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Practical Guide

for *in vivo*

Antimalarial Drug-Efficacy Studies

in the Americas



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Table of Contents

Introduction	1
Preparation of the Protocol.....	2
Number of Study Sites.....	2
Site Selection	3
Study Team	5
Study Start-Up	6
Preparation of Supplies	7
Petty Cash Account.....	7
Record Books and Forms.....	7
Record of Patients with Fever and Malaria	7
Record of Follow-Up Visits	8
Laboratory Notebook.....	9
Petty Cash Account	9
Preparation of the Study Site	10
Patient Enrollment and Follow-Up	10
Preparation and Handling of Blood Smears	13
Labeling of Blood Smears	13
Preparation of Blood Smears	13
Staining	13
Transporting and storing blood smears	14
Microscopes:.....	14
Counters	14
Supervision:	14
Other Laboratory Studies	14
Samples for PCR Studies	14
Samples for Assays of the Concentration of Antimalarial Drugs in the Blood	15
Supervision.....	15
Patient Enrollment	15
Patient Follow-Up	16
Financial Report	16
Closing the Study.....	16
Cost of <i>in vivo</i> Studies.....	17
Preparation of the Database.....	17
Appendix 1: List of Field Supplies Needed for an <i>in vivo</i> Study of 60 Patients	18
Appendix 2: Form to Summarize Blood Smear Results	20

Introduction

Resistance of *Plasmodium falciparum* to antimalarial drugs is one of the most serious challenges facing national malaria control programs in the Americas. At present, *P. falciparum* is resistant to both chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) throughout the Amazon Basin and to CQ alone on the Pacific Coast of South America. Additionally, in recent years several investigators have reported cases of *P. vivax* therapeutic failure to CQ. In response to the public health threat of drug resistance, several ministries of health in the region have begun studies to map the distribution and intensity of *P. falciparum* and *P. vivax* resistance to antimalarial drugs within their borders. Peru and Bolivia have finished their baseline studies and have already changed their first-line treatments for uncomplicated *P. falciparum* infections. These countries are also establishing surveillance systems for drug resistance at sentinel sites throughout the country.

Although a variety of methods have been used by ministries of health to evaluate antimalarial drug resistance, *in vivo* drug efficacy studies are generally considered the method of choice because their results correlate best with the clinical response of patient to these drugs. The World Health Organization (WHO) has published guidelines for *in vivo* drug efficacy studies, originally intended for use in settings with intense transmission, such as Africa, but more recently with modifications appropriate for areas of low to moderate transmission, such as the Americas. In South America, most of the changes in national malaria treatment policies that have been made during the last 3-4 years have been based on the results of these *in vivo* studies.

While *in vivo* drug efficacy studies do not require sophisticated technology, they are not simple to conduct correctly. They require a well-trained and experienced clinical and laboratory team that closely follows the study protocol. Because of the low levels of malaria transmission in the Americas, it may be a challenge to meet the required sample sizes, particularly for *P. falciparum* infections. Also, in regions such as the Amazon Basin, where the population is widely dispersed and highly mobile, it may be extremely difficult to limit the number of patients lost to follow-up to no more than 10-15% of enrolled patients, as recommended by the WHO.

Even though most *in vivo* studies carried out in the Americas during the last 3-4 years have made use of standardized study protocols based on the WHO guidelines, those recommendations do not describe in detail how to carry out a study or how to avoid the pitfalls that can be encountered when working in the field. The purpose of the recommendations in this guide, which is based on recent experiences with antimalarial drug efficacy studies in South America, was to complement to the WHO guidelines and standardized protocols. It is hoped that this guide, together with the generic protocols described below, will contribute to the successful implementation of *in vivo* studies and a surveillance system for antimalarial drug resistance in the Americas.

Preparation of the Protocol

The first step in carrying out an *in vivo* drug efficacy study is preparation of a protocol that will be acceptable to the scientific and/or ethical review committees of the institution responsible for the study and the institution providing financial support. To help investigators in the preparation of protocols specific to their local setting, three generic protocols have been developed. They are based on the WHO recommendations for *in vivo* antimalarial drug efficacy studies of 1996 with modifications made in 1998 for areas with low transmission. They also include the most recent recommendations emanating from a WHO meeting in Geneva in December 2001. The three protocols can be found on-line at Pan-American Health Organization (PAHO) website at the following address: <http://www.paho.org/English/AD/DPC/CD/mal-antimalarials.htm>

1. Chloroquine (CQ) alone, sulfadoxine-pyrimethamine (SP) alone, or a comparison of CQ and SP for uncomplicated *P. falciparum* infections;
2. Mefloquine (MQ) and mefloquine-artesunate (MQ-AS) for uncomplicated *P. falciparum* infections; and
3. CQ for *P. vivax* infections.

To complete the protocol, the local investigator only needs to complete the highlighted sentences and paragraphs in line with the local epidemiology of malaria and drug resistance and the characteristics of the country, region, and site where the study will be conducted.

