Annex 3

Guidelines on the quality, safety and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum*

Introduction and scope

Background

Part A. Guidelines on manufacturing and control

A.1 Recombinant malaria antigens under evaluation
A.2 Terminology
A.3 General manufacturing guidelines
A.4 Control of source materials
A.5 Fermentation
A.6 Single harvests
A.7 Control of purified antigen bulk
A.8 Final bulk
A.9 Filling and containers
A.10 Control tests on final lot
A.11 Records, retained samples, labelling, distribution and transport
A.12 Stability testing, storage and expiry date

Part B. Nonclinical evaluation of malaria vaccines

B.1 Introduction
B.2 Product development and characterization
B.3 Pharmacodynamic studies
B.4 Toxicity

Part C. Clinical evaluation of malaria vaccines

C.1 Introduction
C.2 Clinical studies
C.3 Post-licence investigations

Part D. Guidelines for NRAs

D.1 General
D.2 Release and certification by the NRA

Authors

Acknowledgements

References
Appendix 1
Controlled human malaria infection trials (human-challenge studies) 195

Appendix 2
Methodological considerations: quantification of human immunoglobulin G directed against the repeated region (NANP) of the circumsporozoite protein of the parasite \textit{P. falciparum} (anti-CS ELISA) 197

Appendix 3
Model protocol for the manufacturing and control of recombinant malaria vaccines 199

Appendix 4
Model certificate for the release of recombinant malaria vaccines by NRAs 208

This document provides information and guidance on the development, production, quality control and evaluation of the safety and efficacy of candidate malaria vaccines produced using recombinant DNA technology. Since there is at present no licensed malaria vaccine, this document is written in the form of WHO Guidelines instead of Recommendations, and is intended to facilitate progress towards the eventual licensure of such a vaccine. Guidelines allow greater flexibility than Recommendations with respect to future developments in the field. The parts printed in small type in Part A are comments or examples that are intended to provide additional guidance on the currently most-advanced candidate vaccine. To facilitate the international distribution of vaccines produced in accordance with these Guidelines, a summary protocol for recording test results has been provided in Appendix 3.

\textbf{Note:} Appendices 1–4 are only intended to be illustrative of the thinking and assay methods in place at the time these Guidelines were adopted. They should therefore not be considered as final procedures but rather as evolving approaches.
Introduction and scope

These Guidelines are intended to provide guidance to national regulatory authorities (NRAs) and vaccine manufacturers on the quality (including the production, quality control, characterization and stability), and nonclinical and clinical aspects of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum*.

Various approaches to the development of malaria vaccines are being investigated that employ different production platforms, and target different stages of the life-cycle of malaria parasites. As of 2012, only one candidate vaccine – RTS, S/AS01, a recombinant *P. falciparum* malaria vaccine produced in yeast that targets the pre-erythrocytic stage of the parasite – was under evaluation in phase III clinical trials (1). In early clinical trials, this vaccine demonstrated some degree of efficacy in reducing all episodes of clinical malaria (2–4).

These Guidelines consist of three technical sections – Part A: Guidelines on manufacturing and control; Part B: Nonclinical evaluation; and Part C: Clinical evaluation. These three sections differ somewhat in their scope to reflect the different stages of vaccine development, and the diversity of production platforms and vaccine targets.

Part A of this document focuses on the manufacturing process and the quality-control issues relevant to recombinant antigens. Specific information regarding RTS, S/AS01 vaccine, the vaccine currently under phase III evaluation, is provided in small print. Part B (nonclinical evaluation) and Part C (clinical evaluation) are written to have a wider scope, and may be applicable to the evaluation of other recombinant subunit malaria vaccines that target the pre-erythrocytic or blood-stage of *P. falciparum*. Additionally, the issues discussed in Part C may apply to whole-organism malaria vaccines; however, specific issues for this type of vaccine are discussed in more detail elsewhere (5). The appendices are provided as examples to illustrate the thinking and assay methods in place at the time these Guidelines were adopted. Therefore, they should not be considered as final procedures but as evolving approaches.

Additional and specific considerations for clinical development programmes are necessary for transmission-blocking malaria vaccines because these are intended to reduce malaria transmission by blocking or interfering with the sexual stage of the parasite's life-cycle, and are not expected to prevent malaria disease directly in vaccinated individuals (6).

At present, there is no vaccine licensed for malaria. Some methodological considerations are provided in appendices as examples based on the protocols used by the manufacturer of the most advanced candidate vaccine. These are provided for information only and should not be considered as endorsements of any candidate vaccines. When a malaria vaccine is licensed, the principles...
detailed in these Guidelines may also apply to the evaluation of vaccines for which significant changes to the marketing authorization have been submitted.

This document should be read in conjunction with other relevant WHO Guidelines, including those on the nonclinical (7) and clinical (8) evaluation of vaccines.

It is desirable to apply the 3R principles (reduction, replacement, refinement) to minimize the use of animals for ethical reasons (9). Both manufacturers and the staff of NRAs and national control laboratories (NCLs) are encouraged to further develop and use in vitro assays, and to accumulate more data on their application to the quality control of vaccines. However, the type of testing chosen should be driven by the scientific need for valid and relevant data (10).

This document is a new set of Guidelines, and does not replace a specific earlier version; however, WHO has previously issued documents that have provided guidance on vaccine development. In 1997, WHO developed Guidelines that provided a theoretical and epidemiological framework for malaria-vaccine evaluation (11). In 2002, WHO established a Malaria Vaccine Committee (MALVAC). Under the guidance of MALVAC, and building on the 1997 document, WHO has facilitated a series of consultations on the clinical evaluation of malaria vaccine efficacy. A WHO Study Group on Measures of Malaria Vaccine Efficacy met in 2006 in Montreux, Switzerland (12). There was further elaboration of the methods of analysis for malaria vaccine in field-efficacy studies during the 2008 WHO MALVAC scientific forum (13). The outcomes of these consultations form the basis of the clinical section (Part C) of these Guidelines.

**Background**

**Disease burden of malaria**

Six identified species of the Plasmodium protozoan parasite can infect humans (*P. falciparum*, *P. vivax*, *P. ovale curtisi*, *P. ovale wallikeri*, *P. malariae* and *P. knowlesi*). *P. falciparum* accounts for more than 90% of all malaria-attributable deaths. Vaccine development efforts have focused on *P. falciparum* and to a lesser extent on *P. vivax* (14). Morbidity and mortality from malaria is a consequence of the replication of parasites in red blood cells. Although it is beyond the scope of these Guidelines to discuss in detail the pathophysiological mechanisms by which morbidity and mortality are induced, there is evidence that all of the following can contribute to malaria-related disease: sequestration of infected red blood cells, severe anaemia due to red blood cell lysis, inflammation-related brain pathology, lactic acidosis, and a general shock-like syndrome with hypotension, hypoglycaemia and poor tissue perfusion. The blood stage is established following the injection of the sporozoite form of the parasite by female anopheline
mosquitoes; subsequent development occurs through the liver stage, which is followed by progression into the blood.

The disease burden of malaria is traditionally assessed by estimating the annual number of deaths due to malaria, the number of acute new cases of the disease per year, and the economic costs resulting from the deaths, illnesses and related treatment as well as the loss of productivity. The databases from which these estimates are made vary in accuracy from country to country. However, these annual data afford a global perspective on the malaria-disease burden. As of 2010, WHO estimated that approximately 655,000 deaths per year were attributable to malaria, with the vast majority of these deaths occurring in sub-Saharan Africa, and the majority of the remaining cases occurring in South-East Asia, the Indian subcontinent and South America. Most of the deaths in Africa occur in children who are younger than 5 years, and in primigravid females. The annual number of new cases of clinical episodes of malaria was estimated in 2010 to be 216,000,000 (15). Young children in malaria-endemic countries typically experience several clinical episodes of malaria before they develop immunity, which protects against the more severe forms of the disease. The economic costs of malaria are difficult to estimate, but in some heavily affected countries these costs run to the equivalent of billions of US dollars per year, and negatively impact the country’s gross domestic product by several percentage points (16).

In many African countries substantial malaria-control efforts have been implemented, including the widespread deployment of long-lasting insecticide-treated nets (LLIN) and the use of indoor residual spraying. Studies have indicated reductions in the annual incidence rates of new malaria cases and in deaths due to malaria of ≥ 50% in some settings (15, 17). There is a cost burden associated with maintaining vector-control measures and, in the past, control programmes have been underfunded and interrupted, leading to a corresponding resurgence of malaria transmission and the associated morbidity and mortality.

It is widely believed that substantial reductions in the disease burden of malaria will result from a combination of implementing vector-control measures, using selective chemoprophylaxis, strengthening diagnostic testing, effectively treating people with malaria and, potentially, preventing the disease through immunization. Although immunization may make a contribution, malaria-control efforts are unlikely to rely primarily on vaccination. Therefore, it is within this context of a significant disease burden, highly concentrated among children younger than 5 years of age, and multipronged approaches to malaria-disease control, that consideration of the evaluation of recombinant, stage-specific malaria vaccines will take place.

Life-cycle, vaccine targets and potential vaccine effects

Figure 3.1 illustrates the four distinct stages of the life-cycle of malaria parasites – with each stage providing potential vaccine-antigen targets (18). The pre-
erythrocytic stages (stages 1 and 2 in Figure 3.1) encompass the injection of the sporozoite stage of the parasite by the bite of an infected female anopheline mosquito, and the rapid homing of the sporozoite into the liver cells within a matter of minutes to a few hours. Antigens present on the surface of the sporozoite, such as circumsporozoite protein (CSP), or deployed to the surface of the infected hepatocyte, have been used as pre-erythrocytic-stage candidate vaccines. Immune responses directed at either the sporozoite stage or at the infected hepatocyte could, in theory, prevent the blood-stage infection from developing. Since it is during the blood stage of the infection that all morbidity and mortality occurs, a highly effective pre-erythrocytic vaccine could prevent infection or significantly reduce the disease burden associated with malaria.

Figure 3.1
The life-cycle of *Plasmodium falciparum*¹

¹ Source: Moorthy et al. (19). Used with permission from Elsevier.
After repeated rounds of replication in the hepatocyte, an intrahepatocytic stage develops, termed a hepatic schizont, which then ruptures, releasing thousands of small, round, merozoite forms of the parasite into the venous circulation. These merozoites rapidly invade human erythrocytes (see stage 3 in Figure 3.1). Numerous antigens that are unique to either the merozoite (e.g. the merozoite surface antigens) or to the infected erythrocyte (erythrocyte-associated surface antigens, such as RESA) are potential erythrocytic-stage vaccine antigens, and such vaccines would either prevent the invasion of the erythrocyte by the merozoite, or would target the infected erythrocyte for destruction by the host’s immune system. The net effect of such erythrocyte-stage immune responses could be to limit or ameliorate the blood-stage manifestations of the malaria infection.

Small subsets of infected erythrocytes undergo a developmental switch into the sexual stage of the organisms, termed gametocytes (see stage 4 in Figure 3.1). Although most gametocytes remain within the host erythrocyte until they are taken up during a blood meal ingested by a female anopheline mosquito, some of the infected erythrocytes rupture in the host’s reticuloendothelial system and present gametocyte-specific antigens to the host’s immune system. Vaccines targeting gametocyte stages of the parasite, or targeting the fertilized gamete stage, which is found only in the mosquito midgut after fertilization occurs, may provide transmission-blocking immune responses that could interrupt transmission of the parasite from an infected person to an uninfected person by preventing development of a mature sporozoite in the mosquito.

Combination vaccines containing antigens expressed at different stages of the parasite’s life-cycle may induce an immune response with a broad biological effect. To date, the most successful approaches to inducing protective antimalarial effects have used whole parasites that have been subjected to irradiation while still in the mosquito’s salivary gland and subsequent inoculation of sporozoites by the direct bite of these irradiated mosquitoes. In these experiments it was demonstrated that highly effective infection-preventing immunity could be induced in malaria-naive volunteers (20). Such whole-organism approaches to malaria immunization continue to be explored using various methods, including genetic attenuation of sporozoites and irradiation of sporozoites with subsequent injection by needle and syringe rather than by bite of mosquitoes. However, the results of such efforts have been inconclusive. Additionally, the use of whole-organism vaccines raises safety and standardization issues that are beyond the scope of this document. The focus of these Guidelines is therefore on first-generation recombinant malaria vaccines that have been developed and tested in humans primarily as single-stage and single-antigen constructs.

**Naturally acquired antimalarial immunity**

After repeated natural exposure to *P. falciparum* malaria infections, individuals develop a significantly reduced risk of developing serious illness or dying from
subsequent malaria infections. This acquisition of immunity through natural exposure is sometimes referred to as premunition. In areas of moderate-to-high transmission in malaria-endemic countries, premunition usually develops by the age of 5–7 years, depending on the intensity of malaria transmission. The development of premunition at these ages helps to explain why the preponderance of deaths due to *P. falciparum* malaria is found in children who are younger than 5 years. The mechanisms underlying premunition are not fully understood; however, there are two leading hypotheses. One is that the gradual acquisition of strain-specific immunity occurs; the other is that repeated antigenic exposure, perhaps in conjunction with an age-related immune maturation, is necessary for the development of premunition. This immunity does not prevent future malaria infections, and robust infection-blocking immunity is not thought to occur. Additionally, the immunity acquired during childhood does not protect primigravid women, thus accounting for a spike in malaria-attributable deaths in these women. Premunition (or partial immunity) is also known to wane to a significant degree if an individual migrates out of a malaria-endemic region and ceases to have regular exposure to malaria infection for a number of years. Severe malaria illness and death can occur in people who have migrated out of, and then have returned to, a malaria-endemic area (21), which suggests that premunition requires some level of ongoing re-exposure to critical malaria antigens in order to be maintained at effective, disease-ameliorating levels.

