) can be performed on all patients during the screening procedure. Alternatively, these tests can be limited to febrile patients. If possible, a record book should be kept in which all cases screened are entered, with information on age, sex, address, temperature, blood film, and, if applicable, reason for exclusion from study. This information can be very useful for data interpretation and can provide clues about the rate of transmission.

NOTE: At all times, patient health and safety take precedence over the study procedures. Patients who are obviously severely ill should be cared for immediately as is appropriate for the facility.

A typical screening procedure involves rapid identification of all potential patients coming to the health facility (such as those coming to a paediatric outpatient clinic), measurement of temperature and body weight and height, and recording of basic demographic information (name, age, sex). If a patient's axillary temperature exceeds or equals 37.5 °C, blood should be collected for malaria smear examination and measurement of haemoglobin concentration. Patients who do not meet these basic enrolment criteria are treated by facility staff in accordance with routine practice; however, all clinical and laboratory findings obtained during the screening procedure should be shared with facility staff.

[1] Erythrocyte volume fraction.

5.4 Initial clinical evaluation/ enrolment evaluation

All patients meeting the basic enrolment criteria during the screening procedure should be evaluated in greater depth by clinical staff. Special care should be taken to detect the presence or early signs of febrile diseases other than malaria, as these will probably necessitate exclusion of the patient from the evaluation. Among paediatric populations, the most frequent confounding condition is lower respiratory tract infections: cough or difficult breathing, together with fast breathing, is an indicator for identifying and excluding patients suffering from such conditions. Fast breathing is defined as a respiratory frequency > 50/minute in infants under 12 months of age and > 40/minute in children aged 12–59 months. Other relatively common febrile conditions are otitis media, tonsillitis, measles and abscesses. Patients with these conditions should not be enrolled, but obviously need to be treated both for malaria (if they have parasitaemia) and the other infection as appropriate.

A case record form (Annex 5) should be used to record the general information and clinical observations for each patient

passed from screening into the study sent from screening. Particular care should be taken to record detailed instructions on how to find the patient's home to ensure that follow-up at home is possible should the patient fail to return to the health facility for scheduled visits.

5.5 Informed consent

Formal informed consent should be obtained from all patients meeting the enrolment criteria. The procedure for obtaining consent should conform to international and local guidelines for research on human subjects (*20*). The study should be fully explained to patients or parents/guardians, including potential benefits and risks. A recommended format for an informed consent script can be found in Annex 2.

5.6 Treatment

Patients meeting all enrolment criteria should receive treatment only after they have had the study fully explained to them and have willingly provided their informed consent. All antimalarial treatment should be given by study team members under observation using established treatment regimens for the drug under assessment. If more than one treatment is being studied, patients meeting all enrolment criteria should be randomly assigned to their treatment arm. Although this protocol is not intended to be a comparative clinical trial, such randomization is highly advisable. Randomization is best achieved, and more likely to avoid introduced bias, by strictly following a computer-generated randomization list, although other methods (e.g. coin toss) have been used.

Annex 6 provides treatment schedules for some antimalarial drugs of current interest. Enrolled patients should be observed for at least 30 minutes after treatment to ensure that they do not vomit the medicine. If vomiting occurs within 30 minutes of treatment, the full treatment dose should be repeated. Ancillary treatments, such as antipyretics, may be required and should be provided to patients by the study team. Patients with persistent vomiting (i.e. necessitating more than a single repeat dose) should be excluded from the study and immediately referred to the health facility staff for appropriate management.

Once the complete enrolment and treatment procedure is finished, the patient should be given a schedule for routine follow-up visits. It is also important to ensure that the patient (or patient's parent or guardian) knows that, if symptoms return at any time during the follow-up period, he or she should return immediately to the assessment team for re-evaluation, even if it is not a regularly scheduled follow-up day.

5.7 Recommended duration of follow-up

The recommended duration of follow-up is shorter for areas of intense malaria transmission than for areas with low

to moderate transmission. In both situations, these are *minimum* recommended follow-up periods – programmes that are able to maintain study quality over a longer period of assessment, and have access to the molecular techniques warranted by longer follow-up periods, are encouraged to do so. A detailed discussion of the principal issues involved in selecting an appropriate duration of follow-up for this protocol can be found in Box 3.

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 (polymerase chain reaction (PCR)) to assist in distinguishing recrudescence from reinfection. See Annex 7 for sampling and storing filter paper for molecular marker studies.

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 (14 days minimum) can still provide useful results and may be utilized. Molecular analysis to assist in distinguishing recrudescence from reinfection is highly recommended, but not strictly essential, for studies of more than 14 days' duration conducted in areas of low transmission.

The recommendations of this protocol reflect an attempt to reconcile the benefits of extended follow-up with the practical realities of conducting that extended follow-up, especially in areas of intense malaria transmission. These should be considered minimum recommended follow-up periods; if programmes do in fact have the capacity to conduct quality assessments with extended follow-up, they are encouraged to do so.

5.8 Follow-up schedule

The basic follow-up schedule is presented in table form in Annex 8. The day that the patient is enrolled and receives the first dose of medicine is traditionally designated Day 0. Thereafter, the schedule calls for clinical reassessments to be made on Days 1, 2, 3 and 7, then weekly for the remainder of the follow-up period (i.e. on Day 14 for 14-day assessments and Days 14, 21 and 28 for 28-day assessments). Patients should always be advised to return on ***any*** day during the follow-up period if symptoms return and not to wait for scheduled visit day. Although a minimum set of clinical indicators for follow-up assessments can be found in the example case record form presented in Annex 5, clinical reassessments should be sufficiently thorough to ensure patient safety and should include assessments not only for potential treatment failure but also for potential adverse reactions to the treatment drug.

Blood films for parasite count should be obtained and examined on Days 2, 3, 7 and 14 (and Days 21 and 28 if appropriate) or on any other day if the patient spontaneously returns.

Box 3: Duration of post-treament follow-up

The length of time that is appropriate for assessment of treatment response in vivo has been a topic of recent debate (*21*). Accumulated experience from a number of studies conducted in a variety of settings has raised important observations and issues that are relevant to this debate.

- Studies with shorter follow-up (i.e. <14 days) will underestimate overall treatment failure rates. This is especially true of drugs with longer elimination half-lives. The time to completely clear a drug from the body is six times the elimination half-life (clearance = 6 x $t_{1/2}$). Drug clearance is the parameter that defines the length of the follow-up in the therapeutic efficacy test: for SP, with an approximate sulfadoxine half-life of 100 hours (4 days), clearance will take 6 x 4 = 24 days; follow-up should therefore be for a minimum of 28 days, if possible.
- Follow-up periods longer than 14 days are appropriate for amodiaquine, chloroquine and SP (28 days), for lumefantrine+artemether (42 days), and for mefloquine (63 days) to allow drug levels in the blood to fall below the minimum therapeutic threshold. Any recrudescence of parasites before this threshold is reached would be due to drug resistance; recrudescence after this threshold is reached is not necessarily related to resistance (even sensitive parasites could recrudesce if blood drug levels are subtherapeutic).
- The longer the duration of post-treatment follow-up, the greater the chance that reappearance of parasites may be caused by reinfection rather than recrudescence. Inability to distinguish between reinfection and recrudescence can lead either to an overestimated rate of failure (if all reappearances are assumed to be recrudescences) or to an underestimated rate of failure (if all reappearances are assumed to be reinfections). This risk increases with increasing transmission intensity. More reinfections would be expected to occur between 14 and 28 days post-treatment in an area of intense transmission than in an area of very low transmission, assuming no lingering prophylactic effect of the drug.
- Molecular techniques can help to distinguish recrudescence from reinfection. However, these techniques are not definitive and they require specialized equipment and training that exceed the typical capacity of the malaria control programme. Use of these tests becomes essential when long-duration trials are conducted in areas of intense transmission. Without them, any additional benefit gained from extending the duration of follow-up may be lost as a result of inability to distinguish true treatment failure from reinfection.
- Longer-duration assessments have substantial logistic and cost implications that should not be taken lightly. Even short-duration assessments are prone to invalidation due to high rates of defaulting and loss to follow-up (see section 8). Extending the duration of follow-up requires even greater effort to maintain an acceptably high rate of follow-up completion, as default rates tend to increase with increasing follow-up periods. The additional costs of maintaining a study team in the field for longer follow-up periods, of trying to locate defaulters in the community, and of the molecular testing of samples all need to be weighed against the value of the additional information to the decision-making process of the programme.
- The intended purpose of this protocol is to collect data in support of programmatic decision-making as opposed to conducting research on drug resistance per se. While it may not be an ideal situation, experience has shown that few countries have changed first-line malaria treatment policy at a very early stage in the development of antimalarial drug resistance. Therefore, the added benefit of information gained from extended follow-up must also be weighed against the programme's ability to act on that information.

Additionally, blood films should be obtained whenever parasitological reassessment is requested by the clinical staff for reasons of patient safety. Study patients should be closely monitored in order to minimize risk. Haemoglobin status is typically reassessed on Day 14 (and Day 28, if appropriate). Because many drugs require multiple day dosing, the initial visits are critical not only for the efficacy assessment but also for patient safety; defaulters at this stage will not have received a complete course of treatment and may be at risk. The ultimate success of the study rests on minimizing loss to follow-up.

While patients can be encouraged to return on their own for scheduled follow-up visits, it is essential that provisions be made ahead of time for locating patients at home if they do not attend as requested. This requires obtaining very detailed directions to the home during enrolment and study team members familiar with the community who can be responsible for home visits. The schedule for treatment and follow-up examination given in this protocol must be followed rigorously to ensure data integrity. Patients who fail to appear on Day 1 and Day 2 and miss one dose of the treatment are withdrawn from the study definitively. After Day 3, patients who fail to appear on Day 7 but present on Day 8 (likewise Days 14/15, Days 21/22, and Days 28/29) may still be included in the study group. Deviation from the protocol of more than 1 day cannot be allowed, both for the safety of the patient and for the relevance of the data.

NOTE: Box 2 discusses the use of objectively measured elevations in body temperature and the appropriate situations in which history of fever could be used.

The same fever indicator should be used throughout the assessment. For example, assessments requiring a ***measured fever*** for enrolment should also require a ***measured fever*** for determining whether to collect additional blood smears and for identifying potential treatment failures during follow-up (see section on definitions of response to treatment). Assessments conducted in areas that have chosen to accept the use of a ***history of fever during the preceding 24 hours*** for enrolment, should also use ***history of fever in the preceding 24 hours*** to determine when to take additional blood films and for identification of potential treatment failure. Assessments should not use one fever criterion for enrolment and a different fever criterion for follow-up assessment.

