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GUIDELINES FOR COMMUNITY-BASED TRIALS OF VACCINES AGAINST THE SEXUAL STAGES OF MALARIA PARASITES

PREPARED JOINTLY BY THE WHO DIVISION OF CONTROL OF TROPICAL DISEASES AND THE UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES

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INTRODUCTION

In spite of some setbacks, recent progress in immunology and molecular biology encourages optimism that effective vaccines against malaria will be developed. Three main kinds of vaccine are under development, targeted against different stages in the life cycle of malaria parasites: the pre-erythrocytic stages (sporozoite and liver forms), the asexual blood-stages, and the sexual and sporogonic forms which develop in the mosquito vector. These vaccines are likely to elicit different forms of protective immunity, and some specific issues will arise in the planning of the clinical and epidemiological evaluations of each type. General considerations in the planning of malaria vaccine trials and discussion of the different phases of evaluation through which vaccines must pass were given in (1). Two other WHO documents give guidelines for the field evaluation in malaria-endemic areas of sporozoite and asexual blood-stage vaccines against P. falciparum malaria (2,3). The present document considers the evaluation of vaccines against the sexual stages of P. falciparum or P. vivax in such malaria-endemic areas.

The rationale for the development of sexual-stage vaccines is that they will reduce malaria transmission levels, at least in certain epidemiological situations. Their mode of action is based on antibodies to gametes, ookinetes or zygotes induced by vaccination in the vertebrate host, and ingested by the mosquito vector. Within the mosquito, these antibodies prevent parasite fertilization or development after fertilization. The known target antigens of sexual-stage antibodies are proteins on the surface of gametes, zygotes and ookinetes. They include antigens that are present in gametocytes circulating in the blood of the vertebrate host, which are targets of fertilization-blocking antibodies. In addition, there are antigens which are expressed only after the parasites are ingested in a blood meal which are targets of antibodies that block post-fertilization development. A recent review of research on the characterization and cloning of transmission-blocking antigens is given in (4).

An important feature relevant to the evaluation of sexual-stage vaccines, compared to other kinds of malaria vaccines, is that their impact may be determined indirectly by measuring a functional reduction in mosquito infectivity or oocyst production following the ingestion of parasites and blood from a vaccinated individual.

The kinds of community-based trials discussed in this document are those which should be carried out after initial clinical studies have been completed in both non-endemic and endemic areas. It is assumed that these initial studies will have established basic vaccine safety, immunogenicity and the appropriate dosage (and dosage schedule for multiple dose vaccines). Evidence of efficacy against some endpoints of interest should have been obtained also.

Community-based trials are required to establish the impact that a sexual-stage vaccine has on the transmission of malaria when a high proportion of individuals in a community in a malaria-endemic area are vaccinated. Decisions regarding the widespread usage of sexual-stage vaccines in malaria control programmes are likely to be made on the basis of the results from such trials. It is essential, therefore, that the community-based trials are designed to give an unbiased estimation of the effects of the vaccine. This will require that they are double-blind, randomized and controlled. Because an effect on transmission is unlikely unless a high proportion of the population is vaccinated, the community (transmission unit) must be the unit of randomization, rather than the individual.

Candidate vaccines should be evaluated in several community-based trials to cover the range of epidemiological, ecological and geographical situations in which it is likely that they could be relevant to malaria control.

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A. EFFECTS AND USES OF SEXUAL-STAGE VACCINES

A.1 Possible effects of sexual-stage vaccines

Vaccines that prevent the development of the mosquito stages of *Plasmodia* could interfere with the transmission of malaria in three ways, presented schematically in Figure 1.

i) A decrease in the number of infective mosquitoes should reduce the inoculation rate resulting in a lower rate of malaria infection. If the reduction is large enough this will decrease both morbidity and mortality. A smaller reduction may merely serve to shift the age of disease upward with little overall impact on morbidity or mortality rates.

As with any method which significantly reduces transmission, there is the potential of creating an unstable situation in which malaria morbidity and/or mortality may increase to higher levels than those present at baseline. If population immunity decreases as a result of reduced exposure, even minor increases in transmission as a result of waning of protection over time, inadequate coverage of the population or in-migration of non-vaccinated individuals could lead to malaria epidemics with increased morbidity and mortality.

ii) A decrease in the intensity of infection per mosquito may result in a reduction of sporozoites per inoculum or in a reduction in the number of inocula a mosquito can deliver.

iii) A reduction in antigenic diversity within an inoculum, due to the reduced number of parasites inoculated may modify its effect in the person exposed. Antigenic diversity may also be reduced on a population basis if the vaccine acts selectively against some phenotypes only, leading to the eventual selection of vaccine resistant strains.

At present, only (i) can be estimated by established methods of vector sampling, oocyst and sporozoite determination, and incidence measurement. The effects (ii) and (iii) are possible but speculative. It is important to note that there is some evidence that a low level of antibodies against the sexual stages may enhance transmission, possibly through increased encounter between gametocytes.

A.2 Potential vaccine uses in malaria control

The place of sexual-stage malaria vaccines in malaria control will depend on their individual efficacy, but also on the availability and efficacy of other malaria vaccines, and on how existing malaria control methods are employed. Three potential uses of sexual stage vaccines are discussed below. The first of these, the use of sexual-stage vaccines to reduce malaria transmission, is the focus of this document.

A.2.1 Interruption or reduction of malaria transmission

The impact on malaria transmission by a sexual-stage vaccine will depend on the initial level of transmission, on the efficacy of the vaccine, and on the vaccination coverage achieved. To interrupt transmission requires an effective vaccine coverage (i.e. product of coverage times efficacy) of a proportion of at least $(1 - 1/R_o)$ of the population, where R_o is the basic reproduction rate; e.g. for a basic reproduction rate of 10, the effective coverage required is 90%. In areas where the basic reproduction rate is high, a combination of vector control and vaccination is likely to be required to interrupt transmission. Pre-erythrocytic and asexual blood-stage vaccines could also reduce transmission and, in addition, would give protection against disease.