Although the generic protocols are intended for the evaluation of specific drugs or drug combinations, they can easily be modified for a study of similar drugs. For instance, the CQ/SP generic protocol can be modified for an evaluation of amodiaquine and the MQ-AS protocol modified for a study of SP-AS. Similarly, the length of follow-up can be adjusted to the local epidemiologic situation. If the drug being evaluated is expected to have a rate of parasitological failures $\geq 20\text{-}25\%$, the follow-up period can be limited to 14-days in order to save time and resources. In contrast, if the drug or drug combination to be studied is expected to have a low rate of parasitological failures, follow-up can be extended to 28-days to identify delayed recrudescences of parasitemia and lower levels of resistance.

Number of Study Sites

The number of different sites selected for *in vivo* studies in a given country or region of that country will depend on the epidemiology of malaria and available information about drug resistance in that country, the objective of the studies (baseline studies or studies that are part of a drug surveillance network), and the available resources. Resistance to antimalarial drugs is usually fairly uniform within a geographic area where the epidemiology of malaria is homogeneous. For example, the pattern and intensity of resistance to different antimalarial drugs is quite similar along the Pacific Coast of South America or within the

Amazon Basin. Additionally, the level of resistance to a drug or drug combination usually changes rather slowly and it may take 2-3 years before any appreciable change be detected. Thus, for baseline studies, a maximum of 2-3 sites should be sufficient to map the distribution and intensity of resistance in a given region of a country. In such studies, sites near a country's border are often selected, although this is not absolutely necessary. For studies that are part of a drug resistance surveillance network, one site per zone (that corresponds to a geographic or an ecologic area) should be sufficient and those studies should not have to be repeated any more frequently than every 2-3 years. Ideally, when administrative and ethical differences can be resolved, neighboring countries should try to coordinate their antimalarial drug resistance monitoring by alternating sites between the two countries.

Site Selection

One of the most critical factors for the success of an *in vivo* drug efficacy trial is the selection of the site(s) where the study will be conducted. The principal criteria for selecting a suitable site are:

1. the incidence of falciparum or vivax malaria at the site or in nearby communities;
2. the health infrastructure at the site;
3. access to the area and the means of transportation within the study area for residents and study staff; and
4. the security of the team and the availability of lodging and meals.

Sites for *in vivo* drug efficacy trials should not be selected simply because they are more accessible or more convenient for the study team. It is also important to recognize that Ministry of Health malaria control measures should not be modified or halted, simply because an *in vivo* efficacy trial is planned.

Before starting an *in vivo* study, a senior member of the team should visit the area chosen for the study in order to select the most appropriate site and health facility. During this visit, a courtesy call should be made to local health authorities and health facility records should be reviewed in order to find out which communities report the highest incidence of malaria, the number of malaria cases diagnosed monthly at the different health facilities, and the season of peak transmission. The infrastructure of the health facilities, including potential space for interviewing patients and laboratory work, availability of electricity (with the number of hours/day), telephone or radio for communicating with the study team's headquarters, and transportation services should be examined. In addition, health facility personnel should be interviewed to determine whether residents comply with their follow-up medical visits.

In areas with unstable transmission, such as the Americas, it may be difficult to predict with any degree of accuracy the number of malaria cases that will occur in a given community or month of the year. For this reason, the investigator should not rely completely on malaria incidence data from previous years when selecting sites for an *in vivo* study. Moreover, health facility records generally do not provide all the information necessary to make a decision about the best site for a study, and a simple count of the number of cases of

P. falciparum or *P. vivax* malaria diagnosed each month at a health facility may give a false impression of the number of patients who could be enrolled. Normally, microscopists in ministry of health facilities use a semiquantitative system of grading from $\frac{1}{2}+$ to ++++ to indicate the level of parasitemia, rather than calculating parasite densities. As a general rule, one can assume that " $\frac{1}{2}+$ " is equivalent to about 250 parasites/ μl and "+" is equivalent to 500-1,000 parasites/ μl . However, due to the upper and lower limits of parasitemia in the enrollment criteria, it is likely that at least some of the malaria patients listed in the clinic register will not meet the inclusion criteria. In addition, some of the women will be pregnant and, therefore, ineligible for enrollment. Finally, it is likely that at least some patients will come from communities far from the study site, making follow-up for 14 or 28 days difficult. Taking all of these factors into account, one should probably estimate that no more than 50% of patients with *P. falciparum* or *P. vivax* malaria who are listed in a health center's register can be enrolled in an *in vivo* trial.

From a logistic standpoint, it may be difficult to conduct an *in vivo* study that lasts more than 4-5 months because of the high cost and the difficulty of maintaining a team in the field for an extended period of time. If the duration of a drug efficacy study is to be limited to 4-5 months and a total of 50-60 patients need to be enrolled, a minimum of 3-4 patients will need to be enrolled each week. Assuming that only 50% of infected patients attending the health facility meet the enrollment criteria, at least one patient with malaria per day (i.e. 7-10 patients per week) will be needed in order to achieve the target of 3-4 subjects enrolled per week.