There is one important exception to the above statements. In the definition of early treatment failure, there is an absolute requirement for a ***measured fever*** on Day 3 in order to classify the response as a failure, even if history of fever is used elsewhere. The reason for this is that fever on Day 2 is a frequent occurrence and use of ***history of fever in the preceding 24 hours*** on Day 3 would overestimate early treatment failure.

5.9 Rescue treatment

NOTE: Indications for provision of rescue treatment during a therapeutic efficacy test will be different for assessments conducted in areas of low to moderate transmission from those conducted in areas of intense transmission.

These differences reflect differences in programmatic priorities within different regions. Traditionally, because underlying acquired immunity among individuals with lifetime exposure to intense malaria transmission allows for parasitological failures to occur without concurrent clinical failure, studies done in these areas typically ***do not*** provide rescue treatment for asymptomatic parasitological failures; rescue treatment is given only to clinical failures. The exception is at the end of the follow-up period, when all patients with parasites are given rescue treatment regardless of clinical status. In contrast, programmes in areas of low to moderate malaria transmission typically do not distinguish between parasitological failures and clinical failures (which, because of low levels of immunity, frequently occur together anyway), and studies done in these areas will therefore provide rescue treatment for both asymptomatic parasitological failures or clinical failures.

If an assessment is being conducted in an area of intense transmission and patients with asymptomatic parasitaemia are not being given rescue treatment, it is important for these patients to be monitored closely. This may entail asking a patient to return the next day, even if this is not a regularly scheduled follow-up day, or even admitting the patient for observation. At any time, a patient who is deemed unfit to continue the assessment because of safety concerns may be withdrawn from the assessment and classified in the failure group. If a patient is parasitaemic at a time when monitoring must cease, rescue treatment is recommended to minimize the risk of symptomatic disease emerging in a patient who was enrolled in the study.

"Rescue treatment" is defined as a second-line treatment that is given to a study patient when a treatment failure has been identified (see note above). The choice of this treatment depends on the drug under assessment: ideally, the rescue treatment would use a drug that has an established efficacy and safety record in the case of infections resistant to the treatment under assessment and that may or may not be the officially recommended second-line malaria treatment in the country in question.

Patients experiencing severe deterioration in clinical status during the study should be referred immediately for appropriate inpatient care. However, most study patients should not develop severe illness — in fact, the study design aims to provide sufficiently close patient monitoring to allow for intervention before severe illness can develop.

5.10 Definition of study end-points

A study end-point is the point at which a patient will no longer be followed up within the context of the assessment. Valid study end-points include treatment failure, completion of the follow-up period without treatment failure, loss to follow-up, withdrawal from study (voluntary and involuntary), and protocol violation.

• ***Treatment failure and completion of study without treatment failure.*** For the purposes of this study, treatment classification occurs when an enrolled patient meets the criteria defining one of three possible categories of treatment failure or the criteria for treatment success. These are described in detail in section 5.11.

• ***Loss to follow-up.*** Loss to follow-up occurs when, despite all reasonable efforts, an enrolled patient cannot be found. Examples include failure to find a patient in the community after he or she misses a scheduled follow-up visit or moves out of the study area. Loss to follow-up differs from voluntary withdrawal: voluntary withdrawal suggests a conscious decision to no longer participate in the study, whereas loss to follow-up may have nothing at all to do with a specific decision by the patient to participate or not. Moreover, it may still be possible to ascertain the health status of a person who withdraws from the study, whereas the health status of a patient lost to follow-up is always uncertain.

• ***Withdrawal from study.*** One of the basic requirements for ethical treatment of human subjects is that they are always free to end their participation in the study at any time. A study patient (or a study patient's parent or guardian) who decides not to participate any further in the study is referred to as a voluntary withdrawal. An example of a cause for involuntary withdrawal would development of a concomitant illness that would interfere with the clear interpretation of study outcomes. It is recommended that reinfections identified by PCR be classified as withdrawals rather than as treatment successes. The rationale for this is presented in Box 4.

• ***Protocol violation.*** A protocol violation occurs when a study patient is removed from the study because of an event that does not allow for continued accurate interpretation of response to treatment. Examples include missed treatment dose, detection of a mixed infection during follow-up, or a credible report of additional antimalarial drug use outside the study protocol (such as self-medication).

In all cases, reasons for loss to follow-up, withdrawal, and protocol violation should be recorded and reported in detail. (See also section 7 below on data analysis and interpretation regarding the handling of loss to follow-up, withdrawals, and protocol violations during analysis.)

Box 4: Classification of reinfections identified by PCR

It is recommended that studies that include patient follow-up periods longer than 14 days use molecular techniques to differentiate recrudescences from reinfections. While most of these infections may, in fact, be reinfections, there are two issues that make their appropriate classification problematic. First, the usual PCR techniques used for this purpose are insufficiently sensitive to pick up minority populations of parasites present at Day 0. It is therefore possible that "new" parasites identified during follow-up actually represent recrudescence of parasites from a resistant minority population that was present from the start rather than true reinfections. Second, once a reappearance of parasites has been identified, the patient would receive rescue treatment (in areas of high transmission, this would occur only when in combination with fever; in areas of low to moderate transmission, it would occur regardless of presence of fever). Provision of additional treatment at this point would make further interpretation of follow-up for that patient impossible and potentially "mask" a true treatment failure. In other words, there is a possibility, albeit relatively small, that a recrudescence might have occurred at a later point during follow-up in a patient experiencing, and being treated for, a reinfection.

In either case, classification of all of these reinfections as treatment successes would lead to underestimation of true failure rates. Classifying all reinfections as treatment failures would lead to overestimation of true failure rates (and would render PCR unnecessary). For consistency, it is recommended that reinfections (as well as PCR unclassifiable results) be classified as involuntary withdrawals, as would be the case if the patient had self-treated during follow-up: the implications for bias are essentially the same in both circumstances.

NOTE: The classification system for response to treatment has been changed in this protocol. These changes are aimed primarily at reconciling differences between the previous protocol as it was applied in areas of intense transmission with that used in areas of low to moderate transmission. Additionally, under the previous classification system used in areas of intense transmission, asymptomatic parasitological failures were not specifically identified and reported. These changes allow for more comprehensive reporting of treatment failure.

5.11 Determination of study outcomes

The classification system of response to treatment has been modified (see Annex 9) and now has three categories for treatment failure (*Early Treatment Failure, Late Clinical Failure,* and *Late Parasitological Failure*) and one for treatment success (*Adequate Clinical* and *Parasitological Response*). People familiar with the previous classification system should review the new category definitions carefully, as some subtle but critical changes have been introduced. A description of these changes follows.

• ***Early Treatment Failure.*** There is now only a single uniform definition for *Early Treatment Failure* regardless of intensity of transmission; it has been modified to reflect an absolute requirement for a blood smear to be obtained on Day 2. Previously, in areas of intense transmission, a blood slide taken on Day 2 was required only if fever was present. This change has been made for reasons of study patient safety: waiting until Day 3 to obtain the first follow-up post-treatment blood smear was felt to be too long and risked delays in provision of rescue treatment for patients with unresponsive parasitaemia. There is also an absolute requirement for a measured increase in temperature on Day 3 in the presence of parasitaemia for a response to be classified as an *Early Treatment Failure* (history of fever on Day 3 in the presence of parasitaemia is *not* an acceptable indicator of failure).

• ***Late Clinical Failure.*** There is a single definition of *Late Clinical Failure* regardless of transmission intensity. However, minor variations exist because of differences in recommended duration of follow-up (14 days for intense transmission areas, 28 days for low to moderate transmission areas). Additionally, in areas of low to moderate transmission and under certain circumstances, use of history of fever in the preceding 24 hours, instead of a measured fever, is allowable when necessary (see Box 2).

• ***Late Parasitological Failure.*** Although there is a single definition of *Late Parasitological Failure,* differences exist between transmission areas for the same reasons as stated above. Additionally, this is a new category for people familiar with using the previous protocol in areas of intense transmission. Previously, asymptomatic parasitaemia occurring after Day 4 was included in the category of *Adequate Clinical Response*. However, information on the frequency of parasitological failures is important to understanding drug efficacy and should not be lost.

• ***Adequate Clinical and Parasitological Response.*** The definition of an adequate response to treatment also differed by transmission intensity in the previous protocol. The new definition of an adequate response to therapy reconciles these differences and is applied only to those responses that demonstrate both clinical and parasitological resolution by the end of the follow-up period.

6. Ethical considerations

Therapeutic efficacy tests should always be conducted under the direct supervision of qualified medical personnel. At all times, the safety and welfare of the individual patient should be ensured to the greatest extent possible, and appropriate management of each patient should take priority over the conduct of the test.

While this protocol is intended primarily as a programmatic tool, the study still should be conducted as if it were a research study. In almost all conceivable situations, this would require independent ethical review of the protocol (especially when the drug under study is not currently a recommended malaria treatment in the country in question). Patients under consideration for enrolment should have both the risks and benefits of the study explained thoroughly through a formal informed consent process and their participation should not be coerced in any fashion. The investigators must establish secure safeguards of the confidentiality of subjects' data.

Additionally, all test procedures should be conducted with the patient's health and welfare given priority over continuing in the assessment. Patients have the right to withdraw from the study at any time and for any reason without fear of prejudices.

7. DATA MANAGEMENT AND ANALYSIS

7.1 Data management

Annex 5 provides examples of data collection forms. As mentioned previously, the data on these forms should be considered as the minimum essential variables to consider: individual programmes may want to collect additional information that is relevant to their particular situation.

The primary study coordinator should thoroughly check all data forms on a daily basis, not only for completeness but also to ensure that they are being filled out clearly, that the information collected makes sense, and, most importantly, that study patients are classified correctly and that those who qualify for rescue treatment have received that treatment. Care should also be taken to ensure that samples are correctly labelled and that all laboratory results are reported correctly and promptly.

Use of a computer greatly simplifies data cleaning and analysis, although the process can be done "by hand". If computer-based data entry is used, double-entry validation is strongly recommended to minimize clerical errors in transcription. Double-entry validation is typically done by having two different data entry clerks enter the same information into two separate files, and then comparing the two files. Any differences between the files suggest mistakes in data entry that can be corrected by referring to the original hard-copy data forms.

7.2 Data analysis

NOTE: The recommended method for data analysis represents a major change from the previous protocol.

