

PRACTICAL ASPECTS OF *IN VIVO* ANTIMALARIAL DRUG EFFICACY TESTING IN THE AMERICAS

TRENTON K. RUEBUSH II, WILMER MARQUÍÑO, JORGE ZEGARRA, DANIEL NEYRA, RODOLFO VILLAROEL, JUAN CARLOS AVILA, CÉSAR DÍAZ, AND EFRAÍN BELTRÁN

National Center for Infectious Diseases, Centers for Disease Control and Prevention, U.S. Naval Medical Research Center Detachment, Lima, Peru; Instituto Nacional de Salud Lima, Peru; Programa de Control de Malaria y Otras Enfermedades Metaxénicas Ministerio de Salud, Lima, Peru; Programa Nacional de Control de la Malaria Ministerio de Salud Pública y Previsión Social, La Paz, Bolivia; Servicio Nacional de Erradicación de la Malaria Ministerio de Salud Pública, Quito, Ecuador

Abstract. The World Health Organization (WHO) has developed guidelines for *in vivo* antimalarial drug efficacy testing for *Plasmodium falciparum* and *Plasmodium vivax* in areas with low-to-moderate transmission, such as the Americas. These guidelines are used widely by ministries of health and national malaria control programs to assess the efficacy of their first-line and second-line drugs for the treatment of malaria and to provide the information necessary to update national malaria treatment policies. Following the WHO guidelines, we have conducted *in vivo* efficacy trials with a variety of drugs and drug combinations against *P. falciparum* and *P. vivax* at 13 sites in Peru, Bolivia, and Ecuador. Based on these experiences, we have identified several modifications that we believe should be made in the WHO recommendations to make them more suitable to the relatively low levels of *P. falciparum* transmission in the Americas and to the logistic challenges of carrying out such studies in sparsely populated areas, such as the Amazon Basin. These include changes in inclusion and exclusion criteria, in enrollment and follow-up procedures, and in the measurement of study outcomes.

INTRODUCTION

In 1996, the World Health Organization (WHO) published updated recommendations for *in vivo* antimalarial drug efficacy testing of uncomplicated *Plasmodium falciparum* malaria for areas with intense transmission.¹ These guidelines were based on experiences gained over the previous decade in Africa with a variety of different approaches to *in vivo* drug efficacy testing, which made comparison between results of different studies difficult.^{2–5} Since 1996, the WHO standardized guidelines have been applied broadly across sub-Saharan Africa by ministries of health and individual investigators to document the efficacy of first-line and second-line drugs for the treatment of uncomplicated *P. falciparum* malaria. The guidelines have played a crucial role in helping national malaria control programs modify their national malaria treatment policies^{6,7} and have proved to be highly suitable to the epidemiology of malaria in the region. In 1998, these guidelines were revised for areas with low-to-moderate transmission in the Americas.⁸ Currently, recommendations for *Plasmodium vivax in vivo* drug efficacy testing are being developed by the WHO and the Pan American Health Organization (PAHO) (P. Ringwald, R. Gusmão, personal communication).

Field experience with the *P. falciparum* and *P. vivax* updated guidelines is more limited in the Americas, but increasing numbers of ministries of health are beginning to follow the WHO/PAHO recommendations in designing their drug evaluations. Over 4 years, we have carried out *in vivo* drug efficacy testing for *P. falciparum* and *P. vivax* at multiple sites in Peru, Bolivia, and Ecuador using the WHO/PAHO standardized protocols. As experience was gained during the course of these studies, we assessed several possible modifications in the guidelines. This article describes our experiences and presents suggestions for changes in the current recommendations for *in vivo* antimalarial drug efficacy testing to make them more suitable to the epidemiology of malaria in the Americas and to the logistic challenges of working in areas such as the Amazon Basin.