The WHO guidelines stress the importance of enrolling a representative sample of subjects from the area being studied and recommend not selecting hospitals or health facilities located in urban areas because these health facilities tend to receive patients referred from more peripheral health centers who may have already received antimalarial treatment. In the Americas, it may be difficult to comply with this recommendation because the low levels of transmission and the widely scattered population in areas, such as the Amazon Basin, can severely limit the number of possible study sites and potential subjects. Consequently, investigators may have to choose between a less than ideally representative sample from an area and the total absence of information from that area, if no study is conducted.

As part of the initial exploratory visit to a potential study site and health facility, it is important to examine the infrastructure at the health facility to be sure that the team will have sufficient space for their work, including a quiet, semi-private area for patient interviews and physical examinations, an area for taking, staining, and examining blood smears (which can be part of the health facility laboratory), and a waiting area for patients before they are enrolled and during follow-up. It is also important to consider how to organize best the flow of febrile patients from their arrival at the clinic, to the laboratory for their initial blood smear, and then to the area where the study team is located. If adequate space for the study team is not available at the health facility, it will be necessary to make arrangements nearby, but this will take more time and the additional cost should be taken into account in the study budget.

The initial exploratory visit should also be used also to determine what materials and equipment will be needed, including availability of good quality microscopes and vehicles

and/or boats for follow-up, as well as estimating the amount of money necessary for local expenses. Normally, microscopes in peripheral health facilities are not as good quality or as well maintained as those in National Malaria Control Program laboratories of the research institution conducting the study and it is recommended that the study team bring a microscope with them.

It may also be useful to make visits to several shops in the community to see if photocopying is available locally and whether commodities such as sweets, notebooks, toilet paper, etc., are available, so that they will not have to be transported to the site when the study begins.

When sites selected for an *in vivo* drug efficacy study are distant or access is difficult, several potential additional problems should be kept in mind:

1. supply of materials: sufficient supplies to complete the study should be sent at the beginning of the study;
2. difficulties with regular supervision: an experienced team should be used; and
3. the increased cost of sending and maintaining a research team in such a site.

Study Team

Although *in vivo* studies are not technically difficult to perform, they do require experienced staff able to devote most of their time to the study without other distractions. For this reason, it has proven difficult in most countries for local health center staff to take primary responsibility for the enrollment and follow-up of subjects. Since these workers have to care for other patients, carry out administrative work, and attend meetings, courses, and conferences outside the study site, it is impossible to guarantee the attention necessary for enrolled subjects, particularly when home visits are necessary to trace patients who have not returned for their follow-up visits. However, a senior member of the local health facility team should be invited to take part to learn the procedures of *in vivo* drug trials and with the aim that that individual would participate in future studies at the same site.

For *in vivo* drug efficacy studies that are part of a surveillance system for antimalarial drug resistance and need to be repeated every 2-3 years, the frequent transfer of physicians in rural areas from one site to another makes it extremely difficult to maintain a well-trained local team. For this reason, a national or regional institution in each country will need to maintain a cadre of experienced investigators and laboratory staff who can take responsibility for training and supervising new field teams and local workers when a study is planned. In this way, monitoring of antimalarial drug resistance can be institutionalized in the country.

The minimum team for an *in vivo* study consists of a physician, a nurse or nursing assistant, and a microscopist. Ideally, the physician in charge of the study should have taken part in similar drug efficacy studies before. If no experienced physician is available, the new physician will have to be trained before the fieldwork starts. Later, a more experienced

physician should remain with the new physician in the field during the enrollment and initial 10-14 days of follow-up for the first 5-7 enrolled patients.

Under exceptional circumstances, such as in very isolated regions, it may not be possible for the study physician to be present throughout the entire follow-up period for all patients, but he/she must be present during the enrollment phase. When the physician is not present during part of the follow-up, the remaining study team members should be well aware of the danger signs of severe malaria and know how to manage the patient until he/she can be transferred to a hospital.

The nurse or nursing assistant—who is responsible for weighing patients, administering all doses of the study drugs, and instructing patients about follow-up procedures—can be a staff member of the health facility where the study is being conducted. He/she should be trained and closely supervised by the study physician, particularly in calculating drug dosages and in administering antimalarial drugs, which are often quite bitter, to young children. It is suggested that a second study team member always verify that the dose of study medication is correctly calculated.

A well-trained microscopist, preferably with experience in *in vivo* drug efficacy studies, is key to the success of such studies. If one is not available, a new microscopist should be trained several weeks before the beginning of the study and an experienced microscopist should supervise him/her in the field no less frequently than every 3-4 weeks to evaluate the quality of the blood smears, the species diagnosis, and the parasite density calculations. The most common problems encountered in field settings are thick smears that are too thin or too thick, contamination with bacteria, fungus, or stain crystals, and insufficient dehemoglobinization due to heat fixation of the thick smear. All blood smears should be saved for re-examination later by a more experienced microscopist.

