— 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