It is beyond the scope of these Guidelines to describe the effector mechanisms involved in naturally acquired antimalarial immunity. Significant roles for both humoral and cell-mediated effectors have been demonstrated in animal models, and both humoral and cell-mediated immune responses have been induced in humans after natural malaria infection and exposure to experimental malaria vaccine. No clear correlates of protection have been established for vaccines, although an accumulating body of evidence indicates that antibodies to CSP show some correlation to pre-erythrocytic protection (22), and antibodies to Pfs25, a sexual-stage protein, correlate in animal models with significant transmission-blocking effects (23).

The development of protection against severe disease after natural malaria infection, and the possible role of identifiable and quantifiable effector mechanisms of protection, both lend a positive perspective to the development of effective malaria vaccines. However, the complexity of the parasite and the strong suggestion that naturally induced protection wanes if malaria exposure ceases, pose significant challenges. It is a daunting task to expect a malaria vaccine to produce a better protective response than natural exposure, yet that must be the long-term goal if vaccination is to be the path to achieving sustained control of this disease. Nevertheless, even a partially protective vaccine could have an important role in reducing the burden of malaria disease if it is combined with existing preventive and treatment measures.
Variability in transmission intensity, and effects on clinical disease and acquired immunity

Malaria transmission may be seasonal and generally varies as a function of vector survival and longevity which, in turn, are functions of environmental factors, such as altitude, rainfall and humidity. It has long been recognized that certain areas of the world have intense malaria transmission all year round, whereas other areas have a seasonal pattern of transmission. In areas with a seasonal pattern, transmission may be intense during the transmission season. Although areas can be identified as having a certain level of transmission intensity, malariologists have noted for many years that the actual transmission rates observed tend to be highly local and focal, meaning that malaria transmission and the number of malaria infections identified in a particular area may vary substantially over a rather limited geographical range. The entomological inoculation rate (EIR) is defined as the number of times that an individual is bitten by an infective mosquito in a year. This can be as high as 1000 or more infective bites per year in some areas, but in other malaria-endemic areas the average EIR may be less than 1.

It has been noted that certain clinical manifestations of malaria, such as cerebral malaria, occur more frequently in settings in which transmission is episodic or seasonal, whereas severe and life-threatening anaemia following infection occurs more often in regions where malaria is a year-round threat. If transmission is highly intermittent, a situation may arise in which an entire segment of the population of an area lives through a time when transmission is quite low. Then, perhaps due to changing weather or environmental conditions, transmission may suddenly increase and result in many more-severe cases and deaths than would be expected if transmission had followed a more stable and regular pattern.

The performance of a malaria vaccine may vary according to the seasonal pattern and intensity of transmission. Interpretation of the results of a vaccine trial requires a comprehensive set of baseline data for a given trial location, as well as an understanding of the dynamics of malaria transmission, including the seasonal nature of the disease.

Clinical presentations of malarial disease vary by age, and influence the design of malaria-vaccine trials

The case definitions of an episode of clinical malaria and the methods of diagnosing malaria infection in the context of vaccine trials are addressed in detail later in these Guidelines as well as in related WHO documents (12). Episodes of clinical malaria may present with quite different clinical features, depending on the age of the individual, the intensity of malaria transmission and the clinical stage of the infection. Morbidity due to infection with *P. falciparum*
can range from a mild febrile illness, which is quite difficult to distinguish from many other similar illnesses, to fulminant and life-threatening disease with severe central nervous system stupor and coma, or to a full-blown shock syndrome requiring immediate blood volume support and ancillary supportive measures, or a combination of these. Furthermore, the clinical picture can change within 24 hours, from an illness that appears to be relatively mild to a life-threatening disease. The availability of rapid diagnostic tests can be of great help when expert clinical microscopy is not available. Well designed and carefully executed malaria-vaccine trials must clearly specify how an episode of clinical malaria is to be diagnosed and treated in order to ensure the safety of the subjects, and the validity of the efficacy results.

**Antimalarial vaccines used in conjunction with other control measures**

As noted above, there has been a resurgence of funding for malaria treatment and vector-control programmes, and these programmes show significant promise for decreasing the burden of malaria. Given the modest vaccine efficacy demonstrated to date, malaria-control efforts will have to combine vector-control strategies with immunization strategies once a licensed malaria vaccine becomes available. Phase III study designs will need to document carefully any control measures, such as the use of LLINs, indoor residual spraying or selective chemoprophylaxis programmes, so that the context in which the vaccine’s efficacy was measured can be established; phase III studies also will need to document the comparability of the trial arms, with respect to these factors.

Information is insufficient to predict the public-health benefit of immunizations against malaria that are used in conjunction with the maintenance of other malaria-control measures. Well designed clinical trials can establish the clinical efficacy and confidence intervals for chosen end-points for a malaria vaccine, and can also control for confounding effects from vector-control efforts and programmes aimed at promoting prompt diagnosis and treatment. The longer-term public-health consequences of the simultaneous use of a malaria vaccine and other control measures can be assessed only through post-licensure studies (see section C.3).

**Current phase III candidate malaria vaccines**

The most advanced candidate is the vaccine against *P. falciparum* infection known as RTS,S/AS01. In phase II studies, RTS,S/AS01 has demonstrated efficacy against clinical malaria when given to children aged 5–17 months at first immunization (1, 2, 24). This vaccine, which is based on the *P. falciparum* sporozoite antigen CSP, was developed after a series of clinical trials demonstrated that simpler CSP-based vaccines provided inadequate clinical
efficacy. Furthermore, in addition to using a novel delivery system based on the hepatitis B–malaria antigen fusion protein (see section A.1.1), novel adjuvants have been utilized because RTS,S formulated on aluminium-containing adjuvants alone afforded no protection in human-challenge studies (25). Various RTS,S/adjuvant formulations have been compared in human-challenge studies (see section C.2.2), and the formulation designated as RTS,S/AS01 appeared to provide the greatest protection (26).

Currently, it is envisaged that RTS,S/AS01 may be considered for deployment to those parts of sub-Saharan Africa where *P. falciparum* is the main species of malaria parasite. This document does not consider evaluation of *P. falciparum* vaccines in areas coendemic for *P. vivax* and *P. falciparum*, such as parts of south-east Asia or South America. It is anticipated that trials in coendemic areas would be performed prior to consideration of deployment of a *P. falciparum* vaccine in such areas.

More than 30 *P. falciparum* malaria-vaccine projects are at either advanced preclinical or clinical stages of evaluation (27). As of 2012, RTS,S/AS01 was the only candidate vaccine in pivotal phase III evaluation.

Approaches that utilize recombinant protein antigens and target blood stages are being developed but only pre-erythrocytic vaccine approaches have entered pivotal phase III evaluation.

**Part A. Guidelines on manufacturing and control**

Part A of this document focuses on the relevant issues relating to the manufacturing process and quality control of recombinant subunit vaccines. The primary goal of this section is to outline the general principles that may apply to a number of recombinant malaria antigens. However, given that the pre-erythrocytic *P. falciparum* malaria vaccine (RTS,S/AS01) developed by GlaxoSmithKline Biologicals in conjunction with the Malaria Vaccine Initiative is the most advanced candidate (1), a description of this specific vaccine is provided in section A.1 and, where appropriate, additional specific information on the RTS,S/AS01 vaccine is provided in small print. Information on the general aspects of adjuvant formulations is also included in Part A, and some specific details of the proprietary adjuvant AS01 are indicated in small print. Selected details regarding the production and testing of RTS,S/AS01 vaccine are provided as additional guidance for NRAs that may be asked to review this vaccine in the future; these details illustrate the nature of the manufacturing and testing information that should be provided for other vaccines that are under development.

Quality control during the manufacturing process relies on the implementation of quality systems, such as those known as good manufacturing practice (GMP), to ensure the production of consistent vaccine lots with
characteristics similar to those of lots previously shown to be safe and effective. Throughout the process, a number of in-process control tests should be established (with acceptable limits) to allow quality to be monitored for each lot from the beginning to the end of production. It is important to note that most release specifications are product-specific, and therefore will be approved by the NRA as part of the marketing authorization.

A.1 Recombinant malaria antigens under evaluation

As of 2012, there were no licensed malaria vaccines. However, many candidate vaccines using recombinant subunit antigens were under development.

Recombinant subunit *P. falciparum* malaria candidate vaccines under preclinical or clinical evaluation include antigens expressed at different stages of the parasite’s life-cycle (28). These antigens include the circumsporozoite (CS) antigen, thrombospondin-related adhesion protein (TRAP), merozoite surface protein type 1 (MSP1) and type 3 (MSP3), apical membrane antigen type 1 (AMA-1), and *P. falciparum* mosquito stage antigen (Pfs25). Modifications of these and other antigens have also been explored in attempts to overcome the complexities of obtaining correct folding and secondary structure, or to induce better immune responses (e.g. RTS,S). Combination vaccines containing two or more antigens are also under development.

The development of RTS,S/AS01 vaccine was initiated in the 1980s, and is based on a purified recombinant RTS,S antigen expressed in yeast (*Saccharomyces cerevisiae*) and formulated in a novel adjuvant system named AS01 (29).

The CSP gene from the *P. falciparum* NF54 strain was obtained as a DNA fragment encoding specific portions of the full-length CS (repeat region containing B-cell epitopes and a region containing the T-cell epitopes), and cloned into a suitable vector to produce a fusion protein with the hepatitis B surface antigen (HBsAg) (S antigen). The resulting fusion protein has been designated RTS. The combination of a portion of the CS gene with S antigen gene was selected due to the well known property of the S protein to form particles, and to improve antigen presentation to the immune system (30, 31). To stabilize the recombinant viral-like particles, genes encoding both the RTS fusion protein and the nonfused S antigen are inserted into yeast cells by means of appropriate expression vectors. Thus, the yeast cells produce both the RTS and S proteins, and these spontaneously coassemble into mixed particles that, when purified, comprise the final vaccine antigen (called RTS,S). In summary, the final vaccine antigen is a particle that includes, in defined proportions, the nonfused S hepatitis B antigen and a fusion protein (RTS) that combines the RT portion of the CSP with the S hepatitis B surface antigen.
In addition, the purified RTS,S antigen contains lipid as an integral component of the particle.

The vaccine currently under phase III efficacy evaluation includes the proprietary adjuvant called AS01. This novel adjuvant system contains monophosphoryl lipid A (MPL) and a saponin derived from the bark of the plant *Quillaja saponaria* (QS21) as immunostimulants formulated together with liposomes. Studies have demonstrated that adjuvants capable of enhancing antibody and cellular immunity are required to achieve protection against malaria in human-challenge models (26, 32).

The liquid adjuvant system AS01 is used to reconstitute the lyophilized RTS,S antigen prior to administration. The RTS,S antigen does not adsorb to the adjuvant when the antigen is reconstituted with AS01; thus antigen adsorption is not included among the control tests for this product. The RTS,S/AS01 vaccine is for prophylactic use.

### A.2 Terminology

The definitions given below apply to the terms as used in these Guidelines. They may have different meanings in other contexts.

**Adjuvant:** a substance or a composition of substances that potentiates and/or modulates the immune response to an antigen towards the desired effect.

**Adventitious agents:** contaminating microorganisms of the cell substrate, or materials used in their culture; these may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced.

**Cell bank:** a collection of ampoules containing aliquots of a suspension of cells from a single pool of cells of uniform composition, which are stored frozen under defined conditions.

**Final bulk:** the formulated antigen bulk, prepared from one or more batches of purified bulk, present in the container from which the final containers are filled prior to lyophilization.

**Final lot:** a collection of sealed final containers of lyophilized vaccine that is homogeneous with respect to the risk of contamination during the filling and lyophilization process. Therefore, a final lot must have been filled from a single vessel of final bulk during one working session.

**Master cell bank (MCB):** a collection of containers holding aliquots of a suspension of cells from a single pool of cells of uniform composition, which are stored frozen under defined conditions. The MCB is used to derive all working cell banks for the anticipated lifetime of vaccine production following licensure.

**Production cell culture:** a cell culture derived from one or more containers of the working cell bank and used for the production of vaccines.

**Purified antigen bulk:** the purified antigen prior to the addition of any substances, such as diluents and stabilizers.
**Single harvest**: the biological material prepared from a single fermentation run prior to the downstream (purification) process.

**Working cell bank (WCB)**: a collection of containers holding aliquots of a suspension of cells from a single pool of cells of uniform composition, which have been derived from the MCB, and that are stored frozen under defined conditions (typically below –60 °C for yeast). One or more aliquots of the WCB are used for routine production of the vaccine. Multiple WCBs are made and used during the lifetime of the vaccine product.

A.3 **General manufacturing guidelines**

The general manufacturing recommendations contained in WHO good manufacturing practices for pharmaceutical products: main principles (33) and Good manufacturing practices for biological products (34) apply to the establishment of manufacturing facilities.

A.4 **Control of source materials**

A.4.1 **Yeast cell substrates for antigen production**

The use of any cell substrate should be based on a cell bank system. Only cells that have been approved and registered with the NRA should be used for production. The NRA should be responsible for approving the cell bank. An appropriate history should be provided for the cell bank.

A.4.1.1 **Yeast cells**

The characteristics of the recombinant production strain (host cell in combination with the expression vector system) should be fully described, and information should be given about the absence of adventitious agents (35, 36) and gene homogeneity for the MCBs and WCBs. A full description of the biological characteristics of the host cell and expression vectors should be given. The physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail. These measures should include assessments of the genetic markers of the host cell; the construction, genetics and structure of the expression vector; and the origin and identification of the gene that is being cloned.

The nucleotide sequence of the gene insert and of adjacent segments of the vector, and restriction-enzyme mapping of the vector containing the gene insert, should be provided if required by the NRA. Thorough characterization of the gene product should be done during product development, and documentation should be provided in support of licensure (see Part B, Nonclinical evaluation).