Study Start-Up

Ideally, the study should begin 2-4 weeks before the peak malaria transmission season. In this way, it will be possible to enroll more rapidly the number of patients required to complete the sample size. To achieve this goal, it will be necessary to have up-to-date information on malaria incidence at the site where the study will be conducted. It is important to remember, however, that high transmission season may vary from one year to the next and data from previous years are not always the best guide for scheduling a study. Consequently, beginning 1 to 2 months before the start of the study, the investigator should communicate periodically with the health center personnel to ensure that the number of malaria cases is increasing and that there will be a suitable number of patients for the study. If it is not possible to start enrollment before or at the beginning of the peak transmission season, preparations will have to be made for a study of longer duration.

Preparation of Supplies

The study team should be fully trained and prepared before the study starts. Because of the time that is often needed to purchase and receive supplies, the team should prepare purchase orders well in advance and have everything ready at least 3-4 weeks before the study is scheduled to begin. It is particularly important to ensure that the antimalarial drugs that are going to be used are from a reliable source and of good quality. The study supervisor should record the source and lot number of all antimalarial drugs. If there is any question about the quality of the drugs, it is suggested that a sample be saved for full pharmacological analysis when the study ends. Appendix 1 provides a list of field materials and supplies necessary for a study of 60 patients with a single drug or drug combination. It does not include the cost of medicines nor laboratory supplies and reagents necessary for genotyping parasite strains or detecting point mutations associated with drug resistance.

Depending on the accessibility of the study site and potential transportation difficulties, the team should consider carrying with them to the site all necessary drugs and supplies when the study begins. The local health facility should not be relied on to provide study medications. Based on the report of the investigator who made the initial exploratory visit, a microscope that uses sunlight or electricity (with sufficient replacement light bulbs) will be needed.

Petty Cash Account

Based on the experiences of *in vivo* studies in Peru, Bolivia, and Ecuador, a petty cash fund administered by the physician responsible for the study in the field is extremely useful for the purchase of additional supplies and drugs and reimbursement of patient transportation expenses. Normally these local expenses are not large. If there is easy access to the study site with supervisory visits every 3-4 weeks, \$20-25 should be sufficient. If the site is less accessible and the supervisory visits less frequent, or if local expenses are expected to be higher, a larger sum of \$100-150 will be needed.

Record Books and Forms

To simplify and streamline patient enrollment and follow-up, most experienced investigators make use of record books and forms that are not mentioned, as such, in the study protocol. School notebooks with 50-100 lined pages serve well for this purpose.

Record of Patients with Fever and Malaria

The study team should keep a record of the number of febrile patients and patients with confirmed malaria who are seen daily at the health center where the study is being conducted. The number of febrile patients, the number with confirmed malaria, whether they were enrolled or not, and the reason for exclusion should be recorded. This information

will be helpful for preparing a final report of the study and will allow the team to determine the total population of febrile patients who were screened for enrollment and possible biases in patient enrollment. The following is an example of a page in this record book:

Table N° 1: Record of Patients with Fever and Malaria

Date	First and last name	Age	Sex	Diagnosis (+/-; species)	Enrolled (Yes/No)	Reason for exclusion

Record of Follow-Up Visits

After several subjects have been enrolled, it becomes difficult to remember when specific patients are scheduled to have their follow-up blood smear. For this reason, it is helpful to maintain a running record of follow-up visits. As soon as a patient is enrolled, the physician should record all of that patient's future visits in the notebook. One page is used for each day, and the patient's code number is written below the corresponding day for his/her follow-up visit. An example of a typical page for 7 June, 2002 is given below, in which patients J037 and J038 are scheduled to return for their Day 1 visit, patient J034 for his/her Day 3 visit, patients J026, J027, J028, and J029 for their Day 14 visit, etc. When the patient has been seen, a line is drawn through his/her name to indicate that the follow-up visit has been completed. This record also allows the team to predict and control their daily workload. Because of the critical importance of scheduling follow-up visits on the correct dates, the dates in this notebook must be correct without skipping or repeating dates.

Table 2: Schedule of Follow-up Visits

Day 1* <i>J037**</i> <i>J038</i>	Day 14 <i>J026</i> <i>J027</i> <i>J028</i> <i>J029</i>
Day 2	Day 21 <i>J008</i> <i>J009</i> <i>J010</i>
Day 3 <i>J034</i>	Day 28
Day 7	

*Day of follow-up

**Patient code number

Laboratory Notebook

The microscopist should record his/her daily blood smear results in a bound notebook. At the end of the day, the site physician will use this information to enter the follow-up blood smear results on the case record forms. An example of a page in this record book is given below:

Table 3: Daily Laboratory Record

Patient Code No.	Day	Date	Result	Species	Asexual Parasites	WBCs	Parasites/ μ L	Gametocytes	Gametocytes/ μ L	Comments
J001	D7	22-Jun-02	NPF*							
J002	D7	"	NPF							
J003	D3	"	+	F	33	217	912	0		
J004	D0	"	+	F	295	205	8634	0		
J005	D0	"	+	F	380	201		0		

*NPF = no parasites found

Table 4: Record of First and Second Blood Smear Examinations

ID #	Day	Date	First Exam Asexual Parasites	Second Exam Asexual Parasites	Third Exam Asexual Parasites	Mean	First Exam Gametocytes	Second Exam GAMETOCYTES	Third Exam Gametocytes	Mean	Observations
J001	D0	25-04-01	2163	1934	-	2049	-	-	-	-	
J002	D0	14-05-01	1693	510	594	552	-	-	-	-	
J003	D3	15-06-01	2569	2800	-	2685	5	4		5	
J004	D14	23-06-01	-	-	-	-	125	23	150	138	

Petty Cash Account

To administer the petty cash fund, a notebook to record local travel expenses, supplies, medications or other unexpected purchases should be used. Receipts for study-related expenses should also be saved.