BACKGROUND

The standardized guidelines for *in vivo* antimalarial drug efficacy testing developed by WHO/PAHO are intended primarily for use by ministries of health, national malaria control programs, and investigators involved in applied field research on antimalarial drug resistance. Studies using these guidelines typically are carried out whenever a question arises about the efficacy of the first-line or second-line drugs in a given region or country or if a change in regional or national treatment policy is made and the Ministry of Health wants to monitor the efficacy of the new drugs. The emphasis on the use of standardized methods is to ensure that data collected at 1 site in a country can be compared directly with results from another site or another country. Because many of the institutions using these protocols have only limited staff and resources and because the sustainability of a drug efficacy monitoring system is crucial, an effort has been made to simplify and streamline the guidelines and reduce study costs whenever possible.

The guidelines are intended for outpatient treatment of subjects with uncomplicated malaria and were developed initially for the testing of chloroquine against *P. falciparum* malaria. Since then, their use has been extended to several other drugs, and more recently the guidelines have been modified for testing the efficacy of drugs against *P. vivax*.

Briefly the methods recommended by WHO/PAHO are as follows: Patients attending outpatient clinics of hospitals or health centers with a history of fever or suspected malaria have a screening thick blood smear. If the smear shows a pure infection with the species of *Plasmodium* being studied and a parasite density within the standardized enrollment criteria, the patient's informed consent is requested. Patients who agree to take part in the study have a second thick blood smear taken to confirm their infection and recalculate the parasite density. After a brief medical history and physical examination, patients are treated under observation with a standard dose of the drug being tested, based on the patient's

weight. All drugs being evaluated should come from reliable, quality-controlled sources. Subjects are asked to return daily until all treatment doses of the drug or drug combination have been administered and then on days 3, 7, and 14 (and days 21 and 28 for 28-day trials). On each of the return visits, the patient is questioned about symptoms and adverse drug reactions and has his or her temperature and a repeat blood smear taken.

METHODS

Between March 1998 and October 2001, *in vivo* drug efficacy trials were conducted at 13 sites in Peru, Bolivia, and Ecuador (Table 1). We followed the standardized guidelines of WHO/PAHO for *in vivo* efficacy testing^{1,8} with the following modifications.

Inclusion and exclusion criteria modification:

1. To increase the number of subjects eligible for study, especially in *P. falciparum* trials, the highest acceptable enrollment parasite density of 5,000 parasites/ μ l recommended in the PAHO guidelines for *P. falciparum* studies in the Americas was raised to 50,000 parasites/ μ l. In all trials, patient safety is a primary concern, and the physician-in-charge, who is on site throughout the course of the study, was instructed that even patients with lower parasite densities should be excluded, if they had signs or symptoms of severe malaria or could not tolerate oral medication.
2. To reduce losses to follow-up and avoid the difficulty of having to trace subjects great distances to their homes, the WHO/PAHO recommendations suggest enrolling subjects who have "easy access" to the health facility where the study is being done. We believe this guideline is too vague and generally have excluded from enrollment any patient who lives more than 30–45 minutes by road or river from a study site.
3. At sites in which the drug being evaluated was not part of the currently recommended national treatment policy for *P. falciparum*, a urine pregnancy test was conducted on all women of childbearing age, rather than accepting the patient's history that she was not pregnant as the WHO/PAHO guidelines suggest for studies involving current first-line or second-line drugs. Pregnancy testing was not conducted on women enrolled in studies of chloroquine for the treatment of *P. vivax* because chloroquine is the rec-

ommended drug for *P. vivax* infections in pregnant women.