Both the MCB and the WCB must be maintained in a frozen state that allows recovery of viable cells without alteration of the genotype.
If necessary, the cells should be recovered from the frozen state in selective media such that the genotype and phenotype consistent with the unmodified host and unmodified recombinant DNA vector are maintained and clearly identifiable. Cell banks should be identified and fully characterized by means of appropriate tests. The MCB and WCB should be tested for the absence of adventitious agents according to Part A of WHO General requirements for the sterility of biological substances no. 6 (1973) (35) or by a method approved by the NRA.

Data that demonstrate the stability of the expression system during storage of the recombinant WCB up to or beyond the passage level used for production should be provided to and approved by the NRA. Any instability of the expression system occurring in the seed culture or after a production-scale run should be documented.

A.4.2  **Fermentation medium**

Production fermentation should be performed in a defined culture medium that has been shown to be suitable for the production of relevant antigens with consistent yields. The acceptability of the source(s) of any components used of bovine, porcine, sheep or goat origin should be approved by the NRA. Components should comply with WHO guidance relating to animal-transmissible spongiform encephalopathies (37–39). The NRA should approve any change in the media used.

A.5  **Fermentation**

A.5.1  **Production of cell cultures**

Only cell cultures derived from the WCB should be used for production. All processing of cells should be done in a designated facility in which no cells or organisms are handled other than those directly required for the process.

A.5.1.1  **Control of antigen production up to single harvest**

Microbial purity in each fermentation vessel should be monitored at the end of the production run by methods approved by the NRA. Any agent added to the fermenter or bioreactor to feed cells or to induce or increase cell density should be approved by the NRA.

A.5.2  **Genetic characterization and stability**

Where the plasmid is integrated into the host-cell genome, the presence of the integrated antigen sequences should be confirmed. The DNA sequence of the cloned gene should normally be confirmed from the cell bank stage up to and beyond the usual level of population doubling for full-scale fermentation. Southern blot analysis of total cellular DNA or sequence analysis of the messenger
RNA (mRNA), may be helpful. The copy number, physical state and stability of the vector inside the host cell should be documented (40, 41).

For the RTS,S/AS01 vaccine, documentation of the characterization and stability of the genes encoding for the RT and S fusion protein and the nonfused S protein should be provided in support of licensure.

A.6  
**Single harvests**

A.6.1  
**Storage and intermediate hold times**

During the purification process, all intermediates should be maintained under conditions shown by the manufacturer to ensure they retain the desired biological activity. Hold times should be based on validation studies, and approved by the NRA.

A.6.2  
**Tests on single harvests**

A.6.2.1  
**Sampling**

Samples required for the testing of single harvests should be taken immediately on harvesting, prior to further processing.

A.6.2.2  
**Test for contamination by bacteria and fungi**

Microbial and fungal contamination in the fermentation vessels should be monitored at the end of production, and should be tested for according to Part A, section 5.2 of General requirements for the sterility of biological substances no. 6 (1973) (35) or by methods approved by the NRA.

A.6.3  
**Consistency of yield**

Data on the consistency of yield between runs and during individual production runs should be provided, and the NRA should approve the criteria for determining what constitutes an acceptable production run.

A.7  
**Control of purified antigen bulk**

The purification procedure can be applied to a single harvest or to a pool of single harvests. When applicable, the maximum number of single harvests that may be pooled should be defined on the basis of validation studies, and should be approved by the NRA. Adequate purification may require several purification steps based on different principles (e.g. size, charge or hydrophobicity). The use of procedures that rely on different physicochemical properties of the molecules will minimize the possibility of copurification of extraneous cellular materials. The methods used for the purification of the vaccine antigen should be appropriately validated, and then approved by the NRA. Any agent added to the purification
process should be documented, and its removal should be adequately validated and tested for as appropriate (see section A.7.1.8).

The purified antigen bulk can be stored under conditions shown by the manufacturer to retain the desired biological activity. Intermediate hold times should be approved by the NRA.

A.7.1 Tests on the purified antigen bulk

The purified antigen bulk should be tested using the tests listed below. All quality-control release tests, and specifications for purified antigen bulk, should be validated and shown to be suitable for the intended purpose. Additional tests on intermediates during the purification process may be used to monitor the consistency and yield.

A.7.1.1 Purity

The degree of purity of each purified antigen bulk should be assessed using suitable methods. Examples of suitable methods for analysing the proportion of degradation products and potential contaminating proteins in the total protein of the preparation are polyacrylamide gel electrophoresis (PAGE), optionally followed by densitometric analysis, and high-performance liquid chromatography (HPLC). Specifications should be established based on the formulation that provided acceptable data on safety and efficacy. Specifications should be set during the processes of product development and validation, and established by agreement with the NRA.

For the RTS,S/AS01 vaccine, the RTS and S proteins should be not less than 95% of the total protein in the purified antigen bulk.

A.7.1.2 Protein content

The protein content should be determined by using a suitable method, such as the micro-Kjeldahl method, the Lowry technique or another method.

A.7.1.3 Antigen content

The antigen content of the purified antigen bulk should be determined by an appropriate immunochemical method that measures antigenic activity. An appropriate antigen reference material – of known purity, antigenic activity and protein content – should be included in these assays. The assays should be designed so that the consistency of production can be monitored. This reference material should either be a representative bulk or a highly purified preparation stored in single-use aliquots.

For the RTS,S/AS01 vaccine, the antigenic activity of the RTS,S antigen should be determined by means of an immunological assay, such as the
enzyme-linked immunosorbent assay (ELISA), and a protein-content assay. The antigenic activity is defined as the ratio of the result from the immunological assay to the result from the protein-content test. The test method and acceptable limits should be established in agreement with the NRA.

For the RTS,S/AS01 vaccine, the ratio between the two polypeptides S and RTS should be determined using an appropriate analytical method. The S to RTS ratio should be within a range defined by those lots and shown to have acceptable performance in clinical trials. The limits should be established in agreement with the NRA.

A.7.1.4 Identity

Tests used for assessing other properties of the antigen, such as antigen content or purity, will generally be suitable for assessing the identity of the protein in the bulk. For instance, immunoblots of PAGE separations using antigen-specific antibodies could be used to confirm the molecular identity of the product. The identity testing approach should be defined during the processes of product development and validation, and should be approved by the NRA.

A.7.1.5 Lipids

The lipid content of each purified antigen bulk should be determined using an appropriate method. The methods used and the permitted concentrations of lipid should be approved by the NRA. This test may be omitted after the consistency of the purification process has been demonstrated to the satisfaction of the NRA.

A.7.1.6 Carbohydrates

The carbohydrate content of each purified antigen bulk should be determined using an appropriate method. The methods used and the permitted concentrations of carbohydrates should be approved by the NRA. This test may be omitted after the consistency of the purification process has been demonstrated to the satisfaction of the NRA.

A.7.1.7 Sterility tests for bacteria and fungi

Each purified antigen bulk should be tested for freedom from bacteria and fungi following WHO General requirements for the sterility of biological substances no. 6 (1973) (35) or by using a method approved by the NRA.

A.7.1.8 Tests for agents used during purification or other phases of manufacture

The purified antigen bulk should be tested for the presence of any potentially hazardous agents used during manufacture. The method and the concentration
limits used should be approved by the NRA. These tests may be omitted for routine lot release after it has been demonstrated that the validated purification process consistently eliminates the agents from the purified bulks.

A.7.1.9 Tests for residuals derived from the antigen expression system
The amount of residuals derived from the antigen expression system (e.g. DNA or host-cell proteins) should be determined in each antigen purified bulk using acceptable methods. These tests may be omitted for routine lot release after it has been demonstrated that the validated purification process consistently eliminates the residual components from the bulks to the satisfaction of the NRA.

A.7.1.10 Bacterial endotoxins
Each final purified antigen bulk should be tested for bacterial endotoxins. The method and the concentration limits used should be approved by the NRA. At the concentration of the final formulation of the vaccine, the total amount of residual endotoxins should not exceed that found in vaccine lots shown to be safe in clinical trials or in data from other lots used to support licensing.

A.8 Final bulk
The final bulk may be prepared from one or more purified antigen bulks. Only bulks that have satisfied the requirements outlined in previous sections should be included in the final bulk. The antigen concentration in the final formulation should be sufficient to ensure that the dose is consistent with that shown to be safe and effective in human clinical trials. Formulation is generally based on protein content, but antigen content may be used.

The operations necessary for preparing the final bulk should be conducted in such a manner as to avoid contaminating the product. In preparing the final bulk, any substances that have been added to the product, such as adjuvants, diluents and stabilizers, should have been shown to the satisfaction of the NRA not to impair the safety and efficacy of the vaccine in the concentration used. Until the bulk is filled into containers to prepare the final vaccine or the lyophilized antigen, the final bulk suspension should be stored under conditions that, according to the manufacturer, will ensure that the desired biological activity is retained during the time-limit for holding approved by the NRA.

A.8.1 Tests on the final bulk
Depending on the production process or the characteristics of the vaccine, some tests may be performed on the final bulk rather than on the final product if the NRA agrees.
A.8.1.1 Sterility tests

Each final antigen bulk should be tested for bacterial and fungal sterility according to WHO General requirements for the sterility of biological substances no. 6 (1973) (35) or by using a method approved by the NRA.

A.9 Filling and containers

The general requirements concerning filling and containers given in Good manufacturing practices for biological products (34) apply to vaccine filled in the final form.

Care should be taken to ensure that the materials from which the container and, if applicable, the closure are made do not adversely affect the immunogenicity of the vaccine under the recommended storage conditions.

A.10 Control tests on final lot

Samples should be taken from each final vaccine lot to be tested; these samples must fulfil the requirements of this section. All of the tests and specifications, including the methods used and the permissible limits for different parameters described in this section, unless otherwise specified, should be approved by the NRA. The specifications should be defined based on the results of tests on lots that have been shown to have acceptable performance in clinical studies.

The requirements concerning filling and containers given in WHO Good manufacturing practices for biological products (34) must be met, or an NRA-approved method applied.

The RTS,S vaccine is a two-component vaccine consisting of the RTS,S antigen (lyophilized) and the AS01 adjuvant (liquid). Immediately prior to administration, the full contents of the AS01 vial are withdrawn and added to the antigen vial to reconstitute the RTS,S. During product development and process validation, relevant testing, including evaluation of potential interactions between the antigen and adjuvant, should be conducted on batches of final-container RTS,S antigen lot that have been reconstituted using the final adjuvant system lot. This should be done until consistency has been demonstrated to the satisfaction of the NRA. Routine release testing of only the individual vaccine components (i.e. antigen final container and adjuvant system final container) may be justified when the consistency and compatibility of the antigen and adjuvant have been demonstrated.

Care should be taken to ensure that the materials comprising the container and, if applicable, transference devices and closure systems, do not adversely affect the quality of the vaccine. The manufacturer should provide the NRA
with adequate data to prove that the product remains stable under appropriate conditions of storage and shipping.

A.10.1 **Inspection of containers**
Each container of each the final vaccine-antigen lot should be inspected visually or mechanically, and those containers showing abnormalities should be discarded.

A.10.2 **Appearance**
Visual inspection of the appearance of the vaccine should be described with respect to the form and colour.

The appearance of each of the individual components of the RTS,S/AS01 vaccine should be examined (i.e. the RTS,S final-container lot and the AS01 final-container lot) as should the final reconstituted vaccine. Visual inspection of the reconstituted vaccine may be discontinued when consistency has been demonstrated to the satisfaction of the NRA.

A.10.3 **Identity**
An identity test should be performed on at least one labelled container from each final lot using methods approved by the NRA. The test used for determining the antigen content will generally be suitable for assessing identity. Alternatively, immunoblots using antigen-specific antibodies could also be used to confirm the molecular identity of the product.

A.10.4 **Sterility tests**
Each final vaccine lot should be tested for bacterial and fungal sterility according to the requirements outlined in WHO General requirements for the sterility of biological substances no. 6 (1973) (35) or by using an acceptable method that has been approved by the NRA.

For RTS,S/AS01 vaccine, vials from both the lyophilized final lot and the liquid adjuvant AS01 should be tested for bacterial and fungal sterility.

A.10.5 **General safety test**
Each final lot should be tested in mice or guinea-pigs to confirm the absence of abnormal toxicity using a test approved by the NRA. This test may not be required for routine lot release after the consistency of production has been established to the satisfaction of the NRA.

Each final lot of RTS,S antigen should be tested to confirm the absence of abnormal toxicity upon reconstitution with the proprietary adjuvant system AS01.
A.10.6  **pH**
The pH value of each final batch of containers should be tested. Lyophilized products should be reconstituted prior to testing using the diluent approved by the NRA.

A.10.7  **Pyrogen and endotoxin content**
The vaccine in the final container should be tested for pyrogenic activity through intravenous injection into rabbits. A Limulus amoebocyte lysate (LAL) test may be used in lieu of the rabbit pyrogen test if it has been validated. Similarly, a suitably validated monocyte activation test may be considered as an alternative to the pyrogen test. The endotoxin content or pyrogenic activity should be consistent with levels found to be acceptable in vaccine lots used in clinical trials and approved by the NRA. The test may not be required after the consistency of production has been demonstrated to the satisfaction of the NRA. Lyophilized products should be reconstituted prior to testing using the diluent approved by the NRA.

When testing is performed on RTS,S reconstituted with the adjuvant system, the rabbit test for pyrogens should be performed because the adjuvant may interfere with the LAL test.

A.10.8  **Protein content**
The protein content should be determined using a method approved by the NRA. For some products, the protein content may be calculated using an intermediate from an earlier process if this can be adequately justified and has been approved by the NRA. Specifications should be within the limits for vaccine preparations shown to have acceptable performance in clinical studies.

For the RTS,S/AS01 vaccine, the protein content should be measured in the lyophilized RTS,S antigen.

A.10.9  **Moisture content**
When the antigen is lyophilized, the average moisture content should be determined using methods that have been accepted by the NRA. Values should be within the limits for preparations shown to be adequately stable in stability studies of the vaccine.