The generic protocols include an example of the patient report form, which contains the minimum information needed to ensure that studies are comparable from one country to another, but different investigators may choose to include additional data in the patient report form that they consider pertinent.

Preparation of the Study Site

Normally, on arriving at a new site for an *in vivo* drug efficacy study, at least one day will be needed to prepare the space for the study team. For patient interviews and physical examinations, a room with 3-4 chairs, a table, and a bed or examination table will be required. Since several patients may come in at the same time, a bench or extra chairs where patients and relatives can wait is useful. The microscopist will need a table and two chairs for blood sample taking. Ideally, the location where blood smears are taken should be close to the room where patients are being seen by the physician; however, if samples for measuring drug levels in the blood are being taken, sampling must be done in a separate room from where drugs are administered to avoid contamination. A separate room is not necessary for taking blood samples for PCR analyses but care should be taken that the area has been thoroughly cleaned with a disinfectant.

Because of the difficulty of accurately dividing chloroquine, sulfadoxine-pyrimethamine, and mefloquine tablets into halves and quarters to ensure the correct dose according to the patient's weight, it may be useful to have some divided tablets prepared ahead of time. In addition to the antimalarial drugs used during the study, the team should have other basic medications available, such as paracetamol and dimenhydrinate for nausea or vomiting. A container of water and disposable cups for drug administration should be set up nearby. To avoid patients having to take their medication on an empty stomach, biscuits or crackers should be available.

The study flow diagrams should be attached to the wall in a readily visible location to make it easier for the team to follow the study procedures for each enrolled patient. An example is shown in Appendix 3.

Patient Enrollment and Follow-Up

In most countries in the Americas, the initial selection of patients for study can be simplified because all febrile patients in endemic areas have a blood smear taken for diagnosis. If this is the case, the study microscopist can use the slide taken by the health facility staff to obtain the Day 0 parasite species and density. If the quality of this blood smear is not adequate, it will need to be repeated to obtain an accurate calculation of parasite density. It is recommended that this second blood smear be examined within 24 hours.

According to WHO recommendations for *P. falciparum in vivo* drug efficacy studies, one of the inclusion criteria is a minimum parasite density of 1,000 asexual parasites/ μ l. In the Americas, many patients have low levels of parasitemia when they are first seen and may have to be excluded from the study if this level is used. Consequently, in the studies carried out in Peru, Ecuador and Bolivia between 1999 and 2002, the lower limit for parasite density was set at 250 asexual parasites/ μ l. Reducing the enrollment parasite density more than this would probably make it difficult to detect changes in parasite density after starting treatment.

The physician in charge of the study should talk to each patient on enrollment to assess his/her level of interest in taking part in the study and willingness to return for follow-

up visits. The principal causes for patient withdrawal are living too far from the health center, family problems, or travels. With the highly mobile population of the Amazon Basin, residents may be absent from their villages for a day or more to care for their crops, or for fishing or hunting. Because of the time a study team has to invest in seeking out patients in their homes, it is usually better to exclude such patients from a study, instead of enrolling them and losing them later to follow-up.

Experience has shown that it is preferable to enroll only those patients who are permanent residents of the study community or live no more than 30 to 45 minutes away by road or river. With residents from more distant communities, loss to follow-up rates are higher. Sometimes situations may occur in which residents of other communities who are staying with relatives in the study village come in for treatment. While it may be tempting to enroll such patients, they tend to return to their homes and drop out of the study as soon as they feel better.

There is no upper limit on the number of patients that can be enrolled each day, although experience suggests that generally it is difficult to enroll and follow more than 5 patients per day when this number is enrolled each day. For a less experienced team, it may be better to begin with just 2-3 patients per day and then when they have gained experience, increase the number enrolled daily. A checklist to ensure that all enrollment criteria are met is a useful tool.

To facilitate patient understanding of the consent form, particularly in more complicated studies comparing various antimalarial drugs, a visual aide that explains the rationale and procedures of the study with figures can be prepared.

Patient record forms should be carefully completed in legible handwriting by the study physician. If errors are made in recording data, it should be crossed out with a single line and the correct information added above or below with the initials of the person who made the corrections and the date. The study physician has the responsibility to ensure that the patient record forms are up-to-date with clinical and laboratory information and that they are stored in a secure location.