A.2.2 Protection against morbidity and mortality from malaria

The frequency and size of the infecting sporozoite challenge may be important determinants of infection and progression to clinical disease and death. Therefore, sexual-stage vaccines, either alone or in combination with sporozoite and/or asexual blood stage vaccines, may play a role not only in the reduction of transmission, but also in reducing morbidity and mortality due to malaria.

A.2.3 Protection of pre-erythrocytic or asexual blood-stage vaccines against immune evasion

The use of a transmission-blocking vaccine in combination with a pre-erythrocytic or asexual blood-stage vaccine may prevent the transmission of parasites evading the pre-erythrocytic or asexual blood-stage vaccine through antigenic diversity or variation.

A.3 Modelling the impact of sexual-stage vaccines

Epidemiological simulation modelling could play an important role in the planning and analysis of a sexual-stage vaccine trial. The interpretation of the results of such a trial - which deals with community rather than individual protection - and the prediction of the long-term impact on transmission and disease, will require epidemiological modelling. Since a sexual-stage vaccine will probably be used in combination with drugs, vector control or other vaccines, modelling may be crucial to estimating the impact, and thus the cost-effectiveness, of different combinations. To be of optimal use in trial design and analysis, appropriate simulation models should be developed and tested before a vaccine is subjected to field evaluation.

B. CLINICAL AND LABORATORY STUDIES PRECEDING COMMUNITY BASED TRIALS

Clinical trials are necessary to produce data needed for the planning of community-based field trials, in particular information on safety, acceptability, immunogenicity and preliminary measures of efficacy (e.g. the effect of sera from vaccinated individuals on transmission from infected blood to vectors in membrane feeding experiments, including duration of the effect), related to the age, sex, and past malaria experience of the vaccinee and the dose of vaccine used. Studies will also be required on whether or not revaccination will be necessary.

By the time community trials are planned information should be available on the most appropriate vaccine formulation and method of storage, and whether antimalarial treatment is required before vaccination.

B.1 Objectives of preliminary studies

i) To establish the safety, immunogenicity and efficacy (assessed through membrane-feeding) of the candidate vaccine in non-immunes and immunes;

ii) To establish the safety, immunogenicity, and efficacy of the vaccine assessed through direct mosquito feeding studies of immunized gametocytemic volunteers;

iii) To evaluate simple immunological tests as surrogate measures of protection, validated against efficacy as assessed through membrane-feeding;

iv) To establish whether a current (or past) malaria infection interferes with the response to the vaccine.

B.2 Sequence of studies

Clinical trials conducted in non-endemic areas must be repeated in populations living in endemic areas before proceeding to community-based trials. The extent to which clinical trials are necessary in a particular malaria-endemic area will depend on the availability of information from other trials carried out in areas in which the epidemiology of malaria is similar. For ethical reasons, clinical studies in some special high-risk groups, such as pregnant women and children, should be carried out only in malarious areas. Studies in these groups should be initiated only after safety has been established in adult males and nonpregnant women.

Clinical trials of sexual-stage vaccines to evaluate toxicity, immunogenicity and efficacy are likely to be conducted in different groups in the following sequence:

(i) non-immune adults in a non-malarious area;

(ii) partially-immune adults in one or more endemic areas; done first in non-infected individuals, and subsequently in infected individuals;

(iii) older children, young children, infants and pregnant women, in endemic areas in which community-based trials are planned.

In these investigations the efficacy of the vaccine will be established through functional assays using membrane feeding of mosquitoes, direct mosquito feeding on gametocytemic volunteers, where feasible, and quantifying the frequency and intensity of oocyst and sporozoite infections. Ideally, parasite strains and mosquitoes for these assays should be those native to the areas which are prospective sites for community-based studies.

C. COMMUNITY-BASED TRIALS

Detailed guidelines on the design, conduct and analysis of field trials are given in (6). Specific issues relevant to trials of sexual-stage malaria vaccines are discussed below.

C.1 Objectives of community-based trials

Community-based trials of sexual-stage vaccines should be designed so that the results will be of direct relevance to decisions concerning the place of the vaccines in malaria control. Some potential uses of sexual-stage vaccines have been identified in Section A.2. It is likely that their eventual deployment in control programmes will be in combination with other malaria control measures, possibly including pre-erythrocytic and/or asexual blood-stage vaccines. However, until these other vaccines become available, community-based trials of sexual-stage vaccines should be conducted to evaluate any additional impact they have beyond that due to the existing malaria control activities in the study area. That is, the only substantial change that should be made to the malaria control activities in the context of a trial is the addition of the sexual-stage vaccine in some of the study communities. The two major objectives of the initial trials will be:

1. To measure the effect of a P. falciparum or P. vivax sexual-stage vaccine in vaccinated communities on the sporozoite rate in mosquitoes and the incidence rate of malaria infections, the prevalence and density of parasitaemia, and the morbidity due to malaria in man.

In addition, levels of immunity may be monitored through immunological tests validated in earlier clinical trials, and the infectivity of vaccinated (and unvaccinated) individuals for the local vector may be measured using functional assays.

2. To measure any adverse effects attributable to vaccination

The endpoints of greatest public health importance against which a malaria vaccine may act are disease due to malaria and (in the case of P. falciparum) death, but the main emphasis in early community trials of sexual-stage vaccines should be to assess the impact on malaria infection in the vector and in man. The primary effect of sexual-stage vaccines is to reduce the transmission of malaria parasites in the community and other effects (with the exception of adverse reactions) are secondary. Unless there is a substantial effect on transmission effects on malaria morbidity and mortality are unlikely. Nevertheless, it will be desirable to measure any reduction in morbidity from malaria which may be attributed to vaccination.

The measurement of the impact of vaccination on mortality would require a large study, and this raises several problems. First, with respect to potential adverse effects (in particular those that might appear only upon challenge), it may be imprudent to go quickly to the vaccination of large numbers of individuals. Second, the initial intensity of transmission will be a major determinant of the impact of a sexual-stage vaccine, and one of the objectives of early field trials will be to evaluate the impact over a range of intensities of transmission. To do that with mortality as an endpoint also will be very expensive. Furthermore, a reduction in transmission may produce changes in the population's age-specific immune-status over long time periods, (e.g. there may be slower acquisition of immunity against pre-erythrocytic and asexual blood-stages) and the possible consequences of this, in terms of disease and death, may not be detectable in relatively short-term evaluations. For these reasons, it will probably be preferable to evaluate the impact of sexual-stage vaccines on death after such vaccines have been introduced into control programmes.