The recommended method for analysis of data obtained from this protocol is survival analysis. Computer programmes are available for this method of analysis (for example, EpiInfo 2002, available free via: *www.cdc.gov*, or Excel data analysis sheets, available free via: *www.rbm.who.int*), but calculation by hand is not difficult (see Annex 10).

The benefits of this method of analysis include the use of data from patients who have withdrawn or are lost during follow-up, easy calculation of mean time to failure, and calculation of a reasonably unbiased estimate of failure rates.

To obtain results that can be compared with historical results, it is also recommended that data be analysed using the traditional "per-protocol" approach. This approach retains for analysis only data from patients with evaluable results, i.e. those patients with known efficacy end-points, such as *Early Treatment Failure, Late Clinical Failure, Late Parasitological Failure,* and *Adequate Clinical and Parasitological Response;* data from all other patients are excluded from analysis and do not contribute to the denominator. Nonetheless, if this method is used, the number of patients excluded

from analysis — as well as the reasons for their exclusion — should be reported fully.

Intent-to-treat (ITT) analysis has also been used. In ITT analysis, all patients randomly allocated to a treatment group are retained in analysis whether or not there is an evaluable outcome; that is, they contribute to the denominator if not the numerator. The primary purpose of ITT analysis is to maintain the integrity of the randomization process, which is important for randomized, comparative clinical trials; this protocol, however, is not intended primarily for comparative trials.

7.3 Use of the data for updating treatment policy

The appropriate interpretation of data derived from this protocol has generated considerable debate, and revolves around the simple question of how much treatment failure is too much to justify further use of the drug in question. While it is tempting to define a threshold of failure with a single number above which treatment failure is unacceptable, in practice there is no simple answer to this question — the answer will depend on many factors unique to each situation. For example, the tolerance for treatment failure will increase if the potential alternatives are not readily available to the programme, are too costly for programmatic use, or are associated with serious safety concerns. Programmes in areas of intense transmission have historically had fairly high tolerance for treatment failure, reflected by the fact that relatively few countries in sub-Saharan Africa have changed treatment policy despite reportedly high levels of chloroquine treatment failure. Furthermore, programmes in areas of intense transmission have traditionally excluded asymptomatic parasitological failure from consideration, and have reacted programmatically only to clinical failure rates (as reflected in the design and interpretation of the 1996 WHO standardized in vivo protocol for areas of intense transmission). The rationale behind this approach is related to the effects of acquired immunity and the frequency with which apparently asymptomatic parasitaemia occurs. As more information accumulates regarding the health effects (especially the haematological effects) of apparently asymptomatic parasitaemia, as use of effective preventive interventions (such as insecticide-treated nets) becomes more widespread, and as financial mechanisms evolve to make treatments with high parasitological efficacy programmatically viable, this approach is likely to change.

Conversely, programmes in areas of low transmission have historically had a very low tolerance for any failure and have treated parasitological failure and clinical failure equally (aided, no doubt, by the relative lack of acquired immunity and the generally strong correlation between parasitological and clinical failure). There have been attempts to define such cut-offs, most concerning a transition from chloroquine to SP (*22–24*). WHO

has simplified these earlier attempts into very programmatic terms, which provide a good "rule of thumb" for initiating debate on malaria treatment policy in a given country (*25*). In this scheme (developed primarily for areas of intense transmission), four periods leading to drug policy change are defined: "Grace Period" when clinical failure rates are between 0% and 4%; "Alert" when they are between 5% and 14%; "Action" when they are between 15% and 24%; and finally "Change" when they are 25% and above. Because of the lead time needed to actually change treatment policy (periods often between 12 to 18 months and as long as 10 years have been recorded), the process of change must be started before high levels of treatment failure are reached.

Finally, it should also be noted that many factors influence the development of malaria treatment policy, of which in vivo efficacy is but one. By their very nature, in vivo studies reflect the use of malaria drugs under optimal conditions, i.e. correct diagnosis followed by appropriate dosing with drugs of known high quality given under direct observation, thereby ensuring compliance. Use in practice may be vastly different, so that a highly efficacious drug may have poor programmatic effectiveness. Providers' treatment practices, patients' compliance with recommended drug doses and schedules, drug availability, adverse effects (even minor side-effects), safety in high-risk groups such as infants and pregnant women, and cost are all important factors in determining the best choice of drug for malaria treatment within a programmatic context.

NOTE: During the WHO consultation on the Goal of Antimalarial Treatment Policy in the WHO Africa Region held in Harare, Zimbabwe, 14–15 August 2003, the experts reached the consensus that persistent parasitaemia is associated with increased risk of clinical episode, anemia, and increased gametocyte carriage.

Therefore, the experts agreed that parasitological response should be an additional indicator for the interpretation of the therapeutic efficacy test. The cut-off point for policy change using the standard WHO protocol for high transmission area is now as follows: *Adequate Clinical and Parasitological Response* < 75% (Total Failure ≥ 25%) and *Adequate Clinical Response* < 85% (Clinical Failure ≥ 15%).

The definition of *Adequate Clinical Response* remains the same as in 1996. Using the new classification, it is equal to the sum of *Adequate Clinical and Parasitological Response* and *Late Parasitological Failure.*

Total Failure is equal to the sum of *Early Treatment Failure, Late Clinical Failure* and *Late Parasitological Failure.*

Clinical Failure is equal to the sum of *Early Treatment Failure* and *Late Clinical Failure.*

7.4 Reporting

Proper reporting of results, whether for programmatic use or for publication, is of paramount importance. In order to maximize the usefulness of studies and their comparability between sites and over time, adherence to the details of this protocol is critical. Interpretation, and subsequent comparability, of the results of past in vivo studies has suffered from innumerable protocol modifications. In one extreme example, in vivo studies done in the same general location during the same time frame yielded substantially different results, principally due to modifications to the "standardized" protocol introduced by individual investigators. Situations such as this have led to confusion and, worse, dismissal and disregard of results by decision-makers.

Essential to the proper interpretation of results is complete and proper reporting of both methods used and results. Despite efforts to standardize these methods, differences will remain between studies conducted in areas of intense transmission and those conducted in areas of low to moderate transmission. Similarly, the exact method of analysis should be reported (for example, whether results reflect survival analysis, per-protocol, or intent-to-treat). Deviations from this protocol are strongly discouraged; however, if any deviation is included, its exact nature and the rationale behind it must be clearly stated.

Formal reporting should also include results of data quality assurance, especially of blood smear examination, using the methods described in section 8.

8. TECHNICAL CONSIDERATIONS AND QUALITY ASSURANCE

8.1 Temperatures

Because outcome classifications are dependent on measured body temperatures (especially in areas of high transmission), both thermometers and the temperature-taking technique of the clinical staff should be reviewed. The quality of temperature-taking technique should be ensured through proper training and continuing supervision. Any measured temperature below 36.0 °C should be repeated.

Before the assessment, thermometers should be tested in a water-bath of known temperature (i.e. measured using a reliable thermometer, preferably of laboratory quality, before the study thermometers are tested). If logistically possible, this procedure should be repeated during and at the end of the assessment.

8.2 Body weights

Because dosing is based on body weight, it is important to ensure the reliability of the scales used in the study. The accuracy of scales should be verified before use and at least once during the study.

8.3 Blood slides

8.3.1 *Reagent preparation*

Details of reagent preparation can be found in Annex 11.

8.3.2 *Slide preparation and staining*

Preparation and staining of blood slides follows the procedure outlined in *Basic malaria microscopy, Part 1* (*26*). Fresh Giemsa stain dilution should be prepared at least once each day and possibly more often, depending on the number of slides being processed. The best results are obtained by staining slides for 45–60 minutes in a 2.5–3% Giemsa stain solution. Increasing the concentration of the Giemsa solution can reduce the time needed for staining, but the stain quality (and therefore the quality of the microscopical examination) will be less predictable.

In general, it is best to take two blood slides per patient. One slide can then be rapidly stained (10% Giemsa for 10–15 minutes) for initial screening of patients, while the other is retained. Should the patient subsequently be enrolled, the second slide can be stained more carefully (e.g. 2.5–3% Giemsa for 45–60 minutes). This slower staining method should also be used for all slides obtained during patient follow-up visits. The use of slides with one frosted edge that can be marked or of conventional slides marked with a "permanent" glass writing pen is recommended.

8.3.3 *Examination and interpretation*

• ***Screening smears.*** The thick blood smear for initial screening should be examined by counting the asexual parasites and the white blood cells in a limited number of microscopic fields. Adequate parasitaemia for enrolment requires at least 1 parasite for every

3–4 white blood cells, corresponding to approximately 2000 asexual parasites/μl, for high transmission areas, and at least 1 parasite for every 6–8 white blood cells, corresponding to approximately 1000 asexual parasites/μl, for low to moderate transmission areas.

• ***Enrolment and follow-up smears.*** The second blood smear should be used to calculate the parasite density of enrolled patients. Blood smears taken during patient follow-up should be examined in the same manner.

Parasite density is calculated by counting the number of asexual parasites against a set number of white blood cells (WBCs) — typically 200 or 300 — in the thick blood film, using a hand tally counter. Once a field has been started, it should always be counted to completion; the final WBC count will therefore rarely be exactly 200. If more than 500 parasites have been counted before 200 WBCs have been reached, the count is stopped after the reading of the last field has been completed.

Parasite density, expressed as the number of asexual parasites per microlitre (μl), is calculated by dividing the number of asexual parasites by the number of WBCs counted and then multiplying by an assumed WBC density (typically 6000–8000 WBCs/μl).

The same technique should be employed for establishing parasite counts on each of the subsequent blood film examinations. Parasitaemia is measured by counting the number of asexual parasites against a number of WBCs in the thick blood film. When the number of asexual parasites drops below 10 per 200 WBCs, counting should be done against at least 500 WBCs (i.e. to the completion of the field in which the 500th WBC is counted).

A blood slide can be considered negative when the examination of 100 thick-film fields does not show the presence of asexual parasites. The presence of gametocytes on any enrolment or follow-up slide should be noted, but this information does not contribute to the basic evaluation of the test.

In addition, 100 fields of the second thick film should be examined to exclude mixed infections; in case of any doubt, the thin film should be examined for confirmation. If examination of the thin film is not conclusive, the patient should be excluded from the study after complete treatment.

8.3.4 *Quality control*

Quality control of microscopy involves ensuring that good-quality Giemsa stain is used, that staining procedures adhere to recognized methods, that microscopes are of adequate quality and in good repair, and that the microscopy results are reliable.