For RTS,S/AS01, the moisture content of the vaccine should be measured in the lyophilized RTS,S antigen.

A.10.10  **Potency test**
An appropriate quantitative test for potency should be performed on samples representative of the final vaccine lot. The method used and the data analysis
should be approved by the NRA. The vaccine’s potency should be compared with that of an approved reference preparation. Establishment of the specifications for each product should be based on the response observed in tests on vaccine clinical lots and data from other lots used to support licensing. The specifications for each antigen claimed to contribute to efficacy should be approved by the NRA.

Because no International Standard for the RTS,S antigen is available, the manufacturer should establish a product-specific reference preparation that can be traced to a vaccine lot that has had demonstrated efficacy in clinical trials. Methodological considerations regarding in vivo and in vitro potency assays for RTS,S/AS01 are available on the WHO Biologics web site at: http://who.int/biologicals/vaccines/malaria/en/index.html and will be updated when necessary.

A.10.11 Control tests on the adjuvant

The quality control tests for adjuvants and source materials are specific to the components (e.g. MPL or lipids) and the characteristics of the adjuvant formulation (e.g. water–in-oil emulsions). All of the tests and specifications, including the methods used and permitted concentrations, should be approved by the NRA.

The tests for the proprietary adjuvant system AS01 should be carried out on the final containers of the AS01 adjuvant.

A.10.11.1 Identity and content of adjuvant system components

Components of the final adjuvant system should be identified and quantified using appropriate methods.

A.10.11.2 Adjuvant system quality attributes

Specifications for the relevant quality attributes of the adjuvant system should be set and approved by the NRA. Each component of the adjuvant system should be shown to meet defined purity characteristics.

- For the RTS,S/AS01 vaccine, each final lot of the AS01 adjuvant system should be assayed for average liposome size and size distribution using suitable analytical methods. The methods and limits used should be approved by the NRA.

- The pH of each final lot of the AS01 adjuvant system should be tested.

- Sterility tests should be performed on each final lot of the AS01 adjuvant system to comply with WHO Guidelines (35, 36).
The AS01 adjuvant system in the final container should be tested for pyrogenic activity by intravenous injection into rabbits. The test for pyrogenic activity may not be required after the consistency of production has been demonstrated to the satisfaction of the NRA.

Each final lot of AS01 should be assayed for immunostimulants (i.e. QS21 and MPL) and the content of liposome components. The methods used and the concentrations permitted should be approved by the NRA.

A.11 Records, retained samples, labelling, distribution and transport

The requirements given in WHO Good manufacturing practices for biological products (34) apply. In addition, the label on the carton, the container or the leaflet accompanying the container should state:

- the nature of the cells used to produce the antigen;
- the nature and amount of adjuvant present in the vaccine;
- the volume of one recommended human dose, the immunization schedule, and the recommended routes of administration (this information should be given for neonates, children and immunosuppressed individuals, and should be the same for a given vaccine in all parts of the world);
- the amount of active substance contained in one recommended human dose.

Efforts should be made to ensure that shipping conditions are such as to maintain the vaccine in an appropriate environment. Temperature indicators should be packaged with each vaccine shipment to monitor fluctuations in temperature during transportation. Further guidance is provided in WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (42).

A.12 Stability testing, storage and expiry date

A.12.1 Stability testing

Adequate stability studies form an essential part of vaccine development. Guidance on evaluating vaccine stability is provided in WHO Guidelines on stability evaluation of vaccines (43). In addition to testing the final product, stability testing should include testing at intermediate stages of production.

For the RTS,S/AS01 vaccine, the stability of the final vaccine antigen lot (lyophilized RTS,S antigen) and the adjuvant system (liquid AS01 adjuvant) should be demonstrated at the recommended storage temperatures to the satisfaction of the NRA. The formulation of RTS,S
antigen and the adjuvant system must be stable throughout the claimed conditions for use (e.g. up to 6 hours after reconstitution) when stored according to instructions. Acceptable limits for stability should be agreed with the NRA.

A.12.2 Storage conditions

Storage conditions should be fully validated and approved by the NRA. The vaccine (including the antigen and adjuvant system) should have been shown to maintain its potency for a period equal to the duration from the date of release to the expiry date.

A.12.3 Expiry date

The expiry date should be fixed with NRA approval, and should take into account the experimental data on the stability of the vaccine (including both the antigen and the adjuvant system).

Part B. Nonclinical evaluation of malaria vaccines

B.1 Introduction

The nonclinical evaluation of malaria vaccines should be based on WHO guidelines on nonclinical evaluation of vaccines (7). Prior to the clinical testing of any new or modified malaria vaccine in humans, there should be extensive product characterization, immunogenicity and safety testing, and proof-of-concept studies in animals. The nonclinical testing of vaccines is a prerequisite for the initiation of clinical studies in humans. There is no laboratory test or series of tests that will unequivocally ensure that a newly developed malaria vaccine will be adequately safe and effective. In view of this limitation, manufacturers are expected to provide information describing the approach taken to the collection of supporting evidence, beginning with a comprehensive programme of nonclinical testing, and followed by a progression of clinical evaluations. The extent to which nonclinical studies will be required depends on the type of antigen and the complexity of the formulation, particularly when novel adjuvant systems are employed.

The following sections describe the types of nonclinical information that should be submitted to the NRA. The purpose of the submissions will vary during the product-development process. In some cases, these nonclinical data will be submitted to support the initiation of a specific clinical study; in other cases, the nonclinical data will be included in a marketing-authorization application.

These Guidelines should be read in conjunction with WHO guidelines on nonclinical evaluation of vaccines (7). They are specifically aimed at the nonclinical evaluation of malaria vaccines that are based on recombinant antigens.
in the context of the development of a new vaccine, or when significant changes to the manufacturing process require re-evaluation and re-characterization of a vaccine.

The goal of preclinical testing, defined as the nonclinical testing carried out prior to initiation of any clinical investigations, is to develop a package of supporting data and product information that justifies the move to clinical studies. These data should provide evidence that:

- the vaccine antigens and final product are well defined and thoroughly characterized;
- the vaccine administered to humans is likely to be well tolerated by the target population;
- the vaccine is reasonably likely, on the basis of animal immunogenicity and, when applicable, protection data, to provide protection from clinical malaria.

These issues are discussed in detail below.

Vaccine lots used in nonclinical studies should be adequately representative of those intended for clinical investigation and, ideally, should be the same lots as those used in clinical studies. If this is not feasible, then the lots used in nonclinical studies should be comparable to clinical lots with respect to formulation, physicochemical data, quality characteristics and the stability profile. Details on the design, conduct, analysis and evaluation of nonclinical studies are available in WHO guidelines on nonclinical evaluation of vaccines (7).

Similar considerations should be given to vaccines based on alternative technologies (e.g. viral vectors, DNA or whole organisms). However, it is beyond the scope of this document to provide testing requirements for these vaccine platforms.

**B.2 Product development and characterization**

The general principles of vaccine production, testing and stability are described in WHO guidelines on nonclinical evaluation of vaccines (7). The nonclinical and preclinical testing should include extensive product characterization; however, the nature and extent of the characterization studies may vary according to the stage of development. The testing information obtained during development provides guidance on the product’s characteristics, as well as on the tests and evaluation criteria that are appropriate for quality control, as defined in Part A of this document.

**B.2.1 Strategy for cloning and expressing the gene product**

A full description of the biological characteristics of the host cell and expression vectors used in production should be given. This should include details of: (i) the
construction, genetics and structure of the expression vector; and (ii) the origin and identification of the gene that is being cloned. The physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail.

Data should be provided to demonstrate the stability of the expression system during storage of the WCB, and beyond the passage level used for production. Any instability of the expression system occurring in the seed culture or after a production-scale run – such as rearrangements, deletions or insertions of nucleotides – must be documented. The NRA should approve the system used.

B.2.2 Characterization of the vaccine antigen

The molecular size and integrity of the expressed protein and its composition should be established by techniques such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver staining under reducing and nonreducing conditions, size-exclusion chromatography, and appropriate analyses of any carbohydrate and lipid components or modifications.

The identity of the protein should be established by peptide mapping or terminal amino acid sequence analysis, or both. Following SDS-PAGE, the protein bands should be identified in immunoblots using specific antibodies (e.g. monoclonal antibodies) to confirm the presence of the expected gene product(s). The primary structure of the protein should be further characterized by suitable methods, such as partial amino acid sequence analysis and peptide mapping. Mass spectrometry may be used to confirm the average molecular mass and the presence of the protein in the preparation.

Rigorous identification and characterization of recombinant DNA-derived antigens are required as part of the marketing-authorization application. If applicable and relevant, the ways in which the recombinant antigens differ chemically, structurally, biologically or immunologically from naturally occurring antigens must be fully documented. Such differences could arise during processing at the genetic or post-translational level, or during purification.

B.2.3 Characterization of vaccine formulations

Justification for the selection of the antigen(s) and the adjuvant system, in particular supporting evidence of the adjuvant’s mode of action, should be given. It is important to note that adjuvants are not licensed in their own right but only as a component of a particular vaccine. Evaluations of the vaccine formulation should include proof-of-concept studies that evaluate the enhancement of the desired immune response. Additionally, antigen/adjuvant formulations will need to be fully characterized, and have undergone adequate pharmacotoxicological studies, before clinical evaluation, as discussed in section B.4. Advances in the understanding of the mechanisms that protect against malaria suggest that both
humoral and cell-mediated immunity (22) are important. This, together with the generally poor immunogenicity of recombinant subunit antigens, has led to the development and assessment of various adjuvant systems that are capable of stimulating both adaptive and innate immunity, including a broad range of antibody and cellular immune responses.

Further guidance on the general principles of the nonclinical assessment of adjuvants can be found in the WHO guidelines on nonclinical evaluation of vaccines (7) and in the European Medicines Agency’s Guideline on adjuvants in vaccines for human use (44).

B.3 Pharmacodynamic studies

Immunogenicity studies in animal models can provide important information with respect to the optimization of adjuvant formulations and the evaluation of immunological characteristics of the antigen including, for some antigens, the ability to induce functional antibodies. Animal models of malaria have provided insights into the mechanisms of both pathogenesis and immune protection, and these studies have led to the identification of potential vaccine approaches. However, experience has shown that extrapolating data from animal models to human disease should be done with caution.

B.3.1 Rodent models

Rodent models have frequently provided initial evidence of the immunogenicity or efficacy, or both, of potential malaria vaccines despite the significant limitation that these models are unnatural hosts for human parasites. In contrast to the chronic infection that develops in the natural host and parasite combination, rodent models develop acute, and often lethal, infections. A major potential limitation of these models is that rodents’ immune responses to malarial antigens may be not relevant to natural human infections. Additionally, the lack of standardization in the route of administration, method of challenge and end-points of the numerous rodent models makes comparisons and comprehensive evaluations difficult. Nevertheless, the ability to utilize these models provides mechanisms for the scientific examination of immunological relationships, and facilitates understanding of potential clinical issues. Immunization-challenge models utilizing sporozoites or erythrocytes parasitized with *P. yoelii*, *P. berghei* and *P. chabaudi* are often used in conjunction with vaccine constructs based on these orthologue Plasmodium species. When interpreting data from these studies, the caveats of the evolutionary distance between rodent biology and human biology, as well as between the rodent and human species of Plasmodium, must be considered. Lack of protection in such models is taken as an indication not to progress a given candidate vaccine; and while demonstration of protection does not necessarily predict clinical protection, it provides some
rationale and justification for the clinical development of candidate antigens or vaccine platforms.

In the protection against blood-stage challenge with sporozoites or parasitized red blood cells, the usual end-points measured with lethal and nonlethal strains of parasites are the reduction in peak parasitaemia, prolongation of the prepatent period and protection from mortality.

B.3.2 Nonhuman primate models

The genetic and morphological similarity between humans and nonhuman primates makes nonhuman primates potentially useful models for the evaluation of candidate vaccines. The susceptibility of these primates to human malaria, albeit in modified forms, is their chief advantage over other animal models; however, the nonhuman primate model should not necessarily be given preference over other available models.

The *Aotus* and *Saimiri* species of New World monkeys and the *Macaca mulatta* species (i.e. rhesus macaque) of Old World monkeys are commonly used to examine the immunogenicity and potential efficacy of candidate malaria vaccines. New World monkeys are most useful for their receptivity to *P. falciparum*, *P. vivax* and *P. malariae*. The malaria model in the Aotus monkey is useful for investigations of blood-stage infections of these strains of malaria. In addition, *Aotus* species have been used for the study of mosquito transmission and for susceptibility studies of sporozoite-induced infections and liver-stage studies. The *Saimiri* monkey model provides useful information for investigating *P. vivax* infections and *P. falciparum* blood-stage infection. When compared with humans, New World monkey models often demonstrate a more rapid acquisition of effective immunity, and the development of life-threatening anaemia. Additionally, these nonhuman primates may demonstrate variable parasitological parameters and a tolerance for high parasitaemia. There is a limited set of *P. falciparum* and *P. vivax* isolates and antigenic types adapted for these models; the limitation being that the 3D7 isolate is chloroquine-sensitive.

The rhesus monkey possesses a relatively high degree of homology to humans; however, the rhesus monkey is refractory to most human species of malaria parasites. These monkeys are susceptible to sporozoite or blood-stage-induced infections with *P. knowlesi* (a species for which human infections have been reported) and, when infected with appropriate parasite species such as *P. simiovale* or *P. cynomolgi*, these nonhuman primates can develop chronic infections, semi-immune states, frequent recrudescence and patterns of relapsing infections.

Although nonhuman primates are particularly useful for assessing potential efficacy because of their similarity to humans, the availability, cost and ethical considerations surrounding their acquisition, housing, care and disposition present practical limitations.
B.3.3 Immunological end-points

In the immunization-challenge models, the end-points used to define protection are important and vary among different models. The absence of blood-stage infection or delay in patency is used as a measure of pre-erythrocytic protection in the sporozoite-challenge model. In the same model, liver-stage parasite burden can be measured by the reduction in the number of late liver-stage parasites following challenge.

