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Annex 1
Guidelines on clinical evaluation of vaccines: regulatory expectations

This document provides guidance for national regulatory authorities and vaccine manufacturers on the clinical evaluation of vaccines by outlining the international regulatory expectations applicable to the different stages of vaccine development and for marketing approval. For this reason, the guidance in this document could also be useful for clinical researchers and investigators.

The text is presented in the form of guidelines rather than recommendations because vaccines are a heterogeneous class of agents, and the preclinical and clinical testing programmes will need to be adapted for each individual product. Guidelines allow greater flexibility than recommendations with respect to specific issues related to particular vaccines.

A separate WHO document intended to provide more detailed guidance on preclinical and laboratory evaluation of vaccines is in preparation. This was subsequently established by the 54th meeting, November 2003, of the WHO Expert Committee or Biological Standardization and is to be published in the WHO Technical Report Series. The section of this document that discusses preclinical and laboratory evaluation consequently provides general guidance, but does not define international regulatory expectations in this area.

Introduction

This document provides guidance to national regulatory authorities (NRAs), manufacturers, clinical researchers and investigators on the clinical evaluation of vaccines by outlining the data that should be obtained during the different stages of vaccine development to support an application for marketing approval. This document has been prepared in response to requests from NRAs for assistance in the evaluation of clinical trials, both during the clinical development of a new vaccine and during the regulatory review of dossiers submitted in support of applications for marketing authorization. The NRAs

should have a mandate to review protocols, and when this is necessary to protect the safety of subjects, to require revision of the protocol and/or termination of the trial. This document is intended to provide basic guidance to NRAs on how to achieve these objectives. Because it is common practice for the clinical development programmes and the individual clinical trials to take place in different countries, each NRA should, as far as possible, collaborate with the other regulatory authorities involved to benefit from shared experiences and to align regulatory considerations (*1*).

The World Health Organization (WHO) has made available the following guidelines and requirements that are relevant to the evaluation of vaccines: good clinical practice for trials on pharmaceutical products (*2*), good manufacturing practice for pharmaceutical preparations (*3*, *4*), good manufacturing practice for biological products (*5*), regulation and licensing of biological products in countries with newly developing regulatory authorities (*1*) and guidelines for national authorities on quality assurance for biological products (*6*). Guidelines and recommendations for the production and control of specific vaccines have been reviewed in detail in a series of WHO technical reports (*7*), which should be consulted where applicable but will not be discussed further here. However, there is no existing WHO document that gives guidance on the planning, performance and assessment of clinical studies on vaccines with a regulatory perspective. Specific WHO guidelines that complement this document are available for malaria (*8*) and dengue (*9*) or are in preparation in the case of certain candidate vaccines, such as for human immunodeficiency virus (HIV). Basic standards of care, including details about the cold chain required for transport and storage of vaccines, proper injection techniques for delivery of vaccines and safety of injections have already been described in the WHO manual *Immunization in practice* (*10*).

Guidance on various aspects of clinical trials of vaccines is also available from several other bodies such as the International Conference on Harmonization (ICH), the European Agency for the Evaluation of Medicinal Products (EMEA), the United States Food and Drug Administration (FDA) and the United Kingdom Medical Research Council (MRC). These WHO guidelines are not intended to conflict with, but rather to complement, these other documents (*11–16*, *18–39*).

Regulation of vaccines

Regulatory issues related to a particular candidate vaccine should be considered early in the development process, since compliance with regulatory requirements is the basis for eventual approval. It is strongly recommended that dialogue with the appropriate national regulatory authority be established early on. The national regulatory authority should review the plans for development of the candidate vaccine and clarify requirements for carrying out clinical trials, as well as for marketing approval.

The regulation of vaccines can be divided into three stages: developmental, licensure and postlicensure (*40*). The developmental stage consists of two parts, preclinical research and development, and clinical research and development.

Preclinical testing

Preclinical research and development are carried out in the laboratory using in vitro techniques or, when necessary, in vivo techniques in animals. The data from preclinical and laboratory research include details of the development and production of a vaccine together with reports of control testing, which should be adequate to justify subsequent clinical studies in humans.

Phases of clinical development (I–III)

Clinical trials in humans are classified into three phases: phase I, phase II and phase III and in certain countries formal regulatory approval is required to undertake any of these studies. This approval takes different forms in different countries (e.g. Investigational New Drug Application (IND) in the United States and Clinical Trial Certificate or Clinical Trial Exemption (CTX) in the United Kingdom). This is in addition to ethical clearance which is required for clinical trials in all countries. All studies of human subjects require proper ethical review, in accordance with the Declaration of Helsinki (see http://www.wma.net/e/).

The phase I clinical studies carry out initial testing of a vaccine in small numbers (e.g. 20) of healthy adults, to test the properties of a vaccine, its tolerability, and, if appropriate, clinical laboratory and pharmacological parameters. Phase I studies are primarily concerned with safety. Phase II studies involve larger numbers of subjects and are intended to provide preliminary information about a vaccine's ability to produce its desired effect (usually immunogenicity) in the target population and its general safety. To fully assess the protective

efficacy and safety of a vaccine, extensive phase III trials are required. The phase III clinical trial is the pivotal study on which the decision on whether to grant the licence is based and sufficient data have to be obtained to demonstrate that a new product is safe and effective for the purpose intended.

By the beginning of the phase III stage of development, a vaccine should have been fully characterized and the final manufacturing process, specifications and batch release testing procedures should have been established. An application for market authorization may be submitted to an NRA on the basis of the data from phase III testing and if approved, the vaccine then becomes commercially available in that particular country. If a product contains or consists of genetically modified organisms an environmental risk assessment should also be undertaken and approved by the appropriate agency.

The structure of the clinical development programme must be tailored to the type of vaccine and the antigenic content. For example, the clinical evaluation of a vaccine that contains only novel antigen(s) may of necessity be very different from that of a vaccine that contains one or more previously evaluated antigens. Such factors also influence whether clinical protection trials will be required, whether or not they are feasible, or whether an approval may reasonably be based on immunogenicity data. In all instances, it is the obligation of the applicant to justify the content and structure of the clinical development programme. Pre-submission meetings with regulatory authorities may assist in ensuring that the content of the final data package is likely to be acceptable.

Issues to be considered after the initial licensure

In addition to phase I, II and III studies that may be performed before or after the first licensure of a new vaccine, which are described under other relevant trials as outlined above, the postmarketing period is critical for the collection of data on the safety and effectiveness of a vaccine in large numbers of recipients; these data may come from both active and passive modes of surveillance. Following licensing, there is continued surveillance of vaccinees for adverse events, especially for those rare events that can be detected only in very large numbers of subjects.

Any change in production methods or scale-up following licensing will necessitate further product characterizations to demonstrate equivalence, although the extent of re-characterization required depends on the nature of the changes implemented. Further characterizations should be documented and the NRA should be notified of all

changes. Regulatory authorities should clearly define and implement in their regulations which changes require only a notification and which changes require a formal approval before they can be introduced. This will be decided on a case-by-case basis and, in all instances, regulatory approval for a change must be obtained before the vaccine is used.

Glossary

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

Adverse event
Any untoward medical occurrence in a clinical trial subject to whom a vaccine has been administered; it does not necessarily have a causal relationship with the vaccine/vaccination.

Adverse reaction
A response to a vaccine that is noxious and unintended and that occurs at doses tested in humans for prophylaxis, or during subsequent clinical use, following licensure. The term adverse reaction is usually reserved for a true causal association with a drug or a vaccine.

Attack rate
The proportion of the population exposed to an infectious agent who become (clinically) ill.

Audit
A systematic examination, carried out independently by persons not directly involved in the clinical trial, to determine whether the conduct of a trial complies with the agreed protocol and whether the data reported are consistent with the records on site, e.g. whether data reported or recorded in the case report forms are consonant with those found in hospital files and other original records.

Blinding
A procedure in which one ore more parties to the trial are kept unaware of the treatment assignment(s). Single blinding usually refers to the subject(s) being unaware of the treatment assigned to them, and double blinding usually refers to the subject(s), investigator(s) and, in some cases, data analyst(s) being unaware of the treatment assignment.

Booster vaccination
Vaccination given at a certain time interval (at least 6 months) after primary vaccination in order to induce long-term protection.

Bridging studies
Studies intended to support the extrapolation of efficacy, safety and immunogenicity data from one formulation, population or dose regimen to another.

Case–control study
An observational study in which the exposure to a particular risk factor (the vaccine in the case of vaccine studies) is determined retrospectively, and the effect of this exposure is compared between individuals (the cases) who experience an event (the disease, in vaccine studies) and individuals who do not (the controls).

Case definition
A set of diagnostic criteria that must be fulfilled to confirm a case of a particular disease. Case definitions can be based on clinical criteria, laboratory criteria or combinations of the two.

Case report form
A document used to record data on a subject participating in a clinical trial during the course of the trial, as defined by the protocol. The data should be collected by procedures that guarantee preservation, retention and retrieval of information and allow easy access for verification, audit and inspection.

Cluster
Aggregation of relatively uncommon events or diseases in space and/or time in amounts that are believed or perceived to be greater than could be expected by chance.

Cohort study
A retrospective or prospective study in which the development of a disease or infection, or any other relevant event, is observed over time in a defined group of subjects.

Colonization
The asymptomatic, often transient, presence of a microbe as a part of the normal microflora of a host (e.g. pneumococci on the mucosae of the upper respiratory tract).

Community investigation
A population-based trial in large predefined segments of the population to investigate the impact of a treatment on a preventable infectious disease.

Comparator product
A pharmaceutical or other product (which may be a placebo) used as a reference in a clinical trial.

Contact
An individual who has had contact with an infected person (case) in a way that is considered as having caused significant exposure and therefore a risk of infection.

Control
Any comparator suitable for validation of the trial. The comparator may be either an active treatment or a placebo control.

Equivalence trial
A trial having the primary objective of showing that the response to two or more treatments differs by an amount that is clinically unimportant. Showing that the true treatment difference is likely to lie between a lower and an upper equivalence margin of clinically acceptable differences usually demonstrates this.

Experimental study
A study in which the conditions are under the direct control of the investigator. Such studies may include random allocation of subjects to treatment or control groups and blinding of subject and investigator to the placement status (i.e. whether in the treatment or control group).

Exposure
Having contact with an infectious agent in a way that experience has shown may cause disease.

Foreign clinical data
Clinical data generated outside the target region (i.e. in a foreign region).

Geometric mean titre
Calculation of the average titre for a group of subjects by multiplying all values and taking the *n*th root of this number, where *n* is the number of subjects.

Good clinical practice
A standard for clinical studies that encompasses the design, conduct, monitoring, terminations, audit, analyses, reporting and documentation of the studies, ensures that they are scientifically and ethically sound, and that the clinical properties of the pharmaceutical product (diagnostic, therapeutic or prophylactic) under investigation are properly documented.

Good manufacturing practice
That part of the pharmaceutical quality assurance process which ensures that products are consistently produced and to meet to the quality standards appropriate to their intended use and as required by the marketing authorization. In these guidelines, good manufacturing practice refers to the current good manufacturing practice guidelines published by WHO.

Immunogenicity
The capacity of a vaccine to induce antibody-mediated and/or cell-mediated immunity and/or immunological memory.

Incidence
The number of persons who fall ill with a certain disease during a defined time period.

Informed consent
A subject's voluntary confirmation of his or her willingness to participate in a particular trial, and the documentation thereof. This consent should be sought after giving the subject appropriate information about the trial, including an explanation of its status as research, its objectives, potential benefits, risks and inconveniences, alternative treatment that may be available, and of the subject's rights and responsibilities in accordance with the current revision of the Declaration of Helsinki.

Inspection
An officially conducted examination (i.e. review of the conduct of the clinical trial, including quality assurance, personnel involved, any delegation of authority and audit) by relevant authorities at the site of the trial and/or the site of the sponsor in order to verify adherence to good clinical practice as set out in these guidelines.

Internal control
An additional control arm in a vaccine trial, usually a placebo, which may be required when the efficacy of the active comparator is not adequately established or is known to give inconsistent results.

Investigator
A person responsible for the clinical trial and for the rights, health and welfare of the subjects in the trial. The investigator should have qualifications and competence in accordance with the local laws and regulations as evidenced by an up-to-date curriculum vitae and other relevant credentials. Decisions relating to medical or dental care, and their provision must always be the responsibility of a clinically competent person legally allowed to practice medicine or dentistry.

Minimal risk
A level of risk similar to the risk encountered during an individual's usual daily activities. Minimal risk would apply to activities such as physical examination, venipuncture or urine sample collection.

Non-inferiority trial
A trial with the primary objective of showing that the response to the product under investigation is not clinically inferior to the control vaccine (active or placebo).

Observational studies
Observational studies focus on events, exposures and diseases occurring in the population during their everyday life, not subject to experimental interventions.

Outbreak
The occurrence of two or more linked cases of a communicable disease.

Placebo control
A comparator in a vaccine trial that does not include the antigen under study. In studies of monovalent vaccines this may be an inert placebo (e.g. saline solution or the vehicle of the vaccine), or an antigenically different vaccine. In combined vaccines, this may be a control arm in which the component of the vaccine being studied is lacking.

Post-marketing surveillance
A system for monitoring adverse events following licensure. Postmarketing surveillance can be passive or active and its objectives include, but are not limited to, the following:

— the identification of rare adverse reactions not detected during pre-licensure studies; and
— the identification of risk factors or pre-existing conditions that may promote reactions.

Potency
The quantitative measure of the specific ability or capacity of the product to achieve a defined biological effect.

Pre-exposure trial
A prospective trial in a population expected to be exposed to the pathogen under study within a predefined, relatively short, period.

Prevalence
The number of persons who have a particular disease at a specific time.

Primary vaccination
First vaccination, or series of vaccinations given within a predefined period, with an interval of less than 6 months between doses, to induce clinical protection.

Protocol
A document that states the background, rationale and objectives of the clinical trial and describes its designs, methodology and organization, including statistical considerations, and the conditions under which it is to be performed and managed. The protocol should be signed and dated by the investigator, the institution involved and the sponsor. It can also serve as a contract.

