METHODS FOR SURVEILLANCE OF ANTIMALARIAL DRUG EFFICACY

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ISBN 978 92 4 159753 1

WHO

World Health Organization

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WHO Library Cataloguing-in-Publication Data :

Methods for surveillance of antimalarial drug efficacy.

1.Epidemiologic surveillance - methods. 2.Antimalarials - classification. 3.Drug resistance. 4.Plasmodium - drug effects. 5.Plasmodium - transmission. 6.Malaria - drug therapy. 7.Drug evaluation. 8.Epidemiologic research design. I.World Health Organization.

ISBN 978 92 4 159753 1 (NLM classification: QV 256)

Acknowledgements

This document was prepared for the Global Malaria Programme of the World Health Organization (WHO) by P. Ringwald with the collaboration of A. Barrette and L. Vestergaard.

WHO gratefully acknowledges the helpful comments and suggestions made by K. Baird, U. d'Alessandro, G. Dorsey, Ph. Guérin, R. Price, N. Valecha, M. Warsame and N. White. Financial support for the preparation of this document was provided by the United States Agency for International Development.

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Design and Layout by WHO Graphics
Printed in France.

CONTENTS

INTRODUCTION

Resistance to antimalarial drugs is a major public health problem, which hinders the control of malaria. In order to combat the growing resistance, a surveillance system is needed, which will facilitate monitoring and containment. In 1996, the World Health Organization (WHO) prepared a new protocol for assessing antimalarial drug efficacy in high transmission areas. Since then, WHO has regularly updated the therapeutic efficacy protocol for high transmission areas and validated the therapeutic efficacy protocol for low-to-moderate transmission areas on the basis of feedback from countries and scientific recommendations. The most recent version of the efficacy test protocol was adopted in 2001 (WHO, 2003), but adjustments are required to incorporate the changes recommended by the Technical Expert Group on Malaria Chemotherapy, which met in 2005 and 2008 to discuss the *Guidelines for the treatment of malaria* (WHO, 2006a). The most recent changes, which are incorporated into this document, are:

- applications of the same definitions of treatment responses at all levels of malaria transmission, with slight adjustment of patient inclusion criteria;
- administration of rescue treatment to patients with parasitological treatment failure at all levels of malaria transmission;
- requirement for 28 or 42 days of follow-up as a standard, depending on the medicine tested; and
- requirement for genotyping by polymerase chain reaction (PCR) to distinguish between recrudescence and reinfection.

Recommendations for monitoring the therapeutic efficacy of antimalarial medicines for uncomplicated *P. vivax* malaria are included in the WHO standard efficacy protocol (WHO, 2002). The purpose is to stimulate more routine monitoring of resistance of *P. vivax*, thereby gaining wider experience on which to base further refinement of the protocol. The standard protocol was drawn up and used mainly to test the efficacy of medicines against *P. falciparum*; however, *P. vivax* relapses, and *P. falciparum* does not. This difference requires different approaches to therapeutic evaluation.

1. *PLASMODIUM FALCIPARUM*

1.1 PROTOCOL PREPARATION

To facilitate the work of national malaria control programmes and other organizations involved in routine testing of antimalarial drug efficacy, a template protocol (see Annex 1) has been prepared, which can easily be adapted to meet local conditions and needs while maintaining standardization and interpretation of data. The template protocol translates the standard protocol into a practical format that can be used by national malaria control programmes seeking approval from ethics committees or funding from donors. To complete the protocol, the local investigator only needs to complete the highlighted sentences and paragraphs with the information specific to the study. Although this template has been reviewed by WHO Research Ethics Review Committee, the use of this template does not preclude a scientific and an ethical review.

The WHO standard therapeutic efficacy test provides the minimum essential information for deciding on a malaria treatment policy (WHO, 2005; Vestergaard, Ringwald, 2007). Studies with this basic design can form the basis of a surveillance system for monitoring changes in drug efficacy over time, when conducted periodically in a number of appropriately selected sentinel sites. Although the test does not provide all the scientific data necessary for understanding drug resistance in a given environment, it results in basic data on currently recommended first- and second-line medicines and, where necessary, possible replacement medicines, thereby allowing ministries of health to prepare rational treatment strategies and policies. It is important to stress that the standard protocol is not designed for the evaluation of new or experimental medicines. Evidence from earlier studies indicates that this protocol can also be used to assess drug regimens administered over more than 3 days, such as quinine (7 days), combinations of quinine and tetracycline or doxycycline (7 days), or artemisinin monotherapy (7 days). However, special attention must be taken to assure directly observed treatment (i.e. hospitalization or patient returns to the clinic each day over 7 days).

Considerable emphasis has been placed on maintaining simplicity and practicality. Programmes may collect any additional information that they feel is relevant; however, they are strongly encouraged to collect, at least, the data outlined in the protocol. It is only through the standardization of methods that it will be possible to compare and interpret results over time, within or between regions.

1.2 IMPLEMENTATION AND MANAGEMENT OF THE SURVEILLANCE SYSTEM

Sentinel sites

National malaria control programmes should establish sentinel sites for the surveillance of antimalarial drug efficacy. A limited number of sites is adequate to collect consistent longitudinal data and to document trends. The minimal requirements for establishing a sentinel site are: (i) trained, motivated clinical personnel; (ii) a microscopist; (iii) a laboratory for blood film examination and (iv) knowledge about the level of transmission intensity, as this influences certain protocol decisions. The facility can be community-based or located at a health facility at district level. Patients attending hospitals might have more complex clinical presentations; they may be more likely to have had previous drug failures and may be more difficult to follow up. Thus, whenever possible, monitoring should be done at or close to the community.

The following characteristics should be considered in selecting sentinel sites:

- population density;
- accessibility to and feasibility of supervision;
- epidemiology of malaria, especially intensity and seasonality of transmission; and
- population mobility and migration (especially in border areas).

The sentinel sites should represent all the epidemiological strata in the country. Monitoring can be done either by local personnel at the sentinel site or by a more specialized mobile team; the choice depends on national resources and the availability of trained staff at the sentinel sites.

Although no definitive scientific advice can be given about the number of sites needed, experience suggests that four to eight sites per country will achieve a balance between representativeness and practicality. In deciding on the number of sites, programme managers should consider geographical size, population distribution and density, malaria epidemiology or ecology and other factors deemed to be important to the programme. It is critical to select a 'manageable' number of sites to ensure proper monitoring and supervision.

On the basis of experience rather than science, it is recommended that the efficacy of first- and second-line medicines be tested at least once every 24 months at all sites. For the purposes of comparability, assessments should always be conducted at the same time of year. Some national malaria control programmes conducting sentinel site surveillance prefer to alternate test

sites each year, so that half the sites are tested each year and each site is assessed every other year. Other programmes may find it more manageable to monitor the efficacy of first-line medicines at all sites during the first year and that of second-line medicines the following year.

Budgeting for monitoring antimalarial efficacy

In order to ensure that a country has sufficient resources for adequate monitoring of its antimalarial treatment policy, the following should be budgeted for:

- human resources;
- travel and transport;
- equipment and supplies;
- patient costs;
- technical assistance, supervision, a quality assurance system and data management; and
- laboratory support for genotyping.

The total budget usually depends on the number of sites, but US$ 50 000–60 000 per year is a reasonable estimate, depending on the number of sites and the number of medicines tested. In addition, provision should be made for the necessary training, clinical monitoring to improve the quality of clinical procedures and data collection, management, validation and reporting, which are usually done by a consultant over 2–3 weeks. It is crucial that there be strict adherence to the protocol to ensure data quality.

1.3 PROTOCOL FOR SURVEILLANCE OF THE THERAPEUTIC EFFICACY OF ANTIMALARIAL MEDICINES

Inclusion criteria

- age, 6–59 months, i.e. under 5 years in areas of high transmission, and all patients over 6 months of age in areas of low-to-moderate transmission;
- mono-infection with *P. falciparum* detected by microscopy;
- asexual parasite count of 2000–200 000/µl in areas of high transmission and 1000–100 000/µl in areas of low-to-moderate transmission (Annex 2);
- axillary temperature ≥ 37.5 °C or history of fever during the 24 h before recruitment;
- ability to swallow oral medication;
- ability and willingness to comply with the protocol for the duration of the study and to comply with the study visit schedule;

- informed consent from the patient or from a parent or guardian in the case of children;
- absence of general danger signs in children under 5 years or signs of severe falciparum malaria according to the definitions of WHO (2000);
- absence of severe malnutrition according to WHO child growth standards (WHO, 2006b);
- absence of febrile condition due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhoea with dehydration) or other known underlying chronic or severe diseases (e.g. cardiac, renal or hepatic diseases, HIV/AIDS);
- absence of regular medication, which might interfere with antimalarial pharmacokinetics;
- absence of history of hypersensitivity reactions or contraindication to any medicine being tested or used as alternative treatment; and
- a negative pregnancy test or not breastfeeding.

Sample size

The required sample size is defined as the total number of patients who can be assigned a clinical outcome at the end of the study (i.e. adequate clinical and parasitological response or failure due to recrudescence, after confirmation with PCR correction). After the initial sample size calculation, an additional 20% should be added to account for patients who are likely to be either lost during follow-up, withdraw or are excluded after detection of reinfection with PCR correction (Stepniewska, White, 2006).

For single-arm efficacy studies, which were recommended by the Technical Expert Group on Malaria Chemotherapy in 2008, sample size should be determined using classical statistical methods. The calculation is based on an expected proportion of treatment failures in the study population and the desired levels of confidence (95%) and precision (5%). For example, in the case of a medicine with an expected failure rate of 5%, a confidence level of 95% and a precision level of 5%, a minimum of 73 patients should be enrolled. An additional 20% should be added for the reasons outlined above. If the treatment failure rate is unknown, a clinical failure rate of 50% should be assumed. In order for the study to be representative, a minimum sample of 50 patients is required, regardless of the rates of failure.

Comparative, randomized studies of two or more antimalarial agents are relevant to programmes that are considering the introduction of a specific replacement medicine because the efficacy of the first-line treatment is less than 90% or in order to compare treatment outcomes of first- and second-line medicines in the sentinel sites. In these cases, additional

considerations are required to determine the necessary sample size. For instance, investigators must decide whether to demonstrate a difference between two treatments or 'non-inferiority'. For highly effective treatments, it is more appropriate to show that a treatment is 'non-inferior' or not worse than the standard treatment, i.e. that the difference in failure rate is not higher than a pre-specified non-inferiority margin.

In order to determine the required sample size, the therapeutic efficacy of the currently used treatment must be estimated and the required minimum detectable difference or the non-inferiority margin between the current and the alternative treatment decided. For example, if the aim of the investigators is to detect a difference between the current treatment, which is 90% effective, and an alternative treatment, with an estimated efficacy of 95%, at least 435 patients would be required in each of the two study arms, with a two-sided z test that has a statistical power of 80% and a significance level of 5%. In contrast, if the aim is to demonstrate the non-inferiority (by a margin of 5%) of an alternative treatment to a current treatment known to be 95% effective, at least 299 patients would be necessary in each study arm, with a one-sided test that has a statistical power of 80% and a significance level of 2.5%. As previously noted, an additional 20% should be added to the estimate to account for those patients who will be lost or excluded. Thus, in the example of the study of the difference between two treatments, an additional 87 patients should be added, yielding 522 patients in each study arm. For non-inferiority trials, an additional 60 patients should be added, yielding 359 patients in each study arm.

Several statistical software packages are available that allow calculation of sample sizes for clinical trials. As the design and planning of randomized comparative trials is complex, national malaria control programmes that do not have the required capacity might wish to seek appropriate statistical advice.

Recommended duration of assessment

Studies of directly observed treatment for uncomplicated malaria are prospective evaluations of clinical and parasitological responses on days 0, 1, 2, 3, 7, 14, 21 and 28 (35 and 42). The day the patient is enrolled and receives the first dose of medicine is traditionally day 0. A follow-up of 28 days is recommended as the minimum duration for medicines with elimination half-lives of less than 7 days (amodiaquine, artemisinin derivatives, atovaquone–proguanil, chloroquine, halofantrine, lumefantrine, quinine and sulfadoxine–pyrimethamine). For medicines with longer elimination half-lives (mefloquine, piperaquine), longer follow-up periods are necessary. Although a follow-up period of 42 days is optimal for most medicines, long periods of follow-up for routine monitoring might not always be feasible for

national malaria control programmes. A longer study follow-up increases the risk that more patients will be lost to follow-up, reducing the study's validity and subsequently its sensitivity to reveal the true level of failure. Thus, as a compromise, a 28-day follow-up is recommended as the minimum standard to allow national malaria control programmes to capture most failures with most medicines, except mefloquine and piperaquine, for which the minimum follow-up should be 42 days (Stepniewska et al., 2004).

Medicines to be tested

Each national malaria control programme should monitor its first- and second-line medicines according to national treatment guidelines. In addition, newly registered combination therapies can be monitored to obtain background information for possible policy change.

Classification of responses to treatment

The same definitions of treatment response are now used for all levels of malaria transmission:

Early treatment failure (ETF)

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

Late clinical failure (LCF)

- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of early treatment failure; and
- presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥ 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure (LPF)

- presence of parasitaemia on any day between day 7 and day 28 (day 42) with axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

Adequate clinical and parasitological response (ACPR)

- absence of parasitaemia on day 28 (day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

1.4 DATA MANAGEMENT

Data analysis

The Kaplan-Meier method is that preferred for statistical analysis of data on drug efficacy. The advantage of survival analysis is that it takes into account data on patients who were lost to follow-up or withdrawn from the study, in particular patients with reinfection. All the baseline and follow-up data

collected on a patient until the day of censoring—the last day the patient was observed and was not classified as a failure—can be included in the analysis, even for patients who do not complete the study. This method also allows for calculation of mean time to failure and gives a reasonably unbiased estimate of failure rates. Although Kaplan-Meier analyses can be done manually, use of a computer program to perform the calculations facilitates the analysis and reduces the possibility of errors (Stepniewska, White, 2006).