C.2 Criteria for the selection of study areas

In conducting community-based trials, it is important to bear in mind that it is not appropriate to carry out successive malaria vaccine trials in the same population, as the interpretation of subsequent trials could be greatly complicated by possible effects of previous vaccinations.

C.2.1 Operational criteria

Highly desirable characteristics of potential trial sites include:

(a) An enthusiastic commitment from national authorities to the conduct of the trial. This will increase the likelihood that the results of the trial are used to plan future malaria control strategies and will help in gaining the support and confidence of both community participants and health professionals;

(b) Enthusiasm for the trial and the associated investigations by the study population. The conduct of a trial will involve the population in inconvenience, including the donation of blood specimens. It may also be necessary to recruit key informants to record morbidity and mortality.

(c) Involvement of local national research institutions with interested national (and international) investigators and field and laboratory teams. These institutions should have access to national and international resources to support the trial;

(d) Availability of background data on the epidemiology of malaria (see Sections C.2.2 and C.3);

(e) Reasonable expectation of social and political stability at the national and local levels for the duration of the trial;

(f) A sufficiently well-established health service infrastructure to meet the primary health care needs of the population and to provide for referral of patients to hospital when required;

(g) An effective programme for the administration of existing vaccines against other diseases;

(h) Availability of basic laboratory services in reasonable proximity;

(i) Adequate year-round transportation and communication infrastructures to provide access to the population and for the population to have access to health care services on a year-round basis;

(j) The potential for the site to serve as a field training centre.

C.2.2 Epidemiological criteria

A vaccine should be evaluated in the range of epidemiological situations and geographic areas in which it is likely to be relevant to malaria control. Thus the selection of areas to conduct trials will depend on having good information on the epidemiology of malaria in candidate sites. Also, preliminary studies with the vaccine (see Section B) should have been done at that site or at another one that is sufficiently similar. Further epidemiological criteria include the following:

(a) Relative stability of the human population. Movement of infected people into the study area will lower the expected effect of a vaccine on transmission;

(b) Sporozoite rates. The percentage of man-biting Anopheles with sporozoites in their glands must be high enough to enable a reduction caused by the vaccine to be detectable, but not so high as to "mask" a potentially significant effect on transmission;

(c) Biting habits of vectors. Some vectors come into human contact mostly at places away from the community, e.g. forests, work places. Sexual-stage vaccine trials will be easier to interpret in situations where vector contact is largely limited to community sleeping areas. The use of personal protection measures must be well understood and documented. Age and sex distribution of human infections are helpful in identifying such situations.

C.3 Background information required

In order to design a trial it is necessary to have demographic, entomologic, and parasitologic information from candidate trial sites. Preliminary data collection at the potential sites may be necessary to answer specific questions. In most situations, longitudinal surveys will be required, involving base-line data collection for a full year. The more important data that will be required for planning a sexual stage-vaccine trial are listed below, and are in addition to those listed in Section C.2.

C.3.1 Maps and demography

Mapping of the study areas and a full census of the population should be performed (6). There will be a need to carry out careful monitoring of migration movements of the study population.

C.3.2 Entomology

(1) Species of Anopheles vectors. These should be known with certainty, and not just presumed.

(2) Seasonality of transmission. Most vector populations have an annual cycle dependent on rainfall. Vaccination should be completed and enough time left for the full immune response to have developed before the start of the annual transmission season.

(3) Flight range of vector. This will be essential for the selection of transmission units (see C.4.1), and can be determined by mark-release-recapture experiments. For the purposes of the trial, the shorter the range, the better.

C.3.3 Parasitology

(1) Age and sex specific rates of parasite incidence and prevalence of malaria morbidity. In populations living in areas of high malaria transmission, parasite prevalence and disease due to malaria usually peak in children under the age of 5 years. However, areas where the parasite prevalence is greatest in adults or predominately in one sex, would suggest that transmission may be occupational. Prevalence of antibody to circumsporozoite antigen may correlate roughly with intensity of inoculation;

(2) Age and sex specific incidence rates of malaria morbidity. Data on overall morbidity and mortality due to malaria are also important.

(3) Drug resistance of parasites. Some trial designs will require treatment of some infected subjects (e.g. pregnant women) and the complications of multi-drug resistance should be anticipated.

C.3.4 Malaria control activities

Information should be gathered on ongoing malaria control activities, including personal protection measures and the use of insecticides and repellents, both at home and for agricultural use.

(1) Drug use. Availability and nature of antimalarials (including some antibiotics, e.g. tetracycline) and local practice of self-medication or government drug administration.

(2) Vector control. Use of bednets, interior insecticide spraying, or other methods of vector control, public or private.

C.4 Design

C.4.1 Units of randomization

Selection of units of randomization, or transmission units, for testing a sexual-stage vaccine will depend on spatial considerations. Sexual-stage vaccines act directly on the parasite in the mosquito and a major endpoint for evaluation will be the infectivity rate in Anopheles biting in the selected communities. The unit of application of the vaccine should be the community, or more specifically the transmission unit for malaria. Each community in which the vaccine is administered should be distant from neighbouring communities by more than the vector's probable flight range. Determination of vector flight ranges will be essential and should not be presumed. Often effective ranges of Anopheles are less than 500 metres and transmission is highly focal.

The requirement for separation between units can be met by either selecting naturally isolated areas or creating transmission buffer zones around each community. Buffer zones would be made up of vaccinated individuals around the areas to be evaluated. Similar buffer zones of unvaccinated individuals (eg. given placebo) would be required around control communities. The creation of buffer zones will substantially complicate the trial design but will be highly desirable if natural isolation is insufficient. When a sufficient number of transmission units have been identified (see section 4.4.1) they should be designated to receive the vaccine or placebo in a randomized double-blind fashion.

Depending on the results obtained during baseline studies it may be desirable to stratify villages on the basis of intensity of transmission before randomization (as determined by inoculation rate).