Change in enrollment procedures:

1. In Peru, Bolivia, and Ecuador, it is routine practice to take a thick blood smear for malaria from all febrile patients attending Ministry of Health facilities. As a result, the initial screening of potential subjects could be simplified, and only persons who had a positive blood smear for the parasite species being studied were considered for enrollment. This initial blood smear was reexamined by the study staff, and the parasite density was calculated. If the quality of the initial blood smear was not considered adequate, a repeat blood smear was taken before the patient was enrolled.
2. When evaluating drugs that were expected to have a moderate-to-high rate of parasitologic failures (e.g., chloroquine for the treatment of *P. falciparum* in the Amazon Basin), we used the lot quality assurance sampling technique recommended in the WHO/PAHO protocol for the Americas to determine the sample size.⁹ This method allows the identification of areas in which the prevalence of drug resistance is above a predetermined critical level with much smaller sample sizes (and at a much lower cost) than would be required using more traditional methods for determining the rate of treatment success or failure within narrow confidence limits. In contrast, when evaluating drugs as monotherapy or combination therapy in which <5% parasitologic failures were expected (e.g., mefloquine or sulfadoxine-pyrimethamine plus artesunate for *P. falciparum* or chloroquine for *P. vivax*), we calculated the sample size based on standard approaches to estimating a population proportion with a specified precision and used a power of 80% to obtain a more precise measurement of the prevalence of treatment failures.¹⁰

Changes in follow-up procedures:

1. In areas where it was expected that parasite strains would be partially or highly resistant to the drug or drug combination being studied, a 14-day trial was conducted to simplify follow-up and to reduce study costs. When the drug or drug combination being evaluated was expected to be highly efficacious, follow-up was extended to 28 days to detect less resistant strains.

TABLE 1
Sites of *in vivo* antimalarial drug efficacy studies in Peru, Bolivia, and Ecuador, 1998–2001

Country	Region	Year	Parasite species	Drug(s)	Duration of follow-up (days)	
Peru	Amazon Basin	1998	<i>P. falciparum</i>	CQ, SP	14	
	North Pacific Coast (3 sites)	1999	<i>P. falciparum</i>	CQ, SP, MQ	14	
	Amazon Basin	2000	<i>P. falciparum</i>	CQ, SP	14	
	Amazon Basin	2000	<i>P. falciparum</i>	MQ, MQ/AS	28	
	Amazon Basin			<i>P. vivax</i>	CQ	28
			2000	<i>P. falciparum</i>	MQ	28
				<i>P. vivax</i>	CQ	28
			2000	<i>P. falciparum</i>	SP, SP/AS	28
			2001	<i>P. vivax</i>	CQ	28
Bolivia	Amazon Basin (2 sites)	2001	<i>P. falciparum</i>	MQ, MQ/AS	28	
Ecuador	Pacific Coast (2 sites)	2001	<i>P. falciparum</i>	CQ, SP	28	

CQ, chloroquine; SP, sulfadoxine pyrimethamine; MQ, mefloquine; AS, artesunate.

2. Because the goal of therapy of the national malaria control programs of Peru, Bolivia, and Ecuador is a parasitologic rather than a clinical cure (as is the case in most African countries), all subjects who had a recurrence of parasitemia between days 4 and 28 were treated with an alternative drug and were dropped from further follow-up, whether or not they were febrile.
3. Blood smears were taken from all subjects on day 2 and day 3, instead of day 3 alone, to allow calculation of RIII resistance levels, as recommended by the 1973 WHO *in vivo* drug efficacy protocol.¹¹ The current WHO/PAHO guidelines for the Americas require a day 2 blood smear only if the patient has signs or symptoms of severe malaria or shows clinical deterioration.
4. Because many of the microscopists involved in our trials had had only limited experience counting parasites or calculating parasite densities, training was conducted before the trial began, and frequent supervisory visits were made to each site by a more experienced microscopist to ensure the accuracy of the parasite densities. Instead of requiring that a second microscopist reexamine only a sample of the blood smears taken during the trial as a quality control measure, as recommended in the WHO/PAHO guidelines for the Americas, all blood smears were reexamined and recounted by a second, more experienced microscopist. If there was a difference in species diagnosis, or if the parasite density differed by $\geq 50\%$, between the two, a third microscopist would re-examine the smears for a final species diagnosis. The final parasite density was the mean of the counts of the 2 initial microscopists or, if the parasite density differed by $>50\%$ between them, an average of the 2 closest counts. A minimum of 200 oil immersion fields were examined before a blood smear was considered negative.
5. Because patients are followed for 14 or 28 days, a negative blood smear on a subject with subpatent parasitemia on one visit should become positive by the time of his or her next visit. To reduce the workload on microscopists, the number of parasites per 200 white blood cells was counted to determine parasite density, unless <10 parasites were seen, in which case counting was continued until 500 white blood cells had been observed. Gametocytes were counted per 500 white blood cells. The WHO/PAHO protocol for the Americas recommends that the number of parasites per 500 white blood cells be counted for all blood smears.