The usual per-protocol method can be used in parallel. In the per-protocol method, all patients who cannot be evaluated (i.e. those withdrawn, lost to follow-up or reinfected after PCR correction) are removed from the denominator. For comparisons with previous studies, results from both types of analyses, Kaplan-Meier and per-protocol, can be reported.

Computer-based applications have been prepared by WHO to provide assistance in all aspects of data management and analysis (Annex 3).

Definition of study end-points

A study end-point is the classification assigned to a patient. Valid study end-points include treatment failure (early treatment failure, late clinical failure, late parasitological failure), completion of follow-up without treatment failure (adequate clinical and parasitological response), loss to follow-up, withdrawal from the study and protocol violation.

Results should be expressed as the cumulative success rate (or the cumulative failure rate) and the proportion of adequate clinical and parasitological response (or proportion of early treatment failure, late clinical failure or late parasitological failure) before and after adjustment by PCR. Importantly, even if follow-up periods longer than 28 days are required, the results at day 28 should always be reported in addition to the failure rate at day 42. Guidelines for calculating the cumulative success or failure rate or the proportion of adequate clinical and parasitological response or treatment failure on PCR-corrected results are:

End-point for day X (X = 28 or 42)	Cumulative success or failure rate (Kaplan-Meier analysis)	Proportion (per-protocol analysis)
Adequate clinical and parasitological response at day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7		
• falciparum recrudescence*	Failure	Failure
• falciparum reinfection*	Censored day of reinfection	Excluded from analysis
• other species with falciparum recrudescence	Failure	Failure
• other species with falciparum reinfection	Censored day of reinfection	Excluded from analysis
• other species infection	Censored day of infection	Excluded from analysis
• undetermined or missing PCR	Excluded from PCR-corrected analysis but included as failures for PCR-uncorrected analysis	Excluded from analysis
Loss to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before withdrawal or protocol violation	Excluded from analysis

* As defined by WHO (2008b).

For PCR-uncorrected results, all late clinical failures and late parasitological failures on or after day 7 are considered as failures.

Data interpretation and policy considerations

Once the data have been validated, the national coordination team should forward its recommendations to drug policy-makers for action. A group of experts (with representatives of the national malaria control programme, Ministry of Health, universities, research institutes, national reference laboratory) should be established to review the data. WHO guidelines for the treatment of malaria recommend that first-line treatment should be changed if the total failure rate exceeds 10%; however, efficacy and failure rates should be considered in the context of their 95% confidence intervals. A decline in efficacy can be due to a number of factors, which should be addressed in confirmatory studies of pharmacokinetics and in vitro and molecular studies. It is likely that the results will differ by site: some may have a substantial deterioration in treatment efficacy, while others may

continue to record an acceptable response to the same medicine. The study investigators may therefore have to decide whether specific treatment guidelines should be targeted to the affected areas without changing national policy or guidelines. In addition, they should determine how many sites can be allowed to have unacceptable treatment failure rates before national policy or treatment guidelines are altered.

1.5 SURVEILLANCE IN COUNTRIES WHERE TRANSMISSION INTENSITY IS DECREASING

The introduction of artemisinin-based combination therapy and other control strategies has decreased transmission intensity in many countries, reducing and localizing the risk to defined geographical areas. National malaria control programmes are thus finding difficulty in enrolling representative numbers of patients at sentinel sites. Several suggestions have been made to overcome this problem.

From high to low-to-moderate transmission

In order to increase the number of patients eligible for enrolment, some countries have adopted a minimum parasitaemia limit of 1000/µl. Use of a low minimum parasitaemia cut-off point as an inclusion criterion has, however, two limitations. In areas of high transmission, immune persons are often asymptomatic carriers of low-grade parasitaemia, which can disappear spontaneously, thereby resulting in underestimation of therapeutic failure. Secondly, reading of slides must be very accurate in order to avoid errors in classification of early treatment failure, failure being based on a comparison of parasitaemia on day 0 and on day 2 or 3.

Other changes in the protocol for areas of high transmission include a wider age range, with enrolment of children up to 10 years of age and adults as young as 15. Furthermore, a history of fever in the past 24 h instead of confirmed fever at the time of admission is sufficient for a case to be eligible for inclusion.

From low-to-moderate to low transmission

The lower parasitaemia limit in these areas has been reduced to 250/µl. This reduction carries the same caveats as those for high transmission settings: reading of slides must be very accurate in order to avoid errors in classification of early treatment failure. The main benefit of reducing the lower limit is that it allows for a 30% increase in the number of patients who can be included.

If, in a given country, at least four or five patients are not included at sentinel sites each week over 6 months, other approaches should be considered.

From low to very low transmission

Another way of reducing the required sample size per site is to combine data from single-arm studies conducted in either multiple centres within one country or with neighbouring countries. The statistical implications of this method are being investigated. Studies with molecular markers should be conducted simultaneously with therapeutic efficacy tests, if the molecular markers are known and have been validated (i.e. for mefloquine, sulfadoxine–pyrimethamine and chloroquine).

In areas where transmission is very low, monitoring should be conducted once every 3 years rather than once every 2 years. Studies with molecular markers should continue to be conducted systematically every year, provided the markers are known and validated.

If it is not feasible for a country to conduct therapeutic efficacy tests every 3 years, surveillance should be based on other elements of early warning systems, such as studies with molecular markers or in vitro studies if molecular markers are not known or not validated.

From very low transmission to pre-elimination or elimination

Countries that are in the stage of pre-elimination or elimination must initiate active case detection. All patients (of all ages and parasitaemia levels) should preferably be hospitalized until their parasitaemia and symptoms are resolved. As countries in the pre-elimination or elimination stage are advised to follow up patients for at least 28 days and ensure that no cases are lost to follow-up, data on drug efficacy can easily be collected for almost all patients at the same time. In vitro and molecular markers can also be used for monitoring drug efficacy.

All transmission settings

The prevalence of patients who are positive on day 3 is used as an indirect clinical marker of artesunate resistance on the Thai–Cambodian border. This indicator has not been validated outside those countries and cannot be a replacement for monitoring the efficacy of artemisinin-based combination therapy, which still requires observation of clinical outcomes over a 28-day follow-up (WHO, 2008a).

2. *PLASMODIUM VIVAX*

2.1 IMPLEMENTATION AND MANAGEMENT OF THE SURVEILLANCE SYSTEM

Countries in which *P. vivax* is endemic are strongly encouraged to carry out pilot studies to monitor the efficacy of chloroquine. In areas where both falciparum and vivax are prevalent, existing sentinel sites can also be used to monitor therapeutic efficacy against falciparum and vivax malaria simultaneously and independently. In areas where only vivax is prevalent, sentinel sites should be set up, as described for *P. falciparum*.

2.2 PROTOCOL FOR SURVEILLANCE OF THE THERAPEUTIC EFFICACY OF ANTIMALARIAL MEDICINES

Inclusion criteria

- age over 6 months;
- mono-infection with *P. vivax* detected by microscopy;
- asexual parasite count > 250/µl;
- axillary temperature ≥ 37.5 °C or history of fever during the 48 h before recruitment;
- ability to swallow oral medication;
- ability and willingness to comply with the protocol for the duration of the study and to comply with the study visit schedule;
- informed consent from the patient or from a parent or guardian in the case of children;
- absence of a clinical condition due to vivax malaria (coma, respiratory distress syndrome or severe anaemia) requiring hospitalization (Genton et al., 2008; Tjitra et al., 2008);
- absence of severe malnutrition according to WHO child growth standards (WHO, 2006b);
- absence of a febrile condition due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhoea with dehydration) or other known underlying chronic or severe disease (e.g. cardiac, renal or hepatic disease, HIV/AIDS);
- absence of regular medication, which might interfere with antimalarial pharmacokinetics;

- absence of history of hypersensitivity reactions to any medicine tested or used as alternative treatment;
- absence of glucose-6-phosphate dehydrogenase deficiency when primaquine is being assessed; and
- a negative pregnancy test or not breastfeeding.

Sample size

Classical statistical methods are recommended for determining sample size, on the basis of an expected proportion of treatment failures, desired confidence interval (95%) and precision (5%). In the case of a medicine with an expected failure rate of 5%, a confidence interval of 95% and a precision level of 5%, a minimum of 73 patients should be enrolled. After the initial sample size calculation, an additional 20% should be added to the original estimate to account for patients who are likely to be lost to follow-up or withdrawn.

Recommended duration of assessment

The studies are prospective evaluations of clinical and parasitological responses on days 0, 1, 2, 3, 7, 14, 21 and 28 to directly observed treatment for uncomplicated malaria. The day on which the patient is enrolled and receives the first dose of medicine is traditionally designated as day 0.

Concomitant treatment

Many countries require primaquine therapy for radical cure of infection with *P. vivax*, especially when the risk for reinfection is considered low. Concurrent primaquine therapy certainly improves the activity of chloroquine against resistant blood stage parasites (Pukrittayakamee et al., 1994; Baird et al., 1995), but administration of primaquine concurrently or soon after administration of chloroquine may conceal resistance to chloroquine alone, resulting in underestimation of the risk for therapeutic failure or resistance to chloroquine. Therefore, primaquine therapy should be postponed until after the 28-day follow-up. Nonetheless, if local health policy includes mandatory administration of primaquine with chloroquine, the failure rate should be considered that of the combination regimen. The data will still be useful, even though the rate of chloroquine failure or resistance to chloroquine might be underestimated. If a patient is to receive primaquine, a test for glucose-6-phosphate dehydrogenase deficiency should be conducted to protect him or her from a possible haemolytic reaction. Primaquine is contraindicated for pregnant women and children under 4 years (Hill et al., 2006).

In studies to determine the rate of resistance to chloroquine alone, a full-course of primaquine should be administered at the end of the patient

follow-up. Before primaquine is administered, the patient's risk for relapse is extremely low because of the prophylactic effect of chloroquine; however, if a relapse occurs, careful follow-up, as outlined in this protocol, will prevent unnecessary morbidity.

Alternative treatment

Although the treatment of choice for *P. vivax* is chloroquine with primaquine, resistance to chloroquine is emerging. It is important to monitor the spread of chloroquine resistance in vivax malaria and to monitor the therapeutic efficacy of alternative treatments, in particular artemisinin-based combination therapy.

Classification of therapeutic response

In the initial protocol, the distinction between early and late treatment failure was abandoned, and a single definition of treatment failure was endorsed (WHO, 2002):

- clinical deterioration due to *P. vivax* illness requiring hospitalization in the presence of parasitaemia;
- presence of parasitaemia and axillary temperature ≥ 37.5 °C on any day between days 3 and 28; and
- presence of parasitaemia on any day between days 7 and 28, irrespective of clinical condition.

Treatment success is defined as the absence of parasitaemia on day 28, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of treatment failure. However, since a high prevalence of early treatment failure after chloroquine treatment for *P. vivax* has recently been reported, the same outcome classification used for *P. falciparum* can also be used for *P. vivax* (Ratcliff et al., 2007).

2.3 RESISTANCE TO CHLOROQUINE

Concentrations of chloroquine of 15 ng/ml in plasma and 30 ng/ml in serum have been considered to be the minimum effective for suppressing *P. vivax.* The concentrations in whole blood are usually several times higher than those in plasma or serum. In addition, the metabolite monodesethylchloroquine can also act against *P. vivax*, as it does against *P. falciparum*. It can be assumed that chloroquine-sensitive *P. vivax* is suppressed by whole-blood concentrations of 70–90 ng/ml of chloroquine and its metabolite. The presence of parasites at a blood concentration exceeding 100 ng/ml would indicate chloroquine resistance (Baird et al., 1997), although this requires further investigation.

In order to measure the whole-blood levels of chloroquine to which the

P. vivax parasite would be exposed, blood samples should be collected on filter paper (Whatman® 31ETchr) on day 7 and on the day of failure, or on day 28 in the case of treatment success. If the resources are available, samples can also be collected on day 0. Exactly 100 µl of whole blood should be drawn from a finger-prick into a capillary tube containing heparin or EDTA anticoagulant. The blood should be immediately expelled onto filter paper, air dried and stored individually for up to 24 months in a sealed plastic envelope at ambient temperature. Alternatively, venous blood can be sampled into a vacutainer, and exactly 100 µl of whole blood transferred onto filter paper with a pipette.

2.4 *P. VIVAX* GENOTYPING

Parasite relapse, reinfection and recrudescence cannot be distinguished reliably in infections with *P. vivax*. In studies in which primary and confirmed relapse parasites were genotyped, more than half of the parasites that caused the relapse had a different genotype from those that caused the primary infection (Chen et al., 2007; Imwong et al., 2007). This poses a major obstacle for PCR adjustment in *P. vivax* antimalarial drug trials, because true relapses caused by reactivated hypnozoites cannot be ruled out or confirmed (WHO, 2008b).

2.5 MONITORING THE EFFICACY OF PRIMAQUINE FOR THE PREVENTION OF *P. VIVAX* RELAPSES

Primaquine, an 8-aminoquinoline, is recommended for the prevention of relapse of vivax malaria. Primaquine is active against the exoerythrocytic phase of the parasite, destroying latent hypnozoites and thereby preventing relapse. Although numerous reports from many geographical areas describe primaquine resistance, convincing evidence is often lacking because of the many confounding factors, such as unsupervised therapy, parasite tolerance and the possibility of reinfection (Baird, Hoffman, 2004; Goller et al., 2007).

Relapses may occur as early as 16 days and as late as up to 3 years after the initial infection, even if the blood stage was adequately treated. Therefore, in clinical trials, a treatment with highly effective blood schizonticide with a short half-life such as 7-day quinine or artesunate is necessary to rule out the suppression of early relapses due to the prophylactic effect of a blood schizonticide with a longer half-life.

Primaquine failure is defined as a confirmed positive blood smear for *P. vivax* after treatment with an effective blood schizonticide medicine and primaquine therapy during the follow-up phase in a patient in whom reinfection has been prevented. This follow-up phase varies in the literature from 3 to 12 months. Follow-up should be adapted to the regional characteristics of the parasite.