All enrolled patients should be given a code number, usually beginning with the number 01 or 001. Some investigators prefer to use code numbers for all screened patients and then continue to those same numbers for the patients who are enrolled. Others prefer to assign new code numbers to the enrolled subjects. If two or more *in vivo* studies are being carried out at the same time, the initials of the health facility can be included in the patients' code, for example SJ003 for patient No. 3 from the San Juan Health Center.

To ensure that all patients are seen on the day their follow-up visit is scheduled, it is preferable to schedule follow-up visits during the morning hours. In that way, the study team will know by midday if the patient is going to return or not and it will be possible to visit them in their home during the afternoon. Making home visits in the evening can be difficult, especially in communities without electricity. To facilitate home visits, the address and directions to each patient's home should be carefully recorded on the case record form, including points of reference such as nearby churches, schools, shops, etc. When problems are encountered in tracing a patient, health facility personnel may be of help as they often are

familiar with their patients' residences. If a car and driver are available, the patient could be driven to his/her home after the first clinic visit to be sure that someone in the health facility knows where they live.

To help patients remember the dates of their follow-up visits, a paper with the dates of each visit can be prepared. This paper is also useful to identify patients and ensure that they are rapidly seen by the study team on their return to the health facility.

Ministry of Health San Juan Health Center			
<i>Patient:</i> Juan Peres Ramos			
Follow-up 1 :	Thur. 3/07	return at	8 am ✓
Follow-up 2 :	Friday 4/07	return at	8 am ✓
Follow-up 3 :	Saturday 5/07	return at	8 am ✓
Follow-up 4 :	Wed. 9/07	return at	8 am
Follow-up 5 :	Wed. 16/07	return at	8 am
Follow-up 6 :	Wed. 23/07	return at	8 am
Follow-up 7 :	Wed. 30/07	return at	8 am

Because of the time and effort invested in tracing patients who fail to return for their follow-up visits, it is often cost-effective to reimburse patients for their transportation expenses to and from the health facility. Some investigators also give patients who return on Days 14 and 28 small gifts (for example, a kilo of rice, sugar, or powdered milk, plastic dishes, etc.) as an incentive to return for follow-up visits. These gifts should not be mentioned when the patient first enrolls in the study to avoid influencing his/her decision. When a patient fails to return for a follow-up visit and is traced to his/her home, study personnel should remind the patient to return to the health center for all remaining visits, to ensure that they don't assume that all follow-up visits will be in their homes.

In areas, such as the Amazon Basin, where the population is widely dispersed, it may be impossible to obtain all follow-up visits on exactly the scheduled date. If a patient fails to return for his/her follow-up visit and cannot be found at home, the patient should be seen the next day. The date of follow-up should be noted on the patient's case record form and data can be adjusted when the analysis is done. Due to these difficulties, a variation of +/- one day is acceptable for follow-up visits and blood smears on Days 7, 14, 21, and 28.

Due to the low levels of malaria transmission in the Americas, study teams may have to make special efforts to identify patients for the study. In some villages, community health workers administer antimalarial drugs to febrile patients in their homes and they rarely seek

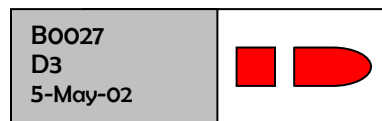
treatment at the local health center. In these situations, an active case search can be conducted, taking a blood smear from all febrile resident and if the blood smear is positive and the patient meets enrollment criteria, they can be invited to take part in the study. Although not all patients with parasitemia are febrile, their level of parasitemia is usually quite low and there is usually little value in taking blood smears from non-febrile patients.

Preparation and Handling of Blood Smears

Labeling of Blood Smears

Adequate identification and storage of thick and thin blood smears is important for the success of the study. Any type of microscope slides can be used, but those with frosted ends are easier to label. Slides should be labeled with the patient's code number, the day of follow-up, and the date. An example of a correctly labeled slide is given below:

Figure 1



where “B” is the code assigned to the study site and “0027” corresponds to the patient’s number, “D3” to the third day of follow-up. All slides, even new ones, should be well washed before using.

Preparation of Blood Smears

Care should be taken to prick the finger firmly to avoid having to repeat the finger-prick. Consider the use of automatic lancets in those studies in which very young children are going to be enrolled. Use one slide per patient and position the thin smear next to the thick smear. This will assist with identification of the species of the parasite in mixed infections, since the parasite morphology can be viewed more easily on thin smears. Once the thick smear is made, the thin smear can be fixed with alcohol taking care that the thick smear does not come into contact with the alcohol. Recently taken blood smears should be kept horizontally until the blood dries and then protected from insects that might eat the blood.

Staining

The daily Giemsa solution should be prepared at the time the slides are going to be stained and slides should be stained as soon as possible after they are made to avoid their becoming heat-fixed in tropical climates. Filter the stain periodically. Use buffer solution if the local water is acidic or alkaline. Giemsa stock solution should always be stored in a dark bottle.