• ***Quality control for Giemsa stain***

To ensure that proper staining results have been achieved, a known positive smear should be included with each new batch of working Giemsa stain. Control slides may be prepared from a patient's blood and stored for future use.

Method. From a patient known to have malaria infection, collect a blood sample in

an EDTA (ethylenediaminetetraacetic acid) blood tube. An ideal blood sample has at least one parasite in every 2–3 fields on a thin blood smear. Make as many thin smears as possible, preferably within one hour of drawing the blood from the patient. Allow the smears to dry quickly, using a fan or blower at room temperature. Fix the smears in absolute (100%) methanol and allow them to dry. Place them, touching back to back, in a box with separating grooves. Label the outside of the box with the species, date and "Giemsa control slides". The slides can be stored at room temperature but will last longer if stored at or below –70 °C. Just before use, remove the slide from the box and allow the condensation to evaporate; label the slide with the date and "+ control". The smear can then be stained and examined to check that the working solution of Giemsa stain is of good quality.

• ***Quality control for microscopic examination and quantification***

The procedure for assessing microscopy results should emphasize the reproducibility of final outcome classifications over reproducibility of exact parasite counts: even two highly experienced microscopists can differ widely in their parasite counts, especially at higher parasite densities. For the purposes of this assessment, discrepant results that lead to a change in outcome classification for a given patient are more important than discrepant results for individual blood slides that make no difference to the ultimate classification.

Method. Ideally, two qualified microscopists should independently read all of the slides and parasite densities will be calculated by averaging the two counts. Blood smears with non-concordant results (differences between the two microscopists in species diagnosis, or differences in parasite density of > 50%) will be re-examined by a third, independent microscopist, and parasite density will be calculated by averaging the two most concordant counts.

If this is not feasible, a random selection of 10% of the enrolled patients or a minimum of 10 randomly selected patients, whichever is the greater, should be selected for rechecking. The second microscopist should be blinded to the patient number, day of follow-up, original results, and outcome. The second microscopist should re-examine all microscope slides from patients selected for quality control and should provide results as if reading the slide for the first time (i.e. determine whether negative or positive and, if positive, provide a parasite count using standard procedures).

After all slides have been reviewed, the new patient-specific data should be reassembled and the results should be used to assign an outcome using the recommended classification system. The new outcome should be compared with the original and discrepancies noted. If more than 10% of the subsample (i.e. 1 patient for a subsample of 10 patients) has non concordant outcomes, all study results should be reviewed.

8.4 Haematological assessment

Haematological assessment of the patient should be done whenever possible by measuring either the haematocrit or the haemoglobin concentration. Measuring haematocrit requires specialized equipment and supplies (such as a centrifuge and microhaematocrit capillary tubes); haemoglobin concentration is most easily measured by using commercially available, portable photospectrometers, but fairly simple laboratory-based assays are also available.

Valid comparisons between Day 0 and Day 14 require either haematocrit values or quantitatively determined haemoglobin levels; semi-quantitative methods, such as the WHO haemoglobin colour scale, are not accurate enough for use in these studies.

In healthy persons, the haematocrit (expressed as a percentage) is roughly 3 times the haemoglobin concentration (expressed in grams per decilitre). This ratio is maintained in normocytic anaemia, but in most of the tropical forms of chronic anaemia the ratio is 3.3:1. Enrolment requires a haemoglobin level above 5.0 g/dl or a haematocrit above 15%.

8.5 Drug quality

The drugs used in a therapeutic efficacy test must be of unquestionable quality. Unfortunately, experience suggests that merely buying test drugs from an otherwise reputable source is insufficient to guarantee quality, as poorly manufactured antimalarial drugs have been identified from many sources.

To the extent possible, programmes should obtain test drugs from WHO or other internationally recognized sources of good-quality drugs. For drugs that cannot be obtained through such sources, it is recommended that the drugs used in a therapeutic efficacy test be tested (for both content and dissolution) by a qualified pharmaceutical laboratory ***before*** studies begin.

9. EVALUATING STUDY VALIDITY

The validity of a study may be compromised by exclusion of a high proportion of enrolled patients from analysis, by too-frequent protocol violations, or by frequent clinical or laboratory mistakes leading to misclassification of outcomes. A primary responsibility of the study coordinator is to maintain vigilance over the quality of all aspects of data collection and study conduct. Mistakes must be identified and corrected rapidly to minimize wasted effort or invalidation of the study results.

In general, if 20% or more of enrolled patients are lost to follow-up or are otherwise excluded due to protocol violations, study validity can be questionable. This is especially true if a high rate of loss to follow-up or exclusion also reduces the sample size to less than the calculated minimum. While the latter problem can be dealt with by enrolling more patients than needed to account for expected drop-out, the former frequently requires tracking patients into the community if they fail to return for follow-up visits, plus constant efforts to ensure the quality of data collection.

It is recommended that misclassification of outcomes be kept below 10% of total sample size. Clinical mistakes can be avoided only by adequate training of clinical staff before the study begins and constant supervision during the study.

Laboratory mistakes should be avoided by staffing the laboratory with experienced and qualified technicians and by following the quality assurance methods described above.

A frequent cause of misclassification of outcomes is poor microscopy — especially inaccurate counting of asexual parasites and subsequent miscalculation of parasite density. Critical mistakes in basic microscopy include misdiagnosis of parasite species and missing mixed infections at enrolment. Accurate parasite counts are critical throughout the study: inaccurate counts on Day 0 can lead to an increase in protocol violations (e.g. enrolling patients with too few parasites) or loss of enrolment efficiency (e.g. excluding patients with adequate numbers of parasites). Misclassification of outcomes can occur when parasite counts are inaccurate on Day 0 and/or Day 3 (when absolute numbers of parasites need to be compared to determine whether ≥ 25% of the Day 0 count remains on Day 3) or during follow-up.

After quality assurance of blood slides using the methods described above, all patient outcomes should be reclassified using the corrected blood slide results. If 10% of patients or more have changes in outcome classifications, the study should be considered invalid.

10. Determination of underlying malaria transmission intensity

The development of clinical immunity depends largely on the frequency of parasite exposure from birth. In areas of stable high-intensity transmission, clinical immunity is acquired early in life, often well before the child's fifth birthday. As the intensity of transmission declines to very low levels and becomes unstable, clinical disease events may be equally common among young children, older children, and adults as populations fail to develop an effective clinical immunity. Under these conditions, the overall frequency of disease, in an average year, is generally low.

In much of sub-Saharan Africa there is intense transmission of *P. falciparum*, clinical immunity is acquired early in life, and the greatest concentration of disease burden falls upon infants and young children. However, the climatic and ecological conditions in some areas of the continent do support exceptionally low-intensity transmission, with a tendency towards periodic epidemics; these areas are typically at high altitude, in arid areas or urban settings.

For the practical purposes of drug-sensitivity testing, it is important to distinguish areas with conditions that result in a slow acquisition of clinical immunity (low to moderate transmission areas) from those where there is relatively rapid acquisition of immunity (high transmission areas). In areas of low to moderate transmission, recruitment of patients for drug-sensitivity testing should reflect the local disease ecology and cover both children and adults (although, even here, children under 5 years of age may be at greater risk for other reasons and would ideally still be a focus of study). In areas of high transmission, sensitivity testing would focus on children aged less than 5 years.

Typically, areas of low-intensity *P. falciparum* transmission would be those that fall between the classical definitions of unstable malaria and stable hypoendemicity. There are a number of ways in which these epidemiological definitions can be described, involving complex parameter estimation through entomological studies (*27*). These approaches are often beyond the scope of the teams investigating drug sensitivity. For practical purposes, investigators should aim to distinguish unstable malaria settings as follows:

• ***Consulting existing data.*** A database containing the results of extensive parasitological and entomological surveys undertaken across the continent has been created by the Mapping Malaria Risk in Africa (MARA) collaboration. Searches can be made of these data at: *www.mara.org.za.* All areas where the prevalence of infection among children aged under 10 years is 10% or less can be regarded as hypoendemic, low intensity or potentially unstable. In addition, MARA has developed a series of maps of modelled malaria risk that can be viewed at the same web site. The country maps identify geographical areas that, according to climatic determinants, are unlikely to support stable endemicity. In general terms, areas with a fuzzy logic value of less than 0.25 (see the web site for details on fuzzy logic modelling) are unlikely to support stable, endemic malaria and can be regarded as potentially unstable

(*28*). The climate models do not provide adequate predictions of urban malaria transmission, as these are often lower in reality than is suggested by the models. Other national databases should also be consulted; for Kenya, for example, a national archive can be found at: *www.kmis.org*.

• ***Empirical estimation of malaria infection risks.*** The simplest and most frequently performed surveys of malaria endemicity are cross-sectional infection prevalence surveys. Depending upon resources, these surveys can be undertaken among randomly selected children (aged 2–9 years) resident in the community surrounding the clinic, or among infants (aged 3–11 months) attending the clinic for routine vaccination or growth monitoring. The latter will provide a lower estimate of the infection prevalence compared with older children but, conversely, might overestimate infection risks if the attendance is associated with illness. To identify areas of 10% prevalence of infection, a sample size of 200 children would allow a 95% confidence interval of 6–14%. Thick and thin blood smears would have to be taken from finger-prick samples following informed consent from the parents or normal guardians of the children. Slides would be examined as described elsewhere in this protocol.

Spleen rate has also been used as a proxy marker of malaria endemicity. Definition of hypoendemic, mesoendemic, hyperendemic and holoendemic malaria in relation to the spleen rate and the parasite rate are given in Annex 12. However, these markers cannot be taken as a reliable index since the spleen and parasites rates are arbitrary and do not capture the seasonal nature of transmission (*27*).

• ***Age distribution of clinical cases.*** A proximate measure of transmission stability could be derived directly from an examination of the clinical case data at the clinic. By summing the number of adult malaria cases (aged ≥ 15 years) and the number of cases aged < 15 years over an entire year or transmission season, the adult : child case ratio can be calculated. A ratio of 1 or greater, i.e. equivalent or greater numbers of adult cases compared with children, would suggest a slow development of clinical immunity in the community and hence low-intensity transmission. This clinical description makes a number of assumptions:
– the community using the clinic is typical of many rural populations in developing countries, with approximately half of the population aged < 15 years;
– there are no age-dependent biases in clinic attendance; and
– clinical diagnosis is of a high standard and includes microscopy.

The last assumption is important because many clinic records pertain to presumptive malaria diagnoses, which may be completely different from true, parasitologically confirmed clinical cases. Where investigators feel that the case definitions are inappropriate for retrospective review, they can mount a limited prospective assessment of clinical malaria among children and adults with improved diagnostics.