Randomization
In its simplest form, randomization is a process by which n individuals are assigned to a test (n_T) or control (n_C) treatment so that all possible groups of size $n = n_T + n_C$ have equal probability of occurring. Thus randomization avoids systematic bias in the assignment of treatment. It also promotes balance with respect to known and unknown prognostic factors that could affect the outcome of interest. While it does not guarantee that treatment groups will be exactly equal with respect to these factors, it does guarantee that any imbalance that occurs arose purely by chance. The process of randomization guarantees the validity of statistical analyses of treatment effect, and (with adequate sample size) allows the detection, or ruling out, of small or moderate treatment differences.

Reactogenicity
Reactions, either local or systemic, that are considered to have a causal relationship to the vaccination.

Reproductive rate
The average number of secondary cases of an infection arising from a single primary case. The measure is inherent to the potential (infectiousness, susceptibility, measures of protection) of a microorganism to spread from person to person in a population.

Secondary attack-rate study
An outbreak investigation in a defined susceptible population. The population to be studied is either a cluster (in an urban or semi-urban setting) or a household (or family). Outbreak investigations may be either observational or experimental. The unit of randomization may be the individual, a household or a cluster.

Sensitivity (statistical)
The probability that a test will detect a disease/condition when it is used on an individual who truly has the disease/condition. It is estimated in a study as the proportion of individuals with positive test results out of all individuals classified by a gold standard as having the disease/condition.

Serious adverse event
An event occurring in connection with the clinical trial that results in death, admission to hospital, prolongation of a hospital stay, persistent disability or incapacity, or is otherwise life-threatening.

Seroconversion
Predefined increase in antibody concentration, considered to correlate with the transition from seronegative to seropositive, providing information on the immunogenicity of a vaccine. If there are preexisting antibodies, seroconversion is defined by a transition from a predefined low level to a significantly higher defined level such as a fourfold increase in geometric mean antibody concentration.

Serological surrogate
Predefined antibody concentration correlating with clinical protection.

Serosurveillance
The surveillance of an infectious disease by measuring disease-specific antibodies in a population or subpopulation.

Specificity (statistical)
The probability of a negative test result when a test is used on an individual who truly does not have the disease/condition. It is esti-

mated in a study as the proportion of individuals with negative test results out of all individuals classified by a gold standard as not having the disease/condition.

Sponsor
An individual, a company, an institution or an organization that takes responsibility for the initiation, management and/or financing of a clinical trial. When an investigator initiates and takes full responsibility for a trial, the investigator has then also assumed the role of the sponsor.

Standard deviation
The measure of the variability of a sample of observations around the mean.

Superiority trial
A trial with the primary objective of showing that the response to the product under investigation is superior to the control vaccine (active or placebo).

Surveillance
The systematic collection, collation and analysis of data and the dissemination of information to those who need to know in order that appropriate action may be taken.

Survey
An investigation in which information is systematically collected. It is usually carried out in a sample of a predefined population group for a defined time period. A survey is not a continuous investigation and may be repeated after a period of time. If repeated regularly, surveys can form the basis of a surveillance system.

Vaccine (protective) efficacy
The reduction in the chance or odds of developing clinical disease after vaccination relative to the chance or odds when unvaccinated. Vaccine efficacy measures direct protection (i.e. protection induced by vaccination in the vaccinated population sample). Vaccine efficacy is calculated according to the following formula:

$$\mathrm{VE} = \left(\frac{Iu - Iv}{Iu}\right) \times 100\% = \left(1 - \frac{Iv}{Iu}\right) \times 100\% = (1 - \mathrm{RR}) \times 100\%$$

where Iu = incidence in unvaccinated population; Iv = incidence in vaccinated population; RR = relative risk

Vaccine effectiveness
The protection rate conferred by vaccination in a specified population. Vaccine effectiveness measures both direct and indirect protection (i.e. protection of non-vaccinated persons by the vaccinated population). Vaccine effectiveness is also determined by vaccination coverage, correlation of vaccine strains with circulating strains and incidence of disease due to strains not included in the vaccine following introduction of the vaccine in that population.

Vaccine failure
The onset of infection or disease, biologically confirmed, in a subject who is supposed to be protected, following completion of age-appropriate immunization as recommended by the manufacturer.

Validation
The action of proving in accordance with the principles of good clinical practice, that any procedure, process, equipment (including the software or hardware used), material, activity or system actually leads to the expected results.

Vector
A carrier, most often an animal or arthropod that transfers a pathogen from an infected person(s) or animal to a susceptible individual.

Scope of the document

Vaccines are a heterogeneous class of prophylactic medicinal products containing antigenic substances capable of inducing specific, active and protective host immunity against an infective agent or toxin, or against other important antigenic substances produced by infective agents. Vaccines for human use contain one of the following: microorganisms inactivated by chemical and/or physical means that retain adequate immunogenic properties; living microorganisms that are avirulent to humans or have been selected for their attenuation whilst retaining immunogenic properties; or antigens extracted from organisms, secreted by them, or produced by recombinant DNA technology. The antigens may be in their native state, detoxified by chemical or physical means and/or aggregated, polymerized or conjugated to a carrier to increase immunogenicity.

This document also covers novel products such as DNA vaccines and live genetically engineered microorganisms used themselves as vaccines or used as carriers for other antigens. However, therapeutic

vaccines (e.g. viral-vector-based gene therapy, tumour vaccines and anti-idiotypic vaccines such as monoclonal antibodies used as immunogens) are *not* considered here.

Part A. Preclinical and laboratory evaluation of vaccines

A.1 General remarks

The preclinical evaluation of a vaccine is a prerequisite for the initiation of clinical trials. Laboratory evaluation should however be continued throughout both the preclinical and clinical phases of vaccine development. This section on preclinical and laboratory testing discusses the general principles for the nonclinical evaluation of vaccines which should be taken into consideration both before and during clinical trials. (A document which deals with the nonclinical and laboratory evaluation of vaccines in more detail has also been prepared by WHO. Established by the 54th meeting, November 2003, of the WHO Expert Committee on Biological Standardization and to be published in the WHO Technical Report Series.)

The primary goal of preclinical testing of a new vaccine product, or a new combination vaccine comprised of previously licensed antigen(s), or vaccines presented in new formulations or new delivery systems, should be to demonstrate that the vaccine is suitable for testing in humans.

Preclinical and laboratory studies are aimed at defining the characteristics (physical, chemical and biological) of a product, including the indicators of safety and immunogenicity in an appropriate animal model. When preclinical testing is performed in animals, there should always be a clear rationale for doing so, and the study should be performed in compliance with Good laboratory practice guidelines (*11*) and with national guidelines on animal experimentation. In addition to establishing the characteristics of the candidate vaccine, preclinical and laboratory studies may also identify possible risks to the vaccinees, and can be used to plan protocols for subsequent clinical studies in human subjects in which safety and efficacy of the candidate vaccine are evaluated.

Close collaboration between the preclinical and the clinical investigators is particularly important in assessing the first results of the administration of vaccines in humans. The clinician, in consultation with the appropriate advisers, has, however, the responsibility of ensuring that the preclinical experiments are adequate in scope and for requesting a full account of all relevant data.

A.2 Production, characterization and quality assurance of candidate vaccines

The basic principles for the production and control of vaccines are set out in the relevant publications in WHO Technical Report Series which cover general requirements (*41–46*). Specific guidelines and recommendations for particular vaccines are also available (*7*) and should be consulted as appropriate. The WHO guidelines and recommendations are often adopted by national regulatory authorities as definitive national requirements. Other useful guidance may be obtained from the documents produced by other bodies (*47*). The characterization, standardization and control of the components, safety and potency of vaccine preparations are key issues during development. The amount of data collected to support clinical studies should increase throughout phases I and II, and product characterization should be completed by the beginning of the phase III stage of development. In-process testing should be performed to ensure adequate control over the manufacturing process and manufacturing consistency. Analytical criteria should be established during product development and used subsequently to evaluate new batches and to establish batch-to-batch consistency. The tests adopted for routine batch release should be a selection of those tests used for the initial characterization of the vaccine. A batch release protocol providing an outline of production and a summary of the test results and establishment specifications should be available for each batch.

Candidate vaccines for clinical trials should be prepared according to good manufacturing practices. The general manufacturing recommendations contained in good manufacturing practices for pharmaceutical and biological products (*3–5*) should be applied by all establishments involved in producing candidate vaccine for clinical studies. Standard operating procedures covering all aspects of production, quality control, storage and distribution should be documented.

Any proposed change in the formulation of a vaccine should be considered carefully both by the manufacturers and NRAs. Some changes in formulation may have a serious effect on the quality, safety and efficacy of vaccines and will subsequently require clinical trials.

Sufficient stability data should be generated to support clinical trials. Accelerated stability data could be used to support preliminary data generated at the normal storage temperature. Further data on stability to support the expiry date of the product for licence should be based on long-term, real-time, stability studies under the real conditions of use. All relevant documentation should be made available to the regulatory authorities.

In accordance with good clinical practice, sufficient samples of each batch of candidate vaccine, together with a record of analyses and characteristics, must be kept for future reference by the manufacturer and ideally a national control laboratory (NCL) for possible subsequent re-testing and investigation. The product should be stored under safe and stable conditions for at least the duration of its anticipated or approved shelf-life and preferably longer.

A.3 Toxicity and safety testing

Toxicity studies in animals may be considered for the assessment of the potential toxic effects of a vaccine in target organs, including the haematopoietic and immune systems as well as to assess systemic toxicity. Such studies may help to identify potential toxicity problems requiring further clinical monitoring. Detailed guidance on toxicological and pharmacological testing may be found in the EMEA *Note for guidance on preclinical pharmacological and toxicological testing of vaccines* (*12*). However, it should be recognized that a suitable animal model may not be available for undertaking toxicological evaluation of candidate vaccines, and such models are not necessarily predictive of human responses the interpretation of the results may be difficult. Furthermore, a classical repeated dose toxicity test as applied to medicines may or may not be applicable for vaccines. Applicability of repeated dose toxicity tests depends on the vaccine dose regimen and the composition of the vaccine. Usually there is no chronic exposure of the subject to a vaccine through repeated administration.

The design and value of repeated-dose toxicity tests should therefore be considered on a case-by-case basis, as should the selection of the animal species used for these investigations. If a vaccine is intended to be clinically tested in women of childbearing age, the need for reproductive toxicity studies and studies of embryo/fetal and perinatal toxicity should be considered on a case-by-case basis. Reproductive toxicity studies, where appropriate, will need to be undertaken before licensing.

Toxicity tests should include:

— an evaluation of the initial safe dose and of subsequent dose escalation schemes relevant to the clinical dose;
— an evaluation of single and repeated doses as appropriate;
— a determination of a set of relevant safety parameters for clinical monitoring;
— a demonstration of potential reversibility of virulence of attenuated vaccine strains;

— a demonstration of the completeness of inactivation for inactivated vaccine strains;
— a demonstration of the completeness of inactivation as well as reversibility to toxicity of toxoids;
— local tolerability studies; and
— an evaluation of the potential of the vaccine antigen(s) to induce antibodies that cross-react with human tissues, where appropriate (e.g. streptococcal vaccine).

Where different routes of administration are proposed, multiple safety and toxicity studies in a suitable animal model should be considered. These should address the specific safety concerns associated with administration of the vaccine by each of the proposed routes. Caution is recommended when extrapolating safety data obtained using one route of administration to other routes.

A.4 Potency and immunogenicity

A.4.1 *Potency*

Where relevant, potency tests should be established during vaccine development and used for routine batch release. Examples of potency assays are challenge models such as the intracerebral mouse test for pertussis and rabies vaccines, and evaluations of infectious units of live attenuated organisms for viral vaccines and bacille calmette-Guèrin (BCG). Ideally, the potency assay should mimic the clinically expected function of the vaccine in humans (as for rabies vaccine). However, in many cases, this is not possible and the assay is based on artificial challenge procedures that assess clinical protection (e.g. potency test for whole cell pertussis vaccine). For polysaccharide vaccines chemical characterization may be sufficient. For products for which little is known about the pathogenic mechanism and or the protective factors, animal testing with subsequent serological evaluation or challenge testing is informative. However, as understanding of the mechanism of protection and immunity to vaccine increases, every effort should be made to replace in vivo potency assays with validated in vitro alternatives based on the biological activity of the product, test systems and novel laboratory methods as they become available.

A.4.2 *Immunogenicity*

Data obtained from the immunization of animals with candidate vaccine preparations will provide valuable information to support a clinical indication. Such studies may include testing in non-human primates, but only if an appropriate disease model is available. Immu-

nogenicity data derived from animal models can help in the selection of the doses, schedules and routes of administration to be evaluated in clinical trials. Preclinical studies should be designed to assess the relevant immune responses, e.g. seroconversion rates, geometric mean antibody titres, or cell-mediated immunity in vaccinated animals. Such studies may also address interference between antigens and/or live viruses. If a vaccine consists of more than one antigen (e.g. acellular pertussis vaccine) the response to each antigen should be evaluated. Immunogenicity studies may include the characterization of antibody class, avidity, affinity, half-life, memory, and potential induction of cell-mediated immunity as well as release of soluble mediators affecting the immune system, as appropriate.

Of primary concern in interpreting the data obtained from such studies should be how closely the animal models resemble the human disease and human immune responses. For example, the demonstration of humoral antibody responses in an animal model to a vaccine delivered mucosally (i.e. oral or nasal) may be irrelevant to the evaluation of the clinically expected secretory and cell-mediated immune response.

Although immunogenicity testing in animals may be necessary during the development of a vaccine to demonstrate its ability to induce an appropriate immune response, an animal immunogenicity test may not always be needed for routine lot release (e.g. *Haemophilus influenzae* type b conjugate vaccine) (*48*).

A.5 Special considerations

A.5.1 *Adjuvants*

Adjuvants may be included in new vaccines to promote appropriate immune responses to particular antigens, or to target a particular immune response. It is important that the adjuvants used comply with pharmacopoeial requirements where they exist, and that they do not cause unacceptable reactogenicity.

Compatibility of the adjuvant(s) with all the antigenic components of the vaccine should be demonstrated. Where relevant, adsorption of all the antigenic components present in the vaccine, should be shown to be consistent on a lot-to-lot basis. Possible desorption of antigen during the shelf-life of the product should be evaluated, reported and specifications set. If a new adjuvant is proposed for use in a vaccine formulation, appropriate preclinical studies are necessary (*12*, *49*). It should be noted that no adjuvant is licensed in its own right, but only as a component of a particular vaccine. If no toxicological data exist for a new adjuvant, toxicity studies of the adjuvant alone should first

be performed. Preclinical animal studies to determine the safety profile of the combination of adjuvant and vaccine should also be undertaken.