Immunogenicity should be measured as humoral, cellular or functional immune responses in the various models. Parasite-specific assays – such as immunofluorescence assays against sporozoites, liver-stage or blood-stage parasites, or a combination of these – or antigen-specific assays – such as ELISA, that measure the quantity and subclass of antibody to recombinant proteins or synthetic peptides – should be used to characterize humoral responses. Cellular responses should examine CD8+ and CD4+ T cells, and Th1 and Th2 responses using assays such as the enzyme-linked immunosorbent spot (ELISPOT), intracellular cytokine staining or multiparameter flow cytometry. Functional immune-response activity may be measured in vitro using methods such as the growth inhibition assay (45) and antibody-dependent cellular inhibition assay. More extensive analyses of the functional activity of immune responses may include the kinetics and duration of CD8+ and CD4+ cells and antibody responses, as well as assessing the quality or fine specificity of the antibody response.

B.4 Toxicity

Toxicology studies should be undertaken on the final vaccine formulation in accordance with WHO guidelines on nonclinical evaluation of vaccines (7). If the vaccine is to be used in adults, additional studies may be needed (e.g. to assess the risk of using a particular adjuvant/antigen combination during pregnancy).

If the vaccine is formulated with a novel adjuvant, nonclinical toxicology studies should be conducted, as appropriate, for the final vaccine (these should include the antigens and the adjuvant). A repeated-dose toxicity study may be used to compare the safety profile of the novel adjuvant with the safety profile of an established vaccine formulation, taking into account existing guidelines (7, 44). If no toxicological data exist for a new adjuvant, toxicity studies of the adjuvant alone may, in some situations, provide useful information; however, consultation with the NRA is encouraged.

If a novel cell substrate (i.e. a substrate that has not been previously licensed or used in humans) is used for the production of the recombinant antigen (46), safety aspects, such as potential immune responses elicited by residual host-cell proteins, should be investigated in a suitable animal model.
Part C. Clinical evaluation of malaria vaccines

C.1 Introduction

Clinical trials should adhere to the principles described in WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (8) and in WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (47), as well as other guidance (48). All clinical trials should be approved by the relevant NRA. This document adheres to the definition of phases of clinical trials as defined in Guidelines on clinical evaluation of vaccines: regulatory expectations (8). Details specific to malaria vaccines, particularly those related to the definitions of phase IIa and phase IIb trials, are included in section C.1.1 below.

The guidance regarding clinical development programmes provided in this section should be viewed in the light of data on the safety, immunogenicity, efficacy and effectiveness of malaria vaccines that may become available in the future. For example, the existence of a licensed malaria vaccine in the future may influence the design of clinical trials of new malaria vaccines in some settings.

C.1.1 Outline of the clinical development programme

No licensed malaria vaccines are currently available, and no immunological correlates of protection have been established that could be used to predict the protection afforded to individuals by vaccination against clinically apparent malaria. Therefore, the protective efficacy of candidate malaria vaccines has to be demonstrated in prelicensure studies of an appropriate design in which subjects in the control groups do not receive the test product.

Before proceeding to large-scale phase III efficacy studies, the evidence regarding safety, immunogenicity and efficacy obtained from nonclinical studies and phase I and phase II clinical studies should support an expectation that a clinically useful degree of efficacy may be achievable without unacceptable adverse effects, when the vaccine is administered using the planned dosing regimen.

As described in the sections that follow, a typical clinical development programme for a candidate malaria vaccine could include the following, using a logical progression between phases:

- extensive nonclinical studies that provide data to support human use (as described in Part B);
- initial safety and immunogenicity studies in healthy adults – i.e. phase I studies (since it is envisaged that malaria vaccines would ultimately be intended for use from infancy, these studies should include adults who are naive to malaria as well as non-naive adults);
- human-challenge studies in naive adult subjects – i.e. phase IIa studies (see Appendix 1);
- safety and immunogenicity data from subjects aged < 18 years who are resident in endemic areas; with progression to the target age group;
- one or more larger clinical studies in endemic areas that evaluate safety and immunogenicity and are of a sufficient size to provide preliminary evidence of efficacy – i.e. phase IIb studies (some of these studies should be conducted in, or at least include some data from, the target age group);
- one or more concomitant immunization trials in the target population that evaluate potential interactions with other vaccines administered on the same schedule;
- one or more randomized controlled studies of sufficient size to provide definitive evidence of protection in at least one pivotal study of efficacy – i.e. a phase III study;
- post-licensure studies of safety and effectiveness – i.e. phase IV studies.

C.2 Clinical studies

C.2.1 Immunogenicity

The assessment of the immunogenicity of candidate malaria vaccines is an essential part of the clinical-development programme (see Appendix 2 for an example). This is needed to underpin:

- the selection of the dose of antigen;
- the inclusion of an adjuvant (if this is proposed);
- the selection of a primary vaccine regimen to be evaluated for efficacy in the target population, including the route of administration and the immunization schedule;
- the potential for boosting immune responses by revaccination should the efficacy studies or effectiveness data, or both, indicate a waning of protection over time;
- the possible need to adjust the dose regimen for subgroups that may have lower immune responses – such as HIV-positive people with low CD4 cell counts or people who are severely malnourished (immune-response data could be obtained from such subgroups in separate studies or during protective efficacy studies as part of an immunogenicity substudy).

Identifying the parameters that may be most sensitive for demonstrating differences in immune responses (e.g. between vaccine formulations and
populations) is also necessary to ensure that clinical studies are well designed and will support:

- changes in manufacture, which may occur during or after licensure, or both, that are considered to have some potential to affect immune responses;
- assessment of the effects of co-administration with other vaccines (i.e. assessment of immune-interference phenomena);
- comparisons between populations (e.g. in different geographical locations) with varied transmission intensities and varied malaria strains in circulation, and with various host factors that could affect immune responses.

As part of the overall evaluation of immunogenicity, it is recommended that sufficient blood samples be obtained during the phase III efficacy studies to enable both a comprehensive exploration of the immune response to malaria vaccines and an evaluation of any correlation there may be between immune responses and protection against clinical malaria.

C.2.1.1 Measurement of immune responses to vaccination

The mechanism(s) of naturally acquired immunity to Plasmodium species, including P. falciparum, are not fully understood. It is thought that humoral immunity is directed against antigens expressed during the asexual blood stages, but seroprevalence studies in residents of endemic areas have indicated low-to-moderate levels of immunoglobulin G (IgG) directed against the P. falciparum circumsporozoite protein (anti-CS), which is the leading pre-erythrocytic vaccine antigen.

Given the many unknowns and uncertainties regarding the immune mechanisms of naturally acquired protection against clinical malaria, it is preferable that a wide range of immunological parameters are assessed when evaluating vaccine immunogenicity. This approach may also facilitate attempts to detect possible correlations between the immune response to vaccination and protection against clinical malaria (49, 50).

There is no animal model or in vitro assay of functional immune responses to malaria vaccines known to correlate with efficacy. Most experience in evaluating the immune response to malaria vaccines has been gained using ELISA to measure antigen-specific IgG. It is expected that, at a minimum, a validated IgG ELISA for determining antibody concentrations to the relevant antigen will be applied as an indication of vaccine immunogenicity. Sponsors are encouraged to explore alternative assays, including the possibility of measuring functional antibody. The selection of assays used to evaluate the human immune response to a vaccine should be justified by the manufacturer. The use of validated
quantitative assays is critical, and the validation report should include a detailed description of the calibration of any in-house references, and of the processing and storage of samples, reference standards and reagents. Data on assay validation should be reviewed and approved by the NRA.

It is recommended that sponsors also conduct an explorative assessment of cell-mediated immunity (CMI). Both ELISPOT (e.g. for interferon gamma) and intracellular cytokine staining have been used to assess CMI, and efforts to standardize these assays are under way in the context of assessing responses to malaria vaccines (51, 52).

Data on immune responses should be obtained throughout the clinical-development programme (see sections C.2.2.1 and C.2.2.2 on phase I–III studies). The blood volume and the number of samples that can be collected from paediatric subjects may limit the number and type of assays that are possible, especially for infants. It may be necessary to prioritize the assays applied to each specimen or use randomized subsets of samples for each assay, or both.

C.2.1.2 Interpretation of immune responses to vaccination

In order to attempt to identify immunological correlates of protection and in the absence of knowledge regarding which immune parameter is most closely associated with protection against clinical malaria, there should be plans in place to utilize the data obtained from prelicensure and post-licensure clinical studies in which immunogenicity and efficacy have been documented.

The basis for assessing responses to different vaccine doses and for comparing antibody levels between vaccination and control groups should take into account derived measures such as:

- seroconversion rates (using an appropriate definition)
- geometric mean concentrations or geometric mean titres
- reverse cumulative distributions (53).

C.2.2 Efficacy

C.2.2.1 Phase I–II studies

The initial (phase I) studies should be sufficient to provide an early indication of whether severe local or systemic adverse events, or both, occur commonly after vaccination. The data on immune responses from such studies should assist in the identification of candidate malaria vaccines suitable for further investigation.

The first studies will most likely enrol healthy adults who are naive to malaria (as assessed using their residence history, medical history and, possibly, serological testing). It is preferable that the initial evaluation includes subjects who reside in non-endemic areas so that they are not at risk of natural infection
with any species of malaria during the study period because this could complicate the assessments of both safety and immunogenicity.

An initial exploration of safety and immunogenicity may also be conducted in healthy adult residents of an endemic area (i.e. including subjects with evidence of pre-existing immunity to malaria). Such a study could provide further reassurance about the safety and immunogenicity of the vaccine before studies progress to younger age groups and larger numbers of subjects who live in endemic areas.

The preliminary data on safety and immunogenicity should support the selection of one or more vaccine formulations (i.e. in terms of antigen doses, and the need for, and amount of, any adjuvant) and regimens to be used in studies that assess efficacy in the target population. To provide a sound basis for future studies, the immunogenicity data should include measurement of responses after sequential doses and, ideally, an exploration of different dose intervals. After a vaccine has been approved, the most practical way to deploy such vaccines may be by incorporating them into the schedule for the Expanded Programme on Immunization (EPI), in which case consideration should be given to the possibility that three doses of the malaria vaccine may not be required to obtain protection. For example, studies could be conducted to compare regimens with different numbers of doses administered at different time points within the EPI schedule.

Human-challenge studies, which are often referred to as phase IIa studies (see Appendix 3), have been instrumental in identifying pre-erythrocytic candidate malaria vaccine formulations for further evaluation. If performed, such challenge studies should be conducted only in highly specialized units that have appropriate expertise and facilities, and only after approval by local authorities, which should include a review of ethical and technical considerations.

The initial evidence of efficacy may be obtained from phase IIb field-efficacy studies. These should generally follow the design principles of phase III efficacy studies, as described below in section C.2.2.2. Phase IIb studies are intended to provide an estimate of protective efficacy that can inform the design of phase III studies. Since phase IIb studies require fewer subjects than phase III studies, there is usually less geographical spread of study sites and less population diversity.

C.2.2.2 Phase III studies

C.2.2.2.1 Overview

Candidate malaria vaccines should be evaluated in randomized double-blind studies in which the safety and efficacy of the vaccine in the vaccinated group are compared with a control group that does not receive the vaccine. If the results of earlier studies have not provided definitive evidence to support the selection of a
single formulation or regimen for the vaccine, then the study design may need to include more than one group assigned to receive the test product. If so, this has implications for the size of the study and the plan for analysis.

The recommendations made in this section may require reconsideration after at least one malaria vaccine has been approved.

Efficacy studies of pre-erythrocytic vaccines should be designed to allow assessment of protection against blood-stage infection and against disease. Studies designed to assess protection against incident infection require pretreatment with antimalarials, and regular cross-sectional surveys to detect new asymptomatic infections.

Phase III studies should include a sufficient number of subjects to ensure that there is adequate power to allow statistically robust conclusions to be drawn from the predefined primary analysis. A double-blind study design should be maintained at least until all data have been collected for the planned primary analysis or a decision has been taken to terminate the study on the basis of predefined stopping rules or safety concerns. In order to avoid the use of placebo injections (at least for some, if not all, visits) the control group may receive an ethically appropriate licensed comparator vaccine that is not expected to have an impact on the risk of malaria.

The use of an independent monitoring committee (consisting of persons independent of the sponsor and the investigators), with an adequately constituted charter, is strongly recommended. This committee should review the safety data that emerge during the study. The committee may also be charged with implementing stopping rules for reasons of unexpectedly low or high efficacy, if this is in accordance with the study protocol (48).

The following factors should be borne in mind when planning the phase III programme.

- All episodes of malaria that meet the case definition described below), and not just the first episode of malaria, should be captured for the duration of the study since many children will experience several clinical episodes of malaria.

- It is most likely that the ultimate target population for primary vaccination will be (or will at least include) infants. Thus, delivery of malaria vaccine may be incorporated into the EPI schedule with or without a need for one or more booster doses. In addition, the target population may include toddlers or older children, and the vaccine may be incorporated into catch-up programmes. Whatever the immunization schedule, the prelicensure and post-licensure studies should evaluate whether efficacy against clinical malaria persists throughout the age range in which the bulk of malaria-related morbidity and mortality occurs. This is important in order to assess
the possibility that vaccination could result in an upward shift in the age at which severe and potentially life-threatening malaria occurs.

- Vaccine efficacy may differ according to transmission intensity, and genetic factors in humans and parasites. Studies should be conducted in settings of both seasonal and year-round transmission, and in settings with a range of transmission intensities. It is desirable that data on efficacy should be obtained from all, or from a representative selection of, areas in which the vaccine may ultimately be deployed. The sponsor may choose to perform separate studies in different geographical areas, or to conduct one large study that includes study sites considered likely to provide representative data. If the latter approach is adopted, a predefined stratification of enrolment by area could be used to support secondary analyses of efficacy by area or by transmission category or type.