Outcome measures modifications:

1. To allow a direct comparison with the results of earlier *in vivo* trials reported from the Americas, we measured the outcomes of trials in terms of the traditional parasitologic

outcomes of sensitive and RI to RIII resistance levels¹¹ and by the newer classification system recommended by WHO/PAHO (early treatment failure, late treatment failure, and adequate clinical response).^{1,8} Parasitologic outcome measures also were thought to be more suitable than those based on clinical findings for trials in the Americas because patients were treated and dropped from the trial as soon as they had a recurrence of parasitemia, regardless of whether it was associated with fever or other symptoms or signs of malaria.

2. Because of the widely dispersed populations in many of the areas where studies were being conducted and the difficulty in obtaining follow-up on exactly those days specified by the WHO/PAHO guidelines, we took a more flexible approach and accepted data from follow-up visits for day 7 and day 14 ± 1 day (i.e., days 6, 7, and 8 and days 13, 14, and 15) and for day 21 and day 28 ± 2 days.

RESULTS AND DISCUSSION

A variety of factors make *in vivo* antimalarial drug efficacy testing in the Americas a challenge, including the relatively low levels of malaria transmission; the inability to predict malaria incidence from year to year even in the same site; the short transmission season; and, in the Amazon Basin, the widely dispersed and highly mobile population with the great distances subjects may have to travel for follow-up. The low levels of malaria transmission in most countries in the Americas increase the logistic difficulty of *in vivo* drug efficacy trials, especially for *P. falciparum*. Even in the Amazon Basin, which traditionally has reported the highest incidence of malaria in the Americas, the intensity of transmission of *P. falciparum* and *P. vivax* has decreased. In Peru, the total number of malaria cases reported for Loreto Region, which makes up most of the Peruvian Amazon, has decreased from 102,153 cases in 1996 to 29,838 in 2000, and the number of *P. falciparum* cases decreased from 34,020 to 7,661.¹² *P. falciparum* now makes up just 25% of all reported malaria cases from the Peruvian Amazon region.

These low levels of transmission greatly increase the difficulty of identifying subjects who meet the enrollment criteria. Table 2 shows the mean number of febrile patients who had to be screened for each subject enrolled, which ranged from 18.7 to 52.6 (mean 29.3) for the *in vivo* trials carried out in Peru during 2000–2001. The duration of the enrollment period in these studies was ≥ 12 weeks in nearly all cases. In comparison, in a series of 6 *in vivo* trials in Zambia, an average of only 2.5 febrile patients had to be screened for each subject enrolled, and the duration of the enrollment period was usually 4–5 weeks.⁶

TABLE 2
Number of patients screened and enrolled and duration of enrollment for selected *in vivo* antimalarial drug efficacy trials, Peru, 2000–2001

Site	Parasite species	No. patients screened*	No. enrolled	Mean no. screened per subject enrolled	Duration of enrollment (wk)
Iquitos (2 sites)	<i>P. falciparum</i>	3,530	115	30.7	16
	<i>P. vivax</i>	2,026	77	26.3	13
Caballococha	<i>P. falciparum</i>	999	18	55.5	13
	<i>P. vivax</i>	999	52	19.2	13
Pampa Hermosa	<i>P. falciparum</i>	916	49	18.7	13
Sullana	<i>P. vivax</i>	2,247	65	34.6	8

* Patients with axillary temperatures $\geq 37.5^\circ\text{C}$ or a history of fever in the previous 2 days.