Preclinical studies should evaluate the combination of adjuvant and antigen as formulated for clinical use. In the case of new adjuvants prepared to replace the well-established aluminium adsorbants in a vaccine already in use, the inclusion of appropriate control groups of animals is important. These groups may include one group receiving the antigen alone, and a group receiving the antigen adsorbed to an aluminium compound.

A.5.2 ***Additives (excipients and preservatives)***

If a new additive such as a preservative or excipient is to be used, its safety should be investigated and documented. If a new preservative is used, its safety as well as efficacy or appropriateness for use in a particular product must be documented. The safety of new additives can be evaluated using vaccine formulations without antigen. However, the compatibility of a new additive with all vaccine antigens should be documented as well as the toxicological profile of the particular combination of antigen(s) and additive in animal models.

A.5.3 ***Other types of product requiring special considerations***

Some types of data and testing are specific for certain types of product, such as genetic stability for recombinant vaccines, data concerning the inactivation and attenuation methods, demonstration of comparability of combination vaccines, contribution of adjuvants and safety/toxicity studies for particular vaccines.

A.5.3.1 Combination vaccines

New combinations of antigens or serotypes should be studied for appropriate immunogenicity in an animal model, if available, before initiation of clinical trials in humans (*13*, *14*). The response and the quality of response to each of the antigens in the vaccine should be assessed. It is preferable to study a new combination in comparison with the individual antigens in animals to determine whether augmentation or diminution of response occurs. Interference between live vaccine strains may also be studied in animal immunogenicity tests.

A.5.3.2 DNA vaccines

Special considerations concerning the production and control of DNA vaccines as well as their preclinical evaluation are covered in WHO guidelines for assuring the quality of DNA vaccines (*42*).

A.5.3.3 Recombinant vaccines

WHO guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology should be consulted (*43*).

A.5.3.4 Synthetic peptide vaccines

Detailed information concerning the production and control of synthetic peptide vaccines, including preclinical safety evaluation is available in guidelines for the production and quality control of synthetic peptide vaccines (*15*, *44*).

A.5.3.5 Live attenuated vaccines

The major concern related to live attenuated vaccines is potential reversion to virulence and the possible transmissibility and exchange of genetic information with wild type or other microorganisms. Every effort should be made to identify markers of attenuation (genetic sequences) which should be used in clinical trials to monitor the results of excretion studies and during clinical evaluation, phase by phase. A specific example of a live attenuated vaccine is the poliomyelitis vaccine, oral (*50*).

Part B. Clinical evaluation of vaccines

B.1 General remarks

Before the start of clinical trials (particularly phase III trials), a sound understanding of the epidemiology of the pathogen or disease of interest in the intended study population is needed. This requires population-based or outbreak evaluations of individuals exposed to, at high risk of, or suffering from, the disease in question. Such studies define disease incidence, the proportion of infected persons who develop clinical disease and the risk of transmission. The understanding of the full clinical spectrum of illness and the optimization of diagnostic criteria as well as definition of the high-risk groups frequently defined by age, gender, ethnic or population group membership, social characteristics as well as geography and seasonality of exposure, is essential for accurate vaccine evaluation. Consideration should also be given to defining laboratory values (e.g. for platelet counts and leukocyte counts) in the intended study population. The use of inappropriate laboratory values often results in too many people failing to meet the "criteria for inclusion". The laboratory values in the protocol should therefore reflect "normal" values in the population in question. In some developing countries, these may differ consider-

ably from those accepted as normal in industrialized countries due to widespread concurrent infections (e.g. with helminths). Seroprevalence studies should also be undertaken, where appropriate, to assess at-risk populations and to evaluate potential protective mechanisms, such as persistence of maternal antibodies. This is particularly important for the evaluation of live attenuated vaccines in infants because pre-existing maternal antibodies can prevent infection with attenuated vaccine strains. The determination of sample size of study population as well as the duration of the trial necessary to achieve a statistically meaningful result with respect to efficacy and safety requires a clear understanding of the incidence of the disease in question. An understanding of the background incidence of various adverse reactions, including those that are specific to the wild type pathogen is essential.

All clinical trials should adhere to the standards described for good clinical practice. The general principles of the WHO guidelines for good clinical practice already in place for trials of pharmaceutical products, also apply to vaccine studies. However, vaccines demand special consideration because:

- Vaccines are given to healthy individuals, mostly children and infants.
- Vaccines are given to prevent disease; this limits tolerability of adverse events.
- Vaccines are biological products which are highly complex substances derived from living materials, and sometimes comprising living organisms. They require specialized assays and testing to assure their quality and safety on a lot-to-lot basis.

Consistency of manufacturing for the vaccine lots used in clinical trials should be demonstrated and well documented. These lots should be adequately representative of the formulation intended for marketing. Clinical data may be required to help to demonstrate manufacturing consistency.

B.2 Methodological considerations

This section describes some methodological considerations common to the different phases of vaccine evaluation. Methodological considerations are vital to the outcome of all clinical studies and they should be given careful attention during the trial design stage. The methods used in all trial protocols should be clearly delineated. Existing effective preventive measures (e.g. bednets for malaria, counselling for HIV) should be continued for trial participants (*2*, *51*).

B.2.1 *Study population*

The initial phase I study is usually conducted in healthy, immunocompetent adults who are at low risk of the infection or complication against which the vaccine protects. Generally, the trial population for phases II and III should be chosen to represent the group that will be the target for the vaccination in an immunization programme. Care should be taken to identify the target population correctly. If a vaccine is intended for children or other vulnerable populations, it should be tested in a small number of subjects from the intended population, usually after at least one phase I study has been completed in healthy adults, but before proceeding to studies in a larger number of subjects from the intended population. Definitive criteria for inclusion or exclusion of subjects in the clinical trial should be established in advance.

B.2.1.1 Inclusion and exclusion criteria for enrolment in the trial

Specific inclusion and exclusion criteria should be defined for each phase of a trial. The subjects enrolled in the trial should be in the required age group, resident within the defined study area(s) during selection, examined by the study physician and able to give their signed informed consent (in the case of children, the consent of the parent(s) or guardian is required). Previous exposure to vaccines and antigens should be recorded for all participants.

Subjects should be excluded from the trial if they do not meet the medical or other eligibility criteria, for example, if they suffer a chronic illness with signs of cardiac or renal failure, suspected progressive neurological disease, uncontrolled epilepsy or infantile spasms, have received other vaccinations within 1 or 2 weeks of administration of the test vaccine, or are receiving long-term treatment with antibiotics. Immune status should also be considered when deciding whether or not an individual may participate in the study (e.g. immunodeficiency, immunosuppression and/or prematurity). Other criteria for exclusion of participants from a study might include a planned move from the study area within the period of follow-up, social and/or language difficulties or other circumstances that interfere with communication and follow up. However, the number of potential participants excluded should be kept to minimum.

Criteria should also be established for contraindications to the administration of a subsequent (second or third) dose of vaccine, if applicable. These might include serious reaction after the first or second dose (e.g. neurological reaction), fever greater than or equal to 40 °C within 48 hours of administration or a generalized allergic reaction within 48 hours of administration.

B.2.2 *Outcome measurement*

The primary end-point should be the most relevant for the disease in the target population.

B.2.3 *Safety*

When safety is the primary end-point in a clinical trial, the adverse event or reactogenicity (local or systemic) considered to be of primary importance should be the major focus in trial design. The safety profile should be representative of, and predictive for, the target population for which the vaccine is to be used in practice (see also B.2.7, monitoring and reporting adverse events).

B.2.4 *Immunogenicity*

In phases I, II and III, immunogenicity data are recorded as an outcome, and in certain circumstances may be used to demonstrate clinical efficacy (see below).

B.2.4 *Efficacy*

In phases II and III, clinical protection outcomes may be measured. Studies in which the end-point is clinical efficacy should be performed in areas where an appropriate impact of active immunization can be expected, and where a controlled trial is feasible. Pre-exposure studies should thus preferably be performed in an area with low endemicity, or in an area with few individuals who have natural long-term protection.

The outcome of a trial is measured as vaccine efficacy and/or vaccine effectiveness. Immunogenicity studies may be sufficient to demonstrate clinical efficacy for vaccines containing a known antigen for which the level of protective antibody is well established (see Correlates of protection, B.7.2.3). If protection cannot be measured as an end-point alternative parameters to be measured should be justified.

B.2.5 *Factors influencing the choice of outcome measurement*

The choice of outcome measurement in a specific trial may be constrained by scientific, logistical, economic or ethical considerations. When a randomized-controlled trial using clinical end-points is not feasible, alternative strategies need to be considered (*52*). The feasibility and validity of such alternative strategies should be considered in the protocol. Evaluation of the feasibility of a serological correlate of protection should address the relationship between the surrogate end-point and the clinical end-point, bearing in mind that this relationship may not necessarily be linear or direct.

B.2.5.1 Vaccine efficacy

Vaccine efficacy could be measured as an outcome of clinical protection and/or as an immunological surrogate end-point based on immunological response. The definition of clinical cases should be given in the protocol (see Case definition and case ascertainment, B.2.6). The inclusion of cases for whom confirmation (e.g. microbiological) was not possible should be justified in the protocol. When relevant, both clinical and serological end-points should be studied and the data presented in the report. The formula by which vaccine efficacy is calculated should be defined and validated (see Glossary) (*53*, *54*).

B.2.5.2 Vaccine effectiveness

The effects of vaccination at the population level depend on the coverage and distribution of the vaccine, as well as on its efficacy in preventing disease and preventing colonization (*54*). In addition to the intrinsic efficacy of the vaccine, its effectiveness depends on the heterogeneity in susceptibility, rates of exposure to infectious agents and protection conferred by the vaccination (*55*). Vaccine effectiveness may also be influenced by time-related changes in protection caused by intrinsic properties of the vaccine (waning of efficacy and boosting) (*54*, *56*, *57*), changes in vaccination coverage, and population characteristics (such as age distribution).

B.2.6 *Case detection, case ascertainment and case definition*

The outcome of trials of clinical protection by a vaccine will depend critically on case definition, as well as on the sensitivity and specificity of case detection and case ascertainment. Sensitivity determines the power of the study, specificity of the predictive value and safety estimate (*54*).

It is essential that the case definitions for the trial end-points be clearly defined at the outset. Case definitions and methods of case detection should be justified and described in the study protocol. The protocol should substantiate and provide a full discussion of the consequences of the anticipated sensitivity and specificity of the case definition. Defined and validated methods should be applied consistently for the duration of the study, at all study sites.

B.2.6.1 Case detection

The methods used for detecting cases should be the same in both vaccinated and unvaccinated populations.

- If attack rates are high, the number of cases in the population of interest may be sufficient to estimate vaccine efficacy accurately in a relatively small population and a relatively short time.

- If attack rates are low, enrolment (sample size) and/or duration of follow-up may need to be increased to detect sufficient cases to enable a precise estimation of efficacy. If this is not possible, other surveillance data may be used to detect other potential cases and subsequently increase the precision of the estimate.

In cohort studies all cases from both the vaccinated and non-vaccinated groups should be included in the analysis. This practice is consistent with the philosophy of "intent-to-treat" (*58*).

In secondary attack rates trials all cases in the target group found in the surveyed household or cluster during the predefined time period should be included, as well as the case which led to the cluster being studied.

Case–control studies use the same case-detection methods as other study designs, but not all cases need be detected.

B.2.6.2 Case ascertainment and case definition

The case definitions should be developed, defined and clearly documented in the study protocol before any efficacy study commences. This ordinarily involves using the efficacy definition(s) in an earlier phase of clinical development. The validity of the diagnosis is most important for an adequate evaluation of the efficacy or safety of a vaccine. When the diagnosis is based on defined clinical criteria, justification and validation of these criteria should be provided. Confirmation of cases using laboratory methods, antigen detection and the clinical signs is necessary to support a clinical case definition.

Specific and sensitive methods properly validated for case ascertainment and consistent use of a reliable and valid case definition are vital to the useful outcome of a study (*59*). Highly specific methods may be needed in certain cases, but are not always available.

Consideration should also be given to defining in the study protocol when and how, in the event of a vaccine failure, the immunological evaluation of study subjects and typing of the infecting microorganism will be performed after unblinding, or as part of planned interim analysis, including where possible:

— evaluation of clustering of cases of the disease in the population with serological and/or microbiological confirmation; and
— information on the antigenic match between vaccine strains or serotypes and circulating strains or serotypes, to provide insight into the possibility of strain or serotype selection.

B.2.7 *Monitoring and reporting adverse events*

An adverse event in a vaccine trial is any untoward medical occurrence in a clinical trial subject administered the vaccine; it does not necessarily have a causal relationship with the vaccine or vaccination. It is critically important, especially in vaccine trials, that adverse events are actively monitored and reported swiftly. The NRA may require the sponsor and/or the investigator to report certain types of adverse events or reactions (e.g. serious or previously unknown events) to itself and to the Independent Ethics Committee. Investigators should report all serious adverse events to the sponsor immediately unless they are identified by the protocol as not needing to be reported immediately. Investigators should also comply with the applicable regulatory requirements related to the reporting of unexpected serious adverse reactions to the NRA and the independent ethics committee. Investigators should be trained adequately for this purpose. After the trial has been completed or terminated, all recorded adverse events should be listed, evaluated and discussed in the final report. Reporting of adverse events should be part of the protocol design.

Standardized methods should be used for investigating and reporting local and systemic adverse events following vaccination. All safety information should be recorded and the procedure for reporting adverse events should be described in the protocols (see guidelines for good clinical practices (2)). The instructions should include details of:

— who is going to make the report (e.g. study investigators or nurses subjects, parents or guardians);
— how the reporting is planned (e.g. using questionnaires or diary cards);
— duration of follow-up; and
— the intervals of reporting (e.g. daily, weekly).

Adverse events following vaccination should be well documented.[a] The report should include evaluation of injection-site reactions (pain, induration, erythema) and systemic events (fever, nausea, malaise, headache, anaphylaxis), at baseline, at pre-specified vaccination times and following vaccination. Any difference in safety profile related to injection site or route of administration should be recorded. For vaccines administered to children and infants, reactions should be recorded both by the parents and by the study investigator or nurse in a structured manner. Parents should be contacted by the study inves-

[a] A useful set of recently established definitions is available at: http://www.brightoncollaboration.org.

tigator or nurse at defined intervals after vaccination to check for any reactions. Before the second and/or third doses (if applicable) parents of infants and children, or the vaccine recipients themselves, should be asked by the study investigator or nurse about reactions to the previous dose. Also, the investigator or nurse should consult the previous vaccination records of the individual in question.