As the available tools for genotyping do not allow investigators to distinguish between vivax relapse and reinfection (see 2.4), the therapeutic efficacy of primaquine should ideally be studied in an environment where there is a no risk for reinfection or at least where the risk is substantially reduced. If the risk for reinfection remains high, the failure rate of primaquine will be overestimated, as it will include failures due to both primaquine inefficacy and reinfection. Because the relapse rates associated with strains prevalent in different geographical areas differ, information on the contribution made by relapses to the failure rate will be useful. If data on the risk for relapse (without primaquine) is available, the efficacy of primaquine can be estimated in an uncontrolled study as the difference between the relapse rate in the study and that recorded previously. If past data are not available, a control group in whom primaquine is contraindicated (e.g. patients with glucose-6-phosphate dehydrogenase deficiency) should be included. Interpretation of the results obtained in the latter type of study is difficult, however, as the two groups are not chosen entirely randomly. Because of the problem of reinfection, the difficulty of finding a valid control group and the many unknown and confounding factors, all studies of the efficacy of primaquine must be interpreted with caution.

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Annex 1. TEMPLATE PROTOCOL FOR THERAPEUTIC EFFICACY TESTS

COVER SHEET FOR THERAPEUTIC EFFICACY TEST PROTOCOL

Title	Efficacy and safety of (name of antimalarial drug(s) or drug combination(s)) for the treatment of uncomplicated *Plasmodium falciparum* malaria in (name of sentinel site(s), (district/province/country(s))
Protocol submission date	(dd/mmm/yyyy)
Protocol number	(unique protocol number/version number)
Principal investigator	(Name, degree) (Institution) Tel: (.....) Fax: (.....) Email: (.....)
Co-investigator(s) (insert additional names as needed)	(Name, degree) (Institution) Tel: (.....) Fax: (.....) Email: (.....)
Medical monitor	(Name, degree) (Institution)
Participating institutions	(Institution name) Tel: (.....) Fax: (.....)
Study dates	From (mmm/yyyy) to (mmm/yyyy)
Sponsor	Ministry of Health (country) (Address.....) Tel: (.....) Fax: (.....) Email: (.....)

SUMMARY

Title: Efficacy and safety of (name of antimalarial drug(s) or drug combination(s)) for the treatment of uncomplicated *Plasmodium falciparum* malaria in (name of the sentinel site(s)), (district/province/country).

Background: (Paragraph describing current first-line and second-line treatment for *P. falciparum* in the country, drug resistance and the rationale for conducting the study).

Objective: To assess the efficacy and safety of (name of the antimalarial drug(s) or drug combination(s)) for the treatment of uncomplicated *P. falciparum* infections in (name of the sentinel site(s)), (district/province/country)

Methods: An antimalarial drug efficacy trial will be conducted in (name of the sentinel site(s)), (country). The participants will be febrile people (specify age range) with confirmed uncomplicated *P. falciparum* infection. Patients will be treated with (name of the antimalarial drug(s) or drug combination(s), dosage and treatment regimen). Clinical and parasitological parameters will be monitored over a (28/42)-day follow-up period to evaluate drug efficacy. The study will be conducted from (month) to (month), (yyyy). The results of this study will be used to assist the Ministry of Health of (country) in assessing the current national treatment guidelines for uncomplicated *P. falciparum* malaria.

1. BACKGROUND

Paragraph describing currently recommended first- and second-line treatment in the country.

Paragraph describing results of previous efficacy studies in the area, recent trends in drug resistance in the country and the rationale for conducting the study.

Paragraph describing the known efficacy and side-effects of the antimalarial drug(s) or drug combination(s) to be tested.

2. OBJECTIVES

The general objective of this study is to assess the therapeutic efficacy and safety of (name of the antimalarial drug(s) or drug combination(s)) for the treatment of uncomplicated *P. falciparum* malaria in (name of the sentinel site(s)), (district/province/country).

The specific objectives are:

- to measure the clinical and parasitological efficacy of (name of the antimalarial drug(s) or drug combination(s)) in patients aged between (minimum

age) and (maximum age) (months/years), suffering from uncomplicated falciparum malaria, by determining the proportion with early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response as indicators of efficacy;

- to differentiate recrudescence from new infection by polymerase chain reaction (PCR) analysis;
- to evaluate the incidence of adverse events; and
- to formulate recommendations and to enable the Ministry of Health to make informed decisions about whether the current national antimalarial treatment guidelines should be updated.

The secondary objectives are:

- to assess the in vitro susceptibility of *P. falciparum* isolates to (name of the antimalarial drug(s));
- to determine the polymorphism of molecular markers for (name of the antimalarial drug(s)) resistance; and
- to determine the blood concentration of (name of the antimalarial drug(s)).

3. METHODS

3.1 Study design

This surveillance study is a one-arm prospective evaluation of clinical and parasitological responses to directly observed treatment for uncomplicated malaria.[1,2] People with uncomplicated malaria who meet the study inclusion criteria will be enrolled, treated on site with (name of the antimalarial drug(s) or drug combination(s)) and monitored for (28/42) days. The follow-up will consist of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the patients will be classified as having therapeutic failure (early or late) or an adequate response. The proportion of patients experiencing therapeutic failure during the follow-up period will be used to estimate the efficacy of the study drug(s). PCR analysis will be used to distinguish between a true recrudescence due to treatment failure and episodes of reinfection.

3.2 Study site

Paragraph describing the study area(s): geographic location, population, routes of access.

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[1] WHO. *Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria*. Geneva, World Health Organization, 2003 (WHO/RBM/HTM/2003.50) (http://www.who.int/malaria/resistance).

[2] WHO. *Method for surveillance of antimalarial drug efficacy*. Geneva, World Health Organization, 2009 (http://www.who.int/malaria/resistance).

Paragraph describing the health facilities in which the study will be conducted and, specifically, how severe malaria cases are to be managed or referred.

3.3 Study population

The population will consist of patients with uncomplicated *P. falciparum* malaria attending the study health clinic who are aged between (minimum age) and (maximum age) (months/years). All adult patients will sign an informed consent form for participation. Parents or guardians will give informed consent on behalf of children. Children over 12 years of age will sign an informed assent form. (Keep those sentences necessary for the age groups included.)

3.4 Timing and duration of study

The study will be conducted during the malaria transmission season, from (month) to (month), (year(s)).

3.5 Inclusion criteria

- age between (minimum age) to (maximum age) (months/years);
- mono-infection with *P. falciparum* detected by microscopy;
- parasitaemia of (minimum)–(maximum)/µl asexual forms;
- presence of axillary or tympanic temperature ≥ 37.5 °C or oral or rectal temperature of ≥ 38 °C or history of fever during the past 24 h;
- ability to swallow oral medication;
- ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule; and
- informed consent from the patient or from a parent or guardian in the case of children.

3.6 Exclusion criteria

- presence of general danger signs in children aged under 5 years or signs of severe falciparum malaria according to the definitions of WHO (Appendix 1);
- mixed or mono-infection with another *Plasmodium* species detected by microscopy;
- presence of severe malnutrition (defined as a child whose growth standard is below –3 z-score, has symmetrical oedema involving at least the feet or has a mid-upper arm circumference < 110 mm);
- presence of febrile conditions due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhoea with

dehydration) or other known underlying chronic or severe diseases (e.g. cardiac, renal and hepatic diseases, HIV/AIDS);

- regular medication, which may interfere with antimalarial pharmacokinetics;
- history of hypersensitivity reactions or contraindications to any of the medicine(s) being tested or used as alternative treatment(s); and
- a positive pregnancy test or breastfeeding (include this criterion only if adults are included);
- unable to or unwilling to take contraceptives (for women of child-bearing age).

3.7 Loss to follow-up

Loss to follow-up occurs when, despite all reasonable efforts, an enrolled patient does not attend the scheduled visits and cannot be found. No treatment outcome will be assigned to these patients. Every effort must be made to schedule a follow-up visit for patients who fail to return to the study site, especially during but also after administration of the study drug. These patients will be classified as lost to follow-up and censored or excluded from the analysis. Patients who are lost to follow-up but who subsequently return to the study site before day 28/42 will not be turned away and will be encouraged to return for check-up visits. The principal investigator will decide whether the patient is to be definitely classified as lost to follow-up on the basis of his or her history or is to be maintained for the analysis.

3.8 Patient discontinuation or protocol violation

Study patients who meet any of the following criteria will be classified as withdrawn.

- withdrawal of consent. A patient may withdraw consent at any time, without prejudice for further follow-up or treatment at the study site.
- failure to complete treatment, due to:
  - persistent vomiting of the treatment. A patient who vomits the study medication twice will be withdrawn from the study and given rescue treatment.
  - failure to attend the scheduled visits during the first 3 days; or
  - serious adverse events necessitating termination of treatment before the full course is completed. A patient can be discontinued from the study if the principal investigator decides so due to an adverse event of adequate nature or intensity. In this case, information on the adverse event and symptomatic treatment given must be recorded on a case report form. If the adverse event is serious, the principal investigator

must notify the sponsor or its designee immediately and follow the reporting procedures described in section 5.3.

- enrolment violation:
  - severe malaria on day 0; or
  - erroneous inclusion of a patient who does not meet the inclusion criteria.
- voluntary protocol violation: self- or third-party administration of antimalarial drug (or antibiotics with antimalarial activity) (Appendix 2);
- involuntary protocol violation:
  - occurrence during follow-up of concomitant disease that would interfere with a clear classification of the treatment outcome;
  - detection of a mono-infection with another malaria species during follow-up; or
  - misclassification of a patient due to a laboratory error (parasitaemia), leading to administration of rescue treatment.

Patients who are withdrawn will nevertheless be followed up until recovery or the end of follow-up, if possible; however, no treatment outcome will be assigned to these patients, and they will be censored or excluded from the analysis. The reasons for discontinuation or protocol violation will be recorded on the case report form.

4. TREATMENT

4.1 Antimalarial treatment

(Name of the antimalarial drug(s) or drug combination(s)) will be administered at a dose of (expressed as mg base per kg body weight, number of daily doses and number of days). The correct drug dosage will be determined from the dosing chart (Appendix 3).

Tablets of (name and formulation in mg base per tablet of antimalarial drug(s)) will be obtained from (name of manufacturer, country) (batch number, expiry date).

All doses of medicine will be administered under the supervision of a qualified member of the staff designated by the principal investigator. The study patients will be observed for 30 min after medicine administration for adverse reactions or vomiting. Any patient who vomits during this observation period will be re-treated with the same dose of medicine and observed for an additional 30 min. If the patient vomits again, he or she will be withdrawn and offered rescue therapy.

4.2 Concomitant treatment and medication that should not be used

Fever over 38 °C can be treated with paracetamol or acetaminophen. Parents or guardians will be instructed in the use of tepid sponging for children under 5 years of age.

Prior treatment with antimalarial drugs will not be considered an exclusion criterion; however, during follow-up, if infections other than malaria require the administration of medicines with antimalarial activity, the patient will be withdrawn from the study. Patients given tetracycline as an eye ointment will not be excluded (Appendix 2). Patients will be withdrawn from the study in the case of self-medication or if an antimalarial drug or an antibiotic with antimalarial activity is administered by a third party.

Adverse events requiring treatment can be treated according to local practice. If there is a clinical indication for any additional medication during the course of the study, including medication given to treat an adverse event related to the study medicine, the name of the medicine, the dosage and the date and time of administration must be recorded on the case report form.

The use of herbal remedies during the study should be avoided, and participants should be encouraged to return to the study site for treatment if they feel unwell. If any herbal remedies are taken during the study, this should be captured on the case report form, under 'study medication administration'.

4.3 Rescue treatment

If a patient vomits twice, he or she will receive parenteral therapy with (name of the antimalarial drug(s) or drug combination, dose expressed as mg base per kg body weight, number of daily doses and number of days) and will be withdrawn from the study.

Women who are found to be pregnant at enrolment will be treated with (name of the antimalarial drug(s) or drug combination, dose expressed as mg base per kg body weight, number of daily doses and number of days) during the first trimester; during the second and third trimesters, (name of the antimalarial drug(s) or drug combination, dose expressed as mg base per kg body weight, number of daily doses and number of days) will be used according to national treatment guidelines.

Any patient with signs of severe or complicated malaria will be hospitalized and will receive parenteral therapy with (name of the antimalarial drug(s) or drug combination, dose expressed as mg base per kg body weight, number of daily doses and number of days) and relevant supportive treatment (include here any additional nationally recommended treatment).

If a patient meets one of the criteria for therapeutic failure, he or she will receive (name of the antimalarial drug(s) or drug combination, dose expressed

as mg base per kg body weight, number of daily doses and number of days) according to current national recommendations. If the patient is reinfected with another malaria species, he or she will receive (name of the antimalarial drug(s) or drug combination, dose expressed as mg base per kg body weight, number of daily doses and number of days) according to current national recommendations.

5. EVALUATION CRITERIA

The study end-point is the classification assigned to a patient. Valid study end-points include: treatment failure, completion of the follow-up period without treatment failure, loss to follow-up, withdrawal from study, and protocol violation. At all times, the well-being of the patient will take priority over his or her continuation in the study.

5.1 Efficacy and safety evaluation

5.1.1 Classification of treatment outcomes

Treatment outcomes will be classified on the basis of an assessment of the parasitological and clinical outcome of antimalarial treatment according to the latest WHO guidelines.[3] Thus, all patients will be classified as having early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response, as defined in Appendix 4.

As parasitological cure is the goal of antimalarial therapy, all study patients who show treatment failure will be given rescue treatment. Follow-up will continue until recovery. The results from these patients do not need to be recorded systematically for the purpose of the surveillance study.

5.1.2 Safety end-points

The incidence of any adverse event will be documented. All patients will be asked routinely about previous symptoms and about symptoms that have emerged since the previous follow-up visit. When clinically indicated, patients will be evaluated and treated appropriately. All adverse events will be recorded on the case report form. Serious adverse events (see definitions in 5.3) must be reported to the sponsor.

5.2 Clinical evaluation

All patients will be evaluated clinically as described below.

5.2.1 Physical examination

A standard physical examination will be performed at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28, (35 and 42). A complete

[3] WHO. *Susceptibility of* Plasmodium falciparum *to antimalarial drugs. Report on global monitoring 1996–2004*. Geneva, World Health Organization, 2005 (WHO/HTM/MAL/2005.110) (http://www.who.int/malaria/resistance).

medical history, demographic information and contact details will be taken at baseline.