Transporting and storing blood smears

Microscope slide folders with a capacity for transporting up to 20 slides are useful for field work because the slides can be protected and kept in a horizontal position while drying. Unstained thick smears should not be left uncovered because they can be damaged by flies and other insects. In addition, in hot environments, it is advisable to stain all slides within one or two days to avoid having the thick smears fixed by the heat. In areas with high humidity, to avoid slides becoming contaminated with fungus, they should be stored in hermetically-sealed plastic boxes. In the field, slides should be stored according to the date they were taken. After finishing with the initial and second examination and all discordant results are resolved, they should be stored grouped by patient in chronological order.

Microscopes:

If possible, use microscopes with self-contained light sources which will allow microscopy to be carried out at all hours, regardless of the availability of sunlight. If the health facility has no electricity, light microscopes will be needed along with appropriate filters. Before going to the field, microscopes should be thoroughly checked to ensure they are functioning.

Counters

Hand-held tally counters or keyboard type counters should be checked to ensure that they are functioning adequately.

Supervision:

Periodic supervision of the laboratory is critical to identify problems and correct errors and should include re-examination of a sample of blood smears to confirm the diagnosis and parasite density. The frequency of supervision will depend on the experience of the study microscopist. For an experienced microscopist, a supervisory visit at the beginning, middle, and end of the study should be sufficient.

Other Laboratory Studies

Samples for PCR Studies

The study laboratorian should be familiar with PCR procedures to ensure that all blood samples are appropriately taken and stored. If whole blood is to be used, it should be taken in microtainer tubes and the cold chain maintained until the samples arrive at the central laboratory where they will be processed. For blood spots made on filter paper, the type of filter paper used is important, since procedures for extraction of DNA are specific for different types of filter paper. The filter paper blood samples should be stored in plastic bags with dessicant to protect them from the humidity.

Samples for Assays of the Concentration of Antimalarial Drugs in the Blood

Samples of blood for these assays can be stored as whole blood or as blood spots on filter paper, depending on the drug being studied. The same precautions as mentioned above for PCR blood samples should be taken.

Supervision

Periodic supervision is critical to the success of *in vivo* studies. The frequency of supervision will depend on the experience of the study team. Ideally, the person carrying out the supervision should have experience in blood smear examination, so that the diagnosis and parasite densities of a sample of thick smears can be confirmed. If the supervisor is not able to do this, an experienced microscopist should participate in the visit.

It is useful to begin the supervisory visit with a general meeting with all members of the study team to explain the purpose of the visit and discuss the methods the study team is using to identify, enroll, treat, and follow-up patients, along with any problems they might have encountered. The supervisor should carefully check each patient report form. If errors are found, they should be reviewed with the physician in charge and if necessary, corrections made by crossing out the error with a single line and writing the correct information above or below with the initials of the physician and the date the correction was made. Use of a checklist makes the supervisory visits easier and ensures that no important aspects are forgotten. The following is an example of a supervisory checklist:

Patient Enrollment

- Does the study team have good rapport with the health center personnel? How are febrile patients being identified? Are febrile patients being recorded in the appropriate notebook?
- Who takes the thick blood smears and where? Are thick smears of good quality in terms of size, thickness, and staining?
- Are inclusion/exclusion criteria being fulfilled?
- Are the reported species diagnoses and parasite densities of the thick smears accurate?
- Are all samples being taken in accordance with the study protocol? (review flow diagram)
- Are samples for molecular studies being taken appropriately?
- Are there missing data on the case report forms?
- Are samples properly labeled and stored in a secure location?
- Are all doses of the study drugs administered under supervision of a member of the clinical staff?
- Is the study team weighing patients and calculating drug doses correctly?
- Are all the ethical criteria being followed as established at the protocol? Are the informed consent being appropriately explained

Patient Follow-Up

- Are patients' addresses and directions to their homes recorded in sufficient detail to find their houses?
- Are the doses of antimalarial drugs correct?
- Is the notebook for follow-up visits up-to-date and correct?
- Are the signs of severe malaria known to all team members? Is the health facility equipped to deal with emergencies caused by severe malaria and referral of patients to other facilities?
- What proportion of patients had to be traced to their homes?
- What are the main causes of loss to follow-up? Is a change in procedures needed to reduce loss to follow-up rates?

Financial Report

- Is the petty cash record complete and up-to-date?
- Do the reported expenses agree with funds spent?
- Is it necessary to increase the amount of the petty cash account?

When the supervisory visit ends, the supervisor should meet with the study team to congratulate them on their work, to correct mistakes and to answer any questions that might have arisen. In most cases, a supervisory visit for an *in vivo* study will take at least 3 days.

The study team should submit brief reports every week or two to the principal investigator. These reports should include the number of malaria cases diagnosed, the number of patients enrolled, the number of treatment failures, the number of patients who have withdrawn and their reasons, and an accounting of the petty cash fund. This report can be sent via fax, e-mail, or telephone, depending on the available means of communication at the study site.

Closing the Study

Before leaving the study site for the last time, a general review of the patient report forms should be carried out to ensure that all information is correct and that nothing has been omitted, such as the patient's age or sex. The study personnel should make sure that all necessary forms, notebooks, and information have been collected for preparation of the final report and any scientific publications that are planned. Equipment and supplies provided by the study team will need to be packed, although it is common practice to leave most of the remaining laboratory supplies at the health facility as a way of thanking the staff for their cooperation. Before leaving the community, the study supervisor should once again thank the local staff of the health facility and provide them with a preliminary overview of the study results. A final social gathering with the local health facility staff is another way to recognize their contribution to the study and help ensure their cooperation in future studies.