11. RECOMMENDATIONS FOR ESTABLISHING A SENTINEL SITE SURVEILLANCE SYSTEM FOR DRUG EFFICACY

The overriding purpose of this protocol is to provide evidence to inform the development of guidelines and/or policies for the treatment of uncomplicated malaria. In practice, most countries have developed and implemented a single, uniform policy for the entire country. In a few countries, treatment guidelines are more closely tailored to the local situation, with two or more different policies for different geographical regions of the country. In either case, it is necessary to have reliable and current efficacy data.

Ideally, antimalarial drug treatment policy and/or guidelines should be developed, reviewed regularly, and updated as needed, on the basis of current evidence obtained from studies using this standardized protocol. As far as possible, treatment guidelines should also reflect local resistance patterns to ensure that efficacious treatment will be provided to the greatest possible proportion of the population.

To accomplish the dual goals of timely and ongoing data collection and local relevance, a sentinel site surveillance system for antimalarial drug efficacy should be established. Although no definitive scientific advice can be given regarding the number of sites needed, experience suggests that a balance between representativeness and practicality can be achieved with four to eight sites. Programmes should increase or decrease this number as needed to account for the size of the country, population distribution and density, differing malaria epidemiology or ecology, and other factors deemed important. When making such decisions, emphasis must be placed on the need for a "manageable" number of sites to ensure proper monitoring and supervision.

Based on accumulated experience rather than definitive science, it is recommended that assessments be conducted at least once every 24 months; for comparability, they should always be carried out at the same time of year. Most programmes conducting sentinel site surveillance find it easiest to alternate test sites, for example testing four sites 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 core group of experts (from the national malaria control programme, ministry of health, universities, institutes of research, national reference laboratory) should be established to coordinate all activities — training, supervision, collection and analysis of data — and to forward recommendations to the drug policy-makers. This core group should ensure the quality of laboratory diagnosis at the sentinel sites and provide continuous logistic support.

The minimal requirements for establishing a sentinel site are the availability of trained and motivated clinical personnel and microscopists, plus a laboratory equipped

for blood film examination. The sentinel site can be at a peripheral facility (true community-based studies of drug efficacy are logistically difficult and expensive) or based at a district-level health facility. Patients seeking health care at hospitals in urban settings may have more complex clinical presentations, may be more likely to have been referred there because of previous drug failures, and/or may be more difficult to find during follow-up. For these reasons, efficacy monitoring should be done at the most peripheral sites possible.

The following characteristics should be considered in the selection of sentinel sites:
– local population density;
– accessibility to the site and feasibility of close supervision;
– epidemiology of malaria, especially transmission intensity and seasonality;
– population mobility and migration (especially in border areas);
– distribution of malaria treatment failures reported by health information system (some areas of the country may be known or suspected to have higher treatment failure rates).

Sentinel sites should be selected to be representative of each major epidemiological stratum 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 national resources and the availability of trained staff at the selected sentinel sites.

Given 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 will not be uniform across sites: some sites may identify a substantial deterioration in treatment efficacy while others continue to record an acceptable response to the same drug. The programme should consider questions associated with this possibility and develop a plan for responding to it.

For example:
• Can specific treatment guidelines be targeted to affected areas without changing national policy or guidelines as a whole?
• How many sites need to show unacceptable treatment failures before national policy or treatment guidelines are altered?
• What happens when one site in the system demonstrates a poor level of treatment success with the existing first-line drug but the national policy or treatment guidelines are not altered because efficacy is higher at the other sites? At what point does it become unethical to continue to evaluate that drug at that site in the future? At what level of treatment failure would this occur? What are the implications for routine treatment of patients at that point?

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Annex 1

Definition of severe malaria[1]

Severe manifestations of *P. falciparum* malaria in adults and children

Prognostic value[a]			Frequency[a]	
Children	Adults		Children	Adults
Clinical manifestations				
+	(?)[b]	Prostration	+++	+++
+++	+	Impaired consciousness	+++	++
+++	+++	Respiratory distress (acidotic breathing)	+++	+
+	++	Multiple convulsions	+++	+
+++	+++	Circulatory collapse	+	+
+++	+++	Pulmonary oedema (radiological)	+/–	+
+++	++	Abnormal bleeding	+/–	+
++	+	Jaundice	+	+++
+	+	Haemoglobinurea	+/–	+
Laboratory findings				
+	+	Severe anaemia	+++	+
+++	+++	Hypoglycaemia	+++	++
+++	+++	Acidosis	+++	++
+++	+++	Hyperlactataemia	+++	++
+/–	++	Hyperparasitaemia	++	+
++	++	Renal impairment	+	+++

[a] On a scale from + to +++; +/– indicates infrequent occurrence.

[b] Data not available.

[1] World Health Organization. Severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, 94(Suppl. 1):1–90.

Annex 2
Informed decision making

Recommendations made by Secretariat Committee on Research Involving Human Subjects (SCRIHS) of the World Health Organization (October 2003).

The consent form has two parts: (*a*) a **statement** describing the study and the nature of the subject's involvement in it, and (*b*) a **certificate of consent** attesting to the subject's consent. Both parts should be written in sufficiently large letters and in simple language so that the subject can easily read and understand the contents. As far as possible, medical terminology should be avoided in writing up the consent form. It should be written in the prospective subjects' mother tongue.

The statement is given or read to each prospective subject. Any questions the subject may have are then answered and, if consent is given, the certificate is signed by the subject or, if consent was verbal, by the staff member who provided the information in the presence of an independent literate witness and ensured that all the information related to the study was understood. By signing, the staff member confirms that consent was given freely. A signed certificate must be obtained in this way for each subject admitted to the study, and a copy must be offered to the subject.

In writing up the **statement** take note of the following points:

- Indicate that this is a research study to distinguish it from routine care.
- Explain why the study is being done and why the subject has been asked to participate.
- Describe, in sequence, what will happen in the course of the study, giving enough detail for the subject to gain a clear idea of what to expect.
- Explain whether or not the study procedures offer any benefits to the subject or to others.
- Explain the nature, likelihood and treatment of anticipated discomfort or adverse effects, including psychological and social risks, if any. Where relevant, include a comparison with risks posed by standard treatments or drugs, and an indication of whether the drug or procedure under investigation bears risks equal to, greater than, or less than the standard. If the risks are unknown or a comparative risk cannot be given it should be so stated.
- Explain what will be done to the "excess" biological samples that will be taken as part of the research protocol – how soon after the research will they be discarded or destroyed or for how long will they be stored, how will they be stored and who will have access to these samples and under what circumstances. Who will have the responsibility to eventually destroy or discard these samples?

- State that all records are to be kept confidential. If absolute confidentiality cannot be guaranteed, explain why this is so. Explain the extent to which the confidentiality of subject-specific information will be protected during the study and in any resulting public disclosures at meetings or in publications. Also state which persons other than the researchers may have access to the records and/or to whom information may be disclosed. State where and in what form subject-specific information will be stored, and when, how and by whom it will be destroyed.

- State that the subject has the right to refuse to participate or withdraw from the study at any time without in any way affecting his/her current or future care. State what alternative treatment or procedure would be available for those who choose not to participate in the study.

- *Contact information*. The name, address and telephone number must be included on the form of the person(s) to be contacted by the research subject if they have questions about, or experience any problems during the course of, the study. These should be persons who are available on site, intimately involved with the research project and easily accessible to research participants, not chairpersons of ethics committees or deans of schools or heads of departments.

- The **certificate of consent,** which should be a part of the information sheet, should begin with a brief summary of the main items from the above statement. It should end with a paragraph such as the following:

  I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand that I have the right to withdraw from the study at any time without in any way affecting my further medical care.

  The informed consent document should be signed by the subject or, when the subject is illiterate, by an independent (preferably literate) witness who provides the information, and who ascertains that it was understood and confirms that consent was given freely. Whenever possible, the witness should be selected by the subject and he/she should not be connected with the research team. Whenever feasible, the recruitment of illiterate subjects should take place in the presence of a literate witness.

- *Statement of Consent for Storage and Future Use of Leftover Specimens and Signatures*. If the protocol calls for the storage and future use of specimens, the consent form should provide the option of either immediate destruction or maintenance and use of specimens for future research on either the same subject or for future research of any type. This section should also provide the option for specimens to either be destroyed after a certain period of time or kept indefinitely, as well as the option for the participant's identity to either be removed or kept with the leftover specimens.

Annex 3
Detection of antimalarial drugs in urine

• Detection of chloroquine

Chloroquine and its metabolite can be detected by an adapted Saker–Solomons test.[1] For this test, 2 ml of urine are mixed in a glass tube with 1 ml of phosphate buffer, pH 8.0, and 0.2 ml of tetrabromophenolphthalein ethyl ester (TBPEE). The tube is capped, vigorously hand-shaken for about 15 seconds, and left to stand for about 15 minutes to allow phase separation before the results are read. A yellow-green colour of the chloroform layer indicates a negative test for chloroquine and metabolites; a red to purple colour of the organic layer indicates a positive result — the shade depends on the concentration of chloroquine and/or metabolites. Comparing the colour obtained in the patient's test with standards containing known concentrations of chloroquine provides semiquantitative results. Other drugs can show cross-reactivity with chloroquine in the Saker–Solomons tests, including quinine, proguanil and, to a lesser extent, mefloquine and pyrimethamine.

– Phosphate buffer stock solution is prepared by mixing 162 g of K_2HPO_4,$3H_2O$ with 5 g of KH_2PO_4 and 500 ml distilled water.
– TBPEE is prepared by dissolving 50 mg TBPEE in 100 ml chloroform, then shaking this mixture with 10 ml of 2 mol/litre HCl.

• Detection of sulfonamides

Lignin test

For this test, two drops of urine are placed on a strip of unbleached paper (blank newspaper or unbleached paper towel). One drop of 10% hydrochloric acid (3 mol/litre) is added to the centre of the moistened area. A yellow or orange colour appearing within 20 seconds denotes the presence of sulfonamides. Although not highly sensitive, the lignin test is adequate for the purposes of this protocol. This test also cross-reacts with other drugs.

Qualitative colorimetric test for sulfonamides in urine

This test is based on the extraction of sulfadoxine (or other sulfonamide drugs) from urine into ethyl acetate followed by the formation of a violet-red Shiff base upon addition of acidic methanolic *p*-dimethylaminocinnamaldehyde. The intensity of the colour is proportional to the sulfonamide concentration in the sample. Semi-quantitative estimates can be made by comparing colour intensities in unknown samples with those in urine samples containing known concentrations of sulfonamide.