5.2.2 **Body weight**

Body weight will be recorded on day 0 to the nearest kilogram on a Salter scale or on a hanging scale for young children. The scales will be properly calibrated. Patients should not wear excessive clothing while being weighed as this can overestimate their true weight. All young children should only wear undergarments while being weighed. The screening weight will be used to satisfy the inclusion or exclusion for nutrition status as well as to calculate the dose (number of tablets) to be administered. The reliability of the scales will be verified before the study begins and checked at regular intervals.

The circumference of the left mid-upper arm will be measured, at the mid-point between the elbow and the shoulder, and will be recorded to the nearest 0.2 cm.

Oedema will be assessed by thumb pressure for 3 seconds on the dorsal surface of both feet.

5.2.3 **Body temperature**

Axillary, oral, tympanic, or rectal temperature will be measured at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28, (35 and 42). Temperature will be measured with a thermometer that has a precision of 0.1 °C. Temperature will also be measured as clinically indicated. If the result is < 36.0 °C, the measurement will be repeated. The same route should be used throughout the study.

The quality of the temperature-taking technique and the thermometers should be assessed regularly. Thermometers should be tested in a water-bath of known temperature before the study begins and at regular intervals thereafter.

5.2.4 **Microscopic blood examination**

Thick and thin blood films for parasite counts should be obtained and examined at screening on day 0 to confirm adherence to the inclusion and exclusion criteria. Thick blood films will be also examined on days 2, 3, 7, 14, 21, 28, (35 and 42) or on any other day if the patient returns spontaneously and parasitological reassessment is required. Specimens will be labelled anonymously (screening number or study number, day of follow-up, date).

A fresh Giemsa stain dilution will be prepared at least once a day and possibly more often, depending on the number of slides to be processed. Giemsa-stained thick and thin blood films will be examined at a magnification of 1000× to identify the parasite species and to determine the parasite density.

Three blood slides per patient will be obtained: two thick blood smears and one thin blood smear. One slide will then be stained rapidly (10% Giemsa for 10–15 min) for initial screening, while the others will be retained. If the patient is subsequently enrolled, the second slide will be stained more carefully (e.g. 2.5–3% Giemsa for 45–60 min), and slower staining will also be used for all slides obtained at follow-up visits. The study number of the patient, the date and the day of follow-up will be recorded either on the frosted edge of the slide or on the glass with a permanent glass pen.

The thick blood smear for initial screening will be used to count the numbers of asexual parasites and white blood cells in a limited number of microscopic fields. The adequate parasitaemia for enrolment is at least one parasite for every three white blood cells, corresponding to approximately 2000 asexual parasites per microlitre, for high transmission areas or at least one parasite for every six white blood cells, corresponding to approximately 1000 asexual parasites per microlitre, for low-to-moderate transmission areas.

The second blood smear will be used to calculate the parasite density, by counting the number of asexual parasites in a set number of white blood cells (typically 200) with a hand tally counter. Once a field has been started, it must be counted to completion; the final number of white blood cells will therefore rarely be exactly 200. If more than 500 parasites have been counted before 200 white blood cells have been reached, the count will be stopped after the reading of the last field has been completed. Parasite density, expressed as the number of asexual parasites per µl of blood, will be calculated by dividing the number of asexual parasites by the number of white blood cells counted and then multiplying by an assumed white blood cell density (typically 6000–8000 per µl).

$$\text{Parasite density (per µl)} = \frac{\text{number of parasites counted} \times (6000\text{–}8000)}{\text{Number of leukocytes counted}}$$

The same technique will be used to establish the parasite count on each subsequent blood film. When the number of asexual parasites is less than 10 per 200 white blood cells in follow-up smears, counting will be done against at least 500 white blood cells (i.e. to completion of the field in which the 500th white blood cell is counted). A blood slide will be considered negative when examination of 1000 white blood cells reveals no asexual parasites. The presence of gametocytes on an enrolment or follow-up slide will be noted, but this information will not contribute to basic evaluation.

In addition, 100 fields of the second thick film will be examined to exclude mixed infections; in case of any doubt, the thin film will be examined for confirmation. If examination of the thin film is not conclusive, the patient

will be excluded from the analysis after complete treatment and follow-up.

Two qualified microscopists will read all the slides independently, and parasite densities will be calculated by averaging the two counts. Blood smears with discordant results (differences between the two microscopists in species diagnosis, in parasite density of > 50% or in the presence of parasites) will be re-examined by a third, independent microscopist, and parasite density will be calculated by averaging the two closest counts.

5.2.5 Genotyping of malaria parasites

In order to differentiate a recrudescence (same parasite strain) from a newly acquired infection (different parasite strain), a genotype analysis will be conducted. This is based on the extensive genetic diversity among the malaria parasite genes *msp1, msp2* and *glurp*.[4] The genotypic profiles of pre- and post-parasite strains are compared.

In order to minimize discomfort to the patient due to repeated finger pricks, two to three drops of blood will be collected on filter paper (specify the type of filter paper) during screening or enrolment and each time blood smears are required according to the protocol on and after day 7.

Specimens will be labelled anonymously (study number, day of follow-up, date), kept in individual plastic bags with desiccant pouches and protected from light, humidity and extreme temperature until analysed. When these conditions cannot be achieved, 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. The PCR technique used will be (describe briefly the method). (Indicate which laboratory will be performing these tests). Paired filter papers will be used for parasite DNA extraction and genotyping only in cases of treatment failure. Unused filter papers will be destroyed immediately after the study.

5.2.6 Pregnancy test (if older children or adults are included in the study)

Female patients of child-bearing age, defined as those who menstruate or are aged over 12 years, will be asked to take a urine pregnancy test before enrolment in the study, because (name of the antimalarial drug(s) or drug combination(s)) is contraindicated during the first trimester. They will also be asked to take a urine pregnancy test on day 28/42 or on early withdrawal from the study.

Female participants of child-bearing age, defined as those who menstruate or are aged over 12 years, and who are sexually active should use barrier

[4] WHO. *Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations.* Geneva, World Health Organization, 2008 (http://www.who.int/malaria/resistance).

contraceptive devices for the duration of the study. Condoms or other appropriate contraceptive will be provided by the investigator or study team at the time informed consent is obtained, with appropriate counselling about the risks of becoming pregnant and exposing the fetus to the study medicines.

5.2.7 Haematological assessment (haemoglobin/haematocrit) (optional)

(Haemoglobin/haematocrit) will be determined on days (indicate the days of sampling) by the (indicate the method used and the quantity of blood sampled for this purpose).

5.2.8 Urinary test for presence of antimalarial drugs (optional)

A urine sample will be collected on day 0 and tested for the presence of various antimalarial drugs and their metabolites. Indicate the medicines screened and the method used. Previous antimalarial treatment is not a criterion for exclusion from the study but will be recorded for further analysis.

5.2.9 In vitro susceptibility of *P. falciparum* isolates (optional)

A blood sample (indicate volume) for in vitro drug testing will be collected on day 0 in order to evaluate in vitro susceptibility of *P. falciparum* isolates to (name of the antimalarial drug(s) or metabolite(s)). Specimens will be labelled anonymously (study number, day of follow-up, date). The in vitro technique used will be (describe briefly the method). (Indicate which laboratory will perform these tests).[5] The results will be expressed as (IC50, IC90 or MIC).

5.2.10 Molecular markers for antimalarial drug resistance (optional)

Two to three drops of blood will be collected on filter paper (specify the type of filter paper) on day 0 (and day of failure) to study the polymorphism or copy number of (list name of gene(s)), which are considered as markers of resistance to (name of the antimalarial drug(s) or metabolite(s)). The technique used will be (describe briefly the method). (Indicate which laboratory will perform these tests). Specimens will be labelled anonymously (study number, day of follow-up, date), kept in individual plastic bags with desiccant pouches and protected from light, humidity and extreme temperature until analysed.

5.2.11 Antimalarial drug blood concentration (optional)

Blood sample(s) (indicate volume) for determining the blood concentration of (name of the antimalarial drug(s) or metabolite(s)) will be collected at (indicate timing). Specimens will be labelled anonymously (study number, day of follow-up, date). The method used will be (describe briefly the method). (Indicate which laboratory will perform these tests).

[5] Basco LK. *Field application of in vitro assays sensitivity of human malaria parasites antimalarial drugs*. Geneva, World Health Organization, 2008 (http://www.who.int/malaria/resistance).

5.3 Safety assessment

Safety will be assessed by recording the nature and incidence of adverse events and serious adverse events. Adverse events will be assessed by direct questioning. An adverse event is defined as any unfavourable, unintended sign, symptom, syndrome or disease that develops or worsens with the use of a medicinal product, regardless of whether it is related to the medicinal product. All adverse events must be recorded on the case report form.

A serious adverse event is defined as any untoward medical occurrence that at any dose:

- results in death, is life threatening;
- requires hospitalization or prolongation of hospitalization;
- results in a persistent or significant disability or incapacity; or
- is a congenital anomaly or birth defect.

'Life-threatening' means that the person was at immediate risk for death; it does not refer to a adverse event that might have caused death if it were more severe. 'Persistent or significant disability or incapacity' means that a person's ability to carry out normal life functions is substantially disrupted.

All serious adverse events occurring during the study must be recorded and reported by the principal investigator to the sponsor, regardless of whether the principal investigator considers the events to be related to the investigated medicine.

The investigator will collect information on all people who become pregnant while participating in this study and will record the information on the appropriate form. The person will also be followed to determine the outcome of the pregnancy. Generally, follow-up will be no longer than 6–8 weeks after the estimated delivery date. Any premature termination of pregnancy will be reported. While pregnancy itself is not considered an adverse event or a serious adverse event, any complication of pregnancy or elective termination for medical reasons will be recorded as an adverse event or a serious adverse event. A spontaneous abortion is always considered a serious adverse event and will be reported as such.

6. STUDY ASSESSMENT

6.1 Screening and enrolment

All patients who meet the basic enrolment criteria (age, fever or history of fever if appropriate, symptoms of malaria, absence of danger signs in children in relation to malaria—child unable to drink or breastfeed, vomiting everything, recent history of convulsions, lethargic or unconscious state, unable to sit or stand, difficulty in breathing—, absence of signs of severe malaria, absence of severe malnutrition, pregnancy) during screening will

be assigned a consecutive number and evaluated in greater depth by clinical staff. In children, care will be taken to detect early signs of febrile diseases other than malaria, as their presence will necessitate exclusion from the evaluation. The most frequent confounding condition is a lower respiratory tract infection: cough or difficult breathing, together with fast breathing, is an indicator for exclusion. Fast breathing is defined as a respiratory frequency > 50/min in infants under 12 months of age and > 40/min in children aged 12–59 months. Other relatively common febrile conditions are otitis media, tonsillitis, measles and abscesses. Patients with these conditions will not be enrolled but should be treated for both malaria (if they have parasitaemia) and the other infection, as appropriate.

The screening record form (Appendix 5) will be used to record the general information and the clinical observations on each patient being screened. If the patient meets the clinical criteria, he or she will be examined for parasitaemia. Once the patient meets all the enrolment criteria, he or she (if adults are included) or a parent or guardian in case of children will be asked for consent to participate in the study.

6.2 Follow-up

Patients who meet all the enrolment criteria will be given a personal identification number and will receive treatment only after the study has been fully explained to them and they have willingly provided informed consent. Any person who decides not to participate in the study will be examined, treated and followed-up by the health facility staff according to the standard of care established by the Ministry of Health.

The basic follow-up schedule is summarized in Appendix 6. A case report form (Appendix 7) and a serious adverse event report form (Appendix 8) will be used to record the general information and clinical observations on each patient enrolled into the study. The appointment schedule will be clearly explained, and a follow-up card with a personal identification number will be provided.

The day a patient is enrolled and receives the first dose of medicine is designated 'day 0'. All antimalarial treatment will be given by a study team member under supervision. Enrolled patients will be observed for at least 30 min after treatment to ensure that they do not vomit the medicine. If vomiting occurs within 30 min of treatment, the full treatment dose will be repeated. Ancillary treatment, such as antipyretics, will be provided if necessary to patients by the study team and documented on the case report form. Patients with persistent vomiting (i.e. necessitating more than a single repeat dose) will be excluded from the study and immediately referred to the health facility staff for appropriate management.

Thereafter, patients are required to undergo regular clinical reassessment. Blood films for parasite counts will be made on days 2, 3 and 7 and then weekly for the remainder of the follow-up period, i.e. on days 14, 21, 28, (35 and 42). Patients will be advised to return on any day during the follow-up period if symptoms return and not to wait for the next scheduled visit day. In particular, parents or guardians should be instructed to bring children to the centre at any time if they show any sign of danger (unable to drink or breastfeed, vomiting everything, presenting with convulsions, lethargic or unconscious, unable to sit or stand, presenting with difficult breathing), if they are still sick or if there is any cause for worry. Clinical reassessment will be sufficiently thorough to ensure patient safety and will include assessment not only for potential treatment failure but also for potential adverse reactions to the medicine. Additionally, blood films will be obtained whenever parasitological reassessment is requested by the clinical staff.

Because many medicines have to be given over several days, the initial visits are critical not only for assessing efficacy but also for ensuring patient safety; defaulters at this stage will not have received a complete course of treatment and may be at risk for clinical deterioration. All reasonable efforts will be made to find defaulters to ensure complete treatment. Similarly, the ultimate success of the study rests on minimizing loss to follow-up. While patients are 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 detailed directions to the home during enrolment, and study team members familiar with the community will be responsible for home visits and means of transport for the patients. The schedule of treatment and follow-up examinations given in this protocol must be followed to ensure data integrity. Patients who fail to return on days 1 and 2 and miss one dose of the treatment will be withdrawn from the study definitively. After day 3, patients who fail to return on day 7 but are present on day 6 or 8 (likewise days 13/15, days 20/22, days 27/29, days 34/36 and days 41/43) may still be included in the analysis. Deviation from the protocol of more than 1 day should, however, be avoided (see also section 3.7).