On finishing the study, one of the senior investigators should also visit the district/regional health authorities to provide them with preliminary results of the study and inform them when the final report can be expected.

The study team should prepare a final report detailing the number of febrile patients examined, the number enrolled, the number excluded with reasons for the exclusions, and the proportion of parasitological and therapeutic failures, according to WHO definitions, which can be found on the WHO website at the following address: http://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_EPH_2002_17/en.

The financial report should also include an accounting of field team expenses by category such as travel, medications, fuel, etc. If there are recommendations for future teams working in the same area, these should be included in the report as well.

Cost of *in vivo* Studies

Based on *in vivo* studies conducted in Peru between 1998 and 2001, a 14-day study that lasts 12 weeks will cost approximately \$9,000, including the hiring of study personnel. A study with a 28-day follow-up study will cost approximately \$1,000 more.

Preparation of the Database

Before entering any of the patient report forms in the database, one of the principal investigators should review each report form for errors such as inconsistencies in data that appear on more than one page (patient's name and sex, weight in relation to age, dose of drug administered based on the patient's weight) and other missing data. This review will also help to standardize terms used to describe symptoms (for example: head pain and headache and the commercial and generic names of drugs).

An *Epi-Info* software package has been prepared for data management, cleaning, and the basic analyses of an *in vivo* study. As soon as field testing is complete, this program will appear on the PAHO Web page. If the study team wants additional analyses, they can be added to the basic program. Patient report forms can be entered in the database by a data clerk or by the personnel who carried out the field study. If the information from the patient report forms will not be double-entered in the database, they should be compared directly with the original forms.

Appendix 1: List of Field Supplies Needed for an *in vivo* Study of 60 Patients

Item	Description	Quantity	Comments
1	Immersion oil (100 ml bottle)	1	
2	Distilled water (1L container)	1	For Giemsa staining
3	Needles for vacutainer tubes (box of 100, 21 x 1 ½ y 20 x ½)	2	
4	Alcohol – ethanol (1L bottle)	2	
5	Cotton (roll of 500 g)	1	
6	Tongue depressor wooden (box of 500)	1	
7	Disposable biohazard bags (box of 100)	1	
9	Slide holder box (wood or acrylic)	10	
10	Disposable table covers		
11	Safety needle holder	1	
12	Digital hand tally counters	2	
13	Biosafety container	1	
14	Microscope slide folders 3x1"	2	
15	Plastic screw cap cups to store drugs	3	
16	Timer	1	
17	Giemsa stain (bottle of 100 ml)	1	
18	Gloves (box of 100), small and medium size	4	
19	Lens paper (box)	60	
20	Microscope slides (box of 50)	35	
21	Disposable lancets (box of 100)	5	
22	Pencils	2	
23	Notebook	1	
24	Ballpoint pen	2	
25	Methanol (50 ml bottle)	1	To fix thin blood smear
27	Microscope slide mailer	5	
29	Paper towels (roll)	5	
30	Toilet paper (roll)	5	To absorb immersion oil from slides
31	Pregnancy test kits	40	If pregnancy tests are conducted
32	Non-sterile disposable plastic pipettes	120	
33	Rack for drying slides	1	

Item	Description	Quantity	Comments
34	Digital thermometer	1	
35	EDTA Vacutainer tubes x2mL	70	For CQ metabolites dosage
36	Urine cups	250	If urine samples are taken
37	Disposable plastic cups	150	
38	Plastic graduated tubes	50	
39	3-ring binder (A4 or letter size)	2	
40	Accordion folder	1	
41	Notebook (100 pages)	3	
42	Pocket calculator	1	
43	Ballpoint pens (black and red)	6	
44	Stethoscope	1	
45	Sphygmomanometer	1	
46	Marker, non-retractable, fine point	3	
47	Pencil sharpener	2	
48	Staples (box)	1	
49	Stapler	1	
50	Transparent tape	1	
51	Scissors	1	
52	Alarm clock	1	
53	Flashlight, use two Size "D" batteries	2	
54	Clipboard, A4 size	3	
55	Ruler, wood, metal-edged	1	
56	Document protector	12	
57	HemoCue B-Hemoglobin Analyzer and Hemocue Blood Hemoglobin Microcuvette X 100	1	Optional, if dosing hemoglobin is planned
58	Ink pad for fingerprints	1	
59	Bath scale	1	If there is no scale at health center
60	Plastic bags 9 x 12"	60	For PCR
61	Silica gel desiccant packet	300	For PCR
62	Filter paper (Whatman grade 3)	60	If Microtainer is not used for PCR samples
63	Microtainer (box of 50)	1	If filter paper is not used for PCR samples
64	Envelopes (9x12")	300	For PCR
65	Tablet cutter	1	

