



UNDP/World Bank/WHO Special Programme for
Research and Training in Tropical Diseases (TDR)

**GUIDELINES FOR THE EVALUATION OF *PLASMODIUM FALCIPARUM*
VACCINES IN POPULATIONS EXPOSED TO NATURAL INFECTION**

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ABBREVIATIONS

ACD	active case detection (through house to house visit)
ATI	anti-toxic immunity
ATV	anti-toxic vaccine
BSI	(asexual) blood-stage immunity (antiparasitic)
BSV	(asexual) blood-stage vaccine (antiparasitic)
DB	double blind
DB-RCT	double blind randomized controlled trial
EPI	expanded programme on immunization
GMP	good manufacturing practices
MCH	mother and child health
PCD	passive case detection (in a health station, fixed or mobile)
PEI	pre-erythrocytic immunity
PEV	pre-erythrocytic vaccine
RBC	red blood cells
RCT	randomized controlled trial
SOP	Standard Operating Procedures
TBI	transmission-blocking immunity
TBV	transmission-blocking vaccine
VA	verbal autopsy
VE	vaccine efficacy
WBC	white blood cells

INTRODUCTION

The general planning of malaria vaccine trials and the phases in which they are expected to be developed are considered in the report of a meeting held in WHO, Geneva, in February 1985 (WHO, 1985). Three other WHO documents give guidelines for the field evaluation in malaria-endemic areas of sporozoite and asexual blood-stage vaccines against *P. falciparum* (WHO, 1986a, 1989), and of sexual stage vaccines against *P. falciparum* or *P. vivax* (WHO, 1992). The present document combines and updates the three guidelines, but is restricted to *P. falciparum* vaccines. Their combination is justified by their many commonalities. Updating is required in light of the lessons learned from the field trials conducted since their elaboration, in particular the trials of SPf66 (Alonso et al, 1994a & b; Ballou et al, 1995; D'Alessandro et al, 1995; Nosten et al., 1996; Valero et al, 1993; 1996) and of further technical discussions, including the development of guidelines for Good Clinical Practice (GCP) for Clinical Trials (WHO, 1995b). The trials considered here are concerned with efficacy against natural challenge, as well as with safety and immunogenicity.

This document is addressed to national health authorities, in particular to those of malaria-endemic countries interested in the potential use of *P. falciparum* vaccines for the control of malaria, and to research scientists interested in the development and field evaluation of such vaccines. The guidelines may help public health officials, with the assistance of their technical advisers, to make decisions about malaria vaccine trials to be conducted in their countries, including not only field trials, but also the earlier clinical trials required before proceeding to field trials. The guidelines may help research scientists to clarify the technical decisions required before designing an actual field trial protocol.

Basic research towards the development of *P. falciparum* vaccines continues. Candidate antigens and vaccines are reviewed elsewhere (Howard & Pasloske, 1993; Kaslow, 1993; Coppel, 1995; Hoffman, 1996). In this document, the terms *Aimmune@* and *Aimmunity@* indicate resistance to infection, or to some of its clinical consequences, or (in the case of transmission-blocking vaccines) resistance to becoming infective, all these kinds of resistance resulting from previous infection or vaccination. *Aimmune responses@* is used to describe the host's humoral or cellular immune responses which follow exposure to antigens, through infection or vaccination, but which do not necessarily reflect or correlate with a state of protection against infection or its adverse effects. A vaccine's immunogenicity is its capacity to generate immune responses. Vaccine potency is a measure of functional activity, which again may or may not correlate with efficacy (clinical protection) in the target group. A clear rationale for the testing of candidate antigens in humans is required. Vaccines directed primarily at pre-erythrocytic stages (sporozoites or liver-stages), asexual blood-stages, malaria toxin(s), or sexual stages, will be called pre-erythrocytic, blood-stage, antitoxic, and transmission-blocking¹ vaccines respectively (in abbreviation, PEV, BSV, ATV, TBV); different

¹ The phrase *Atransmission-blocking@* will be reserved for vaccines directed primarily at sexual stages, without implying that vaccines directed primarily at other stages (e.g. asexual blood-stages) will have no transmission-blocking effect.

types of vaccines may be combined. A Disease@ will refer to uncomplicated malarial disease, A severe disease@ to severe and complicated malaria.

In spite of the knowledge accumulated since the production of the previous WHO guidelines, some issues remain undecided, indeed controversial. These guidelines will reflect some of these uncertainties, rather than try to settle them. The circulation of the guidelines may contribute to the settlement of some of the pending questions.

This document was developed using contributions and comments from many persons. They are listed in Annex 1.

A. GENERAL BACKGROUND

1. Potential uses of vaccines for malaria control

In order to design meaningful field trials, some speculation regarding the ways different vaccines might be used for malaria control in different situations is necessary because field trials of a vaccine have to help define its place in control. Such speculation is, however, problematic, as long as we know very little about the kind and duration of protection likely to be produced in individuals vaccinated with the kinds of vaccines likely to become available. The following combinations of types of uses, situations and vaccines are more or less plausible.

(a) Inclusion of a malaria vaccine in the Expanded Programme of Immunization (EPI), in situations where significant transmission is expected to continue into the foreseeable future (as is the case in much of tropical Africa). A pre-erythrocytic vaccine giving life-long protection would be ideal, but appears improbable. A blood-stage vaccine and/or antitoxic vaccine will probably be the most appropriate; it might be combined with a pre-erythrocytic vaccine to enhance protection of the individual, and/or with a transmission-blocking vaccine, to protect the blood-stage vaccine against selection of refractory antigenic types. Natural infection would be expected to continue, and natural boosting would be desirable; if baseline transmission is high, the inclusion of a pre-erythrocytic vaccine and/or transmission-blocking vaccine in EPI would presumably leave sufficient boosting. The health benefit may only be known after some 5 to 10 years, allowing for enough vaccinated infants to be followed through the dangerous life period (whose length is inversely related to the intensity of transmission), and even beyond, to allow the detection of any rebound. The inclusion of a malaria vaccine in the EPI raises the following issues: (i) possible interference between the malaria vaccine and the other EPI vaccines; (ii) optimal timing of malaria vaccination; (iii) possibility of adapting the existing EPI schedule accordingly (see WHO 1993); (iv) possible effect on the acceptability of the EPI programme of a vaccine that was partially successful, or perceived as such (because it would not protect against *P. vivax* or non-malarious fevers).

(b) Vaccination of non-immune immigrant settlers before their arrival in an endemic area in which significant transmission is expected to continue. The above considerations regarding vaccine types apply here as well. The health benefit may be known within 2 or 3 years.

(c) Periodic mass vaccination, without time-limit, in the resident population of an endemic area in which significant transmission is expected to continue. The type of vaccine desirable would be the same as under (a) and (b). Sustainability of vaccination will be a major problem.

(d) Mass vaccination(s) as part of a time-limited campaign aiming at the elimination (or near elimination) of malaria. Sustainability of the results achieved would be a major problem. A vaccine of maximum efficacy is desirable, and a combination of pre-erythrocytic vaccine, blood-stage vaccine, antitoxic vaccine and transmission-blocking vaccine may be appropriate.

(e) Mass vaccination as part of the short-term management of a malaria epidemic. The type of vaccine desirable would be the same as under (c), but a very short immunization schedule (e.g. a single injection) would be required.

(f) Protection of non-immune temporary visitors to endemic areas (e.g. travellers, seasonal labour). Demand is likely to increase, given the problems of chemoprophylaxis. The best vaccine would be a very effective pre-erythrocytic vaccine, although a good blood-stage vaccine or antitoxic vaccine could still be worthwhile for this purpose. In contrast with the other uses considered, a relatively short protection may be acceptable, and for some of the visitors (e.g. well-off travellers) a relatively high cost may also be acceptable.

(g) Selective vaccination of pregnant women. Pregnant women - and their offspring - are a high risk group. Their protection by chemoprophylaxis is hampered by problems of coverage and drug resistance and potential drug toxicity. Their protection by vaccination raises several (hopefully soluble) problems, concerning placental malaria, vaccine types, timing of vaccination, and preservation of the kind of natural immunity acquired during the first pregnancy.

Among the 7 types of uses considered, (a), (b) and (f) are probably more plausible, at present, than the others, while (a), (b) and (g) are probably the most important for public health.

2. Efficacy endpoints, cofactors , and the limits of extrapolation

2.1 *Epidemiological background*

Figure 1 is a schematic representation of the relationships among: (1) the five candidate primary efficacy endpoints of malaria vaccine field trials (i.e. incidence of

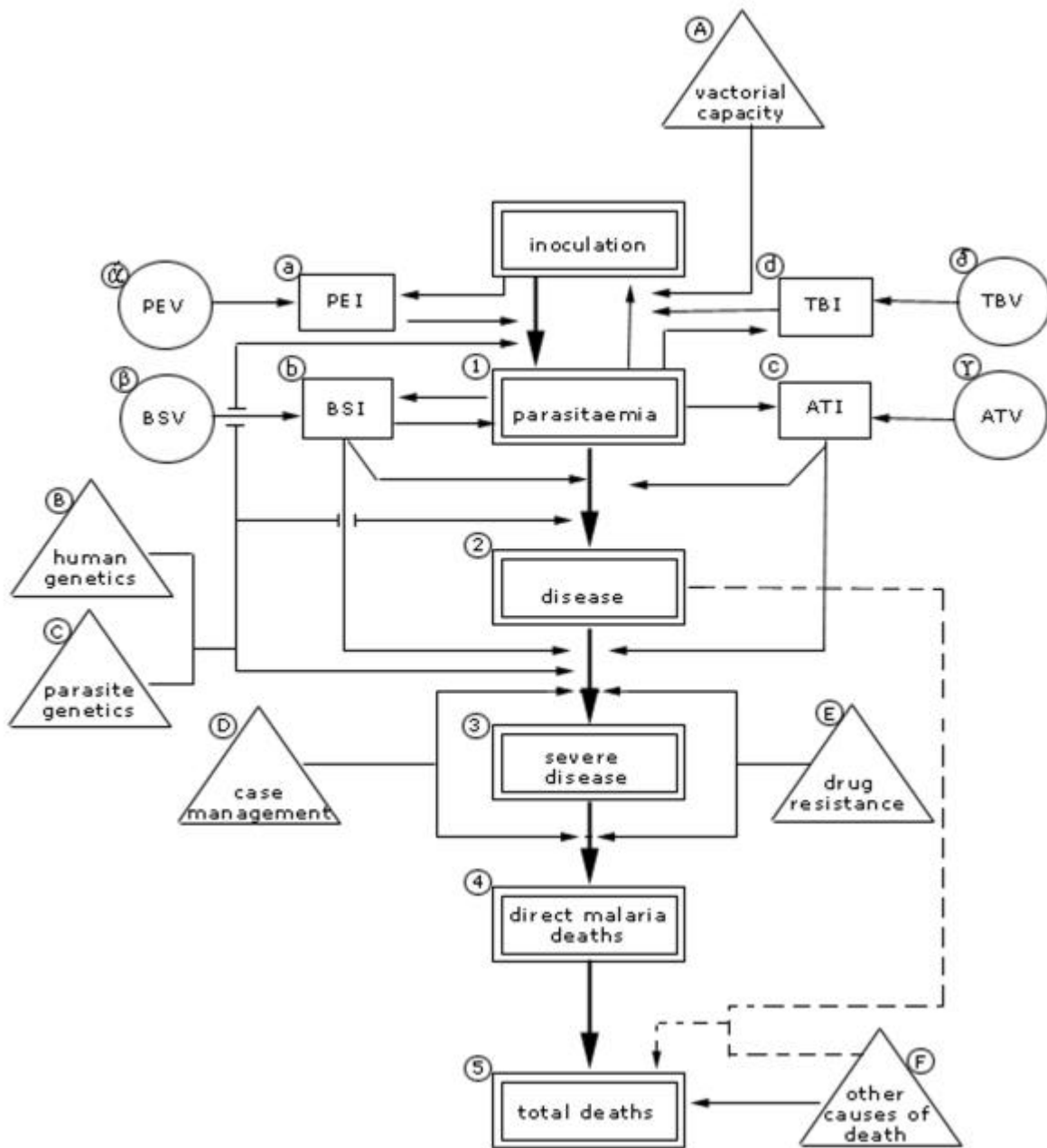


Fig 1 Relationships among endpoints, immunity, malaria vaccines, and cofactors

(1) – (5) the five endpoints whose incidences are the five candidate primary efficacy endpoints

(a) - (d) the four components of protective immunity, i.e. pre-erythrocytic (PEI), asexual blood stage (BSI), anti-toxic (ATI), and transmission-blocking(TBI)

(α) – (δ) four types of malaria vaccines corresponding to the four components of immunity (PEV= pre-erythrocytic vaccines), BSV= asexual blood stage vaccines, etc.)