The procedure for recording adverse events should be defined and carried out at appropriate intervals and for a sufficient duration. Every effort should be made to improve the quality of the reporting of adverse events, for example by the use of standardized forms (e.g. case report forms, subject diaries). Furthermore, such forms should include questions about specific adverse events or findings including qualitative and quantitative parameters, as appropriate. For example, temperature should be measured by pre-specified methods. The forms should also allow for the recording of unsolicited events. Prior instructions for the use of diary cards and follow-up visits or contacts by clinical study staff should be given. All model forms to be used for monitoring should be provided with each protocol.

For some trials, such as large-scale phase II and phase III trials and post-marketing surveillance studies, data safety monitoring boards (DSMBs) need to be in place, to ensure adequate safety monitoring. In special cases DSMBs may also be required for phase I studies (*51*). DSMBs must be independent and preferably linked to the independent ethics committee (see guidelines for good clinical practices). If necessary, a DSMB may initiate a new study to further investigate the nature of the adverse events following vaccination seen in the original trial. In the case of serious adverse events an Institutional Review Board should unblind a study and, if necessary, stop a trial and report its findings to the appropriate NRA. Safety monitoring of trial participants should continue for a defined period after the trial has ended.

Consistency in safety reporting may be improved by increased reporting in the published literature. Issues that pertain to the publication of study data should be considered in the design of study protocols.

B.3 Statistical considerations

B.3.1 *General principles*

Statistical analysis should be based on the recommendations made in relevant WHO documents, where available, and or other suitable guidelines. Early phase trials are often exploratory and may lack the statistical power for definitive inferences. However, if the aim of a study is to provide conclusive information, e.g. the final determination

of the optimal dose for use in a pivotal, phase III trial, then the study should be rigorously designed, powered and statistically analysed, regardless of the phase of investigation. Otherwise, the issues discussed below pertain primarily to phase III trials. Essentially, the recommendations are as follows:

- The procedures for randomization and blinding should be described in the study protocol.
- The primary and secondary objectives of the study should be clearly stated.
- The protocol should state explicitly the outcome variables to be analysed, the null and alternative hypothesis to be tested, the significance level the anticipated power and the statistical methods to be used for assessing each end-point.
- For the evaluation of efficacy, intent-to-treat estimates should accompany traditional per-protocol estimation. Intent-to-treat estimates will include all protocol-defined cases of disease, without regard to completion of vaccine series or compliance with protocol, and will include follow-up from the time of randomization (*58*). The reasons for removal of any subject from the efficacy or safety analysis should be described in detail in the study reports.
- If interim analyses for efficacy are planned, this information should be included in the protocol together with appropriate significance level adjustments to be implemented.
- Statistical estimates should include confidence intervals (*60*).

B.3.2 ***Trial objectives: efficacy and safety***

B.3.2.1 Establishing efficacy

The efficacy of a new vaccine can most convincingly be demonstrated in a randomized, double-blind, placebo-controlled trial based on a clinical disease end-point. The placebo may be an inactive product or a vaccine for a different disease, believed to be ineffective in preventing the disease of interest. This type of trial is called a superiority trial, because the vaccine must be sufficiently superior in efficacy to the placebo to be acceptable (see section B.3.3.1). High specificity of case definition is desired because it is well known that low specificity has a deleterious effect on the ability of a study to estimate vaccine efficacy accurately (*59*). The aim of these trials is not to test a hypothesis regarding efficacy, but rather to estimate efficacy with both a point estimate and the corresponding confidence interval (usually 95%). The size of sample chosen for these trials depends on disease incidence rates in the study population, as well as on the anticipated level of efficacy of the vaccine that is considered to be clinically relevant.

There are, however, situations in which vaccine efficacy cannot be determined from cases of disease. For example incidence of a disease in a population may have been reduced to very low levels by widespread immunization with a previously licensed vaccine. When the serological parameters are known to correlate with clinical protection, evaluation of a new vaccine for the same disease is based on measures of the vaccine's immunogenicity. One or more immune response outcome variables thus serve as "surrogates" for determining efficacy. Since the comparator in this setting is typically the already-licensed vaccine, evaluation of the new vaccine is based on establishing its "non-inferiority" to the licensed vaccine (see section B.3.3.2). Statistical inference of non-inferiority is based on the appropriate confidence interval excluding a pre-specified difference in immune response believed to be clinically meaningful. The size of sample required for establishing non-inferiority of immune response depends upon the variability in the immunogenicity measurements and on the level of efficacy of the comparator vaccine.

B.3.2.2 Evaluating safety

Most vaccine trials are not aimed at testing specific hypotheses regarding adverse events. Consequently, safety assessment is generally characterized by exploratory data analysis. Descriptive statistics are presented and confidence intervals are often informative. *P*-values may be useful for detecting signals of possible vaccine-associated adverse events for further evaluation.

If the detection of a few serious adverse events that have been specified prospectively is the primary focus of a large pre-licensure safety trial, it is advisable to consider a multiplicity adjustment for testing the corresponding small number of hypotheses. This multiplicity adjustment should be accounted for in the determination of the sample size. Otherwise, if there are no a priori hypotheses regarding specific adverse events, meaning that an undetermined number of safety analyses will be performed, adjustment for multiplicity is not generally performed during initial evaluations of the clinical trial data. Signals in the data suggesting possible vaccine-related adverse events may be investigated further for the determination of a potential causal association. However, the effect of multiple testing should be considered before the final decisions are made regarding any safety signals detected. If a serious, unexpected event occurs, prospective monitoring for additional events might be added to the protocol, and formal statistical testing could be implemented. Further general guidance on the statistical evaluation of safety has been published by the International Conference on Harmonization (*39*).

B.3.3 *Study designs (superiority, non-inferiority and two-sided equivalence trials)*

B.3.3.1 Superiority trials

Superiority trials of vaccines are generally based on cases of disease. The control is either a placebo or a vaccine that has no effect on the disease of interest. The purpose of these trials is to estimate the percentage reduction in the incidence rate of disease due to use of the vaccine. The point estimate of this percentage reduction may be obtained by various methods: as a ratio of risks, incidence rates, or hazards (see definition of vaccine efficacy in the glossary). There are also a number of statistical methods for obtaining the confidence interval on vaccine efficacy (*60*).

B.3.3.2 Non-inferiority (one-sided equivalence) trials

A non-inferiority trial of vaccine efficacy is generally designed to show that the use of a new vaccine gives a relative risk, relative incidence rate or relative hazard rate of a disease, infection, etc., when compared to the control, is not greater than a pre-specified clinically relevant quantity. In a non-inferiority trial based on immune response, the relative effect of interest may be a difference in proportions of subjects responding in a pre-specified manner, or a ratio of geometric mean titres or concentrations. For the former, the trial is designed to show that the proportion of subjects responding to the new vaccine is not less than the proportion of subjects responding in the control group by as much as a pre-specified quantity (often 0.10). For the evaluation of titres, the trial may be designed to demonstrate that the ratio of the geometric mean titre (or concentration) of the new vaccine relative to the control is not less than some pre-specified ratio (e.g. 0.50 or 0.67).

The comparative outcome measure for a non-inferiority trial for an adverse event can be either a difference or a ratio of risks. If a ratio is to be obtained, the trial is designed to show that the relative risk of the adverse event occurring with the new vaccine relative to the occurrence in the control is not greater than a pre-specified ratio (e.g. 1.5). If the difference in rates of adverse events, is required, the trial is designed to show that the risk of the adverse event occurring with the new vaccine is not greater than the risk with the control by as much as a pre-specified quantity.

Because non-inferiority evaluations are one-sided, statistical inference is based only on the upper or lower confidence limit, whichever is appropriate for the aim of the study. The null hypothesis (to be rejected) is that the difference between vaccinated and control subjects is greater than the lower or upper equivalence margin. Alterna-

tively, inference may be based on the corresponding one-sided confidence limit.

B.3.3.3 Two-sided equivalence trials

A two-sided equivalence trial, such as might be used to compare two vaccine lots, is designed to show that the outcome measure for one group is similar in both directions to that for another group. The reason that the evaluation of lot consistency is inherently two-sided is that there would be concern if an outcome measure for one lot were either too high or too low when compared to another lot. Such a finding might suggest that the two lots are not similar enough to be considered to be consistently manufactured. The lots are considered equivalent, or consistently manufactured, when a two-sided confidence interval for the appropriate relative effect (e.g. ratio of geometric mean antibody concentrations or relative risk of adverse event) falls entirely within pre-specified limits. The choice of the equivalence margins should be scientifically justified. Thus, statistical inference is based upon both upper and lower confidence limits.

B.3.3.4 Accepted difference or ratio in equivalence and non-inferiority trials

The quantity to be ruled out as the criterion for non-inferiority or equivalence should be based on clinical, laboratory and statistical judgement. It may be based on evidence from previous trials and/or laboratory assay data. In a trial of relative efficacy, the equivalence or non-inferiority criterion should be sufficiently achievable so that, if the new vaccine meets the criterion, it is clear that it will provide an acceptable level of protection from disease. The feasibility of attaining a sample of the appropriate size may also be a factor in the choice of the criterion; the calculated sample size can be very large when the criterion is easily achievable or the variability of the outcome measure is large.

B.3.4 ***Sample size***

The number of subjects participating in a clinical trial must be sufficient to provide a reliable answer to the questions posed. The sample size in a trial of vaccine efficacy should be large enough to allow precise interval estimation of efficacy. Sample size is usually determined by the primary end-point chosen. Generally, the sample size should be large enough to ensure that the lower confidence limit for efficacy will be considerably greater than zero. A sufficiently high lower confidence limit is desirable to ensure a minimal level of vaccine efficacy.

The protocol should clearly explain calculations of sample size required for each primary end-point (immunogenicity, safety and efficacy) and the largest estimate should determine the number of subjects to be enrolled. The amount of information requested prior to licensing and the feasibility of obtaining it need to be carefully balanced.

B.3.4.1 Sample size in non-inferiority/equivalence trials

The sample size should be such that, if a new vaccine is truly non-inferior, there is a high probability that the appropriate confidence interval for the relative effect of interest will not exceed the predefined non-inferiority criterion. Alternatively, for equivalence trials, there should be a high probability that both the upper and lower confidence intervals will fall within the predefined upper and lower equivalence margins. Methods of sample size calculation specially designed for non-inferiority/equivalence trials should be used. Non-inferiority trials of vaccine efficacy based on clinical outcomes usually require much larger samples than placebo-controlled superiority trials or non-inferiority trials based on immunogenicity measurements (*61*).

Undersized superiority trials that give non-significant results will not generally allow any conclusions to be made regarding non-inferiority or equivalence.

Useful information on statistical principles for clinical trials is published by the International Conference on Harmonization (*39*).

B.3.4.2 Considerations underlying sample size determination in efficacy evaluations

The criteria underlying the determination of sample size are based on methodological and statistical considerations, as well as on epidemiological and scientific judgement. Factors to be taken into account include the expected incidence of the disease and its prevalence (endemic spread, epidemic spread, or low-incidence disease). These factors may vary from product to product and from one setting to another.

B.3.4.3 Sample size considerations in immunogenicity evaluations

The evaluation of immunogenicity, when part of an efficacy trial with a clinical end-point, should ideally be conducted in a randomly selected subsample from the population initially enrolled. When immunogenicity is the only primary end-point, it should be studied in individuals representative of the target population. Sample size will depend upon the aim and design of the study, as well as the variability

of the immune response measurements. In certain situations (e.g. when too few subjects are available for immunogenicity testing) additional methodologies could be used in order to increase the number of study subjects. Aspects such as the appropriate choice of control and expected protection rates should always be taken into account.

B.3.4.4 Sample size considerations in safety evaluations

Prior to licensure, comparative studies of common adverse events (e.g. injection site reactions with diphtheria, tetanus, pertussis, whole cell DTPw) require large numbers of subjects to give them sufficient power to detect small differences. The same is true for cohort studies intended to detect serious uncommon adverse events. For evaluation of common local reactogenicity, approximately 300 subjects are needed for each comparison group. However, depending on the type of vaccine, the disease indication, and the target population, enrolment of more than 5000 subjects may be appropriate to provide reasonable assurance of safety pre-licensure in randomized, controlled settings. These numbers are based on a one-sided confidence interval when no adverse events are observed. They increase if one adverse event is observed.

The investigation of uncommon or rare events already occurring in the study population requires long-term prospective population-based surveillance studies. These are often not feasible in pre-marketing trials and such data are obtained from postmarketing surveillance studies. In practice, such events are studied either in retrospective closed cohorts and/or in case–control studies. Valuable sources of information for such purposes are large databases with records of vaccinees. These databases may include several hundreds of thousands of subjects for evaluation.

B.3.5 ***Duration of study***

The impact of a particular vaccination schedule is evaluated by the primary outcome measure of the clinical trial. In principle, all vaccines under development need a long-term evaluation plan. In most confirmatory clinical trials this implies a follow-up period of at least 6 months subsequent to the last vaccination. However, this will depend upon the outcome measurement chosen (i.e. clinical end-point, immunogenicity or safety), the vaccination strategy and the novelty and/or type of the vaccine. Long-term follow-up may be undertaken for the whole study population or in a relevant subset.

For vaccines intended for use in immunization programmes, subjects should be followed up for at least 1 year following the last vaccination to obtain serological and clinical information on the persistence of

protection and the possible need for a booster vaccination. In situations where safety evaluation is a primary outcome, different follow-up periods may be appropriate and should be considered on a case-by-case basis. Fully documented information on follow-up should be obtained for as many individuals enrolled in the trial as possible until all final outcomes are recorded.

B.4 Ethical considerations

For information on the clinical standards and ethical issues to be considered in the design and conduct of vaccine trials, WHO guidelines for good clinical practices should be followed (*2*). Compliance with these standards provides assurance that the rights, safety and well-being of trial subjects are protected, in accordance with the principles that have their origin in the Declaration of Helsinki (*16*). For any study, a review by an independent ethics committee, functioning in accordance with good clinical practice standards, is mandatory (*17*).

To assure protection of the rights of research subjects, the approval of the appropriate independent ethics committee must be obtained before the start of the trial. No subject may be included in a clinical trial without proper informed consent in writing. Informed consent for children should be obtained from their parent or guardian.