7. DATA MANAGEMENT

The principal investigator will ensure that the study protocol is strictly adhered to and that all data are collected and recorded correctly on the case report form. Laboratory and clinical data will be recorded on a daily basis on the case report form designed for the study. Data derived from source documents should be consistent with the source documents, or the discrepancies should be explained. Any change or correction to a case report form should be dated and explained and should not obscure the original entry. All case report forms will be checked for completeness.

After the study has been completed, data will be entered into a database by double independent data entry, according to WHO standard procedures.[6] The trial data will be stored in a computer database, maintaining confidentiality.

The principal investigator is responsible for keeping all screening forms, the case report form and the completed subject identification code list in a secure location.

8. STATISTICAL METHODS

8.1 Minimum sample size

As the treatment failure rate to (name of the antimalarial drug(s) or drug combination(s)) in the area is ___% or unknown, ___% has been chosen. At a confidence level of 95% and a precision around the estimate of 5%, a minimum of ___ patients must be included. With a 20% increase to allow loss to follow-up and withdrawals during the 28/42-day follow-up period, ___ patients should be included in the study per site.

8.2 Analysis of data

(Indicate the software program and version number) will be used for data management and analysis. Data will be analysed by two methods: the Kaplan-Meier method and per-protocol analysis. In addition to the reasons for withdrawal listed in section 3.8, patients will be considered withdrawn from the analysis if the PCR results are unclassifiable or if the results of PCR indicate that the failure is due to reinfection with *P. falciparum* or *P. vivax*.

The final analysis will include:

- a description of all patients screened and the distribution of reasons for non-inclusion in the study;
- a description of all the patients included in the study;
- the proportion of adverse events and serious adverse events in all the patients included in the study;
- the proportion of patients lost to follow-up or withdrawn, with 95% confidence intervals and a list of reasons for withdrawal;
- the cumulative incidence of success and failure rates at day 28/42, PCR-uncorrected and PCR-corrected; and
- the proportion of early treatment failure, late clinical failure, late parasitological failure and adequate clinical and parasitological response at day 28/42, with 95% confidence intervals, PCR-uncorrected and PCR-corrected.

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[6] WHO/GMP. *Standardized data entry for therapeutic efficacy tests*. Geneva, World Health Organization (http://www.who.int/malaria/resistance).

Guidelines on calculating the cumulative success or failure rate, the proportion of adequate clinical and parasitological response and treatment failure are given in Appendix 9.

8.3 Dissemination of results

At the end of the study, the principal investigator will submit a report on the study and its main outcome. This report will be shared with the national malaria control programme and the Ministry of Health.

Indicate if the study will be presented during a scientific meeting or published.

Indicate if the patient data will be included in a global database.

Indicate how the results will be disseminated to the study patients, or, if it is a community-based study, indicate how the information will be shared with the community.

8.4 Amendments to the protocol

After the protocol has been accepted, no change may be made without the agreement of the principal investigator, the sponsor(s) and the institutional review boards.

9. ETHICAL CONSIDERATIONS

9.1 Approval by the national ethical committee

Before the study, official approval to conduct the study will be obtained from (name of institutional review board(s)).

9.2 Informed consent

Patients will be included in the study only if they (if adults included) or parents or guardians of children give informed consent. The consent request, available in (specify language) and translated into (local language), will be read entirely to the patient, parent or guardian. Details about the trial and its benefits and potential risks will be explained. Once any questions have been answered, a signature will be requested on the document (Appendix 10). If the patient is illiterate, a literate witness must sign; if possible, the signatory will be selected by the participant and will have no connection to the research team. The principal investigator must also obtain the assent of children over the age of 12 years, but their assent should be accompanied by the consent of a parent or guardian. A consent statement for the pregnancy test is also required for female participants of child-bearing age who are sexually active.

9.3 Confidentiality

All information on patients will remain confidential and be shared only

by the study team. Unique identifiers will be used for computer-based data entry and blood samples. In all cases, the principal investigator will ensure that screening forms, the case report form and the completed identification code list are kept in locked files.

9.4 Health-care services

Free health care throughout follow-up for any illness related to malaria will be provided to the study patients regardless of treatment outcome; this includes any expenses related to hospital admission and to adverse medicine reactions, if required.

When prospective or actual participants are found to have diseases unrelated to malaria, the principal investigator should advise them to obtain, or refer them for, medical care.

Any person who decides not to participate or who cannot be enrolled into the study because he or she does not meet the criteria will be referred to the health facility staff. Such people will be treated with (name of the antimalarial drug(s) or drug combination, dose expressed as mg base per kg body weight, number of daily doses and number of days) and followed-up according to the standard of care established by the Ministry of Health. The principal investigator will ensure that this antimalarial drug is available at the health centre.

If a patient is withdrawn from the study before he or she has completed the full course of the treatment, the physician must make all necessary arrangements to provide the patient with the full dose of the medicine being tested or with a full course of name of the antimalarial drug(s) or drug combination, dose expressed as mg base per kg body weight, number of daily doses and number of days) also recommended in the national policy.

9.5 Inducement

Subjects shall be reimbursed for their transport to attend all visits to the health centre. Insecticide treated nets will be provided to participants. No other gifts or payments will be made.

9.6 Community

If the study is community-based, mention how the community will be informed and how community participation will be maintained.

10. BUDGET TEMPLATE

Human resources

- professional scientific staff
- technical staff
- local support

Sub-total _____

Travel and transport

Sub-total _____

Equipment and supplies

- equipment
- supplies
- operational costs (space rental, communication)

Sub-total _____

Contingency fees for clinical trials

- ethical review
- registration
- liability insurance

Sub-total _____

Patient costs

Sub-total _____

Technical assistance
(training, support to research institutions, capacity-building)

Sub-total _____

Supervision
(national and consultant)

Sub-total _____

Quality assurance system
(data validation, slides cross-check)

Sub-total _____

Data management
(data entry, data analysis, report writing)

Sub-total _____

Laboratory support
(genotyping)

Sub-total _____

Miscellaneous

Sub-total _____

Grand total _____

11. CURRICULUM VITAE OF THE PRINCIPAL INVESTIGATOR

Family name (surname):	First name:
Place of birth:	Date of birth:
Current nationality:	
Academic qualifications and dates:	
Posts held (type of post, institution/authority, dates chronologically starting with current appointment):	
Selected relevant publications:	

Appendix 1. DEFINITION OF SEVERE FALCIPARUM MALARIA[7]

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

Clinical manifestations

- prostration,
- impaired consciousness,
- respiratory distress (metabolic acidosis),
- multiple convulsions,
- circulatory collapse,
- pulmonary oedema (radiological),
- abnormal bleeding,
- jaundice,
- haemoglobinurea.

Laboratory findings

- severe anaemia (haemoglobin < 5 g/dl, haematocrit < 15%),
- hypoglycaemia (blood glucose < 2.2 mmol/l or 40 mg/dl),
- acidosis (plasma bicarbonate < 15 mmol/l),
- hyperlactataemia (venous lactic acid > 5 mmol/l),
- hyperparasitaemia (> 4% in non-immune patients),
- renal impairment (serum creatinine above normal range for age).

Classification of severe malaria in children

Group 1: children at increased risk for death

- prostration,
- respiratory distress.

Group 2: children at risk for clinical deterioration

- haemoglobin < 5 g/dl, haematocrit < 15%,
- two or more convulsions within 24 h.

Group 3: children with persistent vomiting

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

Appendix 2. MEDICATIONS (WITH ANTIMALARIAL ACTIVITY) THAT SHOULD NOT BE USED DURING THE STUDY PERIOD

- chloroquine, amodiaquine;
- quinine, quinidine;
- mefloquine, halofantrine, lumefantrine;
- artemisinin and its derivatives (artemether, arteether, artesunate, dihydroartemisinin);
- proguanil, chlorproguanil, pyrimethamine;
- sulfadoxine, sulfalene, sulfamethoxazole, dapsone;
- primaquine;
- atovaquone;
- antibiotics: tetracycline*, doxycycline, erythromycin, azythromycin, clindamycin, rifampicin, trimethoprim;
- pentamidine.

* Tetracycline eye ointments can be used.

Appendix 3: DOSING CHART OF (NAME(S) OF ANTIMALARIAL DRUG(S) OR DRUG COMBINATION(S))

Tablets containing _____ mg base of (name(s) of antimalarial drug(s) or drug combination(s))

Body weight (kg)	Number of tablets		
	Day 0	Day 1	Day 2
5			
6			
7			
8			
….			
….			
….			
….			
….			
….			
….			
….			
….			
….			
67			
68			
69			
70			

Appendix 4. CLASSIFICATION OF TREATMENT OUTCOMES[8]

Early treatment failure

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

Late treatment failure

Late clinical failure

- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of early treatment failure;
- presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥ 37.5 °C (or history of fever) in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure

- presence of parasitaemia on any day between day 7 and day 28 (day 42) and axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

Adequate clinical and parasitological response

- absence of parasitaemia on day 28 (day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

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[8] WHO. *Susceptibility of* Plasmodium falciparum *to antimalarial drugs. Report on global monitoring 1996–2004*. Geneva, World Health Organization, 2005 (WHO/HTM/MAL/2005.110) (http://www.who.int/malaria/resistance).

Appendix 5. CASE SCREENING FORM

Case screening form	
Health centre name:	Study number:
Locality:	Patient screening number:
District:	Date of visit (dd-mmm-yyyy):
Province:	

Demographic data

Date of birth (dd-mmm-yyyy): or estimated age: in: ☐ months or ☐ years

Height (cm): Weight (kg):

Sex: ☐ Male ☐ Female

If female, is the patient pregnant? ☐ Yes ☐ No ☐ Not sure

If pregnant, provide the date of the last menstrual period (dd-mmm-yyyy):

Pre-treatment temperature

History of fever in previous 24 h? ☐ Yes ☐ No

Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral

Thick and thin blood smears for estimation of *P. falciparum* parasite counts

Species: ☐ *P. falciparum* ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Were species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, patient is not eligible).**

Approximate number of *P. falciparum* asexual parasites:

Presence of 1–100 parasites / 3–6 white blood cells? ☐ Yes ☐ No **(If no, patient is not eligible)**

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Has a blood sample for PCR been collected? ☐ Yes ☐ No

Haemoglobin: g/dl Haematocrit: %

Urinary analysis (pregnancy test for female patients)

Result of pregnancy test: ☐ Positive ☐ Negative **(If positive, patient is not eligible)**

Inclusion criteria

- age between and months/years
- mono-infection with *P. falciparum* confirmed by positive blood smear (i.e. no mixed infection)
- parasitaemia between and /µl of asexual forms
- measured temperature (depending on method of measurement) or history of fever within previous 24 h
- ability to swallow oral medication
- ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule
- absence of severe malnutrition (defined as per protocol)

Does the patient meet all the inclusion criteria? ☐ Yes ☐ No **(If no, patient is not eligible)**

Case screening form (page 2)	
Exclusion criteria	
• signs and symptoms of severe or complicated malaria requiring parenteral treatment according to WHO criteria (Appendix 1) • mixed or mono-infection with another *Plasmodium* species detected by microscopy • severe malnutrition • febrile conditions caused by diseases other than malaria or other known underlying chronic or severe diseases • regular medication which interferes with antimalarial pharmacokinetics • history of hypersensitivity reactions or contraindications to the medicine tested • positive pregnancy test or breastfeeding • unable to or unwilling to take contraceptives.	
Does the patient meet any of the exclusion criteria? ☐ Yes ☐ No **(If yes, the patient is not eligible)**	
If yes, please specify the reason for exclusion:	
Patient informed consent and assent	
Consent form signed: ☐ Yes ☐ No Assent form signed: ☐ Yes ☐ No	Patient identity number: Date (dd-mmm-yyyy):

	Day										
	0	1	2	3	7	14	21	28	35	42	Any other
Procedure											
Clinical assessment	X	X	X	X	X	X	X	X	(X)	(X)	(X)
Temperature	X	X	X	X	X	X	X	X	(X)	(X)	(X)
Blood slide for parasite count	X		X	X	X	X	X	X	(X)	(X)	(X)
Urine sample	(X)										
Blood for:											
genotyping	X				X	X	X	X	(X)	(X)	X
haemoglobin or haematocrit	(X)					(X)		(X)		(X)	(X)
molecular markers	(X)				(X)	(X)	(X)	(X)	(X)	(X)	(X)
in vitro test	(X)										
antimalarial blood concentration	(X)				(X)	(X)	(X)	(X)	(X)	(X)	(X)
Treatment											
Medicine to be tested	X	(X)	(X)								
Rescue treatment		(X)	(X)	(X)	(X)	(X)	(X)	(X)			(X)

Parentheses denote conditional or optional activities. For example, treatment would be given on days 1 and 2 only for 3-day dosing. On day 1, the patient should be examined for parasitaemia if he or she has any danger signs. Rescue treatment could be given on any day, provided that the patient meets 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 be taken routinely or at the request of the clinical staff.

Day 0

Screening

- clinical assessment, including measurement of weight and height; referral in cases of severe malaria or danger signs;
- measurement of temperature;
- parasitological assessment;
- pregnancy test (if necessary);
- informed consent.

Enrolment

- treatment, first dose;
- blood sampling for genotyping.

Optional

- urinary test to detect antimalarial drugs;
- haemoglobin/haematocrit;
- molecular markers of drug resistance;
- in vitro test;
- antimalarial drug blood concentration.

Day 1

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment in cases of severe malaria or danger signs;
- treatment, second dose or alternative treatment in case of early treatment failure.

Day 2

- clinical assessment; referral in cases of severe malaria or 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, day 28, any other day (day 35 and day 42)

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment;
- alternative treatment in cases of treatment failure;
- pregnancy test at the end of follow-up (if necessary);
- blood sampling for genotyping to distinguish between recrudescence and reinfection in cases of treatment failure after day 7.

Optional (after day 7)

- haemoglobin/haematocrit;
- blood sampling for molecular markers for drug resistance, antimalarial blood concentration.