(A) – (F) additional cofactors (C= parasite genetics, other than drug resistance)

Note: (i) human and parasite genetics (B and C) and their interactions are also likely to affect the development of natural immunity and the inoculation to PEI and parasitaemia to BSI, ATI, TBI) and vaccine-induced immunity (the transitions PEV to PEI, BSV to BSI, etc, (ii) the dotted line indicates that “non-severe” malaria may also kill, with the help of some other cause(s) of death.

parasitaemia, incidence of disease, etc; they may simply be called endpoints, for short); (2) the four components of protective immunity (i.e. pre-erythrocytic immunity, etc); (3) the four corresponding types of malaria vaccines; (4) six additional cofactors (i.e. vectorial capacity, human genetics, etc), each comprising multiple interacting factors; these cofactors are called additional, because the components of protective immunity are also potential cofactors.

However schematic, Fig. 1 is adequate to illustrate the following points: (1) due to the cofactors, extrapolation from impact on a given endpoint (e.g. incidence of disease) to impact on downstream endpoints (e.g. incidence of severe disease) is rather uncertain; (2) the endpoints are more or less remote from the molecular biological targets of the vaccines, and the number of cofactors increases with remoteness; (3) the biological targets of pre-erythrocytic vaccines and transmission-blocking vaccines are clearly upstream from the earliest endpoint (incidence of patent parasitaemia), while blood-stage vaccines and antitoxic vaccines are likely to act both between emergence of parasites from the liver and onset of disease and between onset of disease and the development of severe disease. The relative importance of these two effects is likely to vary among vaccines (especially among blood-stage vaccines, due to the large number of antigens involved).

The relationship between malaria deaths and total deaths will be affected by:

- (a) competition: different causes, each sufficient to kill, compete for the same population;
- (b) interaction: different causes, each insufficient to kill by itself, are sufficient to kill when they coincide in the same person (e.g. the case-fatality rate of malaria is likely to increase in the presence of pneumonia, and vice-versa); and
- (c) aggregation of risks within persons: a person, at higher than average risk of dying from one cause, eg. malaria, is likely to be at higher than average risk of dying from some other causes, eg. measles or gastro-enteritis. In several well-documented cases, effective malaria control (through DDT spraying in Sri Lanka and Guyana; through impregnated mosquito nets or chemoprophylaxis in The Gambia; Greenwood et al., 1987, 1988; Molineaux, 1985; Alonso et al., 1993) prevented many more deaths than the number of deaths previously attributed to malaria in the same populations. This suggests interaction, as defined above, among causes of death (and/or low sensitivity of the diagnosis of deaths due to malaria). The difference between the mortality preventable by malaria control and the mortality attributed to malaria before control may be called indirect malaria mortality. On the other hand, an expected consequence of aggregation (as defined above) would be that the medium- to long-term impact of an intervention on mortality is likely to be smaller than the short-term impact: some of the deaths prevented in the short-term by interventions against one cause of death are in fact only delayed for a relatively short time, due to the competition of other risks and to their aggregation in high-risk individuals. The effects of competition and aggregation are likely to be largest in the least developed areas of the world, which include the most malarious areas.

Table 1 identifies the sources of variation that could affect components of immunity and other cofactors, both independently of vaccination and following vaccination. However conjectural, the table has some important implications: (1) the ratios among endpoints (e.g.

Table 1. Sources of variation that could affect components of immunity and other cofactors (PEI, PEV, etc. defined as in Fig. 1)

COFACTORS	SOURCES OF VARIATION ₁					
	vaccination-independent		vaccination ₂			
Components of immunity	place	time	PEV	BSV	ATV	TBV
pre-erythrocytic (PEI)	+	" ₃	+(8)	! ₄	!	+(9)
blood-stage (BSI)	+	" ₃	+(9)	+(8)	! ₇	+(9)
antitoxic (ATI)	+	" ₃	+(9)	+(9)	+(8)	+(9)
transmission-blocking (TBI)	+	" ₃	+(9)	+(9)	!	+(8)
Other cofactors						
vectorial capacity	+	+	!	!	!	!
human genetics	+	!	!	!	!	!
parasite genetics ₅	+	-	" ₈	" ₈	!	" ₈
drug resistance	+	+	!	!	!	!
case management	+	+	!	!	!	!
other causes of death ₆	+	+	+	+	+	+

1 +, !, and " identify, respectively, probable, improbable, and possible sources of variation of the cofactors; (8), (9) identify the expected effect (increase, decrease) of different vaccines on different components of immunity.

2 In the absence of data, the types of variation expected following vaccination are necessarily hypothetical. The hypothesis is that each type of vaccine is expected to increase the corresponding component of immunity while the other components of immunity are expected either to decrease - through decreased stimulation - or to remain unaffected. Epidemiological observations suggest that the increase in blood-stage immunity associated with aging under continuous exposure, is associated with a decrease in antitoxic immunity.

3 Expected variation inversely related to the stability of the malaria situation, hence to the average vectorial capacity

4 High coverage with an effective blood-stage vaccine might reduce the inoculation rate, hence reduce pre-erythrocytic immunity

5 Other than drug resistance

6 Mortality from other causes of death will change after vaccination if there is competition and aggregation among causes of death (see text)

7 If an increased antitoxic immunity reduces use of antimalarials, it might allow an increase in blood-stage immunity (though increased stimulation)

8 Possible selection of vaccine-refractory genotypes

the ratio of the incidence of disease to that of malaria deaths) could show large geographical

and short-term historical variation; (2) the ratios among endpoints could change progressively, to an unknown extent, after vaccination; (3) while, within a trial, cofactors may be taken care of by randomization (preceded by stratification, if appropriate), the same cofactors are likely to constrain extrapolation from the trial's results to the future or to other geographical areas, and the severity of the constraint is likely to increase with the distance between the vaccine's biological target and the trial's primary efficacy endpoint.

It will eventually be required to compare the efficacy of different vaccines, of similar or different types. Given the geographical variation of potential cofactors, such comparisons will require randomization within local trials. Eligible endpoints include all those that are not upstream from the biological target(s) of any of the vaccines included in the comparison (e.g. a pre-erythrocytic vaccine and an antitoxic vaccine could be compared in terms of incidence of disease, but not in terms of incidence of infection). With respect to extrapolation, one may probably conjecture that the relative efficacy of different vaccines will vary jointly among eligible endpoints and geographical areas (e.g. the relative efficacy of vaccines A & B against disease may not be the same as their relative efficacy against infection, and the relative efficacy of vaccine A - or B - against those two endpoints may not be same in area X as in area Y). The ranking of vaccine efficacies might be more robust, but even that is not certain.

2.2 The selection of a field trial's primary efficacy endpoint

Each field trial requires a single primary efficacy endpoint, namely the endpoint used for the calculation of sample size and the estimation of vaccine efficacy (VE). The above epidemiological background should be taken into account when selecting a trial's primary efficacy endpoint.

One might argue as follows: given (i) the uncertainty of extrapolation from efficacy against early (or mild) endpoints (i.e. infection or disease) to efficacy against later (or severe) endpoints (i.e. severe disease, malaria-specific or total mortality); and (ii) the greater public health importance of the later (severe) endpoints, the primary efficacy endpoint of field trials should be one of the severe endpoints. In particular, where the baseline malaria mortality is probably high, the endpoint of choice should be total mortality, as it is the only way to measure impact on indirect malaria mortality, which can be quite large (see above, A2.1).

There are, however, some (overlapping) epidemiological and ethical constraints:

(1) Epidemiological constraints: (i) trials would have to be relatively long (>5 years), to take into account the probable effect of vaccination on cofactors; that constraint may be manageable, although it would be cumbersome in case several vaccine candidates were to become available within a relatively short time; (ii) as pointed out, the greater the distance between the vaccine's biological target and the trial's endpoint, the larger the number of cofactors, and the narrower the applicability of the results in space-time; and the systematic evaluation of the impact of a vaccine (on a late-severe-endpoint) throughout the existing range of combination of cofactors is unlikely to be feasible.

(2) Ethical constraints: (i) once efficacy has been demonstrated against an early

(mild) endpoint (e.g. incidence of uncomplicated malaria (UM)) it may be ethically questionable to conduct a double-blind randomized controlled trial (DB-RCT) to measure efficacy against a later (severe) endpoint; (ii) this problem might be avoided by including the severe endpoint in early trials (Smith & Hayes, 1991), but that would require a large jump in the number vaccinated, which is questionable with respect to safety, including safety under natural challenge.

A way out of the dilemma may be to plan carefully the respective roles of Phase III and Phase IV trials: (a) Phase III DB-RCT-s against early (mild) endpoints may be adequate for: (i) registration; (ii) ranking of different vaccines; and (iii) decision to undertake Phase IV trials (as scientifically rigorous as possible, e.g. using the stepped wedge design, including randomization, but not DB-RCT) against late (severe) endpoints; (b) Phase IV trials should be conducted only within national programmes committed to use the vaccine, should it satisfy their previously established criteria; (c) because of the wide variation of cofactors, each national programme, before final and generalized adoption of a malaria vaccine, is likely to require its own evaluation of effectiveness - on a limited (though adequate) scale, and on a trial basis - i.e. its own Phase IV trial.

Following the above line of argument, the primary efficacy endpoint of a Phase III field trial should be one that is as close as possible downstream from the vaccine-s molecular biological target(s). For pre-erythrocytic vaccines and transmission-blocking vaccines, the incidence of infection is a clear choice. For blood-stage vaccines and antitoxic vaccines, the incidence of disease (uncomplicated malaria) is probably an acceptable choice, even though part of the vaccine-s molecular biological targets may be further downstream. The later (more severe) endpoints would then be addressed in Phase IV trials. The methods of measurement covered in this document (see below, E) will include all five candidate primary efficacy endpoints.

3. Phases, target groups and study designs of human vaccine trials

3.1 Phases I to IV

Phase I to IV trials are defined in terms of challenge, objectives and registration status (WHO, 1985), as summarized in Table 2. The place of Phase IIa trials (artificial challenge) is debatable. They raise technical and ethical problems, and their predictive value is limited by the obligation to treat relatively early in the course of infection and by the uncertain relationship between artificial and natural challenges; on the other hand, the predictive value of *in vitro* experiments and of animal models is at least equally limited. Phase IIa trials are certainly not indispensable for all vaccines (e.g. they are not applicable to transmission-blocking vaccines); their place and their technical and ethical problems will be addressed in a forthcoming expert consultation. Phase IIb and III trials are field trials *sensu stricto*, and the main topic of the present document; phases IIb and III had originally been distinguished by

Table 2. Human trials of malaria vaccines: phases and their main characteristics

Phases And sequence	Challenge	Objectives		Appropriate design		Scale (number vaccinated)
		primary	secondary	type of comparison	unit of comparison	
I 9	none	safety and immunogenicity	-	DB-RCT ₃	individual	small (tens)
(IIa) ₁ 9	artificial	efficacy	safety and immunogenicity	DB-RCT ₃	individual	small (tens)
IIb - III ₂ 9	natural	efficacy	safety and immunogenicity	DB-RCT ₃	individual or community ₆	medium (hundreds to thousands)
REGISTRATION 9 IV	natural	effectiveness	safety	Astepped wedge ₄ <u>and</u> case-control ₅	unit of management in a national programme individual	large (tens of thousands to hundreds of thousands)

₁ optional; ₂ there is no essential distinction between phases IIb and III; ₃ double-blind randomized controlled trial; ₄ Smith & Hayes, 1991; Smith & Morrow, 1996; ₅ not applicable to transmission-blocking vaccines; ₆ transmission-blocking vaccines require the comparison of communities; for the other vaccine types the comparison of individuals is more efficient

scale (WHO, 1985), but if the scale of all field trials is to be sufficient for testing efficacy, there is no essential distinction between them. Phase IV trials are post-registration trials; they consist of the introduction, on a trial basis, and on a limited scale, of a vaccine in a control programme; they are field trials *sensu lato*, amenable to scientific evaluation, and, as such, an essential component of vaccine evaluation.