In areas with unstable transmission, malaria incidence data from previous years may not always be the best indicator of the suitability of a site for a drug efficacy trial. On the North Coast of Peru, 618 cases of *P. falciparum* were reported from 1 health center between March and July 1999, in contrast to just 98 cases during the same period in 2000 and 56 in 2001. Transmission seasons in the Americas also tend to be short, with peak transmission lasting only 3–4 months, and the beginning of the transmission season may vary considerably from year to year. In the Department of Loreto, which makes up most of the Peruvian Amazon region, *P. vivax* incidence data from 1998 would have suggested that the best time to initiate an *in vivo* trial the following year would be the month of February when, in fact, the number of cases in 1999 did not begin to increase until April (Figure 1). The same was true of the incidence of *P. falciparum* infections, but the differences were not so striking (data not shown).

The widely scattered and highly mobile population in the Amazon region complicate subject enrollment and follow-up. The study team may decide it would be better not to enroll patients from distant villages because of the time and effort it would take to trace them to their homes. Patients who come to a hospital or health center for initial diagnosis and treatment may be unwilling to return for follow-up visits when they begin to feel better.

Because of these logistic difficulties, site selection is crucial to the success of *in vivo* drug efficacy trials in the Americas. Before the trial begins, an investigator should review the monthly incidence of reported malaria from each hospital or health center being considered for the trial, to ensure that (1) there are sufficient numbers of infected patients to meet the sample size requirements, (2) the malaria cases reported from a health facility are patients who have come in person to that health facility from a reasonably circumscribed area (i.e., <30–45 minutes by road or river), and (3) the patients are permanent or semipermanent residents and will remain in the area for the duration of their follow-up. In addition, the adequacy of the clinical and laboratory infrastructure should be

assessed. The time it takes to assess these issues before a trial begins saves considerable effort over the course of the study.

The approach to sample size calculation for *in vivo* drug efficacy trials should depend on the objectives of the trial. Although the lot quality assurance sampling technique is an attractive way to conserve resources while exposing fewer patients to a potentially ineffective drug, its use is most appropriate when evaluating drugs that are thought to have reduced efficacy. In the case of many of the trials we have conducted in the Americas, the drugs being evaluated were expected to be highly efficacious, and a sample size determination by the lot quality assurance sampling approach would have provided a much less exact estimate of drug efficacy rates than traditional methods for sample size determination.

The WHO/PAHO guidelines for *in vivo* drug efficacy trials stress the importance of enrolling a representative sample of subjects from the area being studied.^{1,8} In the Americas, obtaining this sample may be difficult because the usually low levels of transmission and the scattered population in certain areas, such as the Amazon region, can limit severely the number of potential study sites and subjects. Along the 3 major rivers joining the Amazon near the city of Iquitos in Peru, only 1 village out of 103 has a population >1,000. In such cases, investigators may have to weigh the value of obtaining limited but less than ideally representative data on drug efficacy from an area against the total absence of information from that area if no study is conducted.

In areas with low levels of malaria transmission, expanding the enrollment criteria to include patients with lower and higher parasite densities than those recommended in the PAHO guidelines (500–5,000 parasites/ μ l) would increase the number of potential subjects. Concerns have been raised about enrolling subjects with densities <500–1,000 parasites/ μ l because of uncertainties about the quality of microscopic diagnosis and the ability of microscopists to distinguish reliably between rising and falling counts at low parasite densities. Table 3 shows the parasite densities of patients with *P. vivax* and *P. falciparum* infections who attended health cen-