Appendix 7. CASE REPORT FORMS

Case report form: follow-up day 0	
Health centre name:	Study number:
Locality:	Patient identity number:
District:	Date of visit (dd-mmm-yyyy):
Province:	

Demographic data

Date of birth (dd-mmm-yyyy): or estimated age: in: ☐ months or ☐ years

Height (cm): Weight (kg): Sex: ☐ Male ☐ Female

If female, is the patient pregnant? ☐ Yes ☐ No ☐ Not sure **(If yes, patient is not eligible).**

If pregnant, provide the date of the last menstrual period (dd-mmm-yyyy):

Pre-treatment temperature

History of fever in previous 24 h? ☐ Yes ☐ No

Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral

Thick blood smears for estimation of *P. falciparum* parasite counts

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, patient is not eligible).**

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Has blood sample for PCR been collected? ☐ Yes ☐ No

Urinary test for antimalarial drugs

Test used: Test result: ☐ Positive ☐ Negative

Test used: Test result: ☐ Positive ☐ Negative

Prior medication

All prior medication, including natural remedies and homeopathic medicines, taken within the previous 14 days should be reported in this section.
Has the patient taken any prior antimalarial medication? ☐ Yes ☐ No. If yes, please specify below. Either the date of stopping or the 'ongoing' box should be checked.

Medicine name (generic name)	Dates	Ongoing (Yes = ☒)	Total daily dose and unit (e.g. 400 mg)	Route of administration	Indication for use
	Start: Stop:	☐			
	Start: Stop:	☐			
	Start: Stop:	☐			

Case report form: follow-up day 0 (page 2)				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 1				
Study number:				
Patient identity number:				
Date of visit (dd-mmm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No If yes, perform thick blood smear.				
Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 2				
Study number:				
Patient identity number:				
Date of visit (dd-mmm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 3				
Study number:				
Patient identity number:				
Date of visit (dd-mmm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 7				
Study number:				
Patient identity number:				
Date of visit (dd-mmm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
History of fever within previous 24 h? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 14				
Study number:				
Patient identity number:				
Date of visit (dd-mmm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
History of fever within previous 24 h? ☐ Yes ☐ No				
Temperature: °C ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 21

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

History of fever within previous 24 h? ☐ Yes ☐ No

Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral

Thick blood smears for estimation of *P. falciparum* parasite counts

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Has a blood sample for PCR been collected? ☐ Yes ☐ No

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: day ___(any other day that is not part of regular follow-up)				
Study number:				
Patient identity number:				
Date of visit (dd-mmm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
History of fever within previous 24 h? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 35				
Study number:				
Patient identity number:				
Date of visit (dd-mmm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
History of fever within previous 24 h? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral				
Thick blood smears for estimation of *P.falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: final day of follow-up (28/42)

Study number:

Patient identity number:

Date of visit (dd-mmm-yyyy):

Clinical status

Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No

History of fever within previous 24 h? ☐ Yes ☐ No

Temperature: ºC ☐ Axillary ☐ Tympanic ☐ Rectal ☐ Oral

Thick blood smears for estimation of *P. falciparum* parasite counts

Average number of asexual *P. falciparum* parasites/µl:

Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No

Were species other than *P. falciparum* present? ☐ Yes ☐ No

If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*

Has a blood sample for PCR been collected? ☐ Yes ☐ No

Adverse events

Presence of an adverse event? ☐ Yes ☐ No

If yes, name the adverse event:

Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.

Medication administration

Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Urinary analysis (pregnancy test for female patients)

Patients with a positive pregnancy test must be followed up for 6–8 weeks after delivery

Result of pregnancy test: ☐ Positive ☐ Negative Date of test (dd-mmm-yyyy):

If the patient is pregnant, follow-up of the pregnancy is required, including: clinical examination of the infant at birth and 6-8 weeks after birth. Please provide comments below. If needed fill in the serious adverse event report form):

Case report form: final day of follow-up (28/42) (page 2)
Overall assessment
Outcome: ☐ adequate clinical and parasitological response ☐ early treatment failure ☐ late clinical failure ☐ late parasitological failure ☐ lost to follow-up ☐ withdrawn
Outcome occurred on follow-up day: (e.g. 1, 2, 3, 7, 14, …)
PCR: ☐ *P. falciparum* recrudescence ☐ *P. falciparum* reinfection ☐ other species ☐ mixed with *P. falciparum* recrudescence ☐ mixed with *P. falciparum* reinfection ☐ unknown
PCR corrected results: ☐ adequate clinical and parasitological response ☐ early treatment failure ☐ late clinical failure ☐ late parasitological failure ☐ lost to follow-up ☐ withdrawn
Reason for withdrawal:
Other comments:

Appendix 8. SERIOUS ADVERSE EVENT REPORT FORM

Serious adverse event report form	
Health centre name:	Study number:
Locality:	Patient identity number:
District:	Date of visit (dd-mmm-yyyy):
Province:	Follow-up day:
Demographic data	
Date of birth (dd-mmm-yyyy): or estimated age: in: ☐ months or ☐ years	
Height (cm): Weight (kg):	
Sex: ☐ Male ☐ Female	
If female, is the patient pregnant? ☐ Yes ☐ No ☐ Not sure	
If pregnant, provide the date of the last menstrual period (dd-mmm-yyyy):	
Serious adverse event	
Type of event: ☐ Death ☐ Life-threatening ☐ Hospitalization or prolongation of hospitalization ☐ Permanent disability ☐ Congenital anomaly or birth defect Date of occurrence (dd-mmm-yyyy):	
Describe the serious adverse event (include all relevant laboratory results):	
Describe how the reaction was treated:	

Serious adverse event report form (page 2)

Comments (e.g. relevant medical history, drug allergies, previous exposure to similar drugs, other laboratory data, whether reaction abated after stopping the drug, whether reaction reappeared after reintroduction):

Outcome

☐ Recovered completely

☐ Not yet recovered

☐ Recovered with long-term consequences

If patient recovered, provide date of recovery (dd-mmm-yyyy):

Medicines (list the **medicine suspected of causing** the serious adverse event as well as all **concomitant medicines**)

Brand name, batch number, manufacturer name (list suspected medicine first)	Daily dose	Route	Start date	End date	Indications for use

Reporting officer

Name:

Qualification:

Address:

Phone:

Fax:

Email:

Signature: Date:

Appendix 9. GUIDELINES FOR ANALYSIS OF RESULTS

End-point for day X (X = 28 or 42)	PCR-uncorrected results	
	Cumulative success or failure rate (Kaplan-Meier analysis)	Proportion (per-protocol analysis)
Adequate clinical and parasitological response on day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7	Failure	Failure
Other species infection	Censored day of infection	Excluded from analysis
Lost to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before withdrawal or protocol violation	Excluded from analysis

End-point for day X (X = 28 or 42)	PCR-corrected results	
	Cumulative success or failure rate (Kaplan-Meier analysis)	Proportion (per-protocol analysis)
Adequate clinical and parasitological response at day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7		
• falciparum recrudescence*	Failure	Failure
• falciparum reinfection*	Censored day of reinfection	Excluded from analysis
• other species mixed with falciparum recrudescence	Failure	Failure
• other species mixed with falciparum reinfection	Censored day of reinfection	Excluded from analysis
• other species infection	Censored day of infection	Excluded from analysis
• undetermined or missing PCR	Excluded from analysis	Excluded from analysis
Lost to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before protocol violation or withdrawal	Excluded from analysis

* WHO. *Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations*. Geneva, World Health Organization, 2008 (http://www.who.int/malaria/resistance).

Appendix 10. CONSENT AND ASSENT FORMS[9]

Example of an informed consent form for adults

This informed consent form is for adults over xx years of age who attend (indicate name of sentinel site clinic), who have been invited to participate in a study to evaluate the efficacy of (name(s) of the antimalarial drug(s) or drug combination(s)) for the treatment of uncomplicated falciparum malaria.

Name of principal investigator: _____

Name of organization: _____

Name of sponsor: _____

Name of proposal and version: _____

This informed consent form has two parts:

I. Information sheet (to share information about the study with you)

II. Certificate of consent (for signatures if you agree to take part)

You will be given a copy of the full informed consent form.

Part I. Information sheet

My name is X, and I work for the Ministry of Health. We are doing a study on the treatment of malaria. Malaria is a dangerous disease; however, it can be treated with medicine. The purpose of this study is to confirm that the medicine, called (give chemical and trade names of the drug), is still effective for curing malaria.

We are inviting all adults and children aged x–x months or years living in this area to take part in this study.

I am going to give you information and invite you to participate in this surveillance study. Before you decide whether to participate, you can talk to anyone you feel comfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask me, the study doctor or the staff.

Your participation in this study is entirely voluntary. If you choose not to consent, all the services you receive at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services you receive at the clinic will continue.

You will receive x doses of medicine over x days. The medicine, (give chemical name), is recommended by the Ministry of Health. The Ministry regularly conducts studies to make sure the medicine is still working. This

[9] http://www.who.int/rpc/research_ethics/en/

medicine is known to be very effective, but you should know that it has some minor side-effects: (list side-effects).

If we find that the medicine is not working, we will use what is called 'rescue medicine'. This medicine is called (give chemical and trade names of the medicine) and is given over x days. You should know that this medicine has some minor side-effects: (list side-effects).

A small amount of urine will be taken once. It will be tested for the presence of other medicines used to treat malaria in your body. During the follow-up, a small amount of blood will be taken 7/9 times from your finger. You may experience a bit of pain or fear when your finger is pricked. The pain should disappear within 1 day. The blood will be dropped onto a slide or a small piece of paper. The blood samples will be used to study the malaria in your blood. The examination of the blood samples will be done after the study and it will not affect the success of the treatment. Nothing else will be done with your blood.

The study will take place over 28/42 days. During that time, you will have to come to the health facility for 1 hour each day for 7/9 days. At the end of 1 month, the study will be finished. At each visit, you will be examined by a physician.

Today, we will take urine and blood for testing. After the tests, you will receive the first dose of treatment.

On the

- 2nd visit: you will receive the 2nd dose of treatment.
- 3rd visit: you will receive the 3rd dose of treatment plus a blood test.
- 4th, 5th, 6th, 7th, 8th and 9th visits, you will have a blood test.

The medicine can have some unwanted or unexpected effects; however, we will follow you closely and keep track of these effects, if they arise, and of any other problems. We will give you a telephone number to call if you notice anything out of the ordinary or if you have concerns or questions. You can also come to this health facility at any time and ask to see (give name of nurse, doctor). If you experience side-effects, we may use some other medicine, free of charge, which will help to reduce the symptoms or reactions, or we may stop one or more of the medicines. If this is necessary we will discuss it together. You will always be consulted before we move to the next step.

If you decide to participate in this study, any illnesses related to malaria or to the malaria treatment will be treated at no charge to you. Your participation will help us to make sure the medicine is still working, and this will benefit society and future generations. You will be reimbursed (provide a figure if money is involved) for your travel expenses.

We will not share the identity of participants in the study with anyone. The information that we collect from this study will be kept confidential. Any information collected about you will have a number on it instead of your name. Only the study team members will know what your number is, and we will lock that information up.

We will share the knowledge that we get from this study with you before it is made available to the public. Confidential information will not be shared. There will be small meetings in the community, and these will be announced. Afterwards, we will publish the results and make them available so that other interested people may learn from our study.

This proposal has been reviewed and approved by (give name of the institutional review board(s)). This is a committee that makes sure that study participants are protected from harm. If you wish to find about more about the institutional review board, you may contact (give name, address, telephone number).

Part II. Certificate of consent

I have been invited to participate in a study of a medicine used to treat malaria.

I have read the above information, or it has been read to me. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study.

Print name of participant: _____

Signature of participant: _____

Date: _____

(dd/mmm/yyyy)

Witness' signature: (A witness' signature and the patient's thumbprint are required only if the patient is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant and should have no connection with the study team.)

I have witnessed the accurate reading of the consent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of witness: _____
Signature of witness: _____
Date: _____
(dd/mmm/yyyy)

Thumbprint of participant:

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of investigator: _____
Signature of investigator: _____
Date: _____
(dd/mmm/yyyy)

A copy of this informed consent form has been provided to the participant.
_____ (initials of the principal investigator or assistant).

Example of an informed consent form for children or minors

This informed consent form is for parents or guardians of children aged x–x months or years who attend (name of the sentinel site clinic), who have been invited to participate in a study to evaluate the efficacy of (name of the antimalarial drug(s) of drug combination(s)) for the treatment of uncomplicated falciparum malaria.

Name of principal investigator: _____

Name of organization: _____

Name of sponsor: _____

Name of proposal and version: _____

This informed consent form has two parts:

I. Information sheet (to share information about the study with you)

II. Certificate of consent (for signatures if you agree to take part)

You will be given a copy of the full informed consent form.

Part I: Information sheet

My name is X, and I work for the Ministry of Health. We are doing a study on the treatment of malaria. Malaria is a dangerous disease; however, it can be treated with medicine. The purpose of this study is to confirm that the medicine, called (give chemical and trade names of the drug), is still effective for curing malaria.

We are inviting all adults and children aged x–x months or years living in this area to take part in this study.

I am going to give you information and invite you to consent to have your child participate in this study. Before you decide whether you want your child to participate, you can talk to anyone you feel comfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask me, the study doctor or the staff.

Your decision to have your child participate in this study is entirely voluntary. If you choose not to consent, all the services your child receives at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services your child receives at the clinic will continue.

Your child will receive x doses of medicine over x days. The medicine, (give chemical name), is recommended by the Ministry of Health. As the parasites that cause malaria can become resistant to the medicine, the Ministry regularly does studies to make sure the medicine is still working. The medicine

is made by (give company name); it is produced with the trade name (give trade name). This medicine is known to be very effective, but you should know that it has some minor side-effects: (list side-effects).

If we find that the medicine is not working, we will use what is called 'rescue medicine'. The medicine is called (give chemical and trade names of the drug) and is given over x days. You should know that this medicine has some minor side-effects: (list side-effects).

A small amount of urine will be taken once. It will be tested for the presence of other medicines used to treat malaria in your child's body. During the follow-up, a small amount of blood will be taken 7/9 times from your child's finger or heel. Your child may experience a bit of pain or fear when the finger is pricked. The pain should disappear within 1 day. The blood will be dropped onto a slide or a small piece of paper. The blood samples will be used to study the malaria in your child's blood. The examination of the blood samples will be done after the study and it will not affect the success of the treatment. Nothing else will be done with the blood.