3.2 Target groups

For malaria control, the target group for vaccination may vary from the total population to a very limited subgroup (e.g. infants). On the other hand, for ethical reasons, a new product should be tried first in those probably most tolerant of possible toxicity, i.e. healthy adults (excluding pregnant women), while those probably least tolerant of possible toxicity (young children, infants, pregnant women) may be included only later, and only if they belong to a naturally exposed population. Furthermore, before conducting an efficacy field trial in a given population, or population subgroup, it is required to conduct a safety and immunogenicity trial (i.e. a Phase I trial) in the same population or subgroup.

The evaluation of a vaccine will involve a conditional sequence of trials, taking into account phases and target populations and groups. A possible sequence, for a vaccine eventually destined for use in infants, could be (a) Phase I in unexposed adults; (b) Phase IIa (optional) in the same group; (c) Phase I in adult males of an exposed population; (d) Phase IIb-III in the same group; (e) and (f) Phases I and IIb-III in children of the same population; (g) and (h) Phases I and IIb-III in infants of the same population. The sequence could start in (c); the following could be concurrent: (d) and (e), (f) and (g). If infants are, *a priori*, the only eligible target group (e.g. if the EPI is the only feasible method of distribution) the minimum required sequence can be much shorter, e.g. (a), (b), (c): Phase I trials in local adults, children, infants, respectively; (d) Phase IIb-III in infants. The most appropriate sequence will vary among vaccines and epidemiological situations, and requires careful consideration (see below, B 3.2).

3.3 Basic study designs

Table 2 lists the basic study designs appropriate for the different phases. Phases I to III should be based on double-blind, randomized, controlled (DB-RCT) comparisons, either between vaccinated and unvaccinated individuals (I, II or III) or communities (III only). Transmission-blocking vaccine trials demand comparison between communities (or transmission units, see C2, para. 1 & 2), while for pre-erythrocytic vaccines, blood-stage vaccines and antitoxic vaccines both types of comparison are, in principle, eligible. The following considerations apply: (a) if vaccination of the community involves high coverage, the two comparisons are not equivalent, as high coverage may significantly reduce transmission, which may reduce morbidity, but might also have undesirable consequences: loss of the boosting and immunizing effects of natural exposure, increased selection of vaccine-refractory parasites; (b) community vaccination is closer to real-life malaria control (even so, long-term effectiveness is only poorly predictable from field trials *sensu stricto*) (c)

the risk of accidental bias is smaller with comparisons between individuals, because the units of randomization are more numerous, and easier to stratify (or match) by exposure; (d) comparing communities is likely to be more expensive and may last longer (it may be necessary to start with a baseline comparison, before vaccination, for a period of least 1 year); (e) the two comparisons are unlikely to be used sequentially, as proof of efficacy in the individual would make the DB-RCT comparison of communities ethically questionable. In conclusion, the comparison between individuals will be preferred for field trials (*sensu stricto*) of pre-erythrocytic vaccines, blood-stage vaccines and antitoxic vaccines leaving further questions (e.g. long-term effectiveness) for phase IV trials.

In areas of relatively low baseline transmission, the vaccination of a fraction of the community with a pre-erythrocytic vaccine or a blood-stage vaccine might reduce transmission to the point where the trial would lose adequate power. In order to reduce such possible loss of power, it may be prudent to include no more than 25% of a trial community in the vaccinated group (see E 2.3(d); the figure of 25% is arbitrary). If that is still likely to reduce transmission (hence the power of the trial) too much, it would be preferable to reconsider the selection of the study area.

In Phase IV trials standard DB-RCT comparisons are not feasible, but a fairly rigorous evaluation is nevertheless possible, through the use of the stepped-wedge design (involving the randomized stepwise introduction of an intervention in successive population units) (Smith & Hayes, 1991; Smith & Morrow, 1996) and of case-control studies, preferably in combination. Indeed, some important public health questions can only be answered at that stage. The methodology of Phase IV trials of malaria vaccines is beyond the scope of this document and probably requires separate guidelines, including the discussion of study designs.

4. From Phase III trials to a regular vaccination programme: a tentative conditional sequence

However problematic, speculation concerning probable sequences may be useful for planning the further development of malaria vaccines.

(1) Phase III trials

Table 3

	TBV	PEV	BSV	ATV
Design	←————— DB-RCT —————→			
Endpoint*	←———— Infection —————→		←———— Disease —————→	
Unit of comparison	Community	←———— individual —————→		

* for vaccines combining (TBV &/or PEV) with (BSV &/or ATV), the endpoint is disease

(2) If the Phase III trial(s) demonstrate(s) protection, at a predetermined level (e.g. 50%), against the predetermined primary endpoint, with a predetermined confidence (e.g. 95%), identify one or more situations that fulfill 4 criteria: (i) political will to use the vaccine, should it pass the next test (i.e. the Phase IV trial); (ii) programmatic use of the vaccine is likely to be operationally feasible; (iii) it is also likely to yield a measurable epidemiological benefit; and (iv) a rigorous Phase IV trial is probably feasible. Registration/licensing (by an internationally recognized registration authority and by the relevant national authorities), is expected between Phases III and IV. One or more phase I trials (safety and immunogenicity) may have to be repeated in the area(s) selected for Phase IV trial(s). In addition, all available safety follow-up data of phase I-III trials with the same product should be reviewed.

(3) Phase IV trial(s)

Table 4

		TBV	PEV	BSV	ATV
design		←———— Stepped-wedge —————→ plus ←———— case-control —————→			
Endpoint	If baseline mortality is high	←———— Total mortality* —————→ plus ←———— disease —————→ plus ←———— infection —————→			
	If baseline mortality is low	←———— Severe disease ** —————→ plus ←———— disease —————→ plus ←———— infection —————→			

* total (all cause) mortality is preferable to severe disease and malaria-specific mortality, because it is easier to measure (see below, E.1.4 & 5) and because it measures indirect mortality (see above); in situations where it is convenient to measure severe disease or malaria-specific mortality, they may be additional endpoints.

** this endpoint may be waived in situations where it would not be measurable at an acceptable cost.

(4) On the basis of the results of the Phase IV trial(s), national authorities will decide whether or not to proceed with programmatic use of the vaccine.

5. Vaccination, antigenic selection, and interaction among Plasmodium species

(a) in wild parasite populations, many antigens are diverse, and vaccines may be active against only part of the relevant diversity; therefore, vaccination may lead to the selection of refractory antigenic types;

(b) *P. falciparum* probably suppresses other malaria parasites within the host; hence *P. falciparum*-specific vaccines may provoke some deterioration of the epidemiologic situation with respect to the other parasites.

6. Requirements for clinical and field trials and registration (licensing) of a vaccine

6.1 Control of Safety and Efficacy

Ensuring the consistent safety and efficacy of a vaccine has long been recognized as an essential element in a successful disease control programme (Griffiths, 1996). Indeed, the development of appropriate laboratory methods to characterize a vaccine with respect to its component antigens, safety, immunogenicity and potency must be a pre-requisite to the routine clinical use of any new vaccine. When available, an appropriate potency assay may serve as a surrogate marker for clinical protection. However, there are currently no good correlates or surrogate markers of protection against malaria, and their development would reduce the cost of field trials. Adequate product control serves to safeguard vaccinees both against unacceptable adverse effects and inadequate protection.

Special considerations apply to the control of vaccines which do not apply to chemical drugs. This is because of the biological nature of (a) the starting materials, or (b) the manufacturing process and/or (c) the test methods needed to characterize batches of the product. For example, the production of many vaccines involves the culture of cells or micro-organisms, and such systems are inherently variable by nature. Also, vaccines are often highly complex products in molecular terms, and chemical and physical analyses are of only limited value in their characterization. This is in contrast to chemical drugs where definitive chemical analyses can provide an adequate basis for quality assessment. The deleterious effects of drugs are usually based on their chemical nature but experience with vaccines and other biologicals has shown that major problems, or accidents, are usually batch-related and not product-related. This serves to emphasize the need for effective control procedures. Consistency of production is of paramount importance and the demonstration that production lots do not differ from vaccine lots which have been shown to be safe, adequately immunogenic and protective in previous clinical studies is a crucial component of vaccine evaluation and licensing procedures. A vaccine being submitted to a Phase III field study would therefore be expected to have undergone comprehensive characterization.

Regulatory requirements strongly emphasize in-process controls, where tests are carried out on the starting materials and during production, as well as on the final product. The validation of the ability of the manufacturing process to remove unwanted materials is also considered essential. International standards and reference materials play a vital part in the control process, their role ranging from use in specific antigen recognition tests to assays of vaccine toxicity, immunogenicity and biological potency.

6.2 Stages in Regulating Vaccines

The characterization, standardization and control of vaccine preparations during development and clinical testing are key issues, and a well defined candidate vaccine offers by far the best chance of success. If a Phase III clinical trial shows a preparation to be adequately protective, the vaccine must subsequently be produced to the same specifications as the successful preparation. In the case of inadequately defined materials it is never certain whether differences in protection or toxicity, are due to unintentional variations in the vaccine preparations used, suboptimal vaccination schedules, poorly designed trials or differences in target populations. The fact that it took about 50 years from the time of the identification of *Bordetella pertussis* as the causative agent of whooping cough to the licensing of an effective whooping cough vaccine is due largely to the fact that no attempt was made to standardize the preparations used in the many early trials. Only when some degree of standardization occurred did the development of an effective pertussis vaccine become feasible (Griffiths 1988).

At the Phase III stage of a vaccine development it will be expected that comprehensive analysis and characterization of the product will have been undertaken, in order to establish the specifications of the preparation. An acceptable number of consecutive batches of the final formulated product, (say 3-5 batches) and where appropriate, intermediate products, are characterized as fully as possible to determine consistency of composition. Differences between lots are noted, and used for setting limits for routine production when appropriate, the criteria for rejection of harvests and production intermediates should also be defined. Thereafter, for the purpose of batch release following licensing, a more limited series of tests may be appropriate. A clear distinction, therefore, needs to be made between tests performed during the development of a vaccine and tests proposed for use routinely on each production batch of the product. The tests used in routine batch control should be a selection of the tests used to characterize the vaccine initially and for licensing purposes and usually will include tests for identity, immunogenicity, and where appropriate potency.

Changes in production methods at a later date, including scale-up, will necessitate further product characterization to demonstrate equivalence, although the extent of re-characterization will depend on the nature of the changes made. Usually, it is the exact production process, and its size, used to produce vaccine for the Phase III clinical study that will be the one used for the purpose of licensing. Ideally, a Phase III study should be the definitive or pivotal study for licensing.

6.3 Good Manufacturing Practice

Attention is drawn to requirements relating to establishments in which vaccines are manufactured. These can be found in the WHO document Good Manufacturing Practice for Biologicals (WHO, 1992 b). Particular attention needs to be given to the training and experience of persons in charge of production and testing and those assigned to various areas of responsibility in manufacturing establishments. It should be noted that vaccine preparations for Phase III clinical trials also need to be produced under conditions of Good Manufacturing Practice and attention given to developing documented standard operating procedures (SOPs) for both production processes and for testing procedures. These should be introduced as early as possible during the development of a vaccine and be well established by the time Phase III studies are undertaken.