The specific roles and responsibilities of the ethical review boards and regulatory authorities are country-specific.

Special attention should be given to the ethical considerations underlying testing of vaccines in healthy infants, children, pregnant women and the elderly. The use and nature of a placebo should be carefully considered as should the use of human challenge studies. Human challenge studies are appropriate only for selected diseases that have no serious complications or long-term sequelae and for which successful treatment is available. Such studies can provide valuable information on the pathophysiology, clinical manifestations, diagnosis, immunology, treatment response and most importantly protective efficacy of vaccines.

Subjects participating in vaccine trials should not be exposed to unreasonable or serious risks of illness or injury and measures should be in place to ensure that all subjects receive the full benefits of scientific innovation. An adjustment may be needed to an existing national vaccination programme after careful consideration of the possible benefits of innovations. It is important to ensure that economically and socially deprived communities, which are often those at the great-

est risk of disease, are not exploited in conducting research that will be of no benefit to them. Detailed information is available in the ethical guidance documents issued by WHO, Council for International Organizations of Medical Sciences (CIOMS), UNAIDS and other bodies (*17–20*) and these should be consulted as appropriate. Other relevant national or international requirements must also be considered (such as from the US Office for Human Research Protections (OHRP)).

B.5 Phase I studies

If appropriate animal challenge models for the evaluation of immunogenicity or efficacy parameters are available, data from such studies should be provided before starting the clinical trial programme. However, if such models are not available, relevant data from alternative approaches and/or from in vitro testing may need to be considered to provide proof of concept in support of a proposed clinical development plan.

Phase I studies should be undertaken to define acceptable safety and reactogenicity of a vaccine candidate as well as to obtain preliminary information on its immunogenicity (*62*). The dose and method of administration should also be assessed with respect to these parameters. Generally phase I studies are small-scale studies of which the primary focus is the determination of clinical tolerance and safety.

All phase I studies should be conducted in research environments with adequate laboratory support and very carefully monitored. Phase I studies are usually-open label studies and are not randomized with placebo control groups. However, there is a recognized need for controlled trials, even in phase I, to allow at least some comparison of intercurrent common non-vaccine induced events. When possible, the concomitant use of other vaccines or therapeutic agents should be avoided to optimize the safety evaluations. Phase I studies might be conducted in several different age or population groups because of differences in, for example, dose, safety, vaccine schedule, route of administration or disease risk. Where appropriate, laboratory testing (e.g. complete blood count and liver function tests) should be undertaken to establish a baseline database. A short period of evaluation in a clinical research centre or extended observation in a clinic, day-care centre or home environment is recommended for close monitoring of vaccinees. Less intensive phase I trials might involve daily visits by a research nurse to the home or day-care centre or daily return visits by the subject to the clinic.

Live attenuated vaccines (viral or bacterial) are potential causes of clinically significant infections in the recipient or in contacts. Major concerns in the evaluation of a live attenuated vaccine include the possible shedding of the agent, transmission to contacts, potential genetic variability and reversion to a more virulent state. Therefore, such vaccines require intensive investigations in closely monitored clinical settings. Initial studies of candidate attenuated vaccines should be undertaken to make preliminary evaluations of dose ranges, immune responses, clinical signs of infection and reactogenicity (immediate, early and late). Phase I studies may provide preliminary information on shedding, reversion characteristics, transmission to contacts and genetic stability.

Phase I studies may provide data that are useful in the design of further clinical phase studies.

B.6 Phase II studies

Once phase I studies have been successfully completed with a satisfactory outcome, a candidate vaccine should then undergo phase II clinical evaluation. The main distinction between phase I and phase II studies is that phase II studies involve larger numbers of subjects, and are often randomized and well controlled. The outcome measures, however, are often similar. Phase II vaccine trials are intended to demonstrate the immunogenicity of the relevant active component(s) and the safety profile of a candidate vaccine in the target population. Ultimately, the phase II studies should define the optimal dose, initial schedule and safety profile of a candidate vaccine before the phase III trials can begin.

Phase II studies should be undertaken to evaluate multiple variables associated with the host immune response such as age, ethnicity, gender and presence of maternal or pre-existing antibodies. In future trials, genotype may also need to be considered. Other factors to be investigated to determine their influence on immune response include:

— dose of vaccine;
— sequence or interval between vaccine doses;
— number of doses of vaccine; and
— route of vaccine administration.

The duration of immunity, potential need for booster immunizations and qualitative aspects of the immune response may also be investigated. A single study can address several questions, although several

studies are often required to obtain definitive evaluations. If the answer to the scientific question under study will be final, e.g. the determination of the optimal dose to be used in a large phase III efficacy trial, then the phase II trial should be rigorously designed, adequately powered and appropriately analysed to provide conclusive information.

For a live attenuated vaccine, continued active monitoring of specific parameters into the second and third week, or more, post-vaccination is recommended. The duration of follow-up may be determined by a number of factors that may have been identified in the phase I studies including the degree of shedding, transmission and potential reversion characteristics.

The immune responses to vaccine antigen(s) should be carefully evaluated and are a critical part of phase II clinical studies. Such studies are intended to further characterize immune responses elicited by a particular immunogen thought to be relevant to protection, such as level, class, subclass and function of the specific antibodies produced, as well as appearance and duration of adequate antibody titres. Other relevant information such as presence of neutralizing antibodies or cross-reactive antibodies, formation of immune complexes, cell mediated immunity and any interaction that might affect the immune system (e.g. preexisting antibodies, concomitant administration of another vaccine or drugs) should be recorded.

The percentage of responders should be defined and described based on predefined criteria for assessing the immune response (e.g. antibodies and/or cell-mediated immunity). For vaccines for which the immunological correlates of protection are not known, the immunological profile should be studied in detail. Subjects who fulfil immunogenicity criteria (often seroconversion) are regarded as responders (having seroconverted) and the result of an immunogenicity study includes the proportion of responders. For the validation of an immune response, sera should be collected from all participants at regular, predefined intervals throughout the study period. For certain vaccines (e.g. nasally administered vaccines) the investigators should consider whether samples from other body fluids should also be collected. Immunological data from phase II trials should be documented, including geometic mean titre, median, standard deviation, and the range of antibodies in pre and post-vaccination sera (*63*). In the case of vaccines for which the end-point is the induction of antibodies, the immunological data should be presented by dividing the pre- and post-vaccination titres, or antibody concentrations according to arbitrary (or, if known, protective) antibody levels (e.g. 0.01, 0.1

and 1 IU/ml for diphtheria and tetanus antibodies). Presenting reverse cumulative distribution curves may provide additional insight (*64*, *65*). When available, standardized assay methodologies should be used, and details may be found in WHO recommendations, European Pharmacopoeia monographs or US Food and Drug Administration documents. Each assay should be fully documented and consistent use of a validated assay is essential.

B.7 Phase III studies

The phase III studies are large-scale clinical trials designed to provide data on vaccine efficacy and safety. These studies are usually performed in large populations to evaluate efficacy and safety of formulation(s) of the immunologically active component(s). In large-scale efficacy studies of this type, that may enroll many thousands of subjects, serological data are usually collected from at least a subset of the immunized population at pre-defined intervals. It is also important to collect serological data from all persons classified as vaccine failures.

When vaccines containing the same antigens are already in common use and/or the incidence of disease is very low, it may not be feasible to perform a formal study of protective efficacy. In such instances, the phase III trials, although involving larger numbers of persons than previous phases, will be confined to the evaluation of immune responses and comparison with any recognized correlates of protection. However, sometimes there are no established and unequivocal immunological correlates of protection. In such cases, it is important that some attempt should be made to estimate the effectiveness of the vaccine after its licensure and widespread introduction. Phase III trials involve a larger number of subjects than were included in the earlier phases of development and, thus, provide expanded safety assessments.

The duration of follow-up should be determined taking into account the type of vaccine and other relevant factors (e.g. disease incidence, characteristics of immune response to vaccine, and anticipated and safety profile of the vaccine.)

Whether or not a prophylactic vaccine is ultimately accepted as a general public health measure depends upon the availability of clear and definitive evidence that the vaccine is safe and actually able to prevent the infectious disease in question or to significantly reduce the adverse consequences of the disease.

B.7.1 *Considerations for formal trials of protective efficacy*

Vaccine efficacy is the percentage reduction in the incidence rate of a specific disease in vaccinated individuals as compared to that in unvaccinated individuals. Vaccine efficacy measures direct protection (i.e. protection induced by vaccination in the vaccinated population sample).

B.7.1.1 Trial design

Two general approaches can be applied to efficacy studies; they can be either experimental studies or observational studies. The gold standard for assessing the prevention of disease or infection in a phase III trial is the prospective randomized double-blind controlled trial of protective efficacy. This design will control for other variables that might affect disease risk and avoids potential bias in the assessment of end-points. Thus this design maximizes the chance that a difference in disease incidence between two equivalent groups is due to a true effect of the vaccine being evaluated. However, in certain circumstances other approaches may be necessary. Great care should be taken when designing a vaccine trial to maximize efficiency and to eliminate bias. Observational studies of efficacy or effectiveness are usually part of phase IV post licensure studies.

B.7.1.2 Randomized double-blind controlled trials

The most effective efficacy trials are double-blind, randomized and controlled. This design controls for other variables that might affect disease risk by prospectively randomizing groups being studied. Double-blinding is necessary to avoid bias in the assessment of endpoints. The choice and feasibility of blinded, randomized-controlled trials depends on the vaccination strategy and on the demographic and epidemiological characteristics of the study population. The following approaches may be used:

— prospective cohort studies for population-based vaccination strategies; and
— pre-exposure cohort studies in-groups at risk of the target infection (e.g. vaccination for travellers).

A double-blinded evaluation of disease outcomes minimizes potential ascertainment bias and, therefore maximizes the chance that a difference in disease incidence observed between two equivalent groups is due to a true effect of the vaccine being evaluated.

Randomization is necessary to avoid bias in the assignment of the participants to one of the study groups and it permits statistically valid comparisons to be made between different arms of a study. It allows

the detection of small differences between vaccines and comparators; this is particularly important when an active control is used. Non-randomized study designs such as the use of historical controls or case–control studies allow only larger differences to be detected. If possible, these non-randomized approaches should be avoided in phase III trials.

The unit of randomization is usually the individual included in the trial and this is ideally the unit of statistical analysis. In some situations, however, it may be necessary to randomize on the basis of clusters or groups, e.g. school, geographical or political region (*66*). It is important to specify the randomization procedures and to adhere to them. Failure to do so may lead to biased results. Every effort should be made to use randomized well-controlled designs for phase III trials. However, such studies can be technically difficult and the decision to undertake them should be made on a case-by-case basis.

B.7.1.3 Other approaches for obtaining efficacy data

Several alternative types of study may be considered, depending upon the incidence and epidemiology of the disease of interest, the characteristics of the population and the expected efficacy of the vaccine or prophylactic agent. However, the use of designs other than double-blind randomized-well controlled trials to provide efficacy data is allowed only when fully justified. The possible alternative approaches include:

— secondary attack rate study, or household contact study (which can be randomized);
— uncontrolled, open studies (used only to collect additional information on serological responsiveness and tolerance);
— observational cohort studies; and
— case–control studies.

Secondary attack rate study

A secondary attack rate study is a specific type of pre-exposure cohort trial that usually requires smaller sample sizes than other randomized controlled trials. This may be the method of choice in studies of infections with a relatively high secondary attack rate in closed communities and/or susceptible populations (*53*, *67*). The unit to which the intervention is applied may be the individual, family (household) or community (environment) and the unit of randomization will correspond with this. Randomization of groups or clusters rather than of individuals may be preferred in the following situations:

— when a vaccination programme is to be conducted in a geographical area or community
— when it is logistically easier to administer the vaccine to groups than to individuals; and
— when the purpose of the vaccination is to reduce transmission of the infection, where the unit is the "transmission zone" (the area in which humans, vectors and intermediate hosts interact and share a common pool of pathogens).

Groups of subjects (or clusters), the population and the geographical area under investigation should all be defined in the protocol. Data regarding the presence of infecting pathogens and their attack rates are essential. The follow-up period for subjects after contact with the index case may be short; as a minimum it should cover the assumed incubation period and infectious period of the index cases and secondary contacts. The inclusion period for new cases and controls and their contacts should be set at a maximum of 6 months following the detection of the first case. Inclusion over a longer period may introduce bias in favour of vaccine efficacy, because the exposure to the infecting pathogen and thus the risk of infection will be reduced in the vaccinated groups or clusters compared with that in unvaccinated groups or clusters (*54*).

Observational cohort studies

Supportive evidence may be obtained from observational cohort studies if randomized-controlled trials or secondary attack rate trials are not ethically justified, or are not feasible due to low incidence of the disease or there is a requirement for long-term follow-up for the calculation of efficacy. Such studies provide an estimate of the value of a vaccine for operational purposes.

Observational cohort studies in a clinical programme for marketing approval may be considered in those unusual situations in which a double-blind randomized controlled trial is not ethically justified or where the clinical end-point requires long-term follow-up (e.g. hepatitis B vaccination in neonates (see B.9.3.1)), or where the number of individuals is too large to follow up (*69*). However, the absence of randomization is a major limitation (*70*). Where the results of these observational cohort studies are the principal or only evidence of efficacy, careful assessment of the quality of the study and the strength of its results is needed. Seeking the advice of experts in the conduct and evaluation of such studies is recommended. In all cases,

the use of supportive studies should be justified and their relevance to the investigation in question considered.

Case–control studies

Case–control studies may be useful when prospective controlled trials are not feasible due to low incidence of disease (see also case–control studies, section B.9.3.2).

B.7.2 *General considerations for efficacy trials*

B.7.2.1 Size of trial

A vaccine efficacy trial may be based on clinical end-points, incidence of the infection (as in the case of HIV) or, if they exist, on immunological correlates of protection. Efficacy trials based on clinical end-points often require large samples; possibly thousands of subjects in each arm. Large numbers of subjects are needed for the precise estimation of vaccine efficacy if the incidence rate of the disease in the study population is expected to be low. For diseases with a higher incidence (e.g. influenza), smaller sample sizes will often suffice. When an immunological end-point that correlates with clinical protection is used as the primary efficacy end-point, the number of subjects required per arm to provide a statistically adequate evaluation may be considerably smaller e.g. several hundreds per group (see Correlates of protection B.7.2.3). In the case of large trials (e.g. 10 000–50 000 subjects) it may take many months to recruit the subjects who might then need to be followed up for a further 2 or 3 years. Large field trials of this type may simulate conditions in clinical public health practice and evaluate large numbers of subjects in a heterogeneous population. However, trials of this size and duration may be logistically difficult. In all cases, the applicant should provide adequate justification of the size and duration of the trial.