The study will take place over 28/42 days. During that time, your child will have to come to the health facility for 1 hour each day for 7/9 days. At the end of 1 month, the study will be finished. At each visit, your child will be examined by a physician. You may stay with your child during each of the visits and during the procedures.

Today, we will take urine and blood for testing. After the tests, your child will receive the first dose of treatment.

On the

- 2nd visit, your child will receive the 2nd dose of treatment.
- 3rd visit, your child will receive the 3rd dose of treatment plus a blood test.
- 4th, 5th, 6th, 7th, 8th and 9th visits, your child will have a blood test.

The medicine can have some unwanted or unexpected effects; however, we will follow your child closely and keep track of these effects, if they arise, and of any other problems. We will give you a telephone number to call if you notice anything out of the ordinary or if you have concerns or questions. You can also bring your child to this health facility at any time and ask to see (give name of nurse, doctor). If your child experiences side-effects, we may use some other medicine, free of charge, which will help to reduce the symptoms or reactions, or we may stop one or more of the medicines. If this is necessary we will discuss it together. You will always be consulted before we move to the next step.

If you decide that your child will participate in this study, any illnesses

related to malaria or to the malaria treatment will be treated at no charge to you. Your child's participation will help us to make sure the medicine is still working, and this will benefit society and future generations. You will be reimbursed (provide a figure if money is involved) for your travel expenses.

We will not share the identity of participants in the study with anyone. The information that we collect from this study will be kept confidential. Any information collected about your child will have a number on it instead of your child's name. Only the study team members will know what the number is, and we will lock that information up.

We will share the knowledge that we get from this study with you before it is made available to the public. Confidential information will not be shared. There will be small meetings in the community, and these will be announced. Afterwards, we will publish the results and make them available so that other interested people may learn from our study.

This proposal has been reviewed and approved by (give name of the institutional review board(s)). This is a committee that makes sure that study participants are protected from harm. If you wish to find about more about the institutional review board, you may contact (give name, address, telephone number).

Part II: Certificate of consent

I have been invited to have my child participate in a study of a medicine used to treat malaria.

I have read the above information, or it has been read to me. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to my child's participation in this study.

Print name of participant: _____

Print name of parent or guardian: _____

Signature of parent or guardian: _____

Date: _____

(dd/mmm/yyyy)

Witness' signature: (A witness' signature and the thumbprint of the participant's parent or guardian are required only if the parent or guardian is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant's parent or guardian and should have no connection with the study team.)

I have witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to ask

questions. I confirm that the participant's parent or guardian has given consent freely.

Print name of witness: _____
Signature of witness: _____
Date: _____
(dd/mmm/yyyy)

Thumbprint of parent/guardian:

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to ask questions. I confirm that the participant's parent or guardian has given consent freely.

Print name of investigator: _____
Signature of investigator: _____
Date: _____
(dd/mmm/yyyy)

A copy of this informed consent form has been provided to participant's parent or guardian. _____ (initials of the principal investigator/assistant).

An informed assent form will _____ or will not _____ be completed.

Example of an informed assent form

This informed assent form is for children aged 12–x years who attend (indicate name of the sentinel site clinic) and who have been invited to participate in a study designed to evaluate the efficacy of (name of the antimalarial drug(s) of drug combination(s)) for the treatment of uncomplicated falciparum malaria.

Name of principal investigator: _____

Name of organization: _____

Name of sponsor: _____

Name of proposal and version: _____

This informed assent form has two parts:

I. Information sheet (to share information about the study with you)

II. Certificate of assent (for signatures if you agree to take part)

You will be given a copy of the full informed assent form.

Part I. Information sheet

My name is X, and I work for the Ministry of Health. We are doing a study on the treatment of malaria. Malaria is a dangerous disease; however, it can be treated with medicine. The purpose of this study is to confirm that the medicine, called (give chemical and trade names of the drug), is still effective for curing malaria.

We are inviting all adults and children aged x–x months or years living in this area to take part in this study.

I am going to give you information and invite you to participate in this study. You can choose whether you want to participate. We have discussed this study with your parent(s) or guardian, and they know that we are also asking you for your agreement. If you decide to participate in the study, your parent(s) or guardian also have to agree. If you do not wish to take part in the study, you do not have to, even if your parents have agreed. It is your choice. If you decide not to participate, nothing will change; this is still your clinic. Even if you say 'Yes' now, you can change your mind later and it will still be okay. You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. There may be some words you do not understand or things that you want me to explain more because you are interested or concerned. Please ask me to stop at any time, and I will take time to explain.

Interviewer: I have checked with the child, and he or she understands that participation is voluntary. _____ (initials)

You will receive x doses of medicine over x days. The medicine, (give chemical name), is recommended by the Ministry of Health. The Ministry regularly conducts studies to make sure the medicine is still working. The medicine is made by (give company name); it is produced with the trade name (give trade name). This medicine is known to be very effective, but you should know that it has some minor side-effects: (list side-effects).

A small amount of urine will be taken once. It will be tested for the presence of other medicines used to treat malaria in your body. During the follow-up, a small amount of blood will be taken 7/9 times from your finger. You may experience a bit of pain or fear when your finger is pricked. The blood will be dropped onto a slide or a small piece of paper. The blood samples will be used to study the malaria in your blood. The examination of the blood samples will be done after the study and it will not affect the success of the treatment. Nothing else will be done with your blood.

The study will take place over 28/42 days. During that time, you will have to come to the health facility for 1 hour each day for 7/9 days. At the end of 1 month, the study will be finished.

Interviewer: I have checked with the child, and he or she understands the procedures. _____ (initials)

The medicine can have some unwanted effects or some effects that we are not currently aware of; however, we will follow you closely and keep track of any unwanted effects, if they arise, or any other problems. If anything unusual happens to you, we need to know, and you should feel free to call us any time with your concerns or questions. If you get sick or have concerns or questions between scheduled visits to clinic, you should let me or the staff nurse know. You do not have to wait for a scheduled visit. We have also given your parents information about what to do if you are hurt or get sick during the study.

Interviewer: I have checked with the child, and he or she understands the risks and discomforts. _____ (initials)

If you decide to participate in this study, any illnesses related to malaria or to the malaria treatment will be treated at no charge to you. Your participation will help us to make sure the medicine is still working, and this will benefit society and future generations.

Interviewer: I have checked with the child, and he or she understands the benefits. _____ (initials)

Because you live quite far from the clinic, we will give your parents or guardian enough money to pay for the trip here and back and for (whatever other expense is reasonable).

We will not tell other people that you are participating in this study, and

we will not share information about you with anyone who does not work in the study. Information about you that will be collected from the study will be put away, and no one but the study team will be able to see it. Any information about you will have a number on it instead of your name. Only the study team will know what your number is, and we will lock that information up.

When we have finished the research, I will sit down with you and your parent or guardian and tell you about what we learnt. Afterwards, we will be telling more people, scientists and others, about the study and what we found. We will do this by writing and sharing reports and data and by going to meetings with people who are interested in the work we do.

You can ask me questions now or later. You can ask the nurse questions. I have written a number and address where you can reach us or, if you are nearby, you can come and see us. If you want to talk to someone else whom you know, like your teacher, doctor or auntie, that is okay too.

Part II: Certificate of assent

I have been invited to participate in a study of the efficacy of an antimalarial medicine. I have read this information (or had the information read to me), and I understand it. I have had my questions answered and know that I can ask questions later if I have them. I agree to take part in the study. _____ (initials)

or I do not wish to take part in the study and I have not signed the assent below. _____ (initials)

Child's signature (only if the child assents):

Print name of child: _____

Signature of child: _____

Date: _____
(dd/mmm/yyyy)

Witness' signature: (A witness' signature and the child's thumbprint are required only if the child is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant and should have no connection with the study team.)

I have witnessed the accurate reading of the assent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of witness: _____

Signature of witness: _____

Date: _____
(dd/mmm/yyyy)

Thumbprint of the child or minor:

Investigator's signature:

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of investigator: _____

Signature of investigator: _____

Date: _____
(dd/mmm/yyyy)

A copy of this informed assent form has been provided to the participant. _____ (initials of the principal investigator or assistant).

Example of a consent statement for a pregnancy test

I have been invited to participate in a study on the medicine used to treat malaria. I have been asked to supply a specimen of urine at the first visit and at day 28/42 or on the day of withdrawal from the study, all of which will be used for pregnancy testing. I understand that the results of the tests will be kept fully confidential and anonymous. I understand that I must avoid becoming pregnant during the study because the medicine I will be taking would be dangerous for my child. I have discussed the different methods of birth control with my doctor, and I have been offered condoms. I understand that if the test is positive, I will not be eligible to participate in this study.

Participant's signature:

I accept to be tested. _____ (participant's initials) or

I do not want to be tested, and I have not signed the consent form below. _____ (participant's initials)

Print name of participant: _____

Signature of participant: _____

Date: _____
(dd/mmm/yyyy)

Witness' signature: (A witness' signature and the thumbprint of the participant are required only if the participant is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant and should have no connection with the study team.)

I have witnessed the accurate reading of the consent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of witness: _____

Signature of witness: _____

Date: _____
(dd/mmm/yyyy)

Thumbprint of the participant:

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of investigator: _____

Signature of investigator: _____

Date: _____

(dd/mmm/yyyy)

A copy of this consent statement has been provided to participant. _____ (initials of the principal investigator or assistant).

Annex 2. CHARACTERISTICS OF MALARIA ACCORDING TO ENDEMICITY[10]

Characteristic	Endemicity of malaria			
	Hypoendemic	Mesoendemic	Hyperendemic	Holoendemic
Spleen rate (2–9 years)	0–10%	11–50%	> 50%	> 75%
Parasite prevalence (2–9 years)	0–10%	11–50%	> 50%	> 75%*
Endemicity	Low	Moderate	High	
Stability	Unstable		Stable	
Type of epidemic	True	Exaggerated seasonal transmission		
Mapping malaria risk in Africa project (MARA) transmission suitability	0.25		0.75	
Entomological inoculation rate	< 0.25	0.25–10	11–140	> 140

*Parasite prevalence applies to infants in holoendemic area

[10] Adapted from Guintran JO, Delacollette C, Trigg P. *Systems for the early detection of malaria epidemics in Africa. An analysis of current practices and future priorities*. Geneva, World Health Organization, 2006.

Annex 3. STANDARD DATA ENTRY PROCEDURES FOR THERAPEUTIC EFFICACY TESTS

INTRODUCTION

This program was designed by the Global Malaria Programme to facilitate entry and interpretation of data collected in the course of therapeutic efficacy tests. The program, which runs on Excel®, supports double data entry, to reduce the risk for error. The program provides instant information about the criteria for evaluating a patient. It has been designed to ensure that the overall results of the study are interpreted with the methods recommended by WHO. Users with broader experience of Excel® will be able to analyse the results in even more detail. There are two versions, one for a 28-day follow-up and the other for a 42-day follow-up. The program can be used for studies conducted in areas of both high and low-to-moderate transmission.

The program is divided into seven worksheets, each of which is described in detail:

- **Entry 1**
- **Entry 2**
- **Check**
- **Analysis**
- **Kaplan-Meier analysis**
- **Kaplan-Meier analysis, PCR-corrected**
- **Reference guide (summary of this document).**

Note that, in addition to the definitions of the variables to be collected, the text also gives reminders of the WHO protocol for therapeutic efficacy tests, indicated as ▶. The program has a built-in feature that helps to reduce data entry errors, whereby a cell changes colour when an out-of-range value is entered. More detailed explanations are provided below.

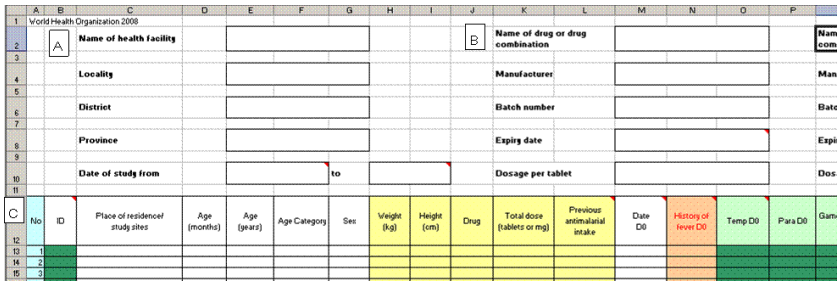
ENTRY 1

The first screen, **Entry 1**, is the main data entry page. Data entry begins here, with entry of information on the:

A. Site

B. Treatment

C. Patient



A. Site

Name of health facility	Health facility name (e.g. health centre, clinic, district hospital)
Locality	Town where the sentinel site is located
District	District where the sentinel site is located
Province	Province where the sentinel site is located
Date of study, from	Month and year when the study started (yyyy-mm)
… to	Month and year when the study ended (yyyy-mm)

B. Treatment

Name of drug or drug combination	Name of drug or drug combination studied
Manufacturer	Name of manufacturer of drug or drug combination studied
Batch number	Batch number of drug or drug combination studied
Expiry date	Expiry date of drug or drug combination studied (yyyy-mm)
Dose per tablet	Dose per tablet (e.g. 153 mg, amodiaquine base)

Note: Separate treatment information is required for each drug. If more than one drug or drug combination was studied, fill in the additional information in the space on the right.

C. Patient

ID	The patient's unique identification (ID) number In accordance with the recommendations of the ethical committee, the patient's name must not appear in a computerized file.
Place of residence/study site	District or town of residence or sentinel site if the study was conducted at several sites
Age (months)	Patient's age in months. This field should be used for studies conducted in areas of high malaria transmission. If age is entered in months, the program will automatically show the age in years in the next column, Age (years). For older patients, it is preferable to go directly to the next column.
Age (years)	Patient's age in years. This field should be used for studies conducted in areas of low-to-moderate malaria transmission and for older patients. For children aged under 5 years (60 months), age should be entered first in the previous column, Age (months).
Age category	This field is calculated automatically from the age information entered in months or years. No data entry is required. There are three possible age categories: < 5, 5–15, adult.