6.4 Independent Batch Release

Although the safety and efficacy of vaccines are primarily the responsibility of the manufacturers, in the interest of public health, vaccines are also subject to batch release by national health authorities. This usually includes independent laboratory evaluation by a national control laboratory which looks especially for trends in quality. However, the extent of laboratory testing by a national control laboratory varies, ranging from examination of manufacturers release protocols to complete laboratory testing for identity, safety, immunogenicity and potency of each lot, as appropriate.

During the development of vaccines, and especially before internationally sponsored Phase III field studies it might be helpful for the vaccine lots to be subjected to independent laboratory evaluation by one or more national control laboratories of international standing (see 6.5).

6.5 Guidelines

WHO requirements or guidelines are available for vaccines and other biologicals of significance and these form the basis for assuring the acceptability of a product globally. Such recommendations provide advice to those responsible for production and control processes and they may be adopted by national health authorities as the basis for national requirements and for licensing.

For vaccines under development, specific WHO, national or pharmacopoeial requirements may not be available and a national health authority will have to agree specifications with the manufacturer on a case-by-case basis during licensing. However, there may be general guidelines on the production and control of recombinant products, such as recombinant DNA-derived vaccines (WHO 1991), DNA-vaccines (WHO 1996a) and synthetic peptide vaccines (In Draft 1996) and these should be consulted. Information about assuring the quality of biologicals in general and on procedures for approving (licensing) products, can be found in "WHO Guidelines for National Authorities on quality assurance for

biological products" (WHO 1992a) as well as in "WHO's Good Manufacturing Practices for Biological Products" (WHO 1992b). WHO's document on "Regulation and licensing of biological products in countries with newly developing regulatory authorities" (WHO 1995a) also contains much useful information, including reference to authorization of clinical trials.

The WHO Guidelines for Good Clinical Practice (GCP) for Trials on Pharmaceutical Products (WHO 1995b) set globally applicable standards for the conduct of biomedical research on human subjects. They should be applied during all stages of product development both prior to and subsequent to product registration and marketing and are applicable, in whole or in part, to biomedical research in general.

B. INFORMATION REQUIRED CONCERNING THE VACCINE BEFORE CONDUCTING AN EFFICACY FIELD TRIAL

The following information should be considered, even though not all of it is indispensable.

1. Product definition (see A. 6 above)

The vaccine must be fully characterized (including manufacture, formulation, quality control and demonstrated consistency of production and stability) and approved for human trial by an experienced registration authority. Acceptable storage conditions must be defined. Route of administration must be specified.

2. Information from preclinical research

(a) *In vitro* observations/experiments concerning the vaccine's molecular biological target(s) and possible mechanism(s) of protection

(b) Safety, immunogenicity and potency, as appropriate in animal models

3. Information from Phase I trials (safety and immunogenicity trials)

3.1 Types of information

(a) Safety & acceptability

The Phase I trials should provide information on the frequency and severity of local reactions at the site of injection after each dose of vaccine and information on the frequency of any general systematic effects such as fever. The latter is particularly important if the vaccine is given to infants.

(b) Immunogenicity

(i) Immunogenicity and/or potency should, preferably, be measured with functional tests which are as close as possible to the vaccine's expected mechanism of protection. Unfortunately, at present, a possibly satisfactory functional test is available only for transmission-blocking vaccines (effect of the vaccinee's serum on the infectivity of gametocytes after membrane-feeding of vectors) and even that test is in need of direct validation. The lack of appropriate functional tests limits the validity of most further information obtained from Phase I trials (i.e. the information listed below (ii), (iv) to (vii), & (c). The development of assays that predict protection is highly desirable and the validation of such assays should be included among the objectives of the field trials.

(ii) If functional tests are available, they are likely to be complicated and expensive, hence generally unsuitable for epidemiological investigations. Therefore, Phase I trials should evaluate simple inexpensive tests (e.g. standard antibody-detection tests) against the best available functional tests.

(iii) Even in the absence of a useful functional assay, it is important to show that a vaccine can induce an immune response, as a test for the integrity of the vaccine and to allow comparisons to be made between batches used in different trials. Measuring antibody, or cellular immune responses, may allow a bad batch to be picked up before a trial is started.

(iv) Duration of immune responses, especially functional ones

(v) Boostability of immune responses by revaccination (optional)

(vi) Effect of current infection (patent parasitaemia), and of its treatment, on immunogenicity

(vii) Possible interaction with current EPI vaccines (indispensable if inclusion of malaria vaccination in the EPI is envisaged) (WHO 1993)

(c) Recommended number, intervals and dosage of inoculations

(d) Possible production of auto antibodies (some *P. falciparum* antigens have homologous domains with some human antigens)

3.2 Populations and target groups

Information from Phase I trials should be applicable to the target population and groups of the efficacy field trial. To ensure this, the Phase I trials should be conducted (i.e., possibly, repeated) in that very population. Before the efficacy field trial in the final target

group (e.g. infants), a sequence of trials may be required, as discussed above (see A.3.2), with appropriate rules for proceeding, or not, from each step to the next.

4. Information from Phase IIa trials (artificial challenge trials)

Given the constraints and limitation of artificial challenge trials (see above), they are likely to be conducted only with some of the vaccines, and must probably be considered optional. When available, they yield further information on the points listed under Phase I, plus new information on:

(a) efficacy, including its relation to challenge dose, its duration and boostability by revaccination or by challenge (optional)

(b) evaluation of simple and inexpensive immunological tests as indicators of protection.

If the artificial challenge trial has been conducted in an unexposed population, its repetition in the target population of the efficacy field trial is probably of low priority: it will be cheaper and faster to go directly to natural challenge, and probably more informative, given the questionable representativity of artificial challenges.

5. Information from prior efficacy field trials (Phases IIb or III) with the same vaccine

Some efficacy field trials may already have been conducted with the same vaccine, perhaps in different epidemiological situations or target groups, or with different endpoints. All these trials should be critically and independently reviewed, before deciding to conduct a new trial, and in order to define its expected contribution, and to design it accordingly.

C. BASIC SPECIFICATIONS OF A PHASE III FIELD TRIAL

In addition to the specification of the vaccine to be tested, the basic specifications of a field trial are the trial's objectives, the basic study design, the target groups, and the sample size.

1. Objectives

The specification of a trial's objectives consists mainly of the identification of its endpoints.

1.1 **Efficacy (Protection)**

1.1.1 *Primary efficacy endpoint*

The primary objective of a trial will be to evaluate protection against a single primary efficacy endpoint, namely the one used for the calculation of sample size and the estimation of vaccine efficacy. The selection of such an endpoint has been discussed above (see A, 2). For Phase III trials of pre-erythrocytic vaccines and transmission-blocking vaccines, the recommended primary efficacy endpoint is the incidence of infection (even though there could be a beneficial effect on other indices of morbidity). For Phase III trials of blood-stage vaccines and antitoxic vaccines the recommended primary efficacy endpoint is the incidence of disease. However, if there are good basic scientific reasons to suspect that a candidate blood-stage vaccine or antitoxic vaccine acts mainly between onset of disease and the development of severe disease, the primary efficacy endpoint should be one of the late (severe) endpoints, preferably the incidence of severe disease. While it is obviously desirable to determine the duration of protection, as well as its boostability by natural infection, the possibility to do so will be constrained by the study design (see below, C.2).

1.1.2 *Secondary efficacy endpoints*

(1) Among the other candidate primary endpoints

(a) Whether the primary endpoint is the incidence of infection or the incidence of uncomplicated malaria (non-severe *P.falciparum* disease), the sample size will be too small to measure efficacy against any of the severe endpoints. It is, nevertheless, recommended to monitor deaths, and to investigate them through medical records and verbal autopsy, and, if possible, to monitor also the incidence of severe disease (severe and complicated malaria), including through the local hospital(s).

(b) Because of potential interference among measurements, protection against infection and protection against disease should not be measured in the same sample (see below, E1.13). Therefore, the addition of either of these two protections, as a secondary efficacy endpoint, requires a second, non-overlapping, population sample. The second sample required will be larger than the first one if the secondary endpoint is disease, smaller than the first one if the secondary endpoint is infection. The inclusion of the secondary endpoint should be weighed against the cost.

(2) Other possible secondary efficacy endpoints

(a) Prevalence and density of parasitaemia (including gametocytes and other Plasmodium species), recommended in all trials

- (b) Parasite density threshold (the parasite density above which an associated fever is generally attributable to malaria): indispensable as an endpoint for antitoxic vaccines; useful to monitor for the other vaccine types, as they might lower antitoxic immunity
- (c) Haemoglobin (or haematocrit), recommended in all trials, wherever malaria-related anaemia is probably common (improvement generally expected, but some speculate that an antitoxic vaccine might lead to higher parasite densities - presumably through reduced consumption of antimalarials - and hence to anaemia).
- (d) Infection in the vector: indispensable as an endpoint for transmission-blocking vaccines; useful to monitor for the other vaccine types, as it provides an independent measure of the challenge
- (e) Infectivity of humans: indispensable as an endpoint for transmission-blocking vaccines; may also be useful for other vaccine types (a blood-stage vaccine may affect the production of gametocytes, an antitoxic vaccine might affect their infectivity)
- (f) Parasite diversity (polyclonality), within isolates
- (g) Analysis of breakthroughs for parasite polymorphisms

1.2 Immunogenicity

1.2.1 Immunogenicity of the vaccine

Immune responses to the vaccine should be measured, using appropriate tests, i.e. adapted to the vaccine's composition, as well as to field conditions. Immune responses tested in the field will be mainly humoral; the assessment should if possible, be epitope-specific by antibody classes and subclasses. A subsample may be adequate for the assessment of immunogenicity, but not sufficient to establish a correlation with protection.

1.2.2 Correlation between immunogenicity and protection

Possible correlations between protection and specific immune responses should be investigated, as they may assist the further development of vaccines (e.g. combination of antigens or optimization of dosage regimens), as well as their further evaluation (e.g. in control programmes) (see also E 1.9, last paragraph).

1.2.3 Evaluation of simplified field tests

Candidate field tests can be evaluated against protection and/or against tests previously shown to measure protection (directly or indirectly).

1.2.4 *Boostability*

It is recommended to evaluate possible boosting, through natural infection, of immune responses to the vaccine.

1.3 **Safety**

The vaccine has been found safe in its early trials, but monitoring for possible side-effects must continue during the field trials (and beyond). In comparison with earlier trials, field trials introduce new potential risk factors: larger numbers, different populations, and exposure to natural infection(s).

1.4 **Possible effect on other human malarias**

It is strongly recommended to monitor the possible effect on the prevalence and density of sympatric human malaria parasites, by including their assessment in the parasitologic surveys included in the trials.

2. **Basic study design**

The only fully adequate design requires a double-blind, randomized, controlled (DB-RCT) comparison between vaccinated and unvaccinated. For reasons already given, the appropriate unit of randomization is the individual for pre-erythrocytic vaccine, blood-stage vaccine and antitoxic vaccine trials, the community (or transmission unit) for transmission-blocking vaccine trials. An ideal transmission unit would consist of a localized human population and a localized vector population, sharing their man-vector contacts exclusively with each other; in real communities there will be variable contamination by other human and vector populations.

Individuals may be stratified, before randomization, by variables likely to affect exposure or susceptibility, eg. residence, age, sex, and, in certain situations, ethnic group or occupation. Such stratified randomization will yield balanced groups, with a high probability. The probability of obtaining balanced groups through randomization of a necessarily small number of communities will not be high. The design can be improved by including an adequate baseline period (eg. 1 year) in the estimation of efficacy (see below, E.3) and by using the baseline data to stratify the communities, (e.g. into pairs of greatest similarity with respect to the primary endpoint).

The duration of a trial is - in principle - predetermined; it enters into the calculation of the sample size (see below, C.4) and ends with the breaking of the code. This may constrain the measurement of the duration of protection: (a) if the vaccine shows efficacy, it might be unethical not to offer vaccination to the control group, so that further protection could not be evaluated; conditions for prolonging the comparison should, as far as possible, be laid down

in advance; (b) the sample size calculated on the basis of the expected incidence during the whole trial period, is unlikely to allow the detection of significant variation of protection in the course of that period. For the same reasons, an adequate evaluation of the boostability of protection by natural infection will be impossible; the trial may, at best, give a hint.