C.4.2 Selection of participants

A major determinant of the efficacy of a sexual-stage vaccine is the coverage attained. All age groups should be considered as potentially infective to the mosquito. Therefore, as far as possible, the trial should not exclude any persons who would be likely to receive vaccination if the vaccine were introduced into a control programme after efficacy had been demonstrated.

It would be prudent, however, to exclude from an initial trial those likely to respond adversely to vaccination or liable to develop episodes of illness which might be difficult to distinguish from adverse reactions. In addition to those who refuse to participate in the trial, it is advisable that individuals be excluded if they have:

(i) any severe acute illness at the time of vaccination
(ii) any severe chronic disease
(iii) severe malnourishment

If substantial numbers of individuals are excluded, an effort might be made to vaccinate them at a later date. If that is not possible, consideration should be given to offering chemoprophylaxis to those excluded, at least for the length of the study. It should be able to calculate the proportion of excluded subjects who could serve as potential reservoirs and which, if large enough, would make a trial unfeasible in that region.

Early clinical trials may show that for effective vaccination, chemotherapy is required at, or shortly before vaccination, to avoid immunosuppression due to current malaria infection. In such circumstances, the trial design may have to be reviewed accordingly, as mass administration of antimalarial drugs may have an impact on transmission and reduce the power of the trial to demonstrate an effect of the vaccine on transmission. Chloroquine is the drug of choice in areas where P. falciparum is sensitive to it and curative doses should be used. In the case of P. vivax, chloroquine treatment may have to be supplemented with a full course of primaquine.

C.4.3 Choice of endpoints

The two most important endpoints in a community-based trial of a sexual-stage vaccine are the incidence of infection in man and the sporozoite rate;

(1) Incidence of infection in man. New infections, which usually occur at a rate lower than the entomological inoculation rate, should be reduced. The relation between entomological inoculation rate and incidence cannot be presumed to be directly proportional because of a number of confounding variables (e.g. levels of immunity). In certain epidemiological situations it may be more appropriate to monitor new cases of malaria by active case detection in vaccinated and control communities;

(2) Vector infectivity rate. The sporozoite rate should fall. Other, related expectations are that the frequency and intensity of mosquitoes with oocyst infected guts will fall, and that the sporozoite load per salivary gland will also fall.

Additional endpoints in which reductions are expected include:

(3) Parasite prevalence and densities. Over a longer term peak prevalence may shift to older ages and densities may increase as acquisition of immunity is delayed.

(4) Circumsporozoite antibody incidence, prevalence and titres.

(5) Malaria morbidity and mortality.

In addition, it is essential to monitor for possible adverse reactions.

C.4.4 Trial size and duration

C.4.4.1 Size

The unit of evaluation must be the community (or the unit of randomization) and communities included in the trial will have been randomly allocated to the vaccination or placebo group.

Transmission levels are likely to vary greatly between communities and the critical factor for the required trial size is the number of communities to be included in the study rather than the number of persons.

The two principal endpoints to be measured for each community are: (i) the rate of new malaria infections in the subgroup which will be treated with a schizonticide at the time of vaccination and monitored for subsequent malarial infection; and (ii) the infection levels in the vector. Sample size computations are illustrated for these two endpoints. Further computations may be appropriate for other trial endpoints, such as the impact of vaccination on malaria morbidity rates.

Conventional statistical methods to estimate the required sample size for a trial require that the levels of the following factors be specified:

(a) the estimated average rate of occurrence of malaria infection in the communities which will not be vaccinated (μ_2);

(b) the minimum protective effect of vaccination (γ) that the trial should have a high probability of detecting;

(c) the probability level (α) at which the null hypothesis (vaccine efficacy = 0) will be rejected. Usually taken as 0.05 or 0.01;

(d) the probability (1-ß) of declaring a significant difference if the true vaccine efficacy is that specified in (b). This is known as the statistical power of the trial.

In general, it is most efficient if the size of the vaccinated and unvaccinated groups are equal and the formulae below assume that this is the case.

The required sample size is calculated as follows:

$$n = (Z_\alpha + Z_\beta)^2 (\sigma_1^2 + \sigma_2^2)/(\mu_1 - \mu_2)^2$$

where n is the number of communities to be included in each of the two groups, μ_1 is the average rate of infection in vaccinated communities. For the purpose of the calculation of the sample size required to detect a vaccine efficacy of γ, it is appropriate to take $\mu_1 = (1 - \gamma)\mu_2$.

Values for Z can be derived from the specified values of α and β using tables of the Normal distribution. For example:

for	$\alpha = 0.05$	$Z_\alpha = 1.96$
	$\alpha = 0.01$	$Z_\alpha = 2.32$
and for 95% power	$\beta = 0.05$	$Z_\beta = 1.64$
for 90% power	$\beta = 0.10$	$Z_\beta = 1.28$
for 80% power	$\beta = 0.20$	$Z_\beta = 0.84$

σ_1 and σ_2 are the standard deviations of the infection rate per community in the vaccinated and placebo groups respectively. They reflect the variability in infection rates between communities, which may be considerable, as well as the variability resulting from the sampling within each community to select the subgroup to be monitored for malaria infection. Since the transmission units will be allocated randomly to the two groups, it is reasonable to assume for the purpose of sample size estimation that $\sigma_1 = \sigma_2 = \sigma$ (even though vaccination may cause σ_1 to be smaller than σ_2).

The two parameters which remain to be estimated before the sample size can be determined are μ_2, the average rate of infection without vaccination, and σ, the standard deviation of the rate between communities. The most satisfactory way to estimate these parameters would be from baseline data collected in the study area concerned. Care should be taken that the estimation of σ is based on infection rates for sub-groups of approximately the same size as will be used in the trial to assess infection rates because σ depends on both the variability between communities and on the average size of the sub-groups.

<u>Example</u>

Suppose that a baseline study has been undertaken to study the rate of malaria infection in the trial area. Several transmission units have been sampled and a fixed proportion of the population of each selected unit has been treated with an effective schizonticidal drug and monitored for the occurrence of malaria infection. Suppose that in this study the rate of infection was of 30% per person year at risk and that the standard deviation of the rate per transmission unit was 8%. If the purpose of the study is to detect a vaccine efficacy of at least 50% with a significance level of 5% and 90% power, then

$$n = (1.96 + 1.28)^2 (0.08^2 + 0.08^2)/(0.3 - 0.15)^2$$
$$= 5.97$$

That is, the trial would require 6 vaccinated and 6 unvaccinated communities to be studied for one year.