[1] Mount DL et al. Adaptations of the Saker-Solomons test: simple, reliable colorimetric field assays for chloroquine and its metabolites in urine. *Bulletin of the World Health Organization,* 1989, 67:295–300.

Reagents

1. Colour reagent solution: 0.3% (m/v) *p*-dimethylaminocinnamaldehyde (DMACNA) and 0.6% (v/v) H_2SO_4 in methanol (for example: 300 mg DMACNA + 0.6 ml H_2SO_4 + 100 ml methanol)
2. Buffer solution: 1.5 mol/litre phosphate buffer, pH = 5.5 (11 g K_2HPO_4,$3H_2O$ + 85 g KH_2PO_4 dissolved and diluted to 500 ml with distilled water
3. SAG-10 silicone antifoam emulsion diluted 50/50 with water
4. Ethyl acetate
5. Sulfadoxine standard: 1mg/ml in ethanol

Procedure

1. Prepare a standard curve by adding appropriate volumes of standard sulfadoxine solution to 2 ml of drug-free urine according to table below:

Sulfadoxine(ppm)	Volume (µl) of 1 mg/ml standard solution
0 (blank)	0
1	2
3	6
7	14

2. Using a pipette, add 2 ml of patient's urine to 0.5 ml ethyl acetate and 0.5 ml buffer solution in a 1-dram clear glass screw-cap vial. Cap the vial and invert 20 times. After the ethyl acetate phase rises to the top, add one drop of SAG-10 defoamer to the acetate phase to break the emulsion formed during inversion. If needed, the vial can be swirled gently to help break up the emulsion. After the ethyl acetate layer has cleared, add 3 drops of the colour reagent to the top of the layer without agitation. The formation of a red-purple ring at the top of the ethyl acetate layer indicates the presence of sulfadoxine. For semi-quantitative results, compare the intensity of the red-purple colour with that produced by standards.

Notes:

1. The colour will fade slowly with time but immediately upon significant agitation or mixing. Fresh standards should therefore be prepared frequently.
2. Vials may be precharged with ethyl acetate and buffer solution.
3. Occasionally, a substance may be present in urine that reacts with the colour reagent at a slower rate than sulfonamide drugs, giving a green-blue colour. This slower colour change does not affect the results of the test.
4. Store colour reagent in a refrigerator to extend its shelf life. Degradation is indicated by the loss of the yellow-orange colour of the solution.

- **Other tests**

Dipstick tests based on a specific reaction of antimalarial drug with monoclonal antibodies have been developed for chloroquine, pyrimethamine, quinine and artemisinin compounds. These tests are more sensitive than the classic colorimetric tests and their results are well correlated with the results of high-performance liquid chromatography (HPLC). HPLC can also assess the presence of antimalarial drugs in biological fluids but the method requires well-equipped laboratories and is beyond the scope of this protocol.

Annex 4
Minimum sample size

If the proportion of treatment failure is already known from earlier studies, the required sample size can be calculated by classical statistical methods for estimating the population proportion. Three pieces of information are needed to determine the proper sample size for estimating a population proportion — the expected population proportion of clinical failures (*P*), the confidence level (usually 95%), and the precision (*d*) (usually between 5% and 10%). In order for the sample to be representative, a minimum of 50 patients should always be included.

If the proportion of treatment failure is unknown, a value of $P = 0.50$ is recommended, since the sample size will be largest and will provide enough observations, irrespective of the actual failure rate value of the true population. Assuming a simple random sample of a community is to be selected, the sample size must be 96 in order to achieve 10% precision with 95% confidence level.

Example

A health department wishes to undertake a therapeutic efficacy test of chloroquine to estimate the prevalence of clinical failure among the local population. Chloroquine is the first-line treatment, and routine follow-up of treated cases suggests that the prevalence of clinical failure is somewhere between 20% and 25%. Dissatisfaction with chloroquine is also reported among the population. How many subjects should be included in the sample so that there is a good chance of the study estimating prevalence to within 10 percentage points of the true value with 95% confidence?

Anticipated population proportion of clinical failures (*P*):	20%
Confidence level:	95%
Precision (*d*):	10 percentage points

The table shows that for $P = 0.20$ and $d = 0.10$ a sample size of 61 would be needed. Sample size must be adjusted for the follow-up losses and withdrawals (expected to be 10% in a study with 14 days follow-up and 20% in studies with longer follow-up).

$n = (1 + 0.10) \times 61 = 67$ or $n = (1 + 0.20) \times 61 = 73$

It is recommended that all the criteria used for the sample size determination are specified when the study results are reported.

	Anticipated population proportion (*P*) – *Confidence level: 95%*									
d	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
0.05	73	138	196	246	288	323	350	369	380	384
0.10	**18**[a]	**35**[a]	**49**[a]	61	72	81	87	92	95	96

[a] In order to be representative, a minimum of 50 patients should always be included.

Annex 5

Example case record forms – (a) 28-day follow-up in low to moderate transmission area

STUDY SITE	Health facility's name:		Town:		District/province:	
PATIENT	Identity number:	Full name:	Age (years):	Sex (M/F):	Weight (kg):	Height (cm):
	Name of guardian:		Contact home address:			
ANTIMALARIAL DRUGS	Drug name:	Manufacturer:	Batch number:	Expiry date:	Total dose (mg base):	
	Previous intake (Y/N/Unknown):	Drug:	Date:	Total dose (mg):	Urinary test (name):	Result:

DAY	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Date															
Danger sign (Y/N)															
History of fever last 24 h (Y/N)															
Axillary temperature (°C)															
Asexual parasite count (per µl)															
Treatment (mg/kg)															
Concomitant treatment															
Possible side-effects of antimalarials															
Observations															

STUDY SITE	Health facility's name:						Town:				District/province:			
PATIENT	Identity number:			Full name:					ANTIMALARIAL DRUGS		Drug name:			
DAY	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Date														
Danger sign (Y/N)														
History of fever last 24 h (Y/N)														
Axillary temperature (°C)														
Asexual parasite count (per µl)														
Treatment (mg/kg)														
Concomitant treatment														
Possible side-effects of antimalarials														
Observations														
Overall assessment:		Early treatment failure (ETF)		Late clinical failure (LCF)		Late parasitological failure (LPF)		Adequate clinical and parasitological response (ACPR)		Withdrawn (WTH)		Loss to follow-up (LOSS)		
Day of assessment:								Reason for WTH						

(b) 14-day follow-up in high transmission area

STUDY SITE	Health facility's name:						Town:					District/province:							
PATIENT	Identity number:	Full name:						Age (years):	Sex (M/F):	Weight (kg):	Height (cm):								
	Name of guardian:							Contact home address:											
ANTIMALARIAL DRUGS	Drug name:		Manufacturer:					Batch number:		Expiry date:	Total dose (mg base):								
	Previous intake (Y/N/Unknown):		Drug:		Date:			Total dose (mg): (mg)		Urinary test (name):		Result:							
DAY	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Date																			
Danger sign (Y/N)																			
Haemoglobin (g/dl)																			
Axillary temperature (°C)																			
Asexual parasite count (per µl)																			
Treatment (mg/kg)																			
Concomitant treatment																			
Possible side-effects of antimalarials																			
Observations																			
Overall assessment:			Early treatment failure (ETF)		Late clinical failure (LCF)		Late parasitological failure (LPF)		Adequate clinical and parasitological response (ACPR)		Withdrawn (WTH)		Loss to follow-up (LOSS)						
Day of assessment:									Reason for WTH										

Annex 6
Treatments

In view of the need for supervision of all treatment doses given during this evaluation, the therapeutic efficacy test system is most suitable for treatment regimens requiring administration of a single dose or of a single daily dose given over 3 days. If drugs have to be given more than once a day, it may be necessary to hospitalize the patients during treatment to ensure that all doses are given correctly. This therapeutic efficacy test is intended only for evaluating the treatment of uncomplicated falciparum malaria, and therefore involves only the orally administered drugs. Detailed information on the various drugs to be used, including doses according to body weight, may be found in the WHO document *The use of antimalarial drugs*.[1]

Drugs and formulations

The drugs employed for therapeutic efficacy testing should be from a reliable, quality-controlled batch; WHO can provide assistance in the procurement of drugs. Drugs should not be used beyond the expiry date mentioned on the package. For purposes of later identification, the manufacturer and batch number of the administered drug should be recorded on the patient record form.

This assessment procedure is appropriate for any standard malaria treatment, including combination approaches. Generally, drugs in current use and those that have been identified as possible alternatives are routinely tested. Protocols intended for assessing new malaria treatments, including new combinations of antimalarial drugs, should be approved by a competent, independent ethical review authority to ensure the protection of patient safety.

The following drugs and formulations are most often evaluated with this efficacy test:

• CHLOROQUINE

Tablets: 100 mg base or 150 mg base (as phosphate or sulfate)
Syrup: 50 mg / 5ml (as phosphate or sulfate)

Treatment with chloroquine

Treatment with chloroquine consists of a 3-day course with the following doses:

Day 0	10 mg base/kg body weight
Day 1	10 mg base/kg body weight
Day 2	5 mg base/kg body weight

In practice, it is difficult to divide the tablets into fractions containing exactly the desired weight-based dose. A range of weight groups are therefore used with the dose regimen adjusted to the nearest manageable fractions of the tablets. The bitter taste of chloroquine often makes it hard to treat infants and young children successfully. Crushing the tablets and mixing them with a little water and sugar on a spoon can often solve this problem; for children with a marked tendency to vomit, the crushed tablets can be mixed with banana or other locally available foods.

[1] *The use of antimalarial drugs. Report of a WHO informal consultation.* Geneva, World Health Organization, 2001 (document WHO/CDS/RBM/2001.33).

• AMODIAQUINE

Tablets: 153 mg base (as hydrochloride)
Tablets: 200 mg and 600 mg base (as hydrochloride) are available but difficult to divide

Treatment with amodiaquine

Amodiaquine treatment follows the same dose regimen as that given for chloroquine above. However, doses of between 25 and 35 mg amodiaquine base/kg over 3 days have been used safely. The most practical dose regimen is 30 mg base/kg divided equally over 3 days.