3. Trial target groups

The trial target group should be clearly defined, eg. children aged 1-4 years at first inoculation, or infants in an EPI programme, etc. The target group of a field trial should preferably be the probable target group of the vaccination programme, but it may be prudent, before conducting a trial in infants, to conduct first a field trial in some other group presumably less fragile, eg. in children aged 1-4 (see A.3.2).

4. Sample size

In any vaccine trial, it is important that the number of participants recruited should be sufficient to provide reliable answers to the main study objectives. If the sample size is too small, an important protective effect may be missed, while if the vaccine is ineffective the confidence limits on the efficacy estimate may be too wide to rule out an important effect. Conversely, unnecessarily large sample sizes waste resources, inconvenience the study population, and may endanger data quality.

Most trials have several objectives, and sample size computations should be carried out for each of these objectives. A minimum requirement, however, is that the trial is large enough to address the primary objective of the trial, and this is the main focus of this section.

As discussed in C1.1, the primary objective of a Phase IIb/III trial is to evaluate the protection conferred by the vaccine against a single primary efficacy endpoint. This endpoint may be infection, mild disease, severe disease or death. In each case, the primary analysis will be based on comparison of the incidence rate of this endpoint (first or only occurrence) in the vaccine and placebo groups.

4.1 *Sample size for individually randomized trials*

Assuming the vaccine and placebo groups are of equal size (generally the most efficient design), Table 5 shows the person-years of observation (y) required in each group if we wish to obtain a significant effect ($P < 0.05$, 2-sided) with 90% power, for various values of the incidence rates in the placebo group (r_0) and vaccine group (r_1). The protective efficacy is $100 \times (1 - r_1 / r_0) \%$.

Table 5. Person-years of Observation Required

Rate (/100y) in placebo group (r_0)	Protective Efficacy		
	25%	50%	75%
0.5	58,800	12,600	4,667
1.0	29,400	6,300	2,333
2.0	14,700	3,150	1,167
5.0	5,880	1,260	456
10.0	2,940	630	233
20.0	1,470	315	117

These figures were obtained using the approximate formula:

$$y = 10.5 (r_0 + r_1) / (r_0 - r_1)^2$$

For 80% power, the factor 10.5 is replaced by 7.84.

Given the duration of follow-up, the number of person-years can be translated approximately into the number of subjects to be recruited. For example, with two years of follow-up, 5,000y requires the recruitment of approximately 2,500 subjects.

Clearly very large sample sizes are needed to evaluate effects on mortality rates, which may be of the order of 1/100y. Much smaller samples may be required for less severe endpoints, for example infection or mild disease, for which rates may exceed 20/100y in highly endemic populations.

Adjustments should be made to the above sample sizes to allow for losses to follow-up, or for any interim analysis planned in the study protocol (Smith & Morrow, 1996).

Example 1

An individually randomized Phase III trial of an ABV is to be conducted among children aged 1-4 years in a rural African population. The primary endpoint is clinical malaria, as detected by PCD through the health unit. Previous data suggest that in the absence of vaccination, about 10% of children are diagnosed with clinical malaria each year. The follow-up period is one year, and 90% power is required to detect an efficacy of 50%.

Reference to the table indicates that around 630 children would be required in each group. Suppose we expect 10% of children to be lost to follow-up over one year, for example due to migration. If losses occur uniformly over the follow-up period, this will result in a shortfall of 5% in person-years. To allow for this, recruitment must be increased by a factor 1/0.95 to give 663 in each group.

4.2 Precision of estimates

In early trials, the main objective may be to establish that a vaccine has any effect, in which case sample size computations based on power considerations seem appropriate. If precise estimates of protective efficacy are required, however, much larger sample sizes may be needed.

Suppose we wish to obtain a 95% confidence interval (CI) for the rate ratio ($R = r_1/r_0$) extending from R/f to Rf . Then the required person-years in each group are given by the formula:

$$y = (1.96/\log_e f)^2 (1/r_0 + 1/r_1)$$

Example 2

In example 1, suppose we require $f = 1.25$, with an expected CI extending from $0.5/1.25$ to 0.5×1.25 , or 0.40 to 0.62 . That is, we wish to estimate the protective efficacy to within about 10%. From Formula (2) we obtain $y = 2,315$ person-years in each group.

With $y = 630$, as obtained from power computations, we obtain $f = 1.53$ and an expected CI from 0.33 to 0.77 . This may or may not be adequate for the purposes of the trial.

4.3 Randomization by community

When allocation is by community, as in a Phase III trial of a TBV, sample sizes must be increased because responses in individuals within the same community are likely to be correlated. Formula (1) therefore has to be adjusted to allow for the intrinsic variation between communities. The number of communities required in each group, c , is given by:

$$c = 1 + 10.5 \left((r_0 + r_1) / y + k^2 (r_0^2 + r_1^2) \right) / (r_0 - r_1)^2$$

where y is now the person-years of observation in each community, and k is the coefficient of variation (SD/Mean) of the (true) incidence rates among the communities in each group.

An estimate of k will sometimes be available from previous data on the same communities, or from a pilot study. If no data are available, it may be necessary to make an arbitrary but plausible assumption about k . For example, $k = 0.25$ implies that the true rates in each group vary roughly between $r_1 \pm 2kr_1$, that is between $0.5r_1$ and $1.5r_1$. In general, k is unlikely to exceed 0.5.

Example 3

In a TBV trial, suppose the primary endpoint is infection, and that a rate of 10/100y is expected in the placebo group as in Examples 1 & 2. About 100 subjects are available in each community, and are to be followed for one year ($y = 100$). We require 90% power if the vaccine reduces the infection rate by 50%.

Assuming $k = 0.25$ we obtain $c = 10.6$, so that 11 communities would be required in each group. The total person-years per group would be 1,100, much higher than the 630 required in an individually- randomized trial. The design effect is about $1,100/630 = 1.7$. With $k = 0.5$, we obtain $c = 20.4$ and a design effect of 3.3.

If communities are randomized after matching or stratification, for example using baseline data, the effect of between-community variation should be reduced, and the above computations will be conservative.

A more detailed discussion of sample size computations is given in Smith & Morrow, 1996.

D. CRITERIA FOR THE SELECTION OF STUDY AREAS, AND BACKGROUND INFORMATION REQUIRED

1. Criteria for the selection of study areas

1.1 *Operational criteria*

Highly desirable characteristics of potential trial sites include:

- (a) Informed and firm commitment from national and local 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) Informed and firm commitment from the study population to the trial and the associated investigations. This commitment should be obtained without Aoversell®, to avoid giving a false sense of security, which might delay treatment. The conduct of a trial will involve the population in inconvenience, including the donation of blood specimens. It will also be necessary to recruit key informants to assist in the collection of morbidity and mortality data.

- (c) Maximal involvement of national research institutions with interested and experienced national investigators and field and laboratory teams. If building up of national experience is required, it should receive high priority.
- (d) Availability of background data on the epidemiology of malaria;
- (e) Reasonable expectation of social and political stability at the national and local levels for the duration of the trial;
- (f) Low expected emigration rate for the duration of the trial (to minimize the drop-out rate)

1.2 *Epidemiological criteria*

1.2.1 General

It is preferable to conduct vaccine trials in situations in which incidence is sufficient to make efficacy relatively easy to measure and in which vaccination is likely to be relevant to malaria control (the two criteria will not always coincide). Potentially eligible situations are likely to be many and varied. Priority for vaccine trials should be given to situations likely to give most information for a given investment. The following populations probably merit highest priority: residents of areas of intense seasonal transmission; residents of areas of intense perennial transmission; non-immune (previously little or not exposed) immigrants of different age-groups settling into areas of intense transmission. The preference for intense transmission is justified on two grounds: challenge and protection are easy to measure and if a vaccine protects against intense transmission, it is likely to protect against less intense transmission, while the reverse may not be true. The inclusion of both seasonal and perennial transmission is justified because they are likely to induce a different pattern of naturally acquired immunity. The justification for including immigrants is that they comprise non-immune subjects of all ages.

1.2.2 Specific for transmission-blocking vaccines

The movement of people or vectors between vaccinated and unvaccinated communities will dilute any effect of the vaccine. The following are therefore required:

- (a) Relative stability of the human population;
- (b) Usual flight range(s) of vector population(s) less than the distance between population units (communities) eligible for allocation to the vaccine or control groups; if existing information is inadequate, the flight-range may have to be determined by mark-release-recapture experiments;

Additional criteria:

- (c) 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;
- (d) Man-vector contact. Some vectors come into human contact mostly at places away from the community, e.g. forests, work places. Transmission-blocking vaccine trials will be easier to interpret in situations where vector contact is largely limited to community sleeping areas;
- (e) Relatively small size of the population units eligible for allocation to either group (to economize resources).

2. Background information required (or desirable)

Background information is required for: (a) verifying that the selection criteria are satisfied; and (b) further specification of the study design (e.g. sample size, timing of vaccination and surveys in relation to transmission, logistics of case detection and surveys). As far as possible, the required background information should be obtained from existing data, which should be retrieved and reviewed; if important information is missing, the collection of new data may be required. Background information is distinct from baseline data *senso stricto* (i.e. pre-vaccination data included in the estimation of vaccine efficacy), such as may be recommended for the evaluation of transmission-blocking vaccines; background information is required whether or not baseline data *senso stricto* are collected, and required before the collection of such baseline data.

2.1 Maps and demography

Mapping of the study areas and a full census of the target population groups should be performed (Smith & Morrow, 1996); the census (to be updated regularly during the trial) provides the denominators necessary for the calculation of rates of infection, disease or death. Data on migration are required to estimate: (a) the fraction likely to be lost to follow-up, a fraction used for adjusting the sample size; (b) the rate of contamination among transmission units in a transmission-blocking vaccine trial. Data on occupation and behaviour may help determine whether man-vector contacts are mostly limited to community sleeping areas, as is desirable for transmission-blocking vaccine trials.

The birth rate and the infant survival curve (e.g. by month of age) are important if vaccination will be timed according to the EPI schedule. The age-specific death rate (all causes) is important if it is to be the primary efficacy endpoint.

2.2 Malaria in the human population

The most important item of the whole background information is the expected incidence of the primary efficacy endpoint; it is crucial for the final adoption of the site and for calculation of the sample size. Other malariometric data (rates of infection, disease, severe disease, specific mortality; prevalence and density of parasitaemia) will also be useful. Analysis of the malariometric data by season will identify the seasonality of transmission, which may affect the timing of vaccinations and surveys. Analysis by age, sex, and - possibly - occupation may help determine whether man-vector contacts are mostly limited to community sleeping areas, as is desirable for transmission-blocking vaccine trials.

2.3 Parasites and antibodies

(a) Sensitivity of malaria parasites to drugs

This information will be required in order to select the chemotherapeutic regimens needed to clear parasitaemia (for measuring the incidence of infection) and to treat malaria in the study area.

(b) Immunological responses induced by natural infection, in persons in different age groups, to the antigens present in the vaccine (required for assessing the vaccine's immunogenicity).

(c) The diversity of antigens, especially those represented in the vaccine, found in the malaria parasites in the study area (not indispensable, but desirable).

2.4 Entomology

Entomological data are useful for all vaccine trials and indispensable for transmission-blocking vaccine trials. The entomological inoculation rate would be useful for all trials. It provides an independent estimate of the challenge and of the seasonality of transmission (which may affect timing of vaccination and surveys). For transmission-blocking vaccine trials, in addition to the inoculation rate and the information listed under selection criteria (see above, E 1.2.2), it is necessary to know the species of the vectors with certainty and precision.