Sex	Patient's sex (M or F)
Weight (kg)	Patient's body weight in kilograms
Height (cm)	Patient's height in centimetres
Drug	Abbreviation of the drug or drug combination assigned to the patient (e.g. CQ for chloroquine, AQ for amodiaquine, SP for sulfadoxine–pyrimethamine, QU for quinine, AS for artesunate, AS+AQ for artesunate+amodiaquine, AS+SP for artesunate+sulfadoxine–pyrimethamine, AS+MQ for artesunate+mefloquine, AM–LU for artemether–lumefantrine)
Total dose (mg or number of tablets)	Total dose in milligrams or number of tablets. If a combination is used, indicate the total doses separated by '/'. For example, for an adult, the total dose in mg of artesunate+amodiaquine is 600/1800. For artemether-lumefantrine, the total number of tablets is 24.
Previous antimalarial intake	Information on previous intake of antimalarial drugs can be obtained by questioning, which is not always reliable, or by urine examination. If questioning is used, select Y (yes), N (no) or U (unknown). If a urinary test is used, select either Pos (positive) or Neg (negative). ▶ Previous administration of antimalarial drugs is not a criterion for exclusion under the WHO protocol.
Date D0	Date on which the first dose was administered (dd/mmm/yy). The day the patient is enrolled and receives the first dose of medicine is traditionally day 0 (D0)
History of fever D0	History of fever on day 0. Select either Y (yes) or N (no). ▶ If patients with a history of fever during the previous 24 h are included, a history of fever should also be used in classifying late clinical or parasitological failure in order to maintain consistency.
Temp D0	Axillary temperature on D0, to one decimal point. The temperature should be between 36 °C and 42 °C. If any other value is entered, the cell will turn red to indicate that the temperature is wrong.
Para D0	Asexual falciparum parasitaemia on D0. ▶ Parasitaemia always refers to falciparum species. Mixed infections detected by light microscopy should be excluded. ▶ The thresholds of parasite numbers for areas of low-to-moderate transmission are between 1000 and 100 000/µl; in areas of high transmission, the thresholds are between 2000 and 200 000/µl. Note: If parasitaemia below 1000 or above 200 000/µl is entered into a cell, it will turn red to show that the value is outside the limits for inclusion, regardless of the area of transmission. Appearance of a red cell does not prevent data from being entered and a classification being obtained.

Gametocytes D0	Presence of gametocytes on D0. Select either Y (yes) or N (no). ▶ To detect the presence of gametocytes, at least 2000 white blood cells should be counted on a thick blood smear.
Danger sign/severe malaria D1	Danger signs of malaria on D1. Select either Y (yes) or N (no). ▶ Depending on the classification, a patient will be considered as having experienced treatment failure if the danger signs are associated with the presence of parasites. For this reason, care must be taken to indicate parasitaemia in the 'Para D1' box for the day corresponding to the appearance of signs of severity; if this is not done, the program will not show the failure.

Note that information on the last five variables is collected throughout the 28 or 42 days. The chart below shows on which day data must be collected. The Excel sheet has a column for each item required, in chronological order.

Item	Day										
	0	1	2	3	7	14	21	28	35	42	Unscheduled*
Danger sign or severe malaria**		•	•	•	•	•	•	•	•	•	•
History of fever	•				•	•	•	•	•	•	•
Temperature	•	•	•	•	•	•	•	•	•	•	•
Parasitaemia	•	•	•	•	•	•	•	•	•	•	•
Gametocytes	•				•	•	•	•	•	•	•

* The unscheduled day must also be defined.

** It is important to fill in complete information on danger signs and parasitaemia, as a patient will be considered to have experienced treatment failure in the analysis only if the danger signs are associated with the presence of parasites on the same day.

Day of LFU	Day of loss to follow-up (LFU), if relevant. Select the day from the drop-down list (D0–D28 or D0–D42) (e.g. if a patient attends on D14 but fails to return on D21, he or she is lost to follow-up on D14)
Day of withdrawal	Day of withdrawal, if relevant. Select the day from the drop-down list (D0–D28 or D0–D42). If a patient is excluded, the grounds for exclusion should be indicated in the 'Observations' column.
Classification	The classification of response (early treatment failure, late clinical failure, late parasitological failure, adequate clinical and parasitological response) is assigned automatically from the results in the previous columns, as per the WHO protocol (see below).
Day of classification	Day of classification appears automatically, determined by the day the patient was lost to follow-up, withdrawn, cured or failed treatment.
PCR	Select from the six options provided: *P. falciparum* (Pf) recrudescence, Pf reinfection, unknown, other species, mixed with Pf recrudescence and mixed with Pf reinfection. ▶ If failure occurs after D7, PCR is mandatory in areas of both high and low-to-moderate transmission, to distinguish between reinfection and recrudescence, to confirm the *Plasmodium* species or to detect mixed infection. Therefore, failure with a species other than *P. falciparum* should also be confirmed by PCR.
Classification, PCR-corrected	The classification of response, PCR-corrected (early treatment failure, late clinical failure, late parasitological failure, adequate clinical and parasitological response) is assigned automatically from the results in the previous columns, as per the WHO protocol (below) and the PCR result. In case of failure after D7, patients whose PCR result is unknown will be excluded from the analysis in accordance with the standardized WHO protocol.
Day of classification, PCR-corrected	The day of classification, PCR-corrected, appears automatically. It is determined by the day the patient was lost to follow-up, withdrawn, cured or failed treatment.
Observations	Any additional information can be recorded here (e.g. description of grounds for exclusion, administration of other treatments, detection by light microscopy of other *Plasmodium* species during follow-up).

WHO classification of treatment outcomes

Early treatment failure

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

Late clinical failure

- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria of early treatment failure; and
- presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥ 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure.

Late parasitological failure

- presence of parasitaemia on any day between day 7 and day 28 (day 42) with axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

Adequate clinical and parasitological response

- absence of parasitaemia on day 28 (day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

12	SUMMARY OF CLASSIFICATION				
13		number	proportion	Lower 95%CI	Upper 95% CI
14	ETF	0	0.000	0.000	0.047
15	LCF	2	0.026	0.003	0.092
16	LPF	8	0.105	0.047	0.197
17	ACPR	66	0.868	0.771	0.935
18	Total analysis	76			
19	WTH	6			
20	LFU	0	0.073		
21	TOTAL	82			
22					
23					
24	SUMMARY OF CLASSIFICATION PCR CORRECTED				
25		number	proportion	Lower 95%CI	Upper 95% CI
26	ETF	0	0.000	0.000	0.051
27	LCF	1	0.014	0.000	0.077
28	LPF	3	0.043	0.009	0.120
29	ACPR	66	0.943	0.860	0.984
30	Total analysis	70			
31	WTH	9			
32	LFU	0	0.114		
33	TOTAL	79			

Summary of classification
Data are analysed automatically from the 'Classification' column; there is no need to enter any additional data. The results reflect all data, including that which has not been PCR-corrected. Results are provided as number of cases, proportion and the 95% confidence intervals (CIs) for the groups 'adequate clinical and parasitological response' (ACPR), 'early treatment failure' (ETF), 'late clinical failure' (LCF) and 'late parasitological failure' (LPF). The total proportions of loss to follow-up (LFU) and withdrawn (WTH) are also shown.

Summary of classification, PCR-corrected
Data are analysed automatically from the 'Classification PCR-corrected' column; there is no need to enter any additional data. The results reflect only data that have been PCR-corrected.

ENTRY 2

This file is identical to **Entry 1**. Data must be entered in **Entry 2** by someone other than that who completed the **Entry 1** worksheet. The consistency between the two files can be verified on the **Check** worksheet.

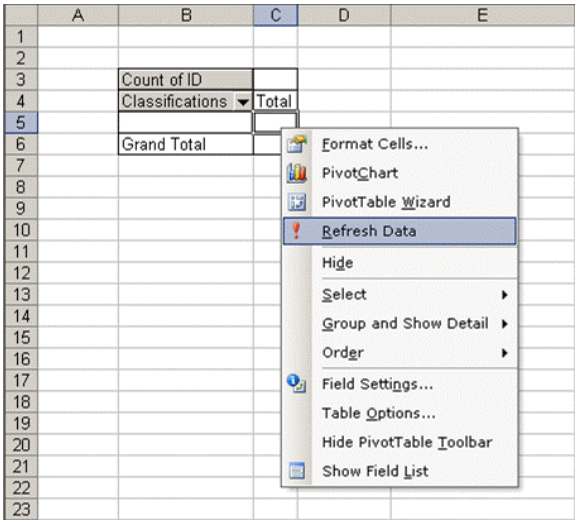
CHECK

This file is used to compare all the data entered in **Entry 1** with those in **Entry 2**. If identical data are entered in the same cell (e.g. column C, row 10) in **Entries 1** and **2**, the word TRUE will appear in the corresponding cell on the **Check** worksheet. If there is a discrepancy between the two sheets, the word FALSE will appear, and the user should check the patient's file to verify the information. Before beginning 'Analysis', all cells should read TRUE.

	A	B	C	D	E	F	G	H	I
13	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE
14	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
15	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE
16	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
17	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
18	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE

ANALYSIS

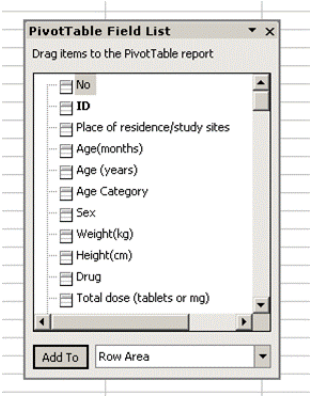
This file allows users to analyse the data more thoroughly. The results are shown as the number of people per group, without the proportion or confidence intervals. All the results are based on the **Entry 1** worksheet. It is critical that the identification numbers for all patients be available so that all data can be taken into account.



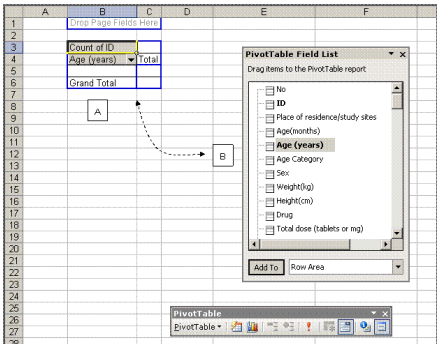
To begin analysis after data entry has been completed or modified, place the cursor over the pivot table in columns B and C, right-click, and select 'Refresh data'. This step should be repeated each time the data are modified in the **Entry 1** file.

The pivot table allows the user to compare the results for all the variables in the file. For example, an analysis can be performed by age category, previous antimalarial intake, drug or study site.

How to work with the pivot table to analyse specific variables:



Click the pivot table to view the field list, shown here.



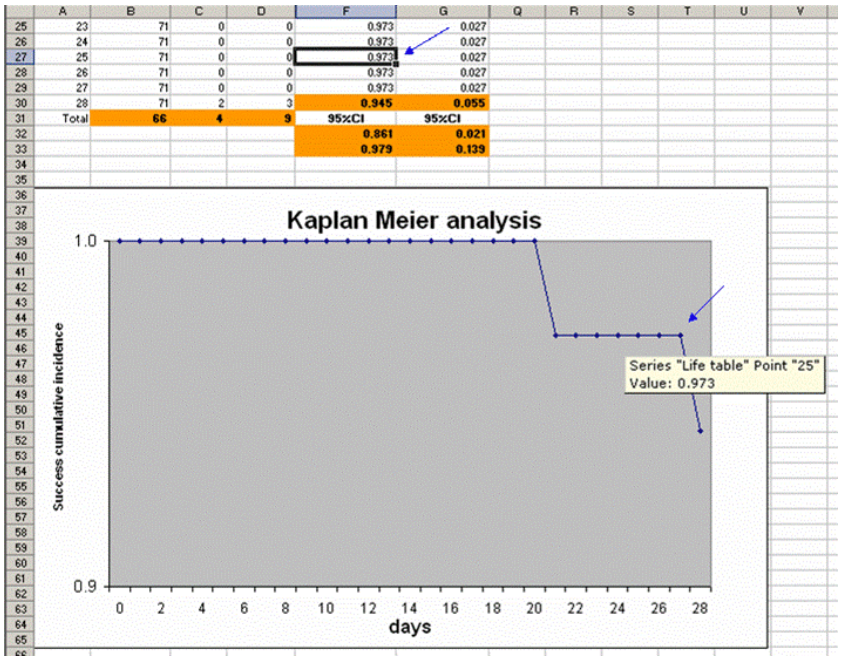
- Select a variable by left-clicking on the variable name in the field list and dragging it to the pivot table (A).
- To remove the variable, do the same in reverse (B).
- Cross-tabulation can be performed by adding more than one variable to the pivot table.

If the field list disappears from the screen, right-click on the pivot table, select 'PivotTable Wizard', click on 'Finish', and the list will reappear.

KAPLAN-MEIER ANALYSIS

A Kaplan-Meier analysis is calculated automatically from data in the **Entry 1** file; there is no need to enter any additional data. The Kaplan-Meier worksheet includes all data, even those that have not been corrected by PCR. The results are expressed as success and failure cumulative incidence, with 95% confidence intervals.

The results are presented on the Kaplan-Meier curve shown on the graph below. When the cursor is placed on a point on the curve, the yellow box indicates the success rate on the corresponding day.



	A	B	C	D	F	G
25	23	71	0	0	0.973	0.027
26	24	71	0	0	0.973	0.027
27	25	71	0	0	0.973	0.027
28	26	71	0	0	0.973	0.027
29	27	71	0	0	0.973	0.027
30	28	71	2	3	**0.945**	**0.055**
31	Total	**66**	**4**	**9**	**95%CI**	**95%CI**
32					**0.861**	**0.021**
33					**0.979**	**0.139**

KAPLAN-MEIER ANALYSIS, PCR-CORRECTED

A Kaplan-Meier analysis PCR-corrected has the same features as on the previous sheet. It is calculated automatically, and no additional data entry is required. The difference is that this sheet reflects only those data that have been PCR-corrected.