• SULFONAMIDE–PYRIMETHAMINE COMBINATIONS

Tablets: 500 mg sulfadoxine + 25 mg pyrimethamine
Tablets: 500 mg sulfalene + 25 mg pyrimethamine

Treatment with sulfonamide–pyrimethamine combinations

Sulfadoxine–pyrimethamine and sulfalene–pyrimethamine are given as a single dose equivalent to 1.25 mg pyrimethamine/kg body weight (up to a maximum adult dose of 3 tablets). If the body temperature exceeds 38.5 °C, the patient should receive one dose of paracetamol immediately and another dose to be taken later at home if the fever persists. Parents/guardians should be instructed to use tepid sponging to reduce fever during the initial 24 to 48 hours. Failure to discuss this issue may lead to perception that treatment is not effective, and patients (or parents/guardians) may seek alternative medication, interfering with the study protocol.

• MEFLOQUINE

Tablets: 250 mg base (as hydrochloride).
Note: The formulation available in USA contains 228 mg mefloquine base.

Treatment with mefloquine

The standard adult dose for treatment of uncomplicated malaria is 25 mg base/kg of body weight. Bioavailability is improved if the patient drinks water or takes some food before drug administration. Febrile children are more likely to have drug-related vomiting, which can be reduced by giving an antipyretic. Tolerance of mefloquine is substantially improved if the drug is given as a split dose with an interval of 6–24 hours.

• ARTEMISININ DERIVATIVES

Treatment with artemisinin compounds and combinations:

Treatment with artemisinin derivatives alone lasts longer than 3 days and is therefore beyond the scope of this protocol. However, artemisinins (particularly artesunate) used in combination with other antimalarial drugs (such as amodiaquine, sulfadoxine-pyrimethamine, mefloquine or lumefantrine) are commonly assessed. The fixed-dose combination of artemether and lumefantrine is given twice daily over 3 days and would probably require hospitalization to ensure correct dosing.

When used in combination with other antimalarial drugs for uncomplicated malaria, artesunate is given at a dose of 4 mg/kg once daily for 3 days. The treatment schedules for the partner drug (whether amodiaquine, sulfadoxine–pyrimethamine or mefloquine) are the same as outlined above. For a combination of artesunate and mefloquine, splitting the mefloquine dose on the second and third day improves tolerability. Additional information regarding proper dosing of other artemisinin-containing drug combinations can be found in the WHO document *The use of antimalarial drugs*.[1]

Other medications

The administration of paracetamol on Day 0, Day 1 and Day 2 is permissible if the patient's condition warrants it. If, during follow-up, infections other than malaria require the administration of drugs with antimalarial activity (such as co-trimoxazole, tetracycline or doxycycline), the patient should be excluded from the study. Patients given tetracycline as eye ointment should not be excluded.

[1] *Ibid.*

Annex 7

Sampling and storing filter paper for molecular marker studies

Recommendations made by working groups during the Workshop on the markers of antimalarial drug resistance: practical, clinical, and epidemiological applications, Geneva 14–16 June 1999.

Several methods are available for collecting infected blood for molecular analysis. Workshop participants have the most experience with IsoCode® stick (Schleicher & Schuell) and 3MM® filter paper (Whatman). Extraction techniques of parasite DNA are similarly simple for both types of sample collection. IsoCode® sticks are more expensive but offer the advantages of standardized sample size and prepackaged storage units. Rigorous direct comparisons of DNA yield have not been undertaken. All samples should be clearly labelled with permanent ink with the date, study identification number, and other pertinent information such as post-treatment day.

IsoCode® sticks should be stored in the individual sealed plastic bags with desiccant provided by the manufacturer. Sites opting to use 3MM® paper should obtain similar small zip-lock bags, such as those used by pharmacies in many countries for dispensing pills, and store filter paper individually with desiccant pouches, which can be obtained in bulk and inexpensively. Storage conditions should protect samples from extremes of temperature and humidity. When such room temperature conditions are not possible, for example in extremely humid environments where air-conditioning is not available, storage in a refrigerator or freezer may be considered, but great care must be taken to protect samples from frost and moisture.

Annex 8

Basic follow-up schedule

	Day 0	Day 1	Day 2	Day 3	Day 7	Day14	(Day 21)	(Day 28)	(Any other day)
PROCEDURES									
Clinical assessment	X	X	X	X	X	X	X	X	X
Temperature	X	X	X	X	X	X	X	X	X
Blood slide for parasites count	X	(X)	X	X	X	X	X	X	(X)
Haemoglobin/ haematocrit[a]	(X)					(X)		(X)	(X)
Urine sample	(X)								
Blood for PCR	(X)					(X)	(X)	(X)	(X) after day 14
TREATMENT									
Drug to be tested	X	(X)	(X)						
Rescue treatment		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

[a] Erythrocyte volume fraction.

NOTES: Parentheses denote conditional or optional activities. For example, treatment on Days 1 and 2 would occur only for drugs requiring 3-day dosing. On Day 1, the patient should be examined for parasitaemia if she or he has any danger signs. Rescue treatment could be given on any day, provided that the patient met the criteria for treatment failure. Extra days are any days other than regularly scheduled follow-up days when the patient returns to the facility because of recurrence of symptoms. On extra days, blood slides may or may not be routinely taken; in areas of intense transmission, slides are typically obtained on extra days only if there is measured fever (or at the request of the clinical staff for reasons of patient safety).

Day 0:

Clinical assessment — referral in case of severe malaria/danger signs
Measurement of axillary temperature
Parasitological assessment
Informed consent — enrolment
Measurement of weight (and height) — treatment, first dose
Optional: haemoglobin/haematocrit, urinary test, blood sampling for PCR

Day 1:

Clinical assessment — referral in case of severe malaria/danger signs
Measurement of axillary temperature
Parasitological assessment in case of severe malaria/danger signs
Treatment, second dose or alternative treatment in case of early treatment failure

Day 2:

Clinical assessment — referral in case of severe malaria/danger signs
Measurement of axillary temperature
Parasitological assessment
Treatment, third dose or alternative treatment in case of early treatment failure

Day 3, Day 7, Day 14, Day 21 and Day 28:

Clinical assessment — referral in case of severe malaria/danger signs
Measurement of axillary temperature
Parasitological assessment
Alternative treatment in case of treatment failure
Optional: haemoglobin/haematocrit (Day 14, Day 28), blood sampling for PCR (any other day on or after Day 14 in case of failure)

Any other day:

Clinical assessment — referral in case of severe malaria/danger signs
Measurement of axillary temperature
Parasitological assessment
Alternative treatment in case of treatment failure

Annex 9

Classification of treatment outcomes

INTENSE TRANSMISSION AREA	LOW TO MODERATE TRANSMISSION AREA
Early Treatment Failure **(ETF)**	
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	**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 **(LCF)** • 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 Clinical Failure*** **(LCF)** • 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 **(LPF)** • 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 Parasitological Failure*** **(LPF)** • 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.*

Annex 10

Calculating Kaplan-Meier survival curves by hand

D	*N*	TF	ex	IR	SCI	FCI
0	65	0	0	1.000	1.000	0
1	65	0	0	1.000	1.000	0
2	65	2	0	0.969	0.969	0.031
3	63	2	1	0.968	0.938	0.062
4	60	0	0	1.000	0.938	0.062
5	60	0	0	1.000	0.938	0.062
6	60	0	0	1.000	0.938	0.062
7	60	4	2	0.931	0.873	0.127
8	54	0	0	1.000	0.873	0.127
9	54	0	0	1.000	0.873	0.127
10	54	0	0	1.000	0.873	0.127
11	54	2	0	0.963	0.841	0.159
12	52	0	0	1.000	0.841	0.159
13	52	0	0	1.000	0.841	0.159
14	52	5	0	0.904	0.760	0.240
Total		15	3			

D = day of test

N_D = number of subjects remaining at risk
= $N_{D-1} - TF_{D-1} - ex_{D-1}$ where D is day of test and D–1 is the day before

TF = incident cases of therapeutic failure

ex = excluded due to loss to follow-up, withdrawal or protocol violation

IR_D = interval risk (at Day 0, IR = 1)
= $[(N_D - ex_D) - TF_D]/(N_D - ex_D)$

SCI_D = cumulative incidence of therapeutic success (at Day 0, $SCI_D = 1$)
= $IR_D \times SCI_{D-1}$ where D is day of test and D–1 is the day before,
e.g. for calculating SCI on Day 14, use IR Day 14 and SCI Day 13

FCI_D = cumulative incidence of therapeutic failure
= $1 - SCI_D$

Annex 11

Reagent preparation

- **Stock 100X Giemsa buffer (0.67 mol/litre)**

Na_2HPO_4	59.24 g
$NaH_2PO,4H_2O$	36.38 g
Deionized water	1000 ml

Method. Autoclave or filter-sterilize (0.2 µm pore). Sterile buffer is stable at room temperature for one year.

- **Working Giemsa buffer (0.0067 mol/litre, pH 7.2)**

Stock Giemsa buffer	10.0 ml
Deionized water	990.0 ml

Method. Check pH before use: it should be 7.2. This working buffer solution is stable at room temperature for about one month.

- **Triton X-100** (5%) – if available

Deionized water (warmed to 56 °C)	95.0 ml
Triton X-100	5.0 ml

Method. Prewarm the deionized water and slowly add the Triton X-100, swirling to mix.

- **Stock Giemsa stain**

Note: Giemsa stain is available commercially, but the following formulation gives more constant results and does not expire.

Glass beads, 3.0 mm	30.0 ml
Absolute methanol, acetone-free	270.0 ml
Certified Giemsa stain powder	3.0 g
Glycerol	140.0 ml

Method. Put the glass beads and the other ingredients, in the order listed, into a clean and dry 500-ml brown-glass bottle. Screw cap onto the bottle tightly. Place the bottle at an angle on a shaker and shake moderately for 30–60 minutes daily, for at least 14 days. Kept tightly closed and free of moisture. Stock Giemsa stain is stable indefinitely at room temperature and, in fact, will improve with age. Before use, shake the bottle. Filter a small amount of this stock stain through Whatman No.1 filter paper into a clean test-tube. Pipette from this tube to prepare the working Giemsa stain in order to keep the stock stain uncontaminated.