Depending on what information is available for a given study area on infection rates and their variability between communities, the size of communities and practical considerations on the difficulties to be expected in vaccination, monitoring for adverse reactions and monitoring for infection, an attempt might be made to determine the optimal balance between the number of transmission units required and the average size of the sub-groups to be monitored for new infections. These statistical aspects are complex and their consideration will require the investigator to seek the assistance of a statistician.

Sporozoite rate

The considerations for determining the sample size required to determine the effect of vaccination on the sporozoite rate are similar to those given above. Again, the critical factor is the number of transmission units to be included, and the sampling design for the entomological evaluation of the effect of vaccination should follow the overall framework as discussed above and involve the same communities.

C.4.4.2 The use of baseline data

The sample size computations illustrated above are based upon a direct comparison of infection rates in vaccinated and control communities. If these rates vary greatly between communities in the absence of vaccination and there is consistent variation from year-to-year (that is, the relative position of a community in a given year, with respect to incidence, is a good predictor of its relative position in the following year) statistical power may be gained by contrasting changes in rates before and after administration of vaccine (or placebo) in the communities in the trial. This may be very good use of baseline data that is likely to have been collected in the year or more before the start of the trial. The computation of the required sample size for such comparisons follows the same lines as given above but information is required on the expected variation (standard deviation) in the changes in rates from year to year in each community. Guidance on sample size determination in such situations should be sought from a statistician.

C.4.4.3 Duration

The most useful vaccines will be those that give high protection for a long period. Thus, if a vaccine is found to protect against malaria transmission during the first year, follow-up of the trial communities should continue, ideally for as long as there is a significant difference between vaccinated and unvaccinated communities, although the maintenance of unvaccinated communities in this situation may raise ethical questions. However, it might be argued that in circumstances where the study communities are subject to close surveillance it would be justifiable to continue follow-up provided that all clinically manifest episodes of malaria were subject to prompt detection and treatment.

Provision should be made to stop the trial at once if there is an unacceptable number of serious adverse reactions. An independent clinical monitor should be appointed for the trial and advising in these circumstances would be one of the major responsibilities of the monitor.

If a vaccine against the sexual-stages is judged efficacious, the opportunity to follow the long-term effects of vaccination in the trial communities should be exploited. In particular, it will be important to determine how long differences in malaria rates persist in the absence of revaccination. Parameters of infection, such as age shifts in prevalence peaks, changes in morbidity patterns, and changes in inoculation rates, should be carefully monitored.

C.5 Conduct

C.5.1 Community and trial preparation

The purposes of the trial and the methods to be used should be discussed with representatives of the communities in which the trial is planned and those who will be eligible for entry into the trial should be properly briefed on possible adverse effects of vaccination and on possible benefits. It should be made clear that participation in the study is voluntary and those refusing to participate will receive all their routine vaccinations and will not be discriminated against in any way. However the community should be aware that the possible beneficial effects of the vaccine are likely to be achieved only if a high proportion of the population agrees to participate.

C.5.2 Measurement of endpoints

Most transmission-blocking parasite vaccine trials will involve making the measurements described below during the pre-intervention stage of the study and after vaccination has been undertaken. Other measurements which would greatly facilitate comparison between trials include measurement of the morbidity and mortality due to malaria (methodology described in document TDR/MAP/AVE/PF/89.5), and characterization of local parasite strains, particularly of vaccine breakthroughs.

The following aspects of measurement require critical review during the preparatory phase of a trial: standardization of all field, clinical and laboratory procedures; quality control monitoring in the trial; reproducibility of measurements within and between field workers and at different times; comparability of procedures and methods with other studies; sensitivity and specificity of diagnostic methods; level of precision required for all measurements to be taken in the trial (not necessarily the highest possible); minimization of the numbers of specimens (e.g. of blood) to be collected; arrangements for the collection, transportation and preservation of specimens; procedures for recording of data and for the analysis of results.

C.5.2.1 Incidence of infection in man

In order to determine the incidence of new *P.falciparum* infections in trial subjects, parasitaemia should be cleared from all subjects included in this aspect of the trial (i.e., a representative sub-sample) by the administration of an effective schizonticidal drug at the time of, or shortly before, vaccination. In the case of *P.vivax*, the schizonticidal treatment should be associated with a full course of primaquine as antirelapse treatment. Once vaccine or placebo has been given, subjects in this cohort should be bled at regular intervals, perhaps once a week or once a month, depending upon the level of malaria transmission, and blood films examined for asexual parasites and gametocytes. In addition, a monitoring system should be established to record clinical episodes and consumption of antimalarials in the cohort. In this way it will be possible to determine the incidence of new infections in vaccinated and control subjects over a defined period. In some communities the development of splenomegaly provides evidence of a recent malaria infection.

The currently accepted method for detecting malaria infections is by microscopic examination of thick and thin blood films. The sensitivity of this technique depends on the volume of blood examined. The most commonly used substitutes for a standard volume of blood are 100 and 200 thick-film microscopic fields. Only a fixed stopping rule (e.g. examine a fixed number of fields, counting whatever parasites are found on the way) allows an unbiased estimation of parasitaemias; flexible stopping rules may underestimate the prevalence of the less dense parasite species or forms (e.g. gametocytes). Currently accepted measurements of density that allow discrimination at high densities involve the counting of parasites either against white blood cells in the thick film or against red blood cells in the thin film. In transforming such relative counts into numbers per volume, it is usual to assume a fixed white or red blood cell count in the study population. Parasite density is measured most accurately by combining a red blood cell count with a parasite count made on a thin blood film but this approach is only suitable for infections with a high level of parasitaemia. Notwithstanding efforts to standardize all procedures involved, their performance usually varies between investigators and with the same investigator over time, and this variation must be allowed for in the study design.