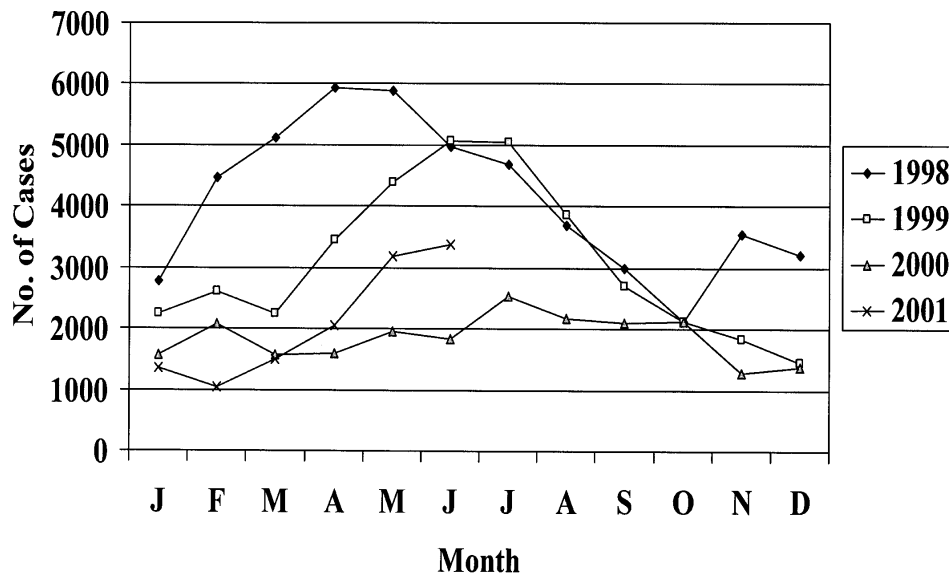


FIGURE 1. *Plasmodium vivax* malaria in the Peruvian Amazon region, 1998–2001.

TABLE 3
Parasite densities of febrile patients attending health facilities in Peru during *in vivo* studies, 2000–2001

Site	Total	<i>P. falciparum</i> (parasites/ μ l)					<i>P. vivax</i> (parasites/ μ l)					
		<250	250–499	500–999	1,000–4,999	\geq 5,000	<250	250–499	500–999	1,000–4,999	\geq 5,000	
Pampa Hermosa	52	3 (6.1%)	4 (7.7%)	4 (7.7%)	17 (34.7%)	24 (46.2%)	—	—	—	—	—	
Cabalococha	28	12 (42.8%)	0 (0%)	1 (3.6%)	5 (17.8%)	10 (35.7%)	64	11 (17.2%)	0 (0%)	4 (6.2%)	22 (34.4%)	27 (42.2%)
Iquitos	164	55 (33.5%)	7 (4.3%)	3 (1.8%)	26 (15.8%)	100 (61.0%)	111	34 (30.6%)	3 (2.7%)	5 (4.5%)	32 (28.8%)	37 (33.3%)
Sullana	—	—	—	—	—	—	60	13 (21.6%)	0 (0%)	1 (1.7%)	21 (35.0%)	25 (41.7%)

ters where we were working during 4 of our *in vivo* trials. A significant proportion of the infected patients attending these health facilities had parasite densities $<500/\mu$ l, and this varied considerably from site to site. Such patients would not be eligible for enrollment according to the existing PAHO guidelines. This is more of an issue with *P. falciparum* than with *P. vivax* in Peru because *P. falciparum* makes up only 25% of all infections, and losing even a few potential patients could prolong enrollment by ≥ 1 week. Because all blood smears in our studies are examined independently by 2 microscopists, one of whom is highly experienced, we feel confident of the species and parasite density results and believe that the lower limit of parasite density could be set at 250 parasites/ μ l, at least for *P. falciparum*.

With regard to the higher end of the parasite density range, restricting enrollment to patients with parasite densities $<5,000/\mu$ l reduces the number of potential subjects by more than a third. Although *P. falciparum* transmission levels are much lower in the Americas than in Africa and patients have less immunity and tolerance to high-density parasitemia, a physician is on site throughout the enrollment and follow-up of each study and is instructed to exclude any patient with signs or symptoms of severe malaria regardless of his or her parasite density. The drugs being tested are not experimental drugs but, in most cases, the same ones that the Ministry of Health already is using or hopes to recommend in the future. Using this approach, we have not encountered problems enrolling patients with parasite densities $\leq 50,000/\mu$ l.