- **Working Giemsa stain** (2.5%)

Working Giemsa buffer	39 ml
Giemsa stain stock	1 ml
Triton X-100, 5%	2 drops – if available

Annex 12

Classification of malaria endemicity

Type	Spleen rates	Parasite rates
Hypoendemicity	0–10% in children aged 2–9 years	Less than 10% in children aged 2–9 years but may be higher during part of the year
Mesoendemicity	11–50% in children aged 2–9 years	11–50% in children aged 2–9 years
Hyperendemicity	Constantly over 50% in children aged 2–9 years, adult spleen rate also high (over 25%)	Constantly over 50% in children aged 2-9 years
Holoendemicity	Constantly over 75% in children aged 2–9 years, adult spleen rate low	Constantly over 75% in infants aged 0–11 months

Low transmission

- A person may attain adolescence before infection is acquired and may escape altogether

Moderate transmission

- Maximum incidence occurs in childhood and adolescence, though still not unusual for adult life to be attained before acquiring infection

High transmission

- By late infancy or early childhood practically all are infected. Little acute illness in adolescents and still less in adults
- Most individuals acquire infection in early infancy, but acute manifestations are less frequent in childhood and are unusual in adults

Annex 13

Standard material required for the therapeutic efficacy test

All equipment and supplies should be of good quality and — while not in use — stored in a way that prevents deterioration. In many countries, the equipment should be available from the country's health services. However, where these supplies and equipment are not readily available, budgetary provision must be made.

EQUIPMENT REQUIRED BY ONE STUDY TEAM

ITEM	*NUMBER REQUIRED*
(1) Clinical	
Stethoscope	1 (per clinician)
Balance	1 (per clinician)
Height gauge	1 (per clinician)
Fever thermometer, electronic	3 (per clinician)
Teaspoons	2 (per clinician)
(2) Laboratory	
Microscope, binocular, w. illumination (or microscope with mirror for situations without electricity)	1
Tally counter	2
Hairdryer (for humid areas and seasons)	1
Laboratory timer	2
Slide box, for vertical storage	10
Slide box, for horizontal storage, WHO type	2
Slide tray, cardboard	2
Drying rack, wood for slides	1
Staining jar, Coplin or horizontal	3
Bottle, screw-cap, plastic, 500 ml	2
Bottle or jerrycan, screw-cap, plastic, 5 l	2
Measuring cylinder, plastic, 500 ml	2
Measuring cylinder, plastic, 10 ml	2
Dropping-bottle, plastic or glass, 50 ml	2
Glass rod, 50 cm, for quick staining	4
(3) General	
Clipboard	1 (per clinician)
File jacket, stiff card	10
Transport box	3

SUPPLIES REQUIRED PER 100 PATIENTS

ITEM	*NUMBER REQUIRED*
(1) Clinical	
Test drug	150 courses/doses
Next-line alternative drug	100 courses/doses
Third-line drug, if applicable	50 courses/doses
Quinine for injection	20 ampoules
Co-trimoxazole	200 tablets

ITEM	*NUMBER REQUIRED*
Paracetamol	200 tablets
Amoxicillin	200 doses
Injection syringes, sterile, disposable, 2 ml	20
Injection syringes, sterile, disposable, 5 ml	20
Injection needles for above, disposable, no.12 or 14	40
Spare battery, for electronic thermometer	3
Plastic cups	3
(2) Patient incentives	
Sweets (bonbons, candy), pack of 100	10
Biscuits, packs of ~50	10
Sugar	1 kg
(3) Laboratory	
Microscope slides, frosted edge, pack of 100	15
Lens tissue, pack of 100	2
Haemolancets, pack of 100	8
Swabs, alcohol (70 %), pack of 100	8
Rubber gloves, disposable, medium size	50 pairs
Rubber gloves, disposable, large size	50 pairs
Pipettes, transfer, disposable, 5 ml, pack of 100	1
Pipettes, transfer, disposable, 1 ml, pack of 100	1
Glass-writing pen, permanent, xylene-proof	5
Plasticine	200 g
Buffer tablets, pH 7.2	20
Immersion oil, bottle of 50 ml	1
Xylene, bottle of 500 ml	1
Methanol, bottle of 500 ml	1
Giemsa stain stock solution, bottle of 500 ml	1
Distilled water	20 litres
Cotton wool, pack of 500 g	2
Syringe, plastic, 10 ml	2
(4) General	
Logbook, A4, 100 pp.	1 (per clinician)
Notepad, A4	4
Ball-point pen, black/blue	10
Ball-point pen, red	10
Adhesive tape, roll, 30 m	2
Toilet paper, roll	10
Patient forms	150
Patient cards	150
Laboratory form (microscopy and haematology)	700

Annex 14

Sample table of contents of a protocol

This annex indicates the essential sections of a protocol for monitoring the efficacy of antimalarial drugs for the treatment of uncomplicated falciparum malaria according to the epidemiological setting. For clarity, consistency and thoroughness, all of the following topics must be included and detailed in the protocol document.

COVER PAGE
- Title
- Principal investigator
- Co-investigators
- Performing institution
- Study site
- Expected start and completion dates
- Sponsor

SUMMARY

BACKGROUND
- Malaria situation in the country
- Previous studies on drug resistance

OBJECTIVES
- General
- Specific

STUDY DESIGN
- Description of the site(s)
- Timing
- Composition of the team
- Drug(s) to be tested:
  - Antimalarial drugs
  - Concomitant treatment
  - Rescue treatment
- Inclusion criteria
  - ***High transmission area***
    - ◊ aged between 6 and 59 months
    - ◊ mono-infection with *P. falciparum*
    - ◊ Parasitaemia in the range 2000 to 200 000/µl
    - ◊ axillary temperature ≥ 37.5 °C
    - ◊ able to come for the stipulated follow-up visits; easy access to the health facility
    - ◊ informed consent of parent/guardian
  - ***Low to moderate transmission area***
    - ◊ patients aged above 6 months
    - ◊ mono-infection with *P. falciparum*
    - ◊ parasitaemia in the range of 1000 to 100 000/µl
    - ◊ axillary temperature ≥ 37.5 °C or history of fever during the previous 24 hours
    - ◊ able to come for the stipulated follow-up visits; easy access to the health facility
    - ◊ informed consent by the patient or by parent/guardian for children

- Exclusion criteria

◊ presence of one or more of the general danger signs or any sign of severe or complicated malaria
◊ presence of mixed infection
◊ presence of severe malnutrition
◊ presence of febrile conditions caused by diseases other than malaria
◊ presence of a severe disease
◊ contraindications related to the antimalarial drugs used, especially history of allergy
◊ pregnancy *(in low to moderate transmission area only)*

- Sample size
  Estimation of the population proportion at a confidence level of 95%

	Anticipated population proportion (*P*)									
d	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
0.05	73	138	196	246	288	323	350	369	380	384
0.10	50	50	50	61	72	81	87	92	95	96

SCREENING AND ENROLMENT PROCEDURES

ETHICAL ISSUES
- Approval by ethical committee
- Informed consent

LABORATORY EXAMINATIONS
- Blood smear for malaria diagnosis
- Haematological assessment (haemoglobin/haematocrit) (optional)
- Urinary test (optional)
- PCR (mandatory in high transmission area if 28-day follow-up)
- Pregnancy test (if adults included)

FOLLOW-UP PROCEDURE

END-POINTS

All patients must be classified as *Adequate Clinical and Parasitological Response, Late Parasitogical Failure, Late Clinical Failure, Early Treatment Failure, Loss to Follow-up,* or *Withdrawn*

High transmission areas

• ***Early Treatment Failure (ETF)***
- Development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitaemia
- Parasitaemia on Day 2 higher than Day 0 count *irrespective of axillary temperature*
- Parasitaemia on Day 3 with axillary temperature ≥ 37.5 °C
- Parasitaemia on Day 3 ≥ 25% of count on Day 0

• ***Late Clinical Failure (LCF)***
- Development of danger signs or severe malaria after Day 3 in the presence of parasitaemia, without previously meeting any of the criteria of *Early Treatment Failure*
- Presence of parasitaemia and axillary temperature ≥ 37.5 °C on any day from Day 4 to Day 14 (Day 28[1]), without previously meeting any of the criteria of *Early Treatment Failure*

• ***Late Parasitogical Failure (LPF)***
- Presence of parasitaemia on Day 14 (or Day 28[1]) 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)***
- Absence of parasitaemia on Day 14 (or Day 28[1]) irrespective of axillary temperature, without previously meeting any of the criteria of *Early Treatment Failure* or *Late Clinical Failure* or *Late Parasitological Failure*

[1] *If 28 day follow-up*

Low to moderate transmission areas

• ***Early Treatment Failure (ETF)***
– Development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitaemia
– Parasitaemia on Day 2 higher than Day 0 count *irrespective of axillary temperature*
– Parasitaemia on Day 3 with axillary temperature ≥ 37.5 °C
– Parasitaemia on Day 3 ≥ 25 % of count on Day 0

• ***Late Clinical Failure (LCF)***
– Development of danger signs or severe malaria after Day 3 in the presence of parasitaemia, without previously meeting any of the criteria of *Early Treatment Failure*
– Presence of parasitaemia and axillary temperature ≥ 37.5 °C on any day from Day 4 to Day 28, without previously meeting any of the criteria of *Early Treatment Failure*

• ***Late Parasitogical Failure (LPF)***
– Presence of parasitaemia on any day from Day 7 to Day 28 and axillary temperature < 37.5 °C, without previously meeting any of the criteria of *Early Treatment Failure* or *Late Clinical Failure*

• ***Adequate Clinical and Parasitological Response (ACPR)***
– Absence of parasitaemia on Day 28 irrespective of axillary temperature without previously meeting any of the criteria of *Early Treatment Failure* or *Late Clinical Failure* or *Late Parasitological Failure*

Reasons for withdrawal of a patient from the study, including voluntary and involuntary withdrawal, and protocol violation

- Withdrawal of consent
- Failure to complete the treatment
- Persistent vomiting of the treatment
- Severe side-effects necessitating hospitalization
- Occurrence during the follow-up of concomitant disease that would interfere with a clear classification of the treatment outcome
- Reinfection with *P. falciparum* during the follow-up
- PCR unclassifiable results
- Detection of another malaria species infection during the follow-up
- Antimalarial (or antibiotics with antimalarial activity) treatment administered by a third party or self-medication with antimalarial (or antibiotics with antimalarial activity)
- Failure to attend all the scheduled visits
- Erroneous inclusion of a patient outside of the inclusion/exclusion criteria
- Severe malaria occurring at Day 0
- Misclassification of a patient due to a laboratory error (parasitaemia) leading to the administration of the rescue treatment

ANALYSIS
- Per protocol, life table

QUALITY CONTROL
- Temperature, body weight, slides, drugs, data (case record form, data entry in computer, analysis), laboratory assessments

DISSEMINATION OF THE DATA
- Raw data on database
- Report

BUDGET

ANNEXES
- Definition of severe malaria
- Follow-up schedule
- Dosing table for antimalarial drugs
- Screening form
- Case record form
- Informed consent (translated in prospective subjects' mother tongue)