The following procedures are considered essential when the finding of malaria parasites in blood films is used as a criterion for assessing the efficacy of a malaria vaccine:

(a) standardization of laboratory procedures within a trial and, as far as possible, between trials;

(b) taking of blood films in duplicate;

(c) "blind" microscopic blood film examination; (i.e. the microscopist should not know if a slide is from a vaccinated or unvaccinated individual or from a febrile or afebrile subject)

(d) preservation of blood films for further reference;

(e) quality control by re-examination of a coded sample of blood films;

The use of newer diagnostic techniques, such as DNA or RNA probes, QBC and antigen detection assays, might also be considered. Whilst these cannot be used at present to replace microscopic examination, they may become valuable supplements, permitting rapid, objective and specific evaluation of large numbers of blood samples.

C.5.2.2 Vector infectivity rate and inoculation rate.

Preference should be given to man-biting *Anopheles* captured at a minimum of two sites in each transmission unit. Sampling error will be reduced by using multiple collection sites per unit. Collection times should correspond to those of natural biting activity or all-night, and collection sites should be located where man-vector contact is known to be highest. To take into account daily variations in activity, several consecutive nights per month should be scheduled and collections in all transmission units done simultaneously, if possible.

Infectivity, that is the presence of sporozoites in the salivary glands, can be measured by dissection or biochemically by using stage specific monoclonal antibodies in an ELISA. Since the biochemical methods assay unassociated antigen as well as whole sporozoites, false positives will be reduced by testing only the fore thorax and head of captured mosquitoes.

Besides speed, the advantages of the biochemical methods are that they can be used to estimate sporozoite load and determine species. New methods of detecting parasites in mosquitoes are being developed using stage specific RNA expression which might prove extremely useful in analyzing large numbers of infected mosquitoes. Measurement of oocyst infection must be done by dissection; this is not necessary for wild-caught mosquitoes. Preferably, parasite determinations should only be done on parous female _Anopheles_. Resting mosquitoes can also be tested (in conjunction with blood meal analysis) but inoculation rates can only be calculated from _Anopheles_ caught biting man.

There are concerns regarding the utilization of "man-biting" techniques to capture mosquitoes for entomological analysis in field studies. It is clear that in some areas this technique is the only means of estimating true biting behaviour of vectors, as other methods, including the use of "double net" do not provide data representative of the natural situation. Offering collectors prophylaxis may be sufficient in areas of chloroquine sensitivity, however, where other drugs must be used, the benefits must be weighed against the potential adverse effects. This is particularly important in areas where adults have not acquired sufficient levels of immunity to reduce risk of acute episodes of disease.

When it is not critical to have precise host preference or biting-frequency data, other methods of assessing mosquito behaviour or estimating vector populations should be considered (e.g. CDC light traps). Only when representative data are imperative should man-biting techniques be considered. The collectors should be offered chemoprophylaxis and/or be monitored very carefully for acute disease, which should be treated promptly.

C.5.2.3 Prevalence of infection in man

Changes in the prevalence of parasitaemia should be followed longitudinally (about once a month) in a representative random sample of the population which is different from the sample used to assess the incidence of infection. Parasitological methodology is the same as in the previous section.

C.5.2.4 Measurement of clinical malaria

A universally agreed definition of clinical malaria does not exist and the criteria used to make this diagnosis may vary from area to area. Provided that the relevant measurements are made, it may be possible to study the effects of a vaccine using definitions of clinical malaria based on more than one criterion, for example, as defined by fever accompanied by high or moderate levels of parasitaemia.

Clinical episodes of malaria in trial subjects may be detected by either active or passive case detection procedures. It is likely that most trials will involve both approaches, the degree of emphasis placed on each will be determined by the access that those in the trial have to treatment facilities. In communities where most patients with even minor illnesses report to a single or a small number of treatment facilities, records from these clinics may provide all the information required on morbidity in a trial. In less privileged communities more active case detection will be required. One approach that has proved successful in other malaria intervention studies is a weekly visit to each trial subject, during which a brief morbidity questionnaire is completed, the subject's temperature is recorded and a blood film is made if a raised temperature is found. The level of temperature chosen to indicate abnormality should be determined in preliminary studies; it will be influenced by both the route by which the temperature is recorded and by the characteristics of the study community. In previous studies in malaria endemic areas of Africa, an axillary temperature in the range of 37.5 - 38.0° has been found to provide a useful cut-off point. Electronic digital thermometers provide a convenient and safe way of measuring temperature in field trials.

The severity of clinical attacks of malaria identified by active and passive case detection techniques, for example, an inability to work or attend school, should be recorded according to pre-determined criteria. Unless a trial is very large, it is unlikely that the incidence of severe or complicated malaria, such as cerebral malaria, could be used as an end-point.

In areas where malaria is highly endemic, the detection of fever and malaria parasitaemia in the same child does not necessarily mean that malaria parasites were responsible for the child's fever, as a significant proportion of all children in the community will have malaria parasitaemia at any one time. The occurrence of a large number of cases of fever accompanied by parasitaemia, but in which malaria parasites were not the cause of the fever, could dilute the apparent protective effect of a malaria vaccine which had its main effect on fever and illness caused by malaria parasites rather than on the prevalence of infection. It might be possible to make some correction for this potential bias if measurements of the prevalence of parasitaemia were made in randomly selected afebrile trial subjects at various points during the post-vaccination observation period.

C.5.3 Monitoring of other relevant parameters

C.5.3.1 Measurement of side-effects

The measurement of side-effects should be considered in relation to the time following vaccination at which side-effects are expected. Current or past *P. falciparum* or *P. vivax* infection may increase the risk and severity of side-effects, so that early trials in non-endemic countries may not be adequate predictors. Side-effects should be assessed in all phases of vaccine trials.

Vaccinated and control (placebo vaccinated) persons should be observed immediately after vaccination to identify acute reactions. A follow-up visit to the homes of the study population should be made within a few days to identify early reactions, such as pain and abscess at the vaccination site, and constitutional symptoms, including fever. This can be done most readily by placing a project worker in each study community.

Delayed toxicity, including immunopathological phenomena, may not become apparent for many months, and observations for side-effects may have to continue beyond the period of vaccine protection and for long enough to cover the first expected challenge infection. Both case detection and population surveys should be used.