Because of the low numbers of patients who may meet enrollment criteria, we occasionally have had to go to considerable lengths to identify subjects, and this may affect the representativeness of the study population. In 1 study, when it became apparent that many febrile patients already had received antimalarial drugs from a village health worker before coming in to the health center for diagnosis and, as a consequence, had low parasite densities, active case detection was conducted in nearby communities. Any parasitemic individual who was detected was invited to participate in the study. If the individuals agreed, enrollment procedures were carried out in their village. In the same study, when patients came in to the health center for diagnosis and treatment too late in the day to return to their homes that afternoon, we paid for lodging and meals overnight.

According to the WHO/PAHO guidelines, interpretation of study results may be jeopardized if $>10\%$ of subjects are lost to follow-up.^{1,8} Although this percentage is appropriate for studies in areas with high levels of transmission, where adequate numbers of subjects can be identified from a rela-

tively small catchment area and with a follow-up period limited to 14 days, we do not believe that it is realistic in the Amazon Basin or for trials with a 28-day follow-up. Table 4 shows the loss-to-follow-up rates for patients enrolled in the trials conducted in Peru between 1998 and 2001. Despite all of our efforts to reduce loss to follow-up to a minimum, including routinely reimbursing all transportation expenses for subjects who returned on their own, tracing subjects to their homes who failed to return, and providing small gifts (e.g., a kilo of sugar or rice) to subjects who did return on days 14 and 28, our lost-to-follow-up rates for trials in the Amazon region were routinely $>10\%$. Occasionally, we have gone to even greater lengths to obtain follow-up, as in the case of 2 subjects enrolled in trials on the North Coast of Peru, for whom arrangements were made for them to be seen by Ministry of Health physicians in the capital, $>1,000$ km away, when they left the study area before follow-up was complete. Based on our experiences, we believe that a 15% loss-to-follow-up rate would be more realistic for 28-day trials and for trials in sparsely populated areas, such as the Amazon Basin. Although failure to return for follow-up visits could influence the trial's results, the primary purpose of these studies is to determine if a drug is efficacious or not, depending on a Ministry of Health's definition of acceptable efficacy. Reducing dropout rates to $<15\%$ or even 10% might increase one's confidence in the results of a trial, but it would be unlikely to change the overall results of the trial or the decision to change or continue with the existing treatment policy.

We also have encountered difficulties in obtaining follow-up examinations and blood smears on exactly the days specified by the WHO/PAHO guidelines. In general, follow-up was easier at the sites on the north coast because patients tended to live closer to health centers. In these trials $\geq 99\%$ of patients were seen on day 7 for their scheduled day 7 follow-up visit, and this close adherence to the study schedule

TABLE 4
Subjects lost to follow-up in *in vivo* antimalarial drug efficacy trials, Peru, 1999–2001

Site	Parasite species	Length of follow-up (days)	No. enrolled	Lost to follow-up
Cabalococha	<i>P. vivax</i>	28	52	7 (13.5%)
	<i>P. falciparum</i>		18	2 (11.1%)
Iquitos	<i>P. vivax</i>	28	77	10 (13.0%)
	<i>P. falciparum</i>		115	13 (11.3%)
Pampa Hermosa	<i>P. falciparum</i>	14	49	1 (2.0%)
Sullana	<i>P. vivax</i>	28	65	5 (7.7%)
	<i>P. falciparum</i>		197	7 (3.6%)

changed little through day 28. In contrast, although the methods we used to ensure follow-up in the Amazon Basin were identical, 98% of subjects returned on day 7 for their scheduled day 7 follow-up visit, and this percentage decreased to 92% by day 28. Although a strict interpretation of the WHO/PAHO guidelines would suggest that subjects who did not return exactly on the day of their scheduled visit should be excluded from analysis, the difficulties we have encountered in obtaining follow-up visits have caused us to become more flexible in our approach to data analysis, and we now accept data from day 7 and day 14 ± 1 day (i.e., from day 6, 7, or 8 and from day 13, 14, or 15) and for day 21 and day 28 ± 2 days.