- The concomitant use of other malaria interventions should be documented.

C.2.2.2.2 Design and analysis

The following section discusses essential features of study design that have the potential to affect estimates of efficacy and to influence the extrapolation of the results to non-study populations. These features include:

- the study population
- adjunctive measures
- case-ascertainment methods
- case definitions that have pre-specified:
  - clinical criteria (including severity of illness)
  - laboratory criteria (including sensitivity and specificity)
- approaches to analyses.

C.2.2.3 Study population

The protocol-defined selection criteria should aim to enrol a study population that is as representative as possible of the target population in which the vaccine is expected to be used. It may be appropriate to exclude persons with severe concomitant disease, including severe malnutrition, but subjects who are only mildly malnourished should not be excluded. A risk assessment should be made of the suitability of a given vaccine construct for administration to those with varying degrees of immunodeficiency. It is recommended that asymptomatic HIV-positive subjects should not be excluded unless this is deemed necessary due to the nature of the vaccine construct.
C.2.2.4 Adjunctive measures

In general, treating study subjects with antimalarial agents immediately prior to vaccination is not encouraged since this would not reflect the expected mode of deployment of a malaria vaccine. If pretreatment is used, the pharmacology of the antimalarial agents administered must be well understood, and the plasma half-lives should be sufficiently short to ensure that no impact on the estimate of vaccine efficacy would be expected.

Sponsors and investigators have an obligation to ensure that effective antimalarial chemotherapy is available to treat any subjects who develop clinical malaria during the study. Throughout the study, it is essential to document, as far as is possible, all antimalarial therapy administered to subjects, including the use of intermittent preventive treatment. It is recommended that study sites should not include sites where a significant number of people with malaria might be treated without contact with investigators (e.g. by the purchasing of antimalarial medicines).

The distribution of bednets to study subjects and the indoor residual spraying of their homes should take place in accordance with the policy of the national malaria-control programme. Sponsors and investigators should liaise with the national malaria-control programme to determine whether LLIN should be supplied as part of the study, or whether the programme will ensure distribution through local channels. Sponsors should endeavour to ensure that access to and use of LLIN are maximized throughout the study, given that the use of LLIN is known to reduce malaria morbidity and mortality in endemic settings (54).

To the extent that it is possible, it is desirable to document use of LLIN during the study both for individual subjects and at the community level in order to allow an assessment of the value of the malaria vaccine in the context of LLIN. Information on other issues (e.g. any entomological control measures) that may have an impact on the rates of clinical cases of malaria should be recorded at each study site.

C.2.2.5 Case definition

The following criteria apply to the evaluation of pre-erythrocytic vaccines. Immune responses to blood-stage vaccines are intended to reduce asexual parasite density, and it is therefore possible that the relationship between fever and parasite density could be altered in those receiving blood-stage vaccines. Thus the following criteria are not acceptable for the evaluation of blood-stage vaccines, and research is continuing to try to develop appropriate case definitions for blood-stage vaccines.

Clear definitions of clinical malaria and severe malaria are critical. However, the definition of clinical malaria is not straightforward because in
malaria-endemic settings a child will often have parasitaemia and a coincidental fever that is not caused by parasites in the blood.

A case of clinical malaria should satisfy the clinical diagnostic criteria and should fulfil the relevant parasitological criteria for defining clinical malaria or severe malaria as follows.

C.2.2.2.6 Clinical diagnostic criteria

The recommended definition of a case of clinical malaria is the presence of fever, defined as an axillary temperature of ≥ 37.5 °C, in a child presenting to a health-care facility with an illness consistent with malaria. This definition has been used in many field-efficacy studies of malaria vaccines. It should be noted that it is not the Brighton Collaboration’s definition of fever (55), which is applicable to post-immunization events.

Cases of severe malaria represent a subset of all cases of clinical malaria and are characterized by the presence of at least one of the following:

- prostration
- respiratory distress
- Blantyre coma score ≤ 2
- seizures (two or more)
- hypoglycaemia (blood glucose < 2.2 mmol/L)
- anaemia (haemoglobin < 5g/dl)
- acidosis (base excess ≤ –10.0 mmol/L)
- lactate ≥ 5.0 mmol/L

without any of the following:

- pneumonia (based on clinical assessment and culture)
- meningitis (based on examination of cerebrospinal fluid)
- bacteraemia (based on blood culture)
- gastroenteritis (based on clinical assessment).

All cases of severe malaria should be included with the clinical malaria cases. This case definition of severe malaria is intended to apply to infants and young children. The low incidence of severe disease in adults would most likely preclude measurement of efficacy against severe disease in adults.

C.2.2.2.7 Parasitological criteria for case definitions

If the vaccine is expected to prevent only \textit{P. falciparum} malaria then the case definition should incorporate this restriction. If the vaccine has the potential to
prevent malaria due to other species of Plasmodium, the definition should be adjusted accordingly.

Parasitological diagnosis is based on a parasite density threshold quantified by microscopy. In high-transmission settings the threshold for defining a case should be derived by using recent historical data from each study area that is appropriate to the age group and case-detection system. Within each study, a single threshold should be selected that is considered to be sufficiently specific to all study areas. For a study conducted in low-transmission settings, it may be that the presence of a specific number of parasites defines a case.

Various methods are applied to calculate parasite density from microscopic observations (56). The acceptable method(s) should be predefined in the protocol, and a quality assurance scheme and laboratory accreditation system should be in place to ensure standardization among sites and consistency of data.

C.2.2.8 Case ascertainment

The case-detection system has an important bearing on the interpretation of vaccine efficacy. Either active case detection (ACD) or passive case detection (PCD) may be used.

In phase IIb efficacy studies with a relatively modest number of study subjects, the use of ACD that includes regular home visits by study staff may be appropriate and, depending on the study setting, PCD may also be appropriate. ACD may identify higher numbers of cases of malaria than PCD.

In phase III efficacy studies in which, on suspicion of malaria, study subjects present to designated health-care facilities (i.e. children are taken by caregivers) for diagnosis and treatment, PCD systems may be acceptable.

In both instances it is important that clinical episodes of malaria are captured from the time of the first vaccination onwards, and that the time of onset of clinical malaria is carefully documented to allow for determination of the time elapsed since the last dose of vaccine.

C.2.2.9 End-points and analyses

For each study, the statistical analysis plan (SAP), defining the primary, secondary and any exploratory analyses that are envisaged, should be finalized before unblinding the treatment assignment; ideally, the SAP should be submitted with the protocol. The SAP submission date should allow sufficient time for regulatory review, as well as provide some assurance that finalization of the SAP was not influenced by interim analyses, whether scheduled or unscheduled. If analyses of specific subsets of the study population are warranted, they should be prespecified in the SAP along with methods of analysis. Similarly, if any ad hoc analyses are added, the addition should be justified.
C.2.2.10 Primary analysis of efficacy

It is recommended that the primary analysis compares the rates of all episodes of clinical malaria between vaccinated and unvaccinated groups because this approach best reflects the impact of the vaccine on a community’s burden of disease.

Alternatively, sponsors may choose to predefine the rates of all episodes and the rates of the first (or only) episodes per participant as coprimary end-points.

The protocol and SAP should specify and define all study cohorts to be evaluated, and should justify the set that will be used for each type of analysis (efficacy, immunogenicity and safety). In vaccine efficacy studies, a common approach is to predefine the per protocol population as primary, and to base the primary analysis on the number of episodes meeting the case definition as counted from a minimum number of days after the final dose of the course is given. Immunogenicity data, including the kinetics of the antibody response, should be taken into account when defining the minimum time elapsed since the last dose. Such an analysis would be expected to provide an estimate of the maximum vaccine efficacy achievable in the study setting. However, it is essential that adequate sensitivity analyses of efficacy are performed.

The timing of the primary analysis should be carefully chosen to provide a robust comparison between vaccination and control groups that is appropriate to support a decision on licensure. When planning the study, investigators should consider whether the sponsor’s staff, investigators and subjects will or will not be unblinded with respect to vaccination group at the time of unblinding of the study for the purposes of regulatory submission. The study protocol and SAP must clarify exactly who will be unblinded, and when they will be unblinded, taking into account the possibility that it may not be tenable in some settings to continue to withhold vaccine from unvaccinated persons if the results indicate very high efficacy. These issues need to be carefully considered at the study-planning stage.

As a minimum, the primary analysis should not take place until all subjects have completed at least 12 months of follow up after the last dose of vaccine. The minimum period of post-vaccination follow-up before the primary analysis is performed should also take into account the seasonality of malaria at the study sites since it may be desirable to follow up for more than one transmission season. Prior to study initiation, the sponsors should reach agreement with the relevant NRAs with respect to the duration of follow-up that will be completed by all subjects before the primary analysis is performed that is intended to support initial licensure. Further follow-up should then continue (see section on Study duration) regardless of whether there has been unblinding at the participant level. If an interim analysis is planned, the protocol and SAP should define who will carry out the analysis, describe the analysis schedule, and provide the details of the analysis along with any statistical adjustments considered.
An alternative approach is to plan to conduct the primary analysis when a predefined number of cases of malaria have occurred. However, it would also be necessary to prespecify the minimum duration of follow-up before the primary analysis is performed. The relative merits of these approaches need to be considered early in the protocol-planning phase.

Potential covariate factors for efficacy should be identified in the study protocol so that, where possible, the information required to evaluate their effect will be collected during the study. The SAP should specify the covariates that are to be explored, and the methods to be applied.

C.2.2.2.11 Secondary analyses

Some of the data required for secondary analyses will become available only after the study has been unblinded and the primary analysis has been conducted to support initial licensure. The range of secondary efficacy analyses should include at least the following:

- efficacy against all cases of malaria counting from the time of the first dose;
- efficacy against clinical malaria cases based on higher and lower thresholds of parasite density;
- efficacy against cases of severe malaria;
- efficacy as calculated from predefined data pools by study site(s) and area(s) (e.g. sites or areas with comparable transmission rates, with and without seasonality);
- vaccine efficacy against all episodes of malaria by time since the last vaccine dose.

With regard to the last point on vaccine efficacy against all episodes of malaria by time since the last vaccine dose, the precise period will depend on individual trial designs and the total duration of the trial, although periods of 6–12 months will usually be appropriate. An illustrative example is provided in Table 3.1. This analysis addresses the important public-health issue of whether the relative risk of disease varies in vaccinated and unvaccinated individuals according to the time since vaccination. There may be several reasons for such variation, including waning vaccine efficacy and the future risk of disease being influenced – through acquired partial immunity – by prior disease experience. However, the analysis addresses the important issue of the extent to which, at a given time after vaccination, a vaccinated person is at a differential risk of disease when compared with an unvaccinated person. The initial protocol-defined duration of follow-up may require amendment if there is evidence of waning efficacy and a possible need to assess the effect of one or more booster doses.
In addition to the efficacy analyses, investigators should prespecify the planned analyses of immune responses, including comparisons between vaccination and control groups at various intervals.

Table 3.1
Reporting of all malaria episodes with time since vaccination

<table>
<thead>
<tr>
<th>Time period(^a)</th>
<th>Vaccine group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malaria episodes</td>
<td>Person-years at risk</td>
</tr>
<tr>
<td>(0–X months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(X–Y months)</td>
<td></td>
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<td>(Y–Z months)</td>
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<tr>
<td>(0–Z months)</td>
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</tbody>
</table>

\(^a\) Z months is the total duration of the trial; X and Y are intermediate points during the trial. The number of such subdivisions used may be more or less than shown in this table.

\(^b\) The rate is the number of malaria episodes per person-year at risk.

C.2.2.2.12 Exploratory analyses

Exploratory analyses may include:

- analyses of associations between antibody concentrations and efficacy
- analyses of associations between cell-mediated immune responses and efficacy
- malaria-related admissions to hospital
- all episodes of hospitalization from any cause
- mortality attributed to malaria
- all-cause mortality
- anaemia
- changes in the prevalence of malaria infections.

An exploration of any possible correlation between immune responses and protection against clinical malaria requires a specific plan to collect and analyse a sufficient number of serum samples from subjects in the vaccination and control groups. There are several possible methods that may be applied to these types of exploratory analyses, and it is recommended that advice should be obtained from appropriate experts in this field when developing the protocol.
C.2.2.2.13 Study duration

Whatever the timing of the primary analysis discussed above in section C.2.2.2.10 and the decisions taken regarding unblinding, it is recommended that protocols should plan to follow up on subjects, or on randomly selected subsets of subjects, for a prespecified period of time. While the protocol should propose an initial period of follow-up that takes into account feasibility issues, this may require amendment depending on the early results of the study, possible evidence of waning protection, and the extent of the post-licensure programme to assess effectiveness (see section C.3). If it becomes necessary for subjects assigned to the unvaccinated group to receive the candidate malaria vaccine before the planned end of follow-up, the vaccinated cohort should still be followed for cases of malaria.

Where there is the possibility of waning efficacy over time, there are advantages in identifying a randomized subset of study subjects (from both the vaccination and control groups) to give prior consent to receive booster doses at timed intervals after completing the initial series. This would allow for evaluation of the safety and immunogenicity of booster doses in the previously vaccinated group relative to the first doses received by the control group. These data would be especially valuable if longer-term follow-up eventually indicates waning efficacy, since data would already be available on the safety and immunogenicity of a booster dose.

In addition to following up on cases of malaria, the study population (or, in some cases, predefined subsets of the study population) should be followed up for safety, including assessing the incidence of serious adverse events, and, depending on the vaccine composition, specific events of interest. For example, if the vaccine incorporates a novel adjuvant there may be a theoretical reason to document any possible cases of autoimmune disease occurring in the longer term.