C.5.3.2 Immunological assays

It is unlikely that it will be possible to make extensive immunological measurements in all those in a field trial. However, it will be essential to make some immunological measurements in selected subjects to confirm that the batch of antigen(s) used in the vaccine induces an appropriate immune response, for example an antibody response to one or more important epitopes. It will also be important to determine in some subjects the duration of the immune response induced by vaccination, and the possible effects of boosting by natural infection by measuring immune responses in vaccinated and control subjects at various times after vaccination.

Circumsporozoite antibody can be rapidly measured in 0.02 ml of serum using an ELISA. This can be collected as a blood spot on filter paper or in a heparinized haematocrit tube from finger puncture at least twice during the study: once just prior to the transmission season and once just after.

C.5.3.3 Drug utilization

Drug utilization during the period of post-vaccination follow-up should be assessed by (a) direct questioning of trial participants (b) review of dispensary records and those of shops selling drugs within the study area (c) random urine assays, e.g. recently developed dip-stick Dot-ELISA assays, for the anti-malarial drugs which are known to be used frequently in the trial community.

C.5.3.4 Malaria control measures

The use of other recent malaria control measures in the study areas should be monitored. This would cover personal protection measures including the use of insecticides, repellents, coils and bed nets.

C.5.3.5 Demographic surveillance

During the trial, all births and deaths in trial communities should be recorded, to the extent possible, and records should be kept of migration to or from the study area. Depending on local conditions and habits, some idea of what the short term movements of the study populations are, should be obtained. This will be particularly important if there are substantial movements of subjects between transmission units.

C.6 Analysis

Data processing and analysis should be a continuous process throughout the trial period and should not be delayed until after the completion of all data collection, as ongoing analysis will often detect systematic errors or omissions in data collection at an early stage in a trial. Initially the aim of the analysis is to assess the quality and consistency of the data collected, to improve the general understanding of the epidemiological situation in the study area by providing information on seasonal variation in vector densities, treatment seeking behaviour, drug availability, etc., and to detect unexpected epidemiological changes which may warrant ad hoc investigations to document their causes at the time when this is still possible.

An analytic plan which characterizes randomization and drop out patterns, prioritizes each primary and secondary endpoint for parameters of safety and efficacy, identifies general and specific statistical methods to be employed, and establishes a level of significance (alpha) must be adopted prior to breaking the study code. The plan should be filed with an external agency not directly involved with the study, and must be adhered to once the data analysis begins.

Once the code identifying vaccinated and unvaccinated communities has been broken, an analysis should be undertaken on the comparability of the two sets of communities in terms of demographic characteristics, vector densities, access to health services, utilization of antimalarials and other factors of relevance to study objectives. Of particular interest is the comparability of pre-vaccination information on malaria infection rates in man and in the vector. In case of great differences between the two groups, it may be necessary to use the pre-vaccination data as a correction factor in the analysis of the observed post-vaccination differences in infection levels between the vaccination and placebo groups. Ideally any such differences will have been taken into account, at least in part, in the design of the trial when allocating communities to the vaccinated or unvaccinated groups (see section C.4.1).

The main focus of the analysis will be on the infection levels in man and vector during the study period and the comparison of these between the vaccinated and unvaccinated groups. The preferred index in man is the malaria infection rate in the cohort treated with a schizonticide at the time of vaccination. This rate is defined as the number of persons who became infected during a specified period X, divided by the sum of time at risk for all persons in the cohort who were free of infection at the start of period X and subsequently monitored for malaria infection. For a person who did not get infected during period X, the time at risk is simply equal to X; for a person who became infected the time at risk is equal to the time between the start of the period and the time of first detection of infection. The preferred entomological index for assessing the effect of vaccination is the sporozoite rate and not the entomological inoculation rate (EIR) which is subject to variations in the vector density (even though the EIR is the most important variable for the comparative analysis of entomological data on transmission and the incidence of infection in man). One specific objective of the analysis is to measure whether a difference in infection levels between vaccinated and unvaccinated groups (if any) remains constant during the study period or whether it tends to diminish.

Finally a formal statistical comparison should be made of the observed differences between the vaccinated and placebo groups in the two main outcome variables, i.e. the infection rate in man for the total study period and the sporozoite rate for the total study period. The statistical significance of the difference between the two groups should be tested and confidence limits should be calculated for the estimated efficacy of the vaccine. The statistical procedures involved in these calculations are described in detail in chapter 14 of (6).

C.7 Ethical considerations

Trials of sexual-stage vaccines are subject to the same ethical constraints as are trials of any new vaccine. The design and implementation of a trial should conform to both national and international ethical standards. Prior to implementation, the study design should be reviewed by a properly constituted local or national ethics committee, which must include representatives of the groups to be vaccinated, responsible health authorities, and technical experts. There must be informed consent from those who participate in the trial and a written statement must be made of how it is to be obtained, even if it is not proposed that written consent is to be given by individual participants. There must be no pressure to participate and no differences between services offered to acceptors and non-acceptors. Health services for treatment of vaccine reactions and malaria infection and adequate referral and follow-up capability must be available. An independent clinical monitor should be designated and given the authority to break the trial code for any of the communities involved, and recommend that vaccination be stopped should there be severe adverse reactions associated with the vaccine. Trials of sexual-stage malaria vaccines should be carried out only in communities that are likely to be included in vaccination programmes, should the vaccine prove effective.

Before embarking on a field trial, an assessment of the safety of a candidate vaccine will have been made in subjects from both non-endemic and endemic areas. Only those candidate vaccines that have acceptable levels of safety and are produced according to internationally recognized manufacturing practices, such as those recommended by WHO, should proceed to community-based trials.

The mode of action of sexual-stage vaccines raises some special ethical issues, which do not arise for other vaccines against malaria or other diseases. In particular, sexual-stage vaccines offer no direct benefit to the individual who is vaccinated, but they are designed to prevent that person from transmitting malaria to others. Benefits to individuals are only likely if a high proportion of individuals in a community are vaccinated. Thus, there is a danger that investigators will be inclined to put undue pressure on community members to ensure a high level of participation. Furthermore, it will be necessary to vaccinate adults, who may be at relatively little risk of severe malaria in areas of intense transmission. They may benefit little from the vaccination but will be exposed to any adverse effects of vaccination. This issue will be of importance if the adverse effects of the vaccination are other than minor. Care should be taken to explain these issues when informed consent is sought.