On rare occasions, we have encountered situations in which subjects have been dropped from a trial after just 1 or 2 parasites are reported on a follow-up blood smear, then later, when the same blood smear is reexamined by a more experienced microscopist, no parasites are found. To avoid such situations, we believe it would be worthwhile to ask subjects with <5 parasites observed on a follow-up blood smear to return the next day for a repeat smear, before making the decision to treat and drop the patient from the trial or continue follow-up. This assumes that the patient is clinically well and follow-up within 24 hours can be ensured.

Although it has been suggested that local Ministry of Health staff in hospitals or health centers can enroll, treat, and follow up subjects in *in vivo* drug efficacy trials as part of their routine clinical responsibilities, in our experience this usually does not work well because of the heavy workload of most health care workers at peripheral levels. Instead, we prefer to use staff who are able to work full-time on the study, whether they are from the local health district or contracted especially for the study. The team should be led by a physician and microscopist who are experienced in *in vivo* trials and be supplemented with a nurse, laboratory staff, and a driver or boatman from the local hospital or health center. If a trial is to be carried out completely by local staff, frequent, direct supervision from the central level is crucial.

Based on our experiences, we believe that a single, standardized protocol for *in vivo* drug efficacy testing that covers *P. falciparum* and *P. vivax* and makes provisions for areas with all levels of transmission would simplify greatly the conduct and interpretation of studies. This generic protocol should detail the minimum data required for an *in vivo* drug efficacy trial. Individual investigators would be free to collect whatever additional information they choose because adherence to the standardized protocol would ensure that their data could be compared with data of other investigators.

The cost of *in vivo* drug efficacy trials in the Americas tends to be considerably higher than in Africa, where many more patients can be enrolled over a much shorter period. The average cost per trial conducted in the Amazon region of Peru, assuming 60 patients enrolled, a 13-week enrollment period, and a 14-day follow-up, was \$9,668. With a 28-day follow-up, the cost increases to \$11,114. With higher levels of transmission and an enrollment period of only 10 weeks, the same 2 trials would cost \$8,262 and \$9,707. On the north coast of Peru, where the population is not so widely dispersed and follow-up is easier, each trial would cost a few hundred dollars less. These costs include salaries for a physician and a microscopist for the duration of the trial but do not include the cost of the study medication.

One way to reduce the overall cost of such trials is to con-

duct 2 trials simultaneously. On several occasions, we have taken advantage of *P. falciparum* trials in which enrollment was expected to be slow to carry out a *P. vivax* trial at the same time. A similar approach could be taken with other types of studies, such as an evaluation of the sensitivity and specificity of a rapid diagnostic test for malaria, in which the patient population for both studies is similar enough to permit the 2 studies to be carried out simultaneously and with the same staff.

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Authors' addresses: Trenton K. Ruebush II and Jorge Zegarra, NM-RCD, Unit 3800, APO AA 34031, USA. Wilmer Marquiño, Instituto Nacional de Salud, Calle Capac Yupanqui 1400, Jesús María 11, Lima, Peru. Daniel Neyra, Programa de Control de Malaria y Otras Enfermedades Metaxénicas, Ministerio de Salud, Avenida Salaverry, Cuadra 8 S/N, Jesús María, Lima, Peru. Rodolfo Villaroel and Juan Carlos Avila, Programa Nacional de Control de la Malaria, Ministerio de Salud y Previsión Social, Calle Capitán Ravelo 2199, La Paz, Bolivia. César Díaz and Efraín Beltrán, Servicio Nacional de Erradicación de la Malaria, Calle S/N (frente a Ciudadela Naval Norte), Guayaquil, Ecuador.

Reprint requests: Trenton K. Ruebush II, NMRC, Unit 3800, APO AA 34031, USA, Telephone: 0051-1-561-2733, Fax: 0051-1-561-3042, E-mail: ruebush@namrid.sld.pe

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