A cohort should also be followed up for immunogenicity. This cohort will need to be identified at the time of randomization to obtain consent for additional blood samples to be taken.

C.2.2.2.14 Concomitant administration with other vaccines

Studies that evaluate co-administration of the candidate malaria vaccine with routine childhood immunizations that deliver antigens are encouraged; such immunizations include diphtheria–tetanus–whole-cell pertussis (DTwP) or diphtheria–tetanus–acellular pertussis (D'TaP) vaccines, *Haemophilus influenzae* type b (Hib) vaccine, oral poliovirus vaccine (OPV) or inactivated poliomyelitis vaccine (IPV), and hepatitis B virus vaccine. Depending on the study site and the schedule for the candidate vaccine, there may be co-administration with other vaccines, including conjugated pneumococcal vaccine, conjugated meningococcal vaccine, oral rotavirus vaccine and measles vaccine.
It is desirable to obtain some information on the possible effects of co-administration on the safety and immunogenicity of the candidate malaria vaccine before commencing large-scale efficacy studies. The effect of malaria vaccination on the immune response to other vaccines is of interest, as is the effect of vaccination with other vaccines on the immune response to the malaria antigen. If necessary, further co-administration studies could be performed in parallel or following completion of phase III efficacy studies. The principles of the design of such studies are discussed in WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (8).

C.2.3 Safety evaluation
The prelicensure assessment of vaccine safety is a critically important part of the clinical programme, and should be developed to meet the general principles described in WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (8). The assessment of safety, with appropriately defined objectives, should be part of the studies described above. Such studies should include a comprehensive assessment of adverse events. Particular attention should be paid to the possible occurrence of specific adverse events that have been associated with vaccines of similar composition.

The minimum acceptable size of the safety database at the time of approval should take into account the vaccine's composition (including all antigens and any adjuvants), whether novel antigens are present, the platforms or recombinant vectors, and the severity of the infectious diseases being prevented.

In the field of blood-stage malaria vaccines, many of the immunological targets are ligand–receptor interactions that mediate adhesion to or invasion of erythrocytes, or both. Clinical evaluation of such vaccines should include haematological monitoring for adverse events, such as anaemia or haemolysis.

Additionally, the dossier should include consideration of safety evaluations in high-risk individuals who may benefit from vaccination. Safety in these groups is often assessed in post-marketing studies (see section C.3); however, a pre-specified plan for such studies is often required at the time of application for marketing authorization.

C.3 Post-licensure investigations
Many malaria vaccines either include novel adjuvants or are based on novel recombinant vector systems. As is the case for all vaccines, there must be adequate systems of pharmacovigilance in place at the time of initial licensure and subsequent to it. In the post-licensure period, longer-term follow-up of subjects enrolled into prelicensure studies will continue. For malaria vaccines intended primarily for use in populations in developing countries, this places a
major emphasis on the strengthening of pharmacovigilance systems in order to
detect rare adverse events that may not have been detected during the prelicensure
phase. For many vaccines, large pharmacovigilance databases generated in high-
income countries may become available after licensure. This may not be the case
for some malaria vaccines. In addition, there will be a need to conduct specific
studies of effectiveness.

In particular, there will be a need to evaluate the vaccine’s effectiveness
during routine vaccination programmes as a result of the unknown validity of
extrapolating an estimate of vaccine efficacy from a phase III study to other
populations, areas and transmission settings. There are several possible factors
that may have an impact on the level of protection (and, hence, the benefit) that
can be achieved by a malaria vaccine, including:

- transmission intensity
- the use of other preventive measures such as LLIN and indoor
  residual spraying
- the health-care system, including the availability of access, diagnosis
  and treatment.

The degree to which the immunization of different age groups does or
does not reduce malaria transmission is an additional piece of information that
is important when assessing the benefit conferred by a malaria vaccine. Where
the anticipated effects on transmission have not been demonstrated prelicensure,
it may be appropriate to design specific post-licensure effectiveness studies to
address this issue. Such studies would most probably be cluster-randomized (6).

In addition, if a malaria vaccine were to confer only a limited duration of
protection, it is possible that widespread use in routine vaccination programmes
could result in deferment of malaria-associated morbidity to an older age range
compared with the pre-vaccine situation. Furthermore, it is unlikely that the
possible need for booster doses can be fully addressed until several years after
the vaccine has been in widespread use. Such decisions would not usually be
based on waning immune responses alone, which would be an expected finding.
Therefore, the overall plans for monitoring the effectiveness of the vaccine should
be adequate for assessing shifts in the demographics and number of malaria cases
over time.

The manufacturer has a responsibility to assess the vaccine’s safety and
effectiveness following initial approval of a new malaria vaccine. At the time of
first licensure, there should be adequate plans in place regarding these activities
and these should conform to applicable legislation. The geographical areas or
countries in which such data can be collected will depend on where and when the
vaccine is introduced into routine programmes. As far as possible, manufacturers
should plan to collect data in specific areas and countries that have been
selected on the basis of their similarity and lack of similarity (in terms of the factors mentioned above and any others that seem important) to regions where the phase III studies were performed. Basic principles for the conduct of post-licensure studies (e.g. that they are intended to provide estimates of effectiveness) and safety surveillance are outlined in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (8).

The collection of reliable and comprehensive data on safety and effectiveness must involve close cooperation between manufacturers and public-health authorities in the areas and countries selected. Preapproval and post-approval discussions between vaccine manufacturers responsible for placing the product on the market and national and international public-health bodies are essential for adequate planning. There may be a need to select areas and countries in which the strengthening of pharmacovigilance functions has occurred or is continuing.

**Part D. Guidelines for NRAs**

**D.1 General**

The general recommendations for NRAs and NCLs given in Guidelines for national authorities on quality assurance for biological products (57) and in Guidelines for independent lot release of vaccines by regulatory authorities (10) apply. These Guidelines specify that no new biological substance should be released until consistency in manufacturing and quality have been established. The detailed production and control procedures, as well as any significant change in them that may affect the quality, safety or efficacy of the malaria vaccine, should be discussed with and approved by the NRA.

Consistency in production has been recognized as an essential component in the quality assurance of malaria vaccines. In particular, NRAs should carefully monitor production records and the results of quality-control tests on clinical lots as well as from a series of consecutive lots of the vaccine.

**D.2 Release and certification by the NRA**

A vaccine should be released only if it fulfils all national requirements or satisfies Part A of these Guidelines, or both (10).

A protocol based on the model given in Appendix 3, signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for release of the vaccine for use.

A statement signed by the appropriate official of the NRA should be provided if requested by a manufacturing establishment, and should certify whether the lot of vaccine in question meets all national requirements as well as Part A of these Guidelines. The certificate should provide sufficient information
about the product. A model certificate is given in Appendix 4. The official national release certificate should be provided to importers of the vaccine. The purpose of the certificate is to facilitate the exchange of vaccines between countries.

Authors

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The first draft was discussed at a WHO working group meeting held on 4–5 October 2010 in Geneva, Switzerland attended by: Dr L. Chocarro, LC Plus Consulting, Canada; Dr P. Corran, honorary position at the London School of Hygiene and Tropical Medicine, England; Dr J.R. Daugherty, United States Food and Drug Administration Center for Biologics Evaluation and Research, USA; Mr A. Mitangu Fimbo, Tanzania Food and Drugs Authority, United Republic of Tanzania; Professor B. Genton, Swiss Tropical and Public Health Institute, Switzerland; Dr I. Hansenne, Scientific Institute of Public Health, Belgium; Mr M.D. Jere, Pharmacy Medicines and Poisons Board, Malawi; Mr E. Karikari-Boateng, Food and Drugs Board Ghana, Ghana; Dr R. Leblanc, United States Food and Drug Administration Center for Biologics Evaluation and Research, USA; Dr B.D. Meade, Meade Biologics, USA; Dr P. Milligan, London School of Hygiene and Tropical Medicine, England; Dr P. Neels, Federal Agency for Medicinal and Health Products, Belgium; Mr J. Pandit, Ministry of Medical Services, Kenya; Ms M.H. Pinheiro, European Medicines Agency, England; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, England; Professor P. Smith, London School of Hygiene and Tropical Medicine, England; Professor T. Smith, Swiss Tropical and Public Health Institute, Switzerland; Dr J.W. van der Laan, National Institute for Public Health and the Environment, the Netherlands; Ms F.A. Kaltovich, Malaria Vaccine Initiative, USA (representing the Program for Appropriate Technology in Health); Dr D. Leboulleux, Malaria Vaccine Initiative, France (representing the Program for Appropriate Technology in Health); Mrs M-C. Uwamwezi, GlaxoSmithKline Biologicals, Belgium (representing the International Federation of Pharmaceutical Manufacturers and Associations); Dr P. Vandoolaeghe, GlaxoSmithKline Biologicals, Belgium (representing the International Federation of Pharmaceutical Manufacturers and Associations); Dr D. Wood, Quality, Safety and Standards, World Health Organization, Switzerland; Dr I. Knezevic, Quality, Safety and Standards, World Health Organization, Switzerland; Dr M. Baca-Estrada, Quality, Safety and Standards, World Health Organization, Switzerland; Dr C. Conrad, Quality, Safety and Standards, World Health Organization, Switzerland;
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The draft Guidelines were posted on the WHO web site for public consultation from 16 April to 15 May 2012.

The document WHO/BS/2012.2186 was then prepared by Dr B.D. Meade, Meade Biologics, USA, in coordination with: Dr C. Conrad, Paul-Ehrlich-Institut, Germany; Dr M. Green, United States Food and Drug Administration, USA; Dr H.N. Kang, Quality, Safety and Standards, World Health Organization, Switzerland; Dr R. LeBlanc, United States Food and Drug Administration, USA; Dr P. Milligan, London School of Hygiene and Tropical Medicine, England;
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Further changes were made to document WHO/BS/2012.2186 by the WHO Expert Committee on Biological Standardization, resulting in the present document.

Acknowledgements

GlaxoSmithKline Biologicals is acknowledged for providing information on the manufacturing process and quality control of the RTS,S/AS01 vaccine.

References


Appendix 1

Controlled human malaria infection trials (human-challenge studies)

Human-challenge studies may be conducted to demonstrate a proof of concept or to gain preliminary information on the efficacy of pre-erythrocytic stage candidate vaccines prior to conducting further clinical studies of efficacy. These studies are considered to be phase IIA studies (see section C.2.2.1). Because human-challenge studies present unique considerations, the sponsor should discuss the study-development plan with the relevant local authorities, including committees that review ethical considerations, prior to initiating such studies for either proof of concept or vaccine efficacy.

Sponsors should provide a description of the human-challenge facility; they should identify any other malaria strains, nonmalaria microorganisms, and other mosquito species cultured and processed in the facility; and they should include details of changeover procedures used to prevent contamination among different strains, microorganisms and mosquito species. In addition, details should be provided regarding the controlled environmental conditions under which the parasites and infected mosquitoes are grown in the facility, and the procedures by which the escape of infected mosquitoes into the environment is monitored and prevented. Also, the sponsors should provide the local authorities with copies of the procedures used for parasite culture, mosquito infection, and challenge of human subjects with infected mosquitoes, as well as the results of tests for sensitivity to antimalarial medicines in the parasite strain(s) used for the challenge.

Administration of sporozoite-stage malaria parasites by mosquito bites has been used extensively to test pre-erythrocytic stage vaccines, and has been instrumental in selecting the most advanced candidate. In the most commonly used model, \textit{Anopheles stephensi} mosquitoes feed on either the chloroquine-sensitive NF54 strain of \textit{P.falciparum} or the 3D7 clone of NF54. Between 14 and 21 days after feeding, the mosquitoes are examined for infection by microscopic examination of their salivary glands to ensure a reliable and reproducible challenge. Subsequently, healthy human volunteers, including immunized subjects and nonimmunized controls, are allowed to be bitten by infected mosquitoes. Volunteers must be carefully screened for their suitability for such studies, and they must provide fully informed consent to indicate that they understand the risks and benefits of challenge studies. Following delivery of the malaria parasites, clinical signs and symptoms are monitored, and a thick blood smear is examined to diagnose blood-stage infection. Upon detection of parasites
microscopically, volunteers must be treated without delay with a rapidly curative antimalarial regimen. The validity of the challenge is verified by concurrently challenging the nonimmunized control group, who are expected to develop malaria infection after being bitten by malaria-infected mosquitoes.

Following sporozoite challenge, it is essential that volunteers be closely monitored in a medical facility or in local hotels. Alternatively, adequate monitoring of subjects at their homes during this phase of the study may be achieved using a combination of technology (e.g. mobile telephones, pagers) and frequent contact between the clinical investigator and the subjects. The monitoring protocol that is to be followed should be specified in the clinical protocol. This should include the criteria to be used to determine whether and when to transfer subjects to an emergency medical facility that has appropriate expertise in the management of malaria.

For the human-challenge study, the sponsor should propose screening and monitoring regimens for cardiac-related adverse events associated with the challenge of subjects with malaria sporozoites or with subsequent antimalarial treatment.

The informed consent form should include information on the previous acute coronary syndrome that occurred in temporal association with a malaria-challenge study in the Netherlands (1). In addition, subjects should be instructed that if such signs or symptoms develop when they are off-site, they should immediately call the clinical investigator or seek care at the nearest appropriate hospital.

Harmonized procedures for designing and conducting controlled human-malaria infection studies are available. They show paramount consideration for safeguarding subjects’ safety and maximizing the comparability of assessments among centres (2). As with all vaccine evaluation methods, new and potentially optimized methods may emerge; these may be considered for adoption in cases in which subjects’ safety and the ability to compare assessments are maintained, and the bridging of results from previous methods to new methods has been demonstrated.

References


Appendix 2

Methodological considerations: quantification of human immunoglobulin G directed against the repeated region (NANP) of the circumsporozoite protein of the parasite P. falciparum (anti-CS ELISA)

Background

This two-step enzyme-linked immunosorbent assay (ELISA) is based on the selective reaction between an antibody and its specific antigen, and allows determination of the titre of antigen-specific antibodies.