2.5 Malaria control activities

(a) Consumption patterns of antimalarials

Sources of antimalarials in the study area should be identified and surveys conducted of consumption patterns of antimalarials (including some antibiotics, e.g. tetracycline and cotrimoxazole) in those in the age groups to be included in a trial. Subjects, or their parents

should be asked what actions they take and where treatment is obtained when they, or their children, are ill. It will be useful to supplement that information by random urine sampling (WHO 1991b)

(b) Protection against mosquito bites and vector control

Use of bednets (impregnated or not), and other personal protection measures, interior insecticide spraying or other methods of vector control, public or private.

2.6 Health services

Coverage and quality of (a) services involved in the diagnosis and treatment of malaria (uncomplicated and severe and complicated); (b) EPI and mother and child health programmes (MCH) (if infants are the target group)

2.7 Other useful information

- (a) The prevalence of genetic factors which affect host responses to *P. falciparum*, in particular Hb-S, thalassaemias, ovalocytosis and G-6-PD deficiency.
- (b) The prevalence of other diseases that might alter responses to malaria or vaccines.

E. METHODS OF MEASUREMENT, IMPLEMENTATION, ANALYSIS

1. Methods of data collection and measurement

The following aspects of measurement require critical review during the preparatory phase of a trial: standardization of all field, clinical and laboratory procedures, including establishment of Standard Operating Procedures (SOPs) and normal values; 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, for validation of data and for the analysis of results. All procedures should satisfy the WHO Guidelines for Good Clinical Practice (WHO, 1995b).

1.1 Parasitaemia

Parasitaemia is a component of several endpoints, including incidence of infection or disease, prevalence and density of infection, and the parasite density threshold. The current method of choice for detecting malaria infections is by microscopic examination of thick

blood films, supplemented or not by the examination of thin films; sensitivity depends on the volume of blood examined, hence the preference for thick films. The most commonly used substitutes for a standard volume of blood are 100 and 200 thick-film microscopic fields (approximately 0.25 and 0.5 FI). Only a fixed stopping rule (e.g. examine a fixed number of fields, counting whatever parasites are found on the way) allows a reliable estimation of parasitaemias; flexible stopping rules will 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 (WBC) in the thick film or against red blood cells (RBC) in the thin film. In transforming the parasite/WBC ratio into number per volume, it is usual to assume a fixed white blood cell count in the study population. Parasite density is measured more accurately by combining each parasite count, made against WBC or RBC with the corresponding cell count (WBC or RBC). An alternative way of measuring parasite density is to count parasites per high power field (Greenwood & Armstrong, 1991). Notwithstanding efforts to standardize all procedures involved, their performance often varies between investigators and with the same investigators over time, and this variation must be allowed for in the study design.

The following procedures are considered essential whenever parasitaemia for assessing the efficacy of a malaria vaccine is used, in any way: (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 whether 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 blind independent re-examination of a coded sample of blood films.

The possible inclusion of newer diagnostic techniques, such as QBC, DNA or RNA probes, possibly including PCR, and antigen detection assays, should also be considered (WHO, 1996c; P. Trigg, 1997). While these cannot at present replace microscopic examination, they may become valuable supplements, permitting rapid, objective and specific evaluation of large numbers of blood samples. Their inclusion (e.g. on a sample basis) in vaccine trials, will help determine their future place.

1.2 Incidence of infection in man

In order to determine the incidence of new *P. falciparum* infections in trial subjects, the standard recommendation is that parasitaemia should be cleared from all subjects in which this endpoint is to be measured, by the administration of a safe and effective schizonticidal drug (WHO, 1994) after the last inoculation of vaccine or placebo. In order to avoid possible interference between treatment and vaccination (see below, E2.3(c)), it is preferable to give the treatment two weeks after the last inoculation. Subjects in this cohort should be bled at regular intervals, perhaps once a week to once a month, depending upon the level of malaria transmission, and blood films examined for asexual parasites and

gametocytes. In addition, an efficient monitoring system (using both active and passive case detection) should be established to record clinical episodes (and consumption of antimalarials) in the cohort. The cases of infection detected in this way between surveys are added to the cases detected by the surveys. In this way it will be possible to determine the incidence of new infections in vaccinated and control subjects over a defined period.

The cases of infection, detected at or between surveys, are removed from the denominator (person-time at risk); if negative at one or more subsequent surveys, they may be returned to the denominator (exact rules to be specified in the trials=protocol; treatment of the asymptomatic infections detected through the cohort surveys is not mandatory). Cases of infection by other *Plasmodium* species should be recorded as such, allowing the analysis to test whether vaccination against *P. falciparum* has an effect on the incidence of patent parasitaemia of the other species.

As treatment may interfere with measurement of infection (or immune responses) and as a completely safe, effective schizontocide may not be available, it would be preferable to measure the incidence of infection without clearing baseline parasitaemia by an initial treatment. This may become possible with the advent of modern methods of isolate characterization.

1.3 Incidence of disease

1.3.1 Case detection

Clinical episodes may be detected by active case detection (ACD) or passive case detection (PCD). ACD may be more or less active, and PCD more or less accessible to the study population, and variation of the method of detection will affect its sensitivity, hence the trials power. Furthermore, variation of the method of detection is likely to affect differentially the detection of different kinds of episodes, at least in areas of intense transmission (hence high immunity): in Papua New Guinea, PCD cases had higher temperatures and parasite densities than ACD cases (Cox et al, 1994); in Senegal, the majority of cases detected by intensive (daily) ACD were self-terminating within 24 to 48 hours and, therefore, unlikely to be detectable by PCD (Trape & Rogier, 1995). There is thus a spectrum of severity even within non-severe malaria, and the efficacy of a given vaccine might vary within that spectrum.

Whether one should use ACD or PCD or both in a particular trial should be influenced by the proportion of cases that would be detected by PCD. If this is high, then it may be sufficient to use PCD alone; if the proportion is only small, with most cases being treated at home or not being treated, it could be dangerous just to rely on PCD alone.

1.3.2 Malaria-attributable morbidity

The diagnosis of clinical malaria rests on clinical suspicion plus parasitaemia. In areas of low transmission (and hence low immunity), asymptomatic parasitaemia is rare and

negligible. However, in areas of high transmission (and hence high immunity) it is common and must be taken into account when determining case definitions for vaccine trials. Even in areas of intermediate intensity of transmission, (of the order of 1 inoculation/person/year) the proportion of asymptomatic parasitaemias can be high (Elhassan et al., 1995) . An appropriate case definition is one with a high specificity (few cases caused by non-malarial illnesses but with concurrent parasitaemia are included) and high sensitivity (few true malaria cases are omitted). The specificity of the case definition is particularly important because vaccine efficacy will be underestimated if cases of non-malarial illness are included. As a rough guideline, investigators might aim for a specificity of at least 80%.

The determination of the sensitivity and specificity of a case definition requires a comparison between the clinically suspect and community controls in terms of parasite prevalence and/or density.

(1) Identification of the clinically suspect

Significant malaria-attributable morbidity has been found among the following: (a) axillary temperature $\geq 37.5^{\circ}\text{C}$ at case detection; (b) temperature $< 37.5^{\circ}\text{C}$ at case detection, but reported fever in the preceding 24 h; (c) reported sick with symptoms other than fever at case detection, but not satisfying (a) or (b) (Hurt *et al*, 1994; Smith T.A. *et al*, 1994 & 1995). Investigators might therefore choose to define the clinically suspect as either (a); (a) or (b); or (a), (b) or (c). The appropriate criteria may depend on the malaria endemicity, the case detection system (ACD or PCD) and on the ages of the trial participants. Where episodes satisfying entry points (b) or (c) are included, the particular entry point used should be recorded separately for each episode.

(2) Identification of community controls

The most convenient source of community controls will be the parasitological surveys, likely to be included in all field trials. Controls should be included irrespective of any symptoms or parasites at the time of survey (an inclusive design, in the terminology of Rodrigues & Kirkwood, 1990). Stratification for age, place of residence, or time-period may be required in the comparison between the parasitological status of the clinically suspect and the controls.

(3) Determining and evaluating case definitions

One of the sets of entry criteria suggested in (1) above is used to define the clinically suspect. In areas of low or moderate malaria endemicity a case definition of clinically suspect plus parasitaemia will be appropriate. In areas of high endemicity the specificity, S , of this case definition is likely to be unacceptably low, and an alternative case definition is appropriate: clinically suspect plus high parasitaemia.

The specificity, S of the case definition of clinically suspect plus parasitaemia depends on the risk, R , of a parasitaemic individual satisfying the entry criteria relative to that of an aparasitaemic one. In the absence of stratification, R is estimated by:

$$R = p_i(1 - p_c) / [p_c(1 - p_i)];$$

where p_i is the parasite prevalence in the clinically suspect and p_c is that in the controls. The malaria attributable fraction among the infected cases, α_i , is then estimated by:

$$\alpha_i = (R - 1) / R;$$

and the specificity of this case definition by:

$$S = (1 - p_i) / (1 - \alpha_i p_i).$$

In areas of high endemicity S may be unacceptably low (<0.8) and/or the estimate of α_i may be close to 0 or negative (Smith et al, 1994). A more specific case definition is obtained by using a parasite density cutoff, so that only the clinically suspect having a high parasite density are included. However, if the chosen cutoff is too high the loss in sensitivity (i.e. number of cases) will reduce the power of the study to detect real effects. Statistical methods for estimating the specificities and sensitivities of different cutoffs have been described by Smith *et al* (1994) and Armstrong Schellenberg *et al* (1994). These methods also provide estimates of the malaria attributable fraction (α_i).

1.3.3 Practical recommendations

Except in studies of anti-toxic vaccines, the investigators should determine a single primary case definition for clinical malaria before the code is broken, so that the choice is not influenced by knowledge of vaccine effects. This should be done using a single entry point and by applying the procedures described in 1.3.2. above to either baseline data from the study area or to data collected during the vaccine trial. Irrespective of whether the primary case definition for the trial is met, clinically suspect cases should be treated for malaria according to locally applicable clinical guidelines.

The primary analyses of efficacy against incidence of clinical malaria should consider only the first episode in each trial participant satisfying the primary case definition. Subsidiary analyses may consider different case definitions, determined using different entry points or different parasitaemia cutoffs.

The primary analysis of the anti-toxic effect of a vaccine will be to consider whether the relationship between the risk of becoming clinically suspect and the parasite density is the same in the vaccine and the placebo groups.

In subsidiary analyses the investigators may wish to include multiple episodes in the same trial participant. Repeated episodes within a short period should be excluded from such analyses since they are likely to reflect treatment failures. An acceptable convention is to exclude repeated episodes within a period of 4 weeks. Studies which consider multiple episodes within the same individual should also take into account the possible statistical dependencies between repeated episodes using appropriate analytical methods.

Without diagnosing individual attacks the total number of malaria attributable episodes can be estimated as $N \cdot \rho_i$ (where N is the total clinically suspect with parasites, and ρ_i is estimated as described in 1.3.2 above). Further subsidiary analyses might use this to consider the overall impact of the vaccine on malaria attributable morbidity rates. Estimates of ρ_i for such analyses should be made separately for placebo and vaccine recipients, since vaccination may well affect the relationship between parasite density and the risk of morbidity.

1.4 Incidence of severe disease

1.4.1 Case detection

Given the uncertainty of verbal autopsy diagnosis of malaria mortality, the use of severe malaria as an outcome of vaccine trials provides a proxy for clinical malaria that could lead to death in the absence of effective secondary level clinical care. Cases of severe, potentially life-threatening malaria can not be realistically detected through active case detection in the community. Passive detection of hospital admissions is therefore the best alternative and the availability of clinical diagnostic facilities enables more precise case definitions. Severe malaria is a rare event relative to the incidence of infection or mild disease in a community. Nevertheless, in many rural settings in Africa with appropriate access to the secondary-level district hospital, a large number of severe disease events are admitted to hospital each year requiring essential clinical services (between 1-3% of all children aged between 1 month and 10 years old).

Paediatric ward surveillance must be linked to continuous demographic surveillance of trial populations to enable the definition of person years exposure to risk and true incidence (by the exclusion of re-admissions) in vaccine and placebo cohorts. An example of how this was developed for studies of severe malaria at Kilifi on the Kenya coast is described by Snow *et al.*, 1994a.