B.7.2.2 Choice of control

The choice of control depends on a number of factors as described below and should always be justified. A "placebo" control in vaccine trials usually denotes the use of a comparator arm that does not include the antigen(s) under investigation. If the antigen of interest is incorporated into a combination vaccine, the control arm may utilize a licensed vaccine that contains all the same antigens except that relevant to the efficacy evaluation. A control arm may also be a vaccine (usually already marketed) indicated for a different infectious disease(s). Finally, an active control is a comparator vaccine indicated for the same infectious disease(s).

Placebo control

Demonstrating the protective efficacy of a new vaccine always requires an appropriate control. For monovalent vaccines, an inert placebo or a vaccine that protects against another disease, but gives no protection against the target disease may serve as the control. Combination vaccines involving a new component for a new infectious disease indication require omission of the new component of the vaccine in the control arm of the study. If the new component is an already-licensed vaccine, or one for which efficacy and safety have already been demonstrated, a placebo-controlled study may not be necessary. The new component may be studied in an interference trial, comparing the simultaneous, but separate, administration (at two different sites of administration) of the new component with the combined administration of the combination vaccine with the new component.

Active control

Vaccines containing a new antigen, or an established antigen with a different formulation (e.g. liquid versus lyophilized; changed adjuvant, excipient or preservative; changed dose of antigen) or that involve a new method of administration (e.g. aerosol as opposed to intramuscular administration of an influenza vaccine) may be investigated in a comparative study using an antigenically similar active control vaccine on which adequate information is available (e.g. stability data).

A placebo control arm for internal validation should be considered when there are factors that may influence the stability and validity of the efficacy measure of the active control, such as vaccine quality; antigenic variation; vaccination coverage and other protective measures, or demographic; epidemiological; socioeconomic and other characteristics of the population.

B.7.2.3 Correlates of protection

In clinical trials where prevention of disease is used as an end-point, considerable effort should be made to establish immunological correlates of protection, in addition. Such correlates are also useful, and may be necessary, for situations in which the conduct of clinical trials using prevention of disease as an end-point cannot be practically or ethically justified. Nevertheless, it is important to recognize that correlates of protection may be difficult or impossible to define.

The following section describes a simple definition of correlates of protection. Immune correlates of protection may be population-

based or individual-based (*71*). Validated and standardized laboratory methods for serological assays are essential.

A commonly used measure of population-based correlates of protection requires the identification of a level of antibody that is achieved by most of the subjects in a protected group (i.e. vaccinated) and is not achieved by the majority of a susceptible group (i.e. unvaccinated). The level of protection correlated with the antibody level of vaccinnees is the vaccine efficacy measured in the phase III trial. For a population-based correlate it is only necessary to measure immunogenicity in a representative and statistically adequate sample of the vaccinated and unvaccinated phase III cohort.

The individual-based correlate of protection involves the measurement of pre-immunization and at least one post-immunization antibody level(s) in all study subjects and relating this to whether they subsequently develop the disease. The objective is to identify a threshold level in a vaccinee that predicts protection. For an individual-based correlate, it is necessary to measure post-immunization antibody levels in the entire phase III cohort. An alternative approach for those subjects who have a defined exposure may based on the measurement of early post-exposure antibody levels before boosting.

Immune responses should always be evaluated as part of a phase III clinical protection study with the aim of identifying immunological correlates of protection. For such an evaluation to be clinically meaningful, validated standardized assays are essential. Methods for the validation and standardization of immunological (antibodies and cell-mediated) correlates of protection should be developed and are vital for ensuring comparability of data between one trial and another. To correlate humoral immune responses to a vaccine with protective efficacy, the qualitative and quantitative relationships should be determined. The recommendations concerning the evaluation of immune responses described in phase II (B.6) should also be applied in clinical protection trials.

B.7.3 *Duration of protection and need for booster vaccinations*

Randomized controlled trials may provide an early indication of likely long-term protection and the need for booster vaccination(s). In addition to the course of antibody response and its relation to clinical outcome, longer-term follow-up of antigenically new vaccines should include critical characteristics of the vaccine that serve as prognostic factors for sustained protection. Therefore, in addition to studying the quality and dynamics of the antibody response, informa-

tion should be obtained on the relative importance of antibody titre, the extent of seroconversion and the induction of immunological memory.

When efficacy trials are completed, controlled follow-up of the entire study population (or a subset), which may extend into the post-licensure period, provides the best opportunity to define with confidence the serological correlate(s) of protection, and the need for, and the timing of booster vaccination(s). If efficacy studies were not possible, subsets of recipients may be followed over time for measurement of serological parameters. However, if there is no established correlate of protection, and if induction of memory is thought to be an important component of immunity, these studies may be inconclusive. For the determination of long-term protection and the potential need for booster vaccination, postmarketing serosurveillance studies may be necessary as it may not be possible or appropriate to prolong a trial beyond the point at which efficacy is established.

B.7.4 *Safety evaluations in phase III trials*

Safety evaluation during clinical development and prior to marketing authorization describes and quantifies the safety profile of a vaccine over a period of time, in a manner that is consistent with the intended use. The safety evaluation should include all subjects enrolled in all trials who receive at least one dose of vaccine, and safety surveillance should begin from the start of enrolment. Data on comparisons with antigenically similar active controls (vaccines used to prevent the same infectious disease) should be provided, if available. Safety issues identified during preclinical testing should be specifically addressed in the phase I, II and III clinical trials. Special considerations should be given to the safety concerns raised in animal studies and to environmental concerns related to vaccines based on genetically modified organisms (*72*).

Frequent adverse events must be thoroughly investigated and special features of the product explored (e.g. clinically relevant interference with other vaccines or drugs and factors leading to differences in effect, such as age or epidemiological characteristics). Obtaining such evidence is often the most difficult task of clinical research and requires large-scale randomized trials that employ clinical, epidemiological, biostatistical and laboratory methods. It is important to have a prospective definition and an order of prioritization for adverse outcomes. The difficulty of conducting such trials is usually determined by the incidence of infection and disease and the ability to establish a specific clinical or laboratory diagnosis for the disease in

question. This, together with the expected vaccine efficacy, is what determines sample size.

Randomized studies must have sufficient power to provide reliable rates of common (>1/100 and <1/10) adverse events, and to detect less common, but not necessarily very rare (<1/10 000) adverse events (*30*).

For the earlier phases of the study, a specific monitoring plan with a timetable and methods should be specified in the protocol for all subjects (see methodological considerations). When adequate safety data are available from phase I and II trials, it may be acceptable in the phase III study to actively monitor only a subset of subjects (e.g. several hundred per group) to quantify common and non-serious local and systemic events in the trial participants. For the rest of the phase III participants, active monitoring could focus on the identification of significant and/or unexpected serious events (e.g. hospitalization and death).

B.7.5 ***Serious adverse events***

A serious adverse event is an event that is associated with death, admission to hospital, prolongation of a hospital stay, persistent disability or incapacity, or is otherwise life-threatening in connection with the clinical trial. All reported serious adverse events should be described in detail and the following information recorded:

— patient's study number or identification number;
— study identification;
— type of adverse event;
— how long after the vaccination the adverse event occurred;
— patient characteristics, including any underlying diseases, concomitant vaccinations or drugs;
— actions taken, e.g. therapy administered; and
— course of the adverse event including duration, outcome and investigator's assessment of causality.

The possibility of biological plausibility and/or a causal relationship with the vaccination should be considered and investigated in every case, although attributing causality is often difficult for events that occur anyway in the study population background (such as sudden infant death syndrome). Active monitoring of serious adverse events reported after completion of immunization is of major importance, because serious adverse events should be evaluated following a specific pattern.

Prior to licensure, both the applicant and the regulatory authority need to consider whether any reports of adverse drug reactions raise sufficient concern to warrant a suspension (perhaps only temporary) of product development. Additional clinical safety studies may be needed to confirm the relationship between the vaccine and the adverse event, and to establish precise incidence.

The duration of monitoring of study subjects following a serious adverse event depends upon the specific characteristics. Standard case report forms should be drawn up and used to record information on adverse events. Such forms should be used from phase I onwards.

Some serious adverse events following vaccination may be too uncommon to be observed in clinical trial programmes undertaken for marketing approval. Therefore, to obtain a more precise insight into the risk–benefit balance of the vaccine, a postmarketing surveillance programme should be implemented. In addition, specific postmarketing studies are often performed.

B.8 Bridging studies

Bridging studies within the context of this document are studies intended to support the extrapolation of efficacy, safety and immunogenicity data from one formulation, population, formulation and dose regimen to another. The need for performing bridging studies should be considered carefully and justified in the protocol. The end-points for clinical bridging studies are usually the relevant immune responses and clinical safety parameters.

Various methods may be used, depending on the purpose of the study. These are considered below.

B.8.1 *Design and extent of a clinical bridging study*

The clinical bridging studies (to support comparability with respect to the manufacturing process, change in product composition, or a new dose, route or schedule for immunization) should ordinarily be randomized controlled trials. As a minimum these studies should have adequate power to establish comparability of the relevant immune responses (see non-inferiority, section B.3.3.2) and to detect common adverse events. Additional comparative safety data may be needed to support extensive changes, such as a change in antigen composition in a new combination vaccine.

Clinical bridging studies to support extrapolation of efficacy data for a vaccine from one population to another are not randomized. However, for the outcomes to be valid it is important to minimize relevant

confounding variables. The composition and manufacturing process of the vaccine administered to study subjects should be as similar as possible (e.g. using the same lot for all subjects if available). The nature and extent of a bridging study are determined by the likelihood that vaccine efficacy may vary according to ethnic factors, manufacturing changes or changes in dosing schedule. Such studies are not required when it is sufficiently clear from pharmaceutical and preclinical experience that a change in the manufacturing process will not alter clinical efficacy or safety (e.g. specifications for quality control and lot release are not changed and therefore physicochemical characterization may be sufficient).

A controlled immunogenicity study may suffice (provided the serological correlate for clinical protection is validated) if regions are ethnically dissimilar, provided extrinsic factors are similar. An immunogenicity study will also help to select the appropriate schedule (i.e. the most protective) taking into account the incidence of the disease to be prevented (*73*). Controlled bridging trials using clinical endpoints are necessary when there has been a change of manufacturing process or manufacturing site resulting in a new product, the preclinical efficacy and safety data relating to the already-licensed product are no longer applicable; and a serological correlate for protection is not established.

Such studies would also be required in the target region when:

— the vaccine may be influenced by ethnic differences in the target population, and extrinsic factors are dissimilar;
— there is uncertainty regarding the appropriate dose regimen because local immunization schedules and/or antigenic doses differ from those used in trials conducted elsewhere;
— there is insufficient confidence in accepting the results of randomized controlled trials carried out elsewhere; or
— the vaccine is antigenically new in the region of the target population.

To minimize confounding factors related to the assays, the sera from different groups should be tested at the same time using the same assays, personnel and laboratory conditions. For studies that are not randomized or are not blinded with regard to subject enrolment (e.g. population bridging studies), special efforts should be made to avoid bias in sample testing. This may be achieved by appropriate coding of samples which will avoid any identification that distinguishes a separate group and sequential testing by group.

B.8.2 ***Situations in which bridging studies may be required***

B.8.2.1 Bridging studies for change in manufacturing process

Changes made to the product composition (e.g. adjuvants or preservatives) or manufacture (process, site or scale) after the efficacy trial and prior to approval, or after licensing, may have a significant impact on safety and/or efficacy. Any proposed change in the production of a vaccine must be shown by the manufacturer to result in a product equivalent to that used in preclinical (or earlier clinical) testing. Such changes should be evaluated on a case-by-case basis to determine the supporting data required to demonstrate comparability of the "new" product with the previous version. An additional clinical study comparing the new version to the previous versions may or may not be required.

B.8.2.2 Bridging studies for new dosing schedules

Comparability with the original vaccine is also a concern when changes have been made in the immunization schedule, dose and/or route of administration (e.g. change from subcutaneous to intramuscular administration). In most cases, these changes should be supported by a clinical bridging study. The vaccine should be studied in the most conservative situation (the most restrictive), i.e. where the least response is expected. The most restrictive schedule should be applied in the initial clinical trials (youngest age at first dose, and smallest interval between doses), to make extrapolation to other schedules possible. This approach will allow the extrapolation to less conservative vaccination schedules without additional trials. For example, it is easier to extrapolate from a 2, 3, 4 schedule to a 3, 4, 5 schedule than the other way around.

B.8.2.3 Bridging studies for a new population

There are many situations in vaccine development where a new population has important differences from the trial population in which efficacy was established. The ability to extrapolate the data is particularly important when it is not feasible to repeat an efficacy trial with clinical end-points.

Population bridging studies address the concern that the safety and/or efficacy profiles of a vaccine in a particular target population may differ from those observed in the population studied in the original efficacy trial. The question of efficacy may be addressed by showing that the relevant vaccine-elicited immune response in the new population is similar to that in the population studied in the original efficacy trial. Thus, retaining sera and other relevant samples from the original efficacy trial for such comparisons is important, and this

requirement should be taken into account in the planning of efficacy trials.

Clinical bridging studies are justified only when ethnic or other factors specific to the target population exist, and when the studies do not unnecessarily duplicate clinical studies or delay the supply of important vaccines to populations requiring them. Ethnic factors may be genetic, physiological (intrinsic), or epidemiological, cultural and environmental (extrinsic). Cultural characteristics include the nature of the health care infrastructure and available resources (*21*).

B.8.2.4 Bridging studies for safety

A bridging study for safety may be necessary when there are special safety concerns in the target population.

- Bridging efficacy studies may provide safety data when the power of the study is sufficient to assess the rates of common adverse events. A limited safety study might precede the clinical bridging study to ensure that serious adverse events do not occur at a high rate.
- A special safety study is required if an efficacy bridging study is not needed, or when the efficacy study does not provide adequate safety information, including when:
  — there is an index case (the individual in whom the event was first reported) or cases of a serious adverse event in foreign clinical data (generated outside the target region);
  — there are differences in reporting of adverse events elsewhere;
  — insufficient data on safety in the target population are available from an efficacy bridging study;
  — the safety profile cannot be extrapolated from foreign data to the target population; or
  — immunization schedules and/or antigenic doses differ from those used in foreign trials.