An additional feature of community-based trials of sexual stage vaccines is that randomization must be at the level of the community rather than the individual. To ensure the "double-blindness" of a trial it will be desirable that those in communities which are randomized to be "unvaccinated" should be given a placebo preparation (identical in appearance to the vaccine). While it is possible to evaluate the impact of a vaccine without double-blindness it is difficult to be sure in such circumstances that the evaluation is not subject to bias. An acceptable and preferable alternative to a placebo preparation might be another vaccine which would be of benefit to the individual in "unvaccinated" communities but which would have no impact on malaria.

D. EVALUATION IN CONTROL PROGRAMMES

The effectiveness of sexual-stage vaccines in reducing malaria transmission rates will be assessed in community-based trials and on the basis of data obtained from these trials, decisions will be made regarding the incorporation of sexual-stage vaccines into malaria control programmes. It must be recognized, however, that the findings in such trials may not predict effects that will be produced when use of the vaccine(s) becomes more widespread. First, the way vaccines are applied in controlled trials may be better than would be the case in control programmes. In trials greater efforts may be made to ensure high coverage and more care may be taken with storage and administration of the vaccine. Second, the effect of the vaccine is likely to be greater if the area included in the vaccination programme is enlarged (as migration into and out of the area will become less of a problem). What the overall impact of these two effects on transmission rates will be is difficult to predict and it will be important, therefore, to design the implementation of the introduction into control programmes in such a way that the impact on malariometric parameters can be assessed. One way of achieving this is to phase the introduction of the vaccine into different areas in such a way that before and after measures can be contrasted and comparisons can also be made between areas with and without the vaccine at the same time. A way in which this might be achieved is the use of a "stepped-wedge" introduction of the vaccine as described in Chapter 2 of (6).

D.1 Post-trial evaluation

If a vaccine against the sexual-stages is judged efficacious, the opportunity to follow the long-term effects of vaccination in the trial communities should be exploited. In particular, it will be important to determine at what intervals booster vaccinations may be required, by monitoring immune response parameters. Parameters of infection, such as age shifts in prevalence peaks, changes in morbidity patterns, and inoculation rates, should be carefully monitored for as long as differences between trial and control units are discernible.

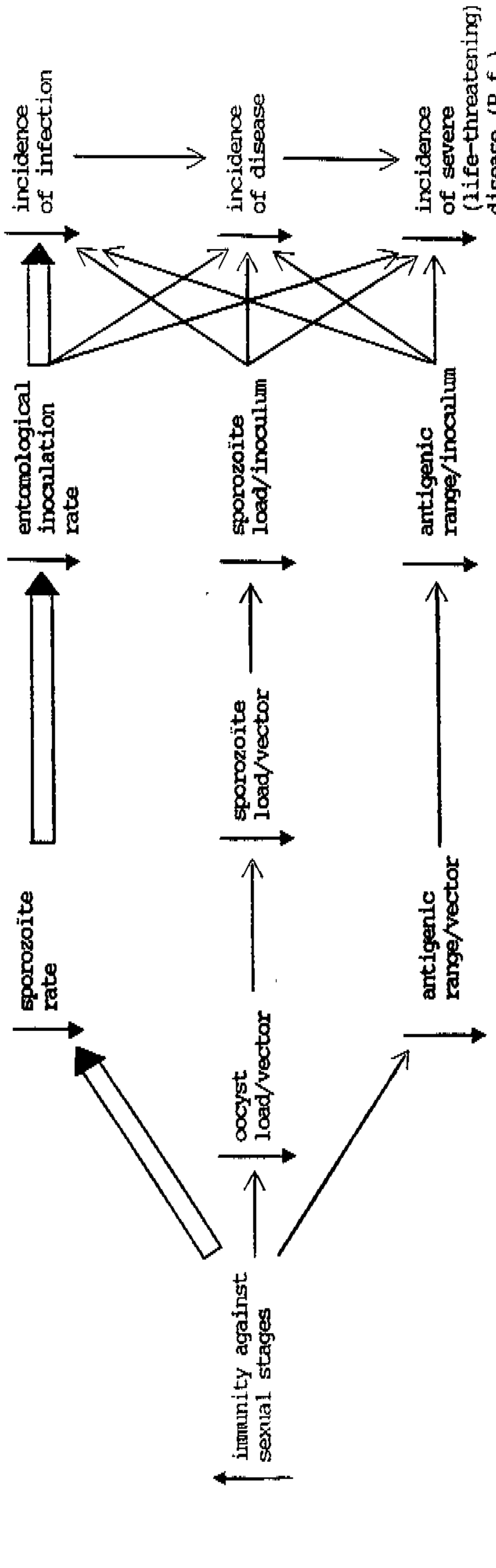
D.2 Confounding variables

(1) Geographical constraints. It cannot be assumed that the vaccine's effect will be the same in all transmission situations. It may have, for example, a more marked effect where transmission is relatively low. It will be important that trials be conducted in a variety of situations.

(2) Parasite resistance. Recent research has uncovered an extraordinary variety in several plasmodial antigens. Relatively little is known about the antigens of the sexual stages, but genetic recombination occurs only in the mosquito gut. Consideration should be given to the possibility that a vaccine will sooner or later select phenotypes resistant to the specific vaccine induced antibodies. Monitoring vaccine-induced changes should be an integral part of all vaccine trials.

(3) Natural boosting. It is possible that natural infection in humans will boost response as their vaccine-induced immunity wanes. Theoretically, a successful vaccine should lower transmission to too low a level to produce boosting. Nevertheless, the possibility of natural boosting can be monitored by comparing the decline of antibody titres in the trial population with those in the clinical trials.

FIGURE 1. POSSIBLE EFFECTS OF TRANSMISSION-BLOCKING VACCINES
(↑ = INCREASE ; ↓ = DECREASE)



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