With controlled trials randomized at the individual level, equal access between vaccine and placebo groups is accounted for through randomization within households or villages. Community-randomized controlled trials of transmission-blocking vaccines will necessitate detailed pre-intervention data and the definition of community-based rates of disease pre- and post-intervention to examine rate changes as described recently for trials of insecticide treated bednets (Nevill *et al.*, 1996).

Typically one would select a district hospital utilized by a predominantly rural community. The choice of hospital will depend upon the case burden from well defined catchment populations selected to participate in the RCT surrounding the hospital. Conversely, the choice of trial community should be guided by a careful appraisal of paediatric admission data by residence of the patients prior to the commencement of the study. Clearly utilization of a district hospital during a potentially fatal illness will depend upon ease of access to the hospital. In general the further away from the hospital or transport to the hospital the lower the sensitivity of case detection (Snow *et al.*, 1994b).

More often than not clinical diagnostics, care and informatics require considerable upgrading in normally over-stretched and busy paediatric in-patient facilities. Every paediatric admission requires a detailed demographic history (particularly to link the patient to community-based enumeration of trial recruits); full clinical history from the guardian of the patient; initial blood samples for malaria smears and haematocrits; and a detailed clinical examination following a pre-defined clinical protocol. Further investigations will be required for both clinical management and additional diagnostic support during the child's stay on the paediatric ward, which must be recorded on the child's proforma. It is essential that 24 hour seven days a week clinical cover is provided for this surveillance system to be effective. As with the surveillance for other outcome measures the examining physicians should not be aware of the trial status of the child.

1.4.2 Case definition

Cases and their clinical status must be defined on admission as these will change after admission following appropriate clinical management. Several criteria for clinical severity of malaria can be employed. The simplest level is that of all children whose primary reason for admission to a district hospital is Falciparum malaria. These patients are by definition not regarded by out patient clinical officers as fulfilling their subjective definitions of ambulatory out patient cases of malaria and require more intensive clinical management. However, this definition will include some patients who are admitted for social reasons such as arriving at night and having difficulties in returning home. Stricter definitions have been employed in other studies based mainly upon the WHO guidelines for severe malaria (WHO, 1990). These guidelines were formulated using considerable clinical experience of Thai adults and simpler definitions for African children have since been developed (Marsh *et al.*, 1995). Furthermore, the clinical spectrum of severe malaria manifests itself differently under different epidemiological conditions with the relative contribution of particular pathologies being dependent upon the age-structure of disease in a community (Snow *et al.*, 1994c).

The majority of severe paediatric Falciparum malaria presentations to African district hospitals will encompass one or more of the criteria shown in Table 6. These criteria have been arranged in ascending order of worsening prognosis following diagnosis on admission.

Table 6 also shows the criteria used to define each syndrome during studies of the epidemiology and control of severe malaria at Kilifi district hospital on the Kenyan coast .

Table 6. Diagnostic criteria for severe malaria

- Multiple seizures (Two or more generalized convulsions within a 24-hour period prior to admission).
- Hyper-parasitaemia (20% or more red cells infected with *P. falciparum*)
- Severe malaria anaemia (Haemoglobin less than 5.0 gm/dl with an accompanying peripheral parasitaemia greater or equal to 10,000/FI)
- Prostration (inability to sit unaided or drink)
- Respiratory distress (Deep breathing (acidotic or Kussmaul-s breathing)).
- Cerebral malaria (Inability to localize to a painful stimulus assessed after one hour following a seizure or administration of anti-convulsants and after correction of hypoglycaemia.

Strict criteria are required to standardize clinical definitions, and training of clinical staff to reduce between observer and trial site variability in definitions of respiratory distress, coma and prostration, should be encouraged. The choice of constellations of severe malaria syndromes will depend upon an assessment of the clinical spectrum of disease in that community through pre-intervention clinical surveillance.

1.5 Detection and investigation of deaths

Detection and investigation of all deaths is required in all trials, even if mortality is not the primary efficacy endpoint, and even though the sample size may be insufficient to test efficacy against death. The most appropriate method for determining mortality among trial subjects will be determined by local circumstances. In some situations, for example among migrants involved in a large industrial project, any deaths will be notified to project management or medical personnel and, thus, a passive surveillance system, adequate to determine mortality rates, will exist. In other communities, including many areas of rural Africa, a more active mortality surveillance system will be required. This may be set up by stationing a reporter in each community in the trial area, possibly a member of the community, whose responsibilities will include the identification and reporting of any deaths among study subjects. In areas where post-mortem examinations are not feasible, some information on cause of death may be obtained through A verbal autopsy@i.e. by asking questions of the bereaved family about the final illness and circumstances of death. If mortality from malaria, as established by the verbal autopsy technique, is used as an end-point for a vaccine trial, it is essential that the criteria used in attributing death to malaria should be clearly established before the start of the trial and subsequently adhered to. Verbal autopsy is difficult to standardize and to validate, and the estimation - necessarily indirect - of its sensitivity and specificity is problematic; those difficulties may be greater with malaria than with some other causes of death (e.g. measles) (Snow *et al.*, 1992; Alonso *et al.*, 1987; Todd *et al.*, 1994). If mortality is selected as the primary efficacy endpoint of a malaria vaccine trial, it should preferably be total (i.e. all cause) mortality, while cause-specific mortality (as measured by verbal autopsy) might be a secondary objective.

1.6 Infectivity of infected persons for vectors

Gametocyte counts should be made routinely. This is an indirect measure of the infectivity of a subject for vectors. Membrane-feeding of laboratory-bred mosquitos on fresh blood taken from an infected individual can provide a more direct measure of infectivity. It is a complex method, probably to be reserved for transmission-blocking vaccine trials. It raises its own sampling problems, not addressed here.

1.7 Human behaviour

The following aspects of human behaviour are likely to affect vaccine trials: use of antimalarials (including treatment-seeking behaviour and self-treatment), use of personal protection measures (eg. mosquito nets), domestic use of insecticides, occupation, migration (especially movement between vaccinated and unvaccinated communities in a transmission-blocking vaccine trial). Information may be obtained through interviews which may be supplemented by direct observation (e.g. of antimalarials, bednets or insecticides available in the home), by surveys of drug providers, by random urine sampling (WHO 1991b). The information may be collected from a population sample, to characterize a situation or its component communities, or from all study subjects, to classify individuals, hence allowing stratified analysis.

1.8 Vector infectivity rate and inoculation rate

1.8.1 Transmission-blocking vaccine trials

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 sites and times should correspond to those of natural biting activity. 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.

The presence of sporozoites in the salivary glands can be measured by microscopy or by stage-specific monoclonal antibodies in an ELISA. Since the immunological methods detect unassociated antigen as well as whole sporozoites, false positives will be reduced by testing only the fore thorax and head of captured mosquitos. Besides speed, the advantages of the immunological methods are that they can be used to estimate sporozoite load and determine species. New methods of detecting parasites in mosquitos are being developed using stage-specific DNA expression products or RNA, which might prove extremely useful in analysing large numbers of infected mosquitos. Measurement of oocyst infection can be done by dissection. For efficiency, it is preferable to do the parasite determinations only on parous *Anopheles*.

There is concern regarding the risk of malaria associated with collections on human baits (usually the same persons are baits and collectors). Such collections are, however,

likely to reflect natural biting behaviour and density much better than any alternative methods (see below), and are therefore recommended for transmission-blocking vaccine trials. The collector-baits should preferably be local adults, presumably immune; as their immunity is only presumed, they should be offered chemoprophylaxis and monitored closely for disease, which should be treated promptly. In areas where drug-resistance complicates prophylaxis and treatment, or where the adults used as collector-baits are expected to have little or no immunity, alternative methods of collection may have to be considered, even for a transmission-blocking vaccine trial. The alternative collection methods include: indoor resting collection, combined with blood meal analysis; CDC light traps; use of a double net protecting the bait.

1.8.2 Pre-erythrocytic vaccine, blood-stage vaccine & antitoxic vaccine trials

The measurement of the sporozoite and inoculation rates, although not indispensable, is useful as an independent estimation of the challenge. Measurement methods are the same as above, but sampling density will be lower, in both time and space, and the preference for collections on human baits will not be as strong.

1.9 Immune responses

The kinds of immune responses it is desirable to measure are considered above. The methods to be used are outside the scope of this document, and will be selected on the basis of the vaccine's composition and the relevant literature, and through consultation with expert malaria immunologists. Immune responses will usually be measured only in a population sample, longitudinally, at selected times, e.g. immediately before the first vaccine inoculation, shortly after the last vaccine inoculation, towards the end of the main transmission season, and at the end of the trial. To study the correlation between immune response and protection (see C. 1.2.2), it may be necessary either to measure immune response to the vaccine in all subjects or to collect sera from all subjects following the last dose of vaccine, but then analyse them on a case-control basis.

1.10 Side-effects

Side-effects should be assessed in all phases of vaccine trials, and the assessment should always be based on a comparison between the vaccinated and the controls. Most information on immediate side-effects, local reactions etc. will have been collected during the Phase I & II studies. It may not be practicable to make detailed side-effects observations during the Phase III trial because numbers are too large. Instead the Phase III trial should concentrate on detecting any relatively uncommon events, for example, fits after vaccination. Vaccinated and control (placebo vaccinated) persons should be observed immediately after vaccination to identify acute reactions. After that the detection of side-effects can be done most readily by placing a project worker in each study community. Current or past

P. falciparum or *P. vivax* infections may increase the risk and severity of side-effects, so that early trials in non-endemic countries may not be adequate predictors. Delayed toxicity, including immunopathological phenomena, may not become apparent for many months, and observations for side-effects must continue beyond the period of vaccine protection. Both case detection and population surveys should be used. The possibility of disease enhancement should be considered. This was a real concern during both The Gambian (D'Alessandro *et al.*, 1995) and Thai SPf66 trials (Nosten *et al.*, 1996), although in the end enhancement was not demonstrated. Malaria vaccines could increase the incidence of either severe disease or disease overall and transmission-blocking vaccines could increase disease transmissibility. Studies need to be designed to pick this up early - for example by reporting regularly all deaths and/or all severe cases of malaria to the monitoring committee. The need for haematological, immunological and biochemical examinations should be assessed on the basis of earlier trials. Extensive testing for urinary protein may permit the detection of incipient renal immunopathological manifestations. Studies requiring venipuncture should be carried out only on a sub-sample of the trial population.

1.11 Demographic surveillance

In addition to the surveillance of deaths and of population movements (see above), birth records are desirable, in particular if the trial involves an EPI-like vaccination schedule.

1.12 Other malaria control measures

Monitoring of individual measures is mentioned above (see 1.8). It is, in addition, required to investigate and monitor the national and local authorities' malaria control activities and plans.

1.13 Possible interference among measurements

The possibility of interference among measurements should be carefully considered before deciding to measure different endpoints in the same population sample.

- (1) Incidence of infection and incidence of disease

The measurement of the incidence of infection through frequent parasitological surveys, supplemented by intensive case detection (active and passive) between consecutive surveys (see above) is likely to detect and treat cases of illness very early, and to detect a large number of otherwise very brief self-limiting clinical episodes. In the other hand, for the measurement of the incidence of disease, one does not necessarily wish to include the mildest (or shortest) end of the clinical spectrum e.g. if one would prefer to count cases of illness detectable by PCD (see above, E1.3.1). It is, therefore, preferable, if both kinds of efficacy (against infection, against disease) have to be measured, to measure them in distinct population samples. If that is too costly, it may be preferable to measure only the efficacy endpoint most appropriate for the vaccine type being tested (infection for pre-erythrocytic

vaccines and transmission-blocking vaccines, disease for blood-stage vaccines and antitoxic vaccines)

(2) Incidence of early endpoints (infection, disease) and incidence of late endpoints (severe disease, death)

Interference of the measurement of an early endpoint with the measurement of a late endpoint is demonstrated by the unexpectedly low mortality, among both vaccinated and controls, in the Gambian SPf66 trial, in which ACD was intensive (MRC Laboratories, The Gambia, Annual Report 1995). A similar observation was made in the Tanzanian SPf66 trial (Alonso *et al.*, 1994b). It is, therefore, preferable, if both kinds of efficacy (against early endpoints, against late endpoints) have to be measured, to measure them in distinct population samples. This does not prevent monitoring of the severe endpoints, for documentary purposes, in the sample used for measuring efficacy against an early endpoint.