B.9 Post-licensure studies and surveillance

Following licensure, when a vaccine is in use, monitoring of its efficacy, safety and quality is referred to as postmarketing surveillance or postmarketing studies (phase IV studies). The purpose of these studies is to monitor the performance of a vaccine in the large target population under conditions of routine use, to detect adverse reactions and to monitor efficacy and effectiveness. In order to obtain more accurate estimates of adverse events and of effectiveness than those from phase III studies, active surveillance and phase IV studies using carefully designed surveys are used. Resource constraints

usually limit such surveys to a subgroup of the population, although for rare diseases it may be necessary to survey the entire population to obtain statistically valid data. Postmarketing studies are planned in study protocols. Although occasionally the designs may be as used in prelicensure trials, in most cases phase IV studies are set up as observational cohort or case–control studies. Whereas phase I, II and III studies make every attempt to standardize subjects, immunizations, evaluations and laboratory studies, it is usually impossible in phase IV studies.

Postmarketing surveillance and studies may be conducted to investigate:

— the optimal use of a vaccine (e.g. age at vaccination, simultaneous administration of other vaccines, changes in the vaccine strains and interchangeability of vaccines);
— efficacy in certain risk groups (e.g. the elderly, immunocompromised patients and patients with certain diseases); and
— maintenance of long-term efficacy and monitoring of long-term safety.

To ensure adequate postmarketing surveillance marketing authorization holders should be committed to presenting a postmarketing surveillance programme at licensure and all national regulatory authorities should endeavour to put in place a system for pharmacovigilance for vaccines. The outcomes of surveillance (assessments of effectiveness, adverse events and quality) should be reported to the national authorities and/or the marketing authorization holder, and they should be published.

Postmarketing surveillance programmes should be appropriate to the disease epidemiology, infrastructure and resources in the target area. Essential standards of efficacy, safety and quality should always be defined before initiating a postmarketing surveillance programme and the programme should include assessment of:

— the impact of the target disease (morbidity and mortality);
— potential of the disease to cause an epidemic;
— whether the disease is a specific target of a national, regional or international control programme; and
— whether the information to be collected will lead to significant public health action.

Ideally, a postmarketing programme should be based on criteria set for a particular vaccine as a part of marketing approval. The essential standards for these should always be defined. To ensure that an

intervention is conducted to an acceptable standard, to identify areas where special attention is required and to ascertain (in cases of vaccine or programme failure) the possible reasons for this failure, each step should be carefully monitored and described in protocols. Important applications of postmarketing surveillance are in the early stages of use of a novel vaccine, or when circumstances change (e.g. the emergence of new antigenic variants of a pathogen) and doubts are raised about the continuing efficacy of the current formulation.

B.9.1 *Safety evaluation*

Postmarketing surveillance may be the only means of detecting long-term or acute events that occur too infrequently to have been revealed by clinical trials. Under specific circumstances active postmarketing surveillance or phase IV studies should be considered to determine the incidence and significance of infrequent and rare emerging serious events following immunization with the vaccine under investigation. With respect to safety, the intent of a phase IV study is to detect the rarer or unexpected events that may not have been seen in the smaller phase II or phase III studies because of their limited statistical power. Rare events are often idiosyncratic; a causal relationship is difficult to establish and this usually cannot be done prior to licensure.

Surveillance for the collection of safety data may be conducted by active or passive processes, and may be directed at an entire population or at a subgroup. In practice, a mixture of these processes is often used. Voluntary reporting of adverse events (passive surveillance) is the most often used. It is effective in detecting severe or lethal events and unusual clinical responses. The true rate of incidence of adverse events, particularly of those that do not have distinctive manifestations, is likely to be considerably underestimated.

Targeted studies of a specific adverse event are usually case–control studies or retrospective studies on exposure cohorts linked to historical controls (*74*). In retrospective exposure cohorts the event of interest can be studied in a controlled setting using sampled historical data identified prospectively. Postmarketing surveillance for safety evaluation should include information from all possible sources. Databases linked to large patient cohorts are a valuable source of information for investigating serious adverse events (*75*). Collecting data on safety using a structured, planned postmarketing surveillance study may be set as a condition for marketing approval.

B.9.2 *Evaluation of vaccine effectiveness*

Following the evaluation of efficacy in a randomized controlled phase III clinical trial, the effectiveness of a new vaccine in routine practice should be determined (*76*). Studies of effectiveness measure direct and indirect protection (e.g. protection of unvaccinated persons by the vaccinated population (herd immunity)). Vaccine effectiveness is affected by a number of factors, including:

— vaccination coverage of the population;
— immune status of the population;
— correlation of strains used in vaccine production with circulating strains; and
— The incidence of disease due to strains not included in the vaccine following introduction of the vaccine in that population.

If conducted consistently over a prolonged period, postmarketing surveillance allows the longitudinal assessment of efficacy under a range of conditions, and it may disclose variations in vaccine quality. The duration of follow-up of subjects in the postmarketing programme should be described in a protocol. Implementation of an immunization programme in a certain population may necessitate the development of a structured plan for postmarketing serosurveillance to identify changes in disease epidemiology in the target population over time. This may include evaluation of:

— the impact of the programme, through analysis of reported vaccine failures, and (if applicable) assessment of why disease is still occurring;
— whether new immunization strategies are necessary; and
— possible harm caused by replacement disease following the intervention (e.g. other serotypes replacing the serotypes in the vaccine).

A protocol for serosurveillance should be presented at the time of marketing authorization, or implementation of a vaccination programme. A structured plan for executing the programme should be presented, including information on participating institute(s) and intervals of reporting (usually every 6 months, for 5 years).

B.9.3 *Study design*

B.9.3.1 Observational cohort studies

The evaluation of the benefit of a community-based immunization programme requires large-scale surveillance. An observational cohort study, directed at the events, exposures and diseases occurring among vaccinated and unvaccinated members of the target

population under normal conditions may provide an estimate of vaccine effectiveness.

In non-randomized studies, nested household surveys in a random sample of the study population may minimize bias. In some cases randomization from phase III trials may be continued concurrently.

Observational cohort studies may require community-wide sampling. The chosen sample size will depend upon the characteristics of the intervention applied (i.e. whether risk-group intervention, community intervention or traveller immunization).

B.9.3.2 Case–control studies

Case–control studies should be considered in investigating diseases of low incidence or when studying adverse events in response to vaccines when they can be particularly useful (*77*). In order to generate adequate information on vaccine efficacy, population samples should be well defined and representative, and a serological correlate for protection, if available, should be used (see B.7.2.3). The advantages of case–control studies are that they can be small-scale and the follow-up period is short. The main limitations are the potential for (a) selection bias, and (b) information bias. Selection bias is due to lack of randomization and the selection of the control group, especially when the study is not population based. Every effort should be made to include as many cases as possible. All aspects of study design and conduct should be detailed in the study protocol and justified.

B.9.3.3 Stepped wedge design

The stepped wedge design should be considered when previous studies have indicated that the intervention is likely to be beneficial (*51*) and the public health need to introduce the intervention precludes withholding it from a population. The intervention is introduced in phases, group by group, until the entire target population is covered. The groups form the unit of randomization.

B.9.3.4 Outbreak interventions

At the start of an outbreak (or epidemic), the susceptibility of all individuals in the target population to the infecting pathogen is assumed to be equal. The methodological approach chosen to study the effectiveness of the intervention should be appropriate to the size and nature of the outbreak.

- Pre-exposure cohort studies or secondary attack-rate studies are preferred in infections with a high attack rate.
- Case–control studies are useful in studies of diseases with a low incidence or in small isolated outbreaks.

- Community-based cohort studies are unsuitable for short-term evaluation; however, they may be useful for the post hoc evaluation of the performance of a vaccination programme or for long-term follow-up of specific clinical outcomes or safety issues.

In areas where the immunization rate is high, outbreak investigations underestimate vaccine efficacy. The degree of underestimation is related to the extent of the epidemic that triggered the investigation, vaccination coverage in the community and the extent of clustering of vaccination failures in the population.

B.9.4 *Monitoring of postmarketing surveillance*

A postmarketing oversight policy should be established by a national regulatory authority to enable control of product release, periodic inspections, reporting mechanisms, recall of batches, or, if necessary, for revoking marketing approvals, approval of manufacturing changes, and evaluation and approval of new indications and/or dose regimens. General guidelines for continued oversight of vaccines after licensure as described in WHO Technical Report Series 858, should be followed (*1*). Guidance on the operation of epidemiological surveillance and monitoring of adverse events are provided by WHO and other bodies (*37*, *78–80*). Standards for assessment of causality are described in these and other regulatory documents. Targeted monitoring and special studies may be required for certain adverse events (*75*). Monitoring vaccines for use in the Expanded Programme of Immunization should include not only efficacy and safety, but also compatibility with existing vaccines (antigens) used in this programme (*81*). Ideally, this should be considered prior to marketing approval. In addition, the immunization programme and vaccine supply should be considered.

B.10 Special considerations for combination vaccines

A combination vaccine consists of two or more vaccine immunogens in a physically mixed preparation intended to prevent several diseases or to prevent one disease caused by different serotypes (or serogroups) of the same organism (*13*, *14*, *79*). The mixing may occur as a manufacturing step or it may be performed by a health care professional on site before administration according to the package insert instructions. Vaccines mixed ad hoc without regulatory approval are not considered to be combination vaccines.

The main goal of a clinical trial of a combination vaccine is to evaluate the efficacy of each component vaccine, and the safety of the combination, regardless of whether or not the combination consists of

previously marketed or investigational individual component vaccines. The immunogenicity and safety of a new combination should be compared with the effects of simultaneous, but separate, administration of the individual vaccines.

B.10.1 *Efficacy studies*

Once the serological correlates of protection have been validated for each of the antigenic components, consideration should be given to evaluating the efficacy of a new combination vaccine consisting of components already licensed and/or components with proven efficacy using immunogenicity rather than clinical protection end-points. Failing this, prospective controlled clinical studies or alternative approaches such as postmarketing surveillance are required.

Studies of combination vaccines are usually designed and analysed (for efficacy or immunogenicity) as non-inferiority trials, the aim being to demonstrate that the combination is comparable with the individual components. Each of the individual components is expected to add materially to the prophylactic effect of the vaccine (*61*, *79*).

Clinical studies of combination vaccines should:

— have sufficient power to rule out pre-existing differences in response parameters between the study groups;
— use appropriate sample sizes, as for monovalent vaccines (see methodological considerations); and
— consider the clinical consequences of any potential difference observed.

Clinical bridging studies may be needed to facilitate extrapolation of data to a different population or to support a different immunization schedule.

Immunogenicity trials of new combination vaccines to prevent several diseases (multidisease combination vaccines) should be designed to rule out predefined differences in immune responses between the new product and the individual components administered separately. When antibody concentrations following administration of the combined vaccine are less than those observed following separate administration of the individual components or simultaneous administration of the individual of the individual vaccines at different sites, it should be demonstrated that these findings are not clinically relevant. Any change in dose or schedule for individual components should be justified.

For a combination vaccine consisting of several strains or serotypes, the primary end-point for clinical efficacy should be the prevention of disease caused by the different vaccine-type strains, or the ability of the vaccine to modify the course of such disease.

The study should have sufficient power to enable meaningful separate analyses to be made of the prevalent strains or serotypes identified as being of major significance to public health in the target area. The appropriateness of the coverage provided by the individual vaccine components in the target population should be justified e.g., in the case of multivalent vaccine that does not cover all serotypes of the disease such as pneumococcal conjugate vaccine, epidemiological data should be provided to justify the selection of strains for this vaccine. The feasibility of extrapolation from limited numbers of strains or serotypes to other strains or serotypes should be substantiated.

B.10.2 *Safety analysis of combination vaccines*

For the safety evaluation of combination vaccines, as much information as possible should be obtained from randomized, controlled trials. Such studies are usually designed and analysed as non-inferiority trials that aim to demonstrate that the safety of the combination is not inferior to that of the individual components. Where applicable, the controls for the study should be the already marketed vaccines with the same antigen composition. The size chosen for the study groups should take into account differences in rates of common and/or clinically important adverse events. For vaccines intended for infants and children, defining differences in rates of high fever may be especially relevant. Blinding is virtually essential for making valid comparisons and for the accurate determination of the rates of events causally related to vaccination. If blinding of a study is not feasible, the methods used to minimize bias should be described.

The safety and efficacy of new formulations in which reduced doses of some or all of the components of a combination vaccine are necessitated by the volume of the combination of components being too large for safe administration must be demonstrated.

Simultaneous administration of vaccines

For monovalent vaccines intended for simultaneous administration with other vaccines to the target population, any clinically relevant interference with the other vaccines should be ruled out. Immunological interference and adverse safety interactions after simultaneous administration should be compared with the results of separate administration of the (new) vaccine component(s) at different times.

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Appendix

Summary protocol for vaccine evaluations

Title and summary _____

Brief description of the study site(s) _____

Investigators _____

Background and rationale _____

Preclinical and laboratory evaluation of vaccines _____

Summary of product characteristics (details of methods for production and control of candidate vaccine) _____

Primary and secondary objectives _____

Study design _____

— hypothesis _____

— end-points _____

— study plan _____

— trial size _____

— duration of study _____

Study population _____

— inclusion and exclusion criteria _____

Methods and procedures _____

— recruitment of subjects _____

— allocation of subjects _____

— vaccine delivery _____

— follow-up _____

— laboratory methods _____

— statistical plan and analyses _____

Monitoring of the trial _____

— data monitoring _____

— quality assurance of data and laboratory methods _____

Timetable _____

— start and end of recruitment _____

— end of follow-up _____

— date of report _____

References

1. Regulation and licensing of biological products in countries with newly developing regulatory authorities. In: *WHO Expert Committee on Biological Standardization. Forty-fifth report.* Geneva, World Health Organization, 1995, Annex 1 (WHO Technical Report Series, No. 858).
2. *Guidelines for good clinical practices (GCP) for trials on pharmaceutical products.* In: *WHO Expert Committee on the Use of Essential Drugs, Sixth report.* Geneva, World Health Organization, 1995, (WHO Technical Report Series, No. 850), Annex 3.
3. Good manufacturing practices for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second report.* Geneva, World Health Organization, 1992, (WHO Technical Report Series, No. 823), Annex 1.
4. Good manufacturing practice: supplementary guidelines for the manufacture of the investigational pharmaceutical products for clinical trials in humans. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-forth report.* Geneva, World Health Organization, 1996, Annex 7 (WHO Technical Report Series, No. 863).
5. Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second report.* Geneva, World Health Organization, 1992 (WHO Technical Report Series, no. 822), Annex 1.
6. Guidelines for national authorities on quality assurance for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-Second report.* Geneva, World Health Organization, 1992, Annex 2 (WHO Technical Report Series, No. 822).
7. Recommendations and guidelines for biological substances used in medicine and other documents. In: *WHO Expert Committee on Biological Standardization. Forty-ninth report.* Geneva, World Health Organization, 2000, Annex 3 (WHO Technical Report Series, No. 897).
8. *Guidelines for the evaluation of Plasmodium falciparum vaccines in the population exposed to natural infection.* Geneva, World Health Organization 1997.
9. *Guidelines for the evaluation of dengue vaccines in populations exposed to natural infection.* UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. 2002 (TDR/IVR/DEN/02.1).