Based on information provided by the manufacturer, 96-well polystyrene plates are coated with the R32LR protein corresponding to the repeated region (NANP) of the P. falciparum circumsporozoite protein (CSP). Serial dilutions of serum samples are added directly to the plate. Antibodies to R32LR present in serum samples bind to the precoated R32LR.

Antihuman immunoglobulin G (IgG) horseradish peroxidase (HRP) conjugated antibodies are used as detection reagents; a chromogen substrate solution specific for HRP is used as a colorimetric detection system. The optical density is then obtained to quantify anti-CS IgG in serum samples.

The negative control is a pool of serum samples from nonimmunized individuals (without anti-CS IgG). A positive control and standard control (both containing well defined levels of anti-CS IgG) are run on each plate in order to assess the relative titre of each sample and to control the quality of each assay plate. A standard curve is generated by plotting the optical density of the serum standard against its assigned value (e.g. the titre). The optical density measured for a given sample allows extrapolation of its antibody titre.

Validation issues

The assessment of the immune response should be based on measuring the antibody concentration in serum using a validated and standardized assay. The validation studies should be designed to demonstrate that the assay is suitable for the clinical study. The validation report should include a detailed description of the calibration of any in-house references, and the processing and storage of samples, reference standards and reagents. The assay validation data should be reviewed and approved by the NRA.
Assay characteristics

The limit of detection for the assay is 0.2 endotoxin units per millilitre (EU/ml), and the limit of quantification is 0.3 EU/ml. The analytical range has been defined from 0.3 EU/ml to 190 EU/ml. However, if the value of a sample is above the upper limit of the analytical range, the sample is further diluted, and quantified again. Therefore, technically speaking, no upper limit is applied for the anti-CS ELISA. The cut-off of the ELISA is based on the upper limit of the 99.9% one-sided confidence interval of the anti-CS titres in a naive population originating from a non-endemic malaria region. This cut-off has been set at 0.5 EU/ml, and subjects with antibody titres ≤ 0.5 EU/ml are considered to be seropositive.

On the basis of reproducibility experiments, the overall coefficient of variation of the assay has been estimated at 22.5%, which is in the range of coefficients of variation usually observed for reproducible ELISAs. The anti-CS ELISA has been shown to be linear in a range of titres from 1.1 EU/ml to 2440 EU/ml. Data from competition experiments have suggested that the anti-CS ELISA was specific for R32LR and did not cross-react with the AMA-1 antigen or hepatitis B antigen. Those who have used the assay concluded that the anti-CS IgG ELISA is a reproducible and robust method for quantifying anti-CS antibodies in human noninactivated serum.
Appendix 3

Model protocol for the manufacturing and control of recombinant malaria vaccines

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by a manufacturer to the NRA. Information and tests may be added or omitted as necessary, with the authorization of the NRA.

It is possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO Guidelines for a particular product should be given in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that will accompany the vaccine container. If the protocol is being submitted in support of a request to permit importation, it should also be accompanied by a lot-release certificate from the NRA or from the NCL in the country where the vaccine was produced or released stating that the product meets national requirements as well as the recommendations in Part A of this annex.

1. Summary information on finished product (final lot)

International nonproprietary name: ___________________________
Commercial name: ___________________________________________
Product licence (marketing authorization) number: ________________
Country: ___________________________________________________
Name and address of manufacturer: ______________________________
Name and address of product licence holder, if different: ________________

Batch number(s): ___________________________________________
Finished product (final lot): _________________________________
Final bulk: ________________________________________________
Type of container: ___________________________________________
Number of filled containers in this final lot: _______________________
Number of doses per container: _________________________________
Composition (antigen concentration)/volume of single human dose: ________________________________
Target group for immunization: ________________________________
Expiry date: _______________________________________________
Storage conditions: ________________________________________
A genealogy of the lot numbers of all components used in the formulation of the final product should be provided.

The following sections are intended for reporting the results of tests performed during the production of the vaccine, so that the complete document will provide evidence of the consistency of production. Thus, if any test has to be repeated, this must be indicated. Any abnormal results should be recorded on a separate sheet.

2. Control of source materials (section A.4)

The information requested below is to be presented for each submission. Full details on the master seed and working seed lots, and cell banks, are to be presented only upon first submission or whenever a change has been introduced.

**Cell banks**

Source of antigen (expression system): ________________________________

Master cell bank (MCB) lot number and preparation date: ________________________________

Date of approval of protocols indicating compliance with the requirements of the relevant monographs and with the marketing authorization: ________________________________

Manufacturer’s working cell bank lot number and preparation date: ________________________________

Date of approval of protocols indicating compliance with the requirements of the relevant monographs and with the marketing authorization: ________________________________

Production cell lot number: ________________________________

Storage conditions: ________________________________

**Identification of cell substrate**

Method: ________________________________

Specification: ________________________________

Date of test: ________________________________

Result: ________________________________

Nature and concentration of antibiotics or selecting agent(s) used in the production cell culture’s maintenance medium: ________________________________

Identification and source of starting materials used in preparing production cells, including excipients and preservatives (particularly any materials of human or animal origin – e.g. albumin, serum): ________________________________
3. Fermentation (section A.5)
Provide information on cells corresponding to each single harvest.

**Yeast cells**

**Bacteria and fungi**

Method: ____________________________
Media used and temperature of incubation: ____________________________
Volume inoculated: ____________________________
Date of inoculation: ____________________________
Date of end of observation: ____________________________
Result: ____________________________

4. Single harvests (section A.6)

Batch number(s): ____________________________
Date of inoculation: ____________________________
Date of harvesting: ____________________________
Volume(s) of fermentation paste, storage temperature, storage time and approved storage period: ____________________________

**Culture purity or sterility for bacteria and fungi**

Method: ____________________________
Media used and temperature of incubation: ____________________________
Volume inoculated: ____________________________
Date of start of test: ____________________________
Date of end of test: ____________________________
Result: ____________________________

5. Control of purified antigen bulk (section A.7)

Batch number(s) of purified bulk: ____________________________
Date(s) of purification(s): ____________________________
Volume(s), storage temperature, storage time and approved storage period: ____________________________

**Purity**

Method: ____________________________
Specification: ____________________________
Date of test: ____________________________
Result: ____________________________
### Protein content

- **Method:** 
- **Specification:** 
- **Date of test:** 
- **Result:**

### Antigen content/Identity

- **Method:** 
- **Specification:** 
- **Date of test:** 
- **Result:**

### Lipids

- **Method:** 
- **Specification:** 
- **Date of test:** 
- **Result:**

### Carbohydrates

- **Method:** 
- **Specification:** 
- **Date of test:** 
- **Result:**

### Sterility for bacteria and fungi

- **Method:** 
- **Media used and temperature of incubation:** 
- **Volume inoculated:** 
- **Date of start of test:** 
- **Date of end of test:** 
- **Result:**

### Potential hazards – e.g. residual chemical(s) (if relevant)

- **Method:** 
- **Specification:** 
- **Date of test:** 
- **Result:**

### Residual DNA (if applicable)

- **Method:** 
- **Specification:**
Date of test: __________________________________________
Result: __________________________________________

*Bacterial endotoxins*

Method: __________________________________________
Specification: __________________________________________
Date of test: __________________________________________
Result: __________________________________________

6. Final bulk (section A.8)

Batch number(s) of final bulk: __________________________________________
Formulation date: __________________________________________
Batch number(s) of all components used during formulation: __________________________________________
Volume, storage temperature, storage time and approved storage period: __________________________________________

*Sterility for bacteria and fungi*

Method: __________________________________________
Media and temperature of incubation: __________________________________________
Volume inoculated: __________________________________________
Date of start of test: __________________________________________
Date of end of test: __________________________________________
Result: __________________________________________

7. Filling and containers (section A.9)

Lot number: __________________________________________
Date of filling: __________________________________________
Type of container: __________________________________________
Filling volume: __________________________________________
Number of containers filled: __________________________________________
Date of freeze-drying (if applicable): __________________________________________
Number of containers rejected during inspection: __________________________________________
Number of containers sampled: __________________________________________
Total number of containers: __________________________________________
Maximum period of storage approved: __________________________________________
Storage temperature and period: __________________________________________
8. Control tests on the final lot (section A.10)

**Inspection of containers (A.10.1)**
- **Method:** 
- **Specification:** 
- **Date of test:** 
- **Result:** 

**Appearance (A.10.2)**
- **Method:** 
- **Specification:** 
- **Date of test:** 
- **Result:** 

**Identity (A.10.3)**
- **Method:** 
- **Specification:** 
- **Date of test:** 
- **Result:** 

**Sterility tests for bacteria and fungi (A.10.4)**
- **Method:** 
- **Media used and temperature of incubation:** 
- **Volume inoculated:** 
- **Date of start of test:** 
- **Date of end of test:** 
- **Result:** 

**General safety test (unless omission authorized by NRA) (A.10.5)**

**Test in mice**
- **Number of mice tested:** 
- **Volume injected and route of injection:** 
- **Date of injection:** 
- **Date of end of observation:** 
- **Specification:** 
- **Result:** 

**Test in guinea-pigs**
- **Number of guinea-pigs tested:** 
- **Volume injected and route of injection:** 
- **Date of injection:**
Date of end of observation: ____________________________
Specification: ____________________________
Result: ____________________________

**pH (A.10.6)**
Method: ____________________________
Specification: ____________________________
Date of test: ____________________________
Result: ____________________________

**Pyrogen and endotoxin content (unless omission authorized by NRA) (A.10.7)**
Method: ____________________________
Specification: ____________________________
Date of test: ____________________________
Result: ____________________________

**Protein content (A.10.8)**
Method: ____________________________
Specification: ____________________________
Date of test: ____________________________
Result: ____________________________

**Moisture content (A.10.9)**
Method: ____________________________
Specification: ____________________________
Date of test: ____________________________
Result: ____________________________

**Potency test (A.10.10)**
*In vitro assay*
Method: ____________________________
Specification: ____________________________
Date of test: ____________________________
Result: ____________________________

*In vivo assay*
Number of mice tested: ____________________________
Species, strain, sex, ages and weight range: ____________________________
Dates of immunization: ____________________________
Date of test: ____________________________
Vaccine doses (dilutions) and number of animals responding at each dose: ________________

ED\textsubscript{50} of standard vaccine and test vaccine: ________________

Potency of test vaccine versus standard vaccine, with 95% confidence interval of the mean: ________________

Validity criteria: ________________

Date of start of period of validity: ________________

Control tests on the adjuvant (A.10.11)

Identity (A.10.11.1)

Method: ________________

Specification: ________________

Date of test: ________________

Result: ________________

Content of component 1 (A.10.11.1)

Method: ________________

Specification: ________________

Date of test: ________________

Result: ________________

Content of component 2 (A.10.11.1)

Method: ________________

Specification: ________________

Date of test: ________________

Result: ________________

Adjuvant system quality attributes – quality (A.10.11.2)

Method: ________________

Specification: ________________

Date of test: ________________

Result: ________________

Adjuvant system quality attributes – purity (A.10.11.2)

Method: ________________

Specification: ________________

Date of test: ________________

Result: ________________

Adjuvant system quality attributes – sterility tests for bacteria and fungi (A.10.11.2)

Method: ________________

Media used and temperature of incubation: ________________
Volume inoculated: ........................................................................................................
Date of start of test: ........................................................................................................
Date of end of test: ........................................................................................................
Result: ...........................................................................................................................

9. Certification by the manufacturer

Name of the manufacturer ............................................................................................... 
Name of head of production (typed) .................................................................................. 

Certification by the person from the control laboratory of the manufacturing
company taking responsibility for the production and control of the vaccine

I certify that lot no. __________________________ of recombinant malaria vaccine,
whose number appears on the label of the final container, meets all national
requirements and/or satisfies Part A\(^1\) of the WHO Guidelines on the quality, safety
and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and
blood stages of *Plasmodium falciparum* (2014).\(^2\)

Signature ............................................................................................................................
Name (typed) ......................................................................................................................
Date ....................................................................................................................................

10. Certification by the NRA

If the vaccine is to be exported, attach a certificate from the NRA (as shown in
Appendix 3), a label from a final container and an instruction leaflet for users.

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\(^1\) With the exception of provisions on distribution and shipping, which the NRA may not be in a position
to assess.

Appendix 4

Model certificate for the release of recombinant malaria vaccines by NRAs

Lot release certificate
Certificate no. ____________________

The following lot(s) of recombinant malaria vaccine produced by ____________________\(^1\) in ____________________,\(^2\) whose numbers appear on the labels of the final containers, complies with the relevant national specifications and provisions for the release of biological products\(^3\) and with Part A\(^4\) of the WHO Guidelines on the quality, safety and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum* (2014),\(^5\) and complies with WHO good manufacturing practices: main principles for pharmaceutical products;\(^6\) Good manufacturing practices for biological products;\(^7\) and Guidelines for independent lot release of vaccines by regulatory authorities.\(^8\)

The release decision is based on ____________________\(^9\)

The certificate may include the following information:

- name and address of manufacturer
- site(s) of manufacturing
- trade name and common name of product
- marketing authorization number

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\(^1\) Name of manufacturer.

\(^2\) Country of origin.

\(^3\) If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA.

\(^4\) With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.


\(^9\) Evaluation of the summary protocol, independent laboratory testing, or procedures specified in a defined document etc., as appropriate.
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary)
- type of container used
- number of doses per container
- number of containers or lot size
- date of start of period of validity (e.g. manufacturing date) and/or expiry date
- storage conditions
- signature and function of the person authorized to issue the certificate
- date of issue of certificate
- certificate number.

Director of the NRA (or other appropriate authority)

Name (typed) __________________________________________________________
Signature ____________________________________________________________
Date _________________________________________________________________