(3) Incidence of severe disease and mortality

The measurement of the incidence of severe and complicated malaria implies its improved detection and management, which may reduce mortality. However, as detection of severe and complicated malaria will not achieve total coverage, and as it will miss indirect malaria mortality, which can be very large (see above), it may be recommended to measure total mortality (all causes) in the same population sample.

2. Implementation

2.1 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB), including a clinical monitor should be appointed for the trial (see Smith and Morrow, 1996). This should be an independent group that can testify that the trial protocol has been properly followed and that relevant quality control procedures have been operating for the duration of the trial. This Board should be set up before the trial begins rather than once it has started, as unfortunately is often the case (also trials in which this has not been done have often been those which have given rise to greater controversy).

2.2 Trial periods and calendar

The trial will proceed in 3 or 4 periods.

(a) Preparatory period (about 1 year)

Activities to collect background information (see above, D2); to finalize the study design, establish facilities, recruit and train personnel, test and standardize all field, laboratory and data recording methods; to prepare an analytic plan; and to prepare the community for the intervention study.

(b) Baseline period

A baseline period, *sensu stricto*, ie. the collection of baseline data to be included in the estimation of vaccine efficacy, may be considered for transmission-blocking vaccine trials, because they compare communities rather than individuals (see above, C.2); suggested duration: 1 year.

(c) Intervention and evaluation period

The duration of follow-up required, after completion of the immunization schedule, will depend on the trials main objective and primary endpoint, on the target group, and on the local epidemiological situation. Duration most commonly recommended: 1 year.

(d) Final analysis period

This period starts with the locking of the data set and the breaking of the code. It includes final analysis, production of the final report and submission of a manuscript for publication.

2.3 Community 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. Arrangements for informing the community of the results should be agreed upon. In the case of a transmission-blocking vaccine, 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.

2.4 Vaccination

(a) Exclusions

As far as possible, the trial should not exclude any persons who would be likely to receive vaccination if the vaccine were eventually introduced into a control programme. 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 strongly recommended to exclude individuals suffering from any severe acute illness at the time of vaccination. It may also be advisable to exclude those suffering from any severe chronic disease or from severe malnourishment, or giving a history of severe allergic reactions. The

criteria for exclusion should be clearly spelled out in each trial. In a transmission-blocking vaccine trial, high coverage is required. If substantial numbers of individuals are excluded, an effort might be made to vaccinate them at a later date. If the fraction excluded is large, a transmission-blocking vaccine trial will not be feasible in that site.

(b) Season of vaccination

Although season of vaccination is probably irrelevant for control programmes, it may be relevant for early field trials. Thus, residents in areas of seasonal transmission might receive their last dose of vaccine at the beginning of the rains and non-immune immigrants just before entering areas of intense transmission.

(c) Associated chemotherapy

The administration of an effective schizonticide, before vaccination, has been recommended, to prevent any possible immunosuppressive effect of malarial infection (WHO, 1986a, 1989, 1992). However, such systematic treatment is unlikely in a vaccination programme, especially as (due to drug resistance) an efficient, safe and cheap schizonticide may not be available. It may, therefore, be preferable to avoid such systematic treatment in the field trials. It is recommended to investigate the effect of malarial infection, and of its treatment, on a vaccine's immunogenicity, as part of Phase I trials (see above, B3).

(d) Vaccine coverage

In a transmission-blocking vaccine trial comparing vaccinated and control communities, maximum coverage is desirable. In pre-erythrocytic vaccine or blood-stage vaccine trials comparing individuals within communities, high coverage might reduce transmission, hence the power of the trial. It may be prudent not to vaccinate more than 25% of a community's population (see also A 3.3, para 2).

(e) Choice of a control intervention

The control intervention may be a placebo. The best placebo is probably a preparation containing all the vaccine ingredients, with the exception of the antigen(s). The vaccine's carrier and adjuvant would thus be included in the placebo. In principle, all of those ingredients have passed extensive safety testing. Once a vaccine of a certain type, e.g. a pre-erythrocytic vaccine, has demonstrated efficacy, it is likely to become ethically required to test new vaccines, of a similar type, against that vaccine, rather than against a placebo. This will increase the required sample size (see A 2.1, last paragraph). This should be weighed against the possibility that such a control intervention might affect the measurement of trial endpoints.

2.5 Provision for stopping the trial

Provision should be made to stop vaccination at once if it produces an unacceptable number of serious adverse reactions. Advising in these circumstances would be one of the major responsibilities of the Data Safety Monitoring Board. In addition, the analytic plan (see below) may include one or more interim analyses, which may also provide for stopping the trial under specified conditions.

3. Data Analysis

Appropriate methods for the statistical analysis of randomised controlled trials of malaria vaccines are essentially the same as for randomised controlled trials of other drugs and vaccines. However, issues specific to malaria vaccines arise because:

(i) The wide range of possible outcome measures in a malaria vaccine trial means that some of them are likely to show differences by chance between vaccine and control groups.

(ii) A large number of subsidiary questions might be addressed in a malaria vaccine trial.

The investigators should prepare an analytical plan specifying the primary analysis of efficacy, prior to the breaking of the code. This addresses the issue of multiple outcome variables by denoting one primary endpoint only, in such a way that it is clear that the results themselves have not influenced the choice of this measure. The analytical plan should also detail the inclusion criteria, the case definition to be used, and the methods of data analysis.

The first analysis should be the comparison of vaccine and placebo recipients with respect to their baseline characteristics. Variables included in the analysis should include: age, sex, initial parasitological status, and area of residence.

In trials with an adequate sample size, random allocation is likely to achieve comparable groups. For this reason, the primary analysis of protective efficacy should not require any adjustments for baseline differences. In assessing comparability, more emphasis should be given to the size of any differences than to statistical significance, since it is the former that affects the degree of confounding. It is recommended that the investigators should specify in the analytical plan the magnitude of differences or the extent of confounding which will be considered important. If important imbalances are detected, the results should be adjusted for these differences by stratification or by using regression methods.

It will usually be appropriate for the primary analysis to include all individuals who received the full vaccination schedule of either vaccine or placebo, with the exception only of any who were included in error (e.g. people who were subsequently found to have been

ineligible because of errors in dates of birth or residency). Vaccine efficacy is then calculated using a standard formula as:

$$VE = 100 \times (1 - r_1 / r_0)\%$$

where r_1 = incidence rate in vaccine group and
 r_0 = incidence rate in placebo group.

In trials where clinical malaria is the primary outcome and where morbidity surveillance is continuous, incidence rates are calculated by dividing the number of first or only episodes after completion of the vaccination schedule by the total person-time at risk. Following an episode of malaria, the individual should be removed from both numerator and denominator. Children who are lost to follow-up, who withdraw, or who die, should be included up to the date of loss, withdrawal or death. Poisson regression may be used to obtain a confidence interval for the vaccine efficacy, and if indicated, to adjust for important imbalances.

In trials of anti-infection vaccines the primary outcome measure will usually be the time to infection. Incidence in both vaccine and comparison groups is calculated by dividing the number of first or only events after completion of the vaccination schedule by the total visits or survey attendances of the individual up to and including that attendance. Ideally, individuals who fail to attend all surveys contribute to both the numerator (number of infections) and denominator (number of attendances) only up to the first survey which they failed to attend. Again, following an infection, the individual should be removed from both numerator and denominator. The formula for vaccine efficacy is the same as for analyses of the efficacy against clinical episodes. Survival analysis techniques may be used if indicated, to adjust for important imbalances.

Some subjects in a trial may experience more than one clinical malaria episode. In subsidiary analyses it will be of interest to compare overall incidence rates between vaccine and placebo groups. This introduces several complications into the analysis, and it is not recommended that the primary analysis of efficacy should include multiple events for single individuals. If such analyses are conducted, firstly a standard rule must be used to ensure that relapses are not enumerated as separate episodes. One convention is that events during the four week period after an episode are not included, and these four weeks do not contribute to the time at risk. Secondly, testing for statistical significance and deriving confidence intervals on the estimates of vaccine efficacy is not straightforward in this case. The usual statistical inference for comparisons of rates is appropriate for multiple episodes only if it is reasonable to assume that once a subject has experienced one or more episodes he or she is no more or less likely to experience another episode than those who have experienced no episodes up to that time in the same group (vaccinated or unvaccinated). This assumption is rarely valid as susceptibility to disease events is usually very heterogeneous between individuals in a community, even among those of the same age and sex. The appropriate statistical analysis will depend on the extent of this heterogeneity in susceptibility.

In a malaria vaccine trial there are likely to be many supplementary questions of interest, particularly if the vaccine shows a substantial efficacy. A range of data analysis techniques will be needed to address effects on secondary end points, such as parasite densities or levels of anaemia, on side effects, or on immune responses and it is not possible to specify in advance what are likely to be the analyses of particular interest. In reporting the results of these supplementary analyses, particularly those based on sub-group analyses, caution will be needed in view of the large potential number of comparisons. It is important to remember that isolated significant results may well be the result of chance unless P-values are extremely small. A precautionary approach demands that substantial weight should be attached to such results only if they relate to adverse effects of the vaccine.

F. ETHICAL CONSIDERATIONS

Trials of malaria 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 regulations and requirements and international ethical standards as outlined in WHO Guidelines for Good Clinical Practice (GCP) for Trials on Pharmaceutical Products (WHO 1995b) and in International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council of International Organizations of Medical Sciences, CIOMS, Geneva 1993). In countries where national regulations or requirements do not exist or require supplementation, relevant government officials may designate or adopt, in part or in whole, the WHO GCP as the basis on which clinical trials will be conducted. Prior to implementation, the study design must be reviewed by a properly constituted local and 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 difference between services offered to acceptors and non-acceptors. Health services for treatment of possible vaccine-related reactions and malaria infection and adequate referral and follow-up capability must be available. A data and safety monitoring board should be constituted and an independent clinical monitor should be designated and given the authority to break the trial code for any of the individuals or communities involved, and recommend that vaccination be stopped should there be severe adverse reactions associated with the vaccine. For more details see WHO GCP Guidelines.

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, (WHO, 1992b) should proceed to community-based trials.

Prior presumption of vaccine efficacy is desirable, but, in certain cases, the decision to embark on a field trial may have to be based on a relatively weak presumption. Earlier field trials with the same vaccine may have demonstrated efficacy against natural challenge, in a different population or target group. Artificial challenge (Phase IIa) trials may have demonstrated efficacy against artificial challenge, but such trials are likely to be available only for some vaccines. A functional immunologic test may give a strong presumption of efficacy, but at present there is a possibly satisfactory functional test only for transmission-blocking vaccines. For most blood-stage vaccines the only presumption of efficacy may come from immunologic tests or animal models of unknown or limited predictive value.

Ethical considerations may affect the selection of trial communities, endpoints or study design. Trials of malaria vaccines should be carried out only in communities that are likely to be included in vaccination programmes, should the vaccine prove effective. The cases of disease (even mild or incipient) detected as a trial's endpoint must be treated. The treatment of asymptomatic infections is not mandatory. As long as efficacy against natural challenge has not been demonstrated in the target group considered, there should be no ethical objection to a double-blind randomized controlled (DB-RCT) design. To the contrary, other designs are ethically questionable, to the extent that they are likely to give ambiguous, i.e. useless, results.

The mode of action of transmission-blocking vaccines raises some special ethical issues, which do not arise for other vaccines against malaria or other diseases. In particular, transmission-blocking 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.

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An advanced draft version of these Guidelines was reviewed by the WHO TDR Steering Committee on Vaccines for Malaria (IMMAL) and by the Coordinating Committee of the African Malaria Vaccine Testing Network (AMVTN).