10. *Immunization in Practice.* Geneva, World Health Organization 1998, (Global Programme for Vaccines and Immunization. Expanded Programme on Immunization) (document WHO/EPI/TRAM/98.01-11REV.1).

11. Organisation for Economic Co-operation and Development principles on Good Laboratory Practice, Paris, OECD, 1998 (ENV/MC/CHEM (98)17).

12. Committee for Proprietary Medicinal Products. *Note for guidance on preclinical pharmacological and toxicological testing of vaccines.* London, CPMP, 1998 (document CPMP/SWP/465/95).

13. Committee for Proprietary Medicinal Products. *Note for guidance on pharmaceutical and biological aspects of combined vaccines.* London, CPMP 1999 (document CPMP/BWP/477/97).

14. Center for Biologics Evaluation and Research. *Guidance for industry for the evaluation of combination vaccines for preventable diseases: production, testing and clinical studies.* Rockville, MD US Food and Drug Administration, 1997.

15. Center for Biologics Evaluation and Research. *Guidance for industry for the submission of chemistry, manufacturing, and controls information for synthetic peptide substances.* Rockville, MD, US Food and Drug Administration, 1994.

16. World Medical Association, Declaration of Helsinki: Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects. (Available on the Internet at www.wma.net/e/.)

17. *Operational guidelines for ethics committees that review biomedical research.* Geneva, World Health Organization, 2000.

18. *International ethical guidelines for biomedical research involving human subjects.* Geneva, Council for International Organizations of Medical Sciences, 1993.

19. Ethics and research on human subjects — international guidelines. In: Bankovski Z, Levine RJ, eds. *Proceedings of the twenty-sixth CIOMS Conference*, Geneva, 5–7 February 1992.

20. *Ethical considerations in HIV preventive vaccine research.* Guidance document. Geneva, UNAIDS 2000.

21. *Note for guidance on ethnic factors in the acceptability of foreign clinical data* (ICH topic E 5, September 1998). Committee for Proprietary Medicinal Products, 1998 (CPMP/ICH/289/95).

22. *Note for guidance on good clinical practice.* ICH topic E6. Informal discussion on harmonisation of existing and future requirements for vaccine licensing, testing and production, Brussels, Committee for Proprietary Medicinal Products, 1997 (CPMP/ICH/135/95).

23. *Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via medicinal products.* Committee for Proprietary Medicinal Products, 1998 (CPMP/BWP/1230/98 revision).

24. *Note for guidance on quality of biotechnological products: stability testing of biotechnological/biological products* (ICH topic Q5C). Committee for Proprietary Medicinal Products, 1996 (CPMP/ICH/138/95).

25. *Note for guidance on quality of biotechnology products: viral safety, evaluation of biotechnology products derived from cell lines of human or animal origin.* (ICH topic Q5A). Committee for Proprietary Medicinal Products, 1997 (CPMP/ICH/295/95).

26. *Note for guidance on preclinical safety evaluation of biotechnology derived pharmaceuticals* (ICH topic S 6). Committee for Proprietary Medicinal Products, 1998 (CPMP/ICH/302/95).

27. *Note for guidance on specifications: test procedures and acceptance criteria for biotechnological/biological products* (ICH topic Q6B). Committee for Proprietary Medicinal Products, 1999 (CPMP/ICH/365/96).

28. *Causality classification in the European Community.* (EC Directive III/3445/91).

29. *Note for guidance on clinical evaluation of new vaccines.* Committee for Proprietary Medicinal Products, 1999 (CPMP/EWP/436/97).

30. European Commission. *Pharmaceuticals and Cosmetics. Notice to Applicants. A Guideline on Summary of product characteristics.* December 1999.

31. *Note for guidance on population exposure: the extent of population exposure to assess clinical safety* (ICH Topic E1A). Committee for Proprietary Medicinal Products, 1995 (CPMP/ICH/375/95).

32. *Note for guidance on clinical safety data management: data elements for transmission of individual case safety reports* (ICH topic E2B). Committee for Proprietary Medicinal Products, 1998 (CPMP/ICH/287/95).

33. *Notice to marketing authorization holders. Pharmacovigilance guidelines.* Committee for Proprietary Medicinal Products, Draft version, January 1999 (CPMP/PhVWP/108/99).

34. Ethics and Epidemiology: International Guidelines In: Bankowski Z, Bryant JH, Last JM eds. *Proceedings of the twenty-fifth CIOMS Conference.* Geneva, Council for International Organizations of Medical Sciences, 1991.

35. Center for Biologics Evaluation and Research. *Guidance for industry: Providing clinical evidence of effectiveness for human drug and biological products.* Rockville, MD, US Food and Drug Administration, 1998.

36. Center for Biologics Evaluation and Research. *Guidance for industry. How to complete the vaccine adverse event reporting system form (VAERS — 1).* Rockville, MD, US Food and Drug Administration, 1998.

37. *International reporting of adverse drug reactions. Final report of the CIOMS Working group.* Geneva, Council for International Organizations of Medical Sciences, 1990.

38. *International guidelines for ethical review of epidemiological studies.* Geneva, Council for International Organizations of Medical Sciences, 1991.

39. *Note for Guidance on statistical principles for clinical trials* (ICH topic E9). Committee for Proprietary Medicinal Products, 1998 (CPMP/ICH/363/96).

40. Griffiths E, Knezevic I. Assuring the quality and safety of vaccines: regulatory expectations for licencing and batch release. In: Robinson A, Cranage M, Hudson M, eds. *Methods in molecular medicine: vaccine protocols*. Totowa New Jersey, Humana Press, 2003, 353–376.

41. Requirements for the use of animal cells as *in vitro* substrates for the production of Biologicals. In: *WHO Expert Committee on Biological Standardization. Forty-seventh report.* Geneva, World Health Organization, 1998, Annex 1 (WHO Technical Report Series, No. 878).

42. Guidelines for assuring quality of DNA vaccines. In: *WHO Expert Committee on Biological Standardization. Forty-seventh report.* Geneva, World Health Organization, 1998, Annex 3 (WHO Technical Report Series, No. 878).

43. Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology. In: *WHO Expert Committee on Biological Standardization. Forty-first report.* Geneva, World Health Organization, 1991, Annex 3 (WHO Technical Report Series, No. 814).

44. Guidelines for the production and quality control of synthetic peptide vaccines. In: *WHO Expert Committee on Biological Standardization. Forty-eighth report.* Geneva, World Health Organization, 1999, Annex 1 (WHO Technical Report Series, No. 889).

45. General requirements for the sterility of biological substances. In: *WHO Expert Committee on Biological Standardization. Twenty-fifth report.* Geneva, World Health Organization, revised 1973, Annex 3 (WHO Technical Report Series, No. 530); Amendment In: *Expert Committee on Biological Standardization. Forty-sixth report.* Geneva, World Health Organization, 1998, Annex 3 (WHO Technical Report Series, No. 872).

46. WHO guidelines on *Transmissible Spongiform Encephalopathies in relation to biological and pharmaceutical products*. Geneva, World Health Organization, 2003 (document, WHO/BCT/QSD/03.01).

47. Center for Biologics Evaluation and Research. Guidance for Industry. *Content and format of chemistry, manufacturing and controls information and establishment description information for a vaccine or related product.* Rockville, MD, US Food and Drug Administration 1999, **64**:518–519.

48. Recommendations for the production and control of *Haemophilus influenzae* type b conjugate vaccines. In: *WHO Expert Committee on Biological Standardization. Forty-ninth report.* Geneva, World Health Organization, 2000, Annex 1 (WHO Technical Report Series, No. 897).

49. Goldenthal KL et al. Safety evaluation of vaccine adjuvants: national cooperative vaccine development meeting working group. *AIDS Research and Human Retroviruses*, 1993, **9**(suppl. 1):S47–S51.

50. Requirements for poliomyelitis vaccine (oral) (Addendum 1998). In: *WHO Expert Committee on Biological Standardization. Forty-ninth report.* Geneva, World Health Organization, 2000, Annex 2 (WHO Technical Report Series, No. 897).

51. Smith PG, Morrow RH. *Field trials of health interventions*, 2nd ed. Geneva, World Health Organization 1996.

52. **Clemens J et al.** Evaluating new vaccines for developing countries. Efficacy or effectiveness? *Journal of the American Medical Association*, 1996, **275**:390–397.

53. **Giesecke J.** *Modern infectious disease epidemiology.* Oxford, Arnold/Oxford University Press. 1995.

54. **Halloran ME, Struchiner CJ, Longini IM.** Study designs for evaluating different efficacy and effectiveness aspects of vaccines. *American Journal of Epidemiology*, 1997, **146**:789–803.

55. **Anderson RM, Donnelly CA, Gupta S.** Vaccine design, evaluation and community based use for antigenically variable infectious agents. *Lancet*, 1997, **350**:1466–1470.

56. **Durham KL et al.** Estimation of vaccine efficacy in the presence of waning: application to cholera vaccines. *American Journal of Epidemiology*, 1998, **147**:948–959.

57. **Halloran ME, Struchiner CJ.** Causal inference and infectious diseases. *Epidemiology*, 1995, **6**:142–151.

58. **Horne AD, Lachenbruch PA, Goldenthal KL.** Intent-to-treat analysis and preventive vaccine efficacy. *Vaccine*, 2000, **19**:319–326.

59. **Lachenbruch PA.** Sensitivity, specificity, and vaccine efficacy. *Controlled Clinical Trials*, 1998, **19**:569–574.

60. **Ewell M.** Comparing methods for calculating confidence intervals for vaccine efficacy. *Statistics in Medicine*, 1996, **15**:2379–2392.

61. **Blackwelder WC.** Similarity/ equivalence trials for combination vaccines. *Annals of the New York Academy of Sciences*, 1995, **754**:321–328.

62. **Folb P.** In Grady JO, Joubert PH. *Handbook of phase I/II clinical drug trials.* CRC Publishers, 1997.

63. **Horne AD.** The statistical analysis of immunogenicity data in vaccine trials: a review of methodologies and issues. *Annals of the New York Academy of Sciences*, 1995, **754**:329–346.

64. **Reed GF, Meade BD, Steinhoff MC.** The reverse cumulative distribution plot: a graphic method for exploratory analysis of antibody data. *Pediatrics.* 1995, **96**:600–603.

65. **Horne AD.** Statistics, use in immunology. In: *Encyclopaedia of immunology.* London, Academic Press, 1998.

66. **Hayes RJ et al.** MRC Tropical Epidemiology Group. Design and analysis issues in cluster-randomized trials of interventions against infectious diseases. *Statistical Methods in Medical Research* 2000, **9**:95–116.

67. **Orenstein WA et al.** Field evaluation of vaccine efficacy. *Bulletin of the World Health Organization*, 1985, **63**:1055–1068.

69. **Olin P et al.** Randomised controlled trial of two-component, three-component, and five-component acellular pertussis vaccines compared with whole cell pertussis vaccine. *Lancet*, 1997, **350**:1569–1577.

70. **Smith PG, Rodrigues LC, Fine PEM.** Assessment of the protective efficacy of vaccines against common diseases using case control and cohort studies. *International Journal of Epidemiology*, 1984, **13**:87–93.

71. **Siber GR.** Methods for estimating serological correlates of protection. In: Brown, F et al. eds. *Pertussis vaccine trials: Developments in Biological Standardization.* Basel, Karger, 1997, **89**:283–296.

72. Committee for Proprietary Medicinal Products. 1994 *Note for guidance on environmental risk assessment for human medicinal products containing or consisting of GMOs.* (CPMP/III/5507/94).

73. **Fedson DS.** Measuring protection: efficacy versus effectiveness. In: Brown F, Plotkin S, eds. Preclinical and clinical development of vaccines. *Developments in Biological Standardization* 1998, **95**:195–201.

74. **Jefferson T.** Vaccination and its adverse effects: real or perceived. *British Medical Journal*, 1998, **317**:159–160.

75. **Taylor B et al.** Autism and measles, mumps and rubella vaccine: no epidemiological evidence for a causal relationship. *Lancet*, 1999, **353**:2026–2029.

76. **Chen RT, Orenstein WA.** Epidemiologic methods in immunization programmes. *Epidemiology Review*, 1996, **18**:99–117.

77. **Rodrigues LC, Smith PG.** Use of the case-control approach in vaccine evaluation: Efficacy and adverse effects. *Epidemiologic Reviews*, 1999, **21**:56–72.

78. **Falk LA et al.** Testing and licensure of combination vaccines for the prevention of infectious diseases. In: Ellis RW ed. *Combination vaccines: Development, clinical research and approval.* Totowa, NJ, Humana Press, 1999, 233–248.

78. *WHO recommended surveillance standards.* Geneva, World Health Organization, 1997 (document WHO/EMC/DIS/97.1).

79. **Horne AD et al.** Analysis of studies to evaluate immune response to combination vaccines. *Clinical Infectious Diseases*, 2001 **33**(Suppl 4):5306–5311.

79. *Protocols for the evaluation of epidemiological surveillance systems.* Geneva, World Health Organization, 1997 (document WHO/EMC/DIS/97.2).

80. *Surveillance of adverse events following immunization. Field guide for managers of immunization programmes.* Geneva, 1997 (document WHO/EPI/TRAM/93.02 Rev.1).

81. **Aylward B et al.** A framework for the evaluation of vaccines for use in the expanded programme on immunization. *Vaccine*, 1994, **12**:1155–1159.