ANNEX 1

WHO GUIDELINES ON CLINICAL EVALUATION OF VACCINES: REGULATORY EXPECTATIONS

This document provides guidance to National Regulatory Authorities (NRAs) and vaccine manufacturers on the clinical evaluation of vaccines by outlining international regulatory expectations during the different stages of vaccine development and for marketing approval. In this respect, the guidance in this document could also be useful for clinical researchers and investigators.

The text is written in the form of guidelines instead of recommendations in view the fact that vaccines represent a heterogeneous class of agents, and the preclinical and clinical testing programmes will need to be adapted for the product in question. Guidelines allow greater flexibility than Recommendations with respect to specific issues related to particular vaccines.

A separate WHO document is under development to provide more detailed guidance on preclinical and laboratory evaluation of vaccines. 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.

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This document was adopted by the WHO Expert Committee on Biological Standardization at its 52nd meeting. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.
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 in order to support a marketing approval. This document has been developed in response to requests from NRAs for assistance in the evaluation of clinical trials, both during the clinical development of a new vaccine and also during the regulatory review of dossiers submitted in support of applications for marketing authorizations. NRAs are expected to have a mandate to review protocols, and when necessary to protect the safety of subjects, to require protocol revision and/or termination of the trial. This document intends to provide a basis to NRAs to achieve these objectives. Since it is a common practise for the clinical development programmes and for individual clinical trials to take place in different countries, each NRA should, as far as possible collaborate with the other regulatory authorities involved in order to benefit from shared experiences and to align regulatory considerations (4).

Currently the World Health Organization (WHO) has the following guidelines and requirements that are relevant to the evaluation of vaccines: Good Clinical Practice (GCP) for trials on pharmaceuticals products (1), Good Manufacturing Practice (GMP) for pharmaceuticals (2, 81), GMP for biologicals (3), regulation and licensing of biological products in countries with newly developing regulatory authorities (4) and Guidelines for national authorities on quality assurance for biological products (5). Guidelines and recommendations for the production and control of specific vaccines are reviewed in detail in a series of WHO technical reports (6), which should be consulted where applicable but will not be reviewed here. However, there is no previous WHO document that gives guidance on planning, performance and assessment of clinical studies on vaccines with a regulatory perspective. Specific WHO guidelines that complement these general guidelines are available or are under development in the case of certain candidate vaccines, such as
those for malaria (80), dengue (84) and for 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 are already described in WHO manual "Immunization in Practice" (83).

Guidance on various aspects of clinical trials of vaccines is also available from several other bodies such as International Conference on Harmonization (ICH), The European Agency for the Evaluation of Medicinal Products (EMEA), the United States Food and Drug Administration (FDA), the United Kingdom Medical Research Council (MRC). These WHO guidelines are not intended to conflict with but to complement these other documents (7, 15, 18, 19, 21, 23-27, 57-76).

**REGULATION OF VACCINES**

Regulatory issues related to a particular candidate vaccine should be considered early in the development process, since regulatory compliance is the basis for eventual approval. It is strongly recommended that dialogue be established early on with the appropriate National Regulatory Agency which should review developmental plans for 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 (22). The developmental stage consists of two parts, preclinical research and development, and clinical research and development.

**Preclinical testing**

Preclinical research and development is carried out in the laboratory and uses *in vitro* techniques or, when necessary, *in vivo* techniques in animals. Preclinical and laboratory research data 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. In certain countries formal regulatory approval is required in order to undertake clinical phase I, II and III studies. This approval takes different forms such as Investigational New Drug Application (IND) in the United States and Clinical Trial Certificate (CTC) or Clinical Trial Exemption (CTX) in the United Kingdom. This is in addition to ethical clearance which is required in all countries for clinical trials. All human subjects studies require ethical consideration and review, in accordance with the Declaration of Helsinki (see paragraph ethical considerations).

In phase I clinical studies, initial testing of a vaccine is carried out in small numbers (e.g. 20) of healthy adults, to test the properties of a vaccine, 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 obtain preliminary information about a vaccine’s ability to produce its desired effect (usually immunogenicity) in the target population and general safety. To fully assess protective efficacy and safety of a vaccine extensive phase III are required. The phase III clinical trial is
the pivotal study on which licensing 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 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 a market authorization may be submitted to a national regulatory authority on the basis of the phase III data and if approved, the vaccine then becomes available on the market in that particular country. If a product contains or consists of genetically modified organisms (GMOs) an Environmental Risk Assessment should also be undertaken and approved by the appropriate agency.

While stages I through III are described, the actual 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 one that contains one or more previously evaluated antigens. These and other factors also influence whether clinical protection trials will be required, or even whether 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 final data package is likely to be of acceptable content.

Issues to be considered after the initial licensure

In addition to 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 post-marketing period is critical for the collection of safety and effectiveness data in large numbers of recipients. This section discusses both active and passive modes of surveillance. Following licensing, there is continued surveillance of vaccinees for adverse events, especially for rare events that can only be detected in very large numbers of subjects.

Any subsequent change in production methods or scale-up following licensing will necessitate further product characterisations to demonstrate equivalence although the extent of re-characterization depends on the nature of the changes implemented. These should be documented and the NRA notified of all changes. Regulatory authorities should clearly define and implement into regulations what changes require only a notification and what 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.

SCOPE OF THE DOCUMENT

Vaccines are 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: micro-organisms inactivated by chemical and/or physical means that retain adequate immunogenic properties; or living micro-organisms 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, polymerised or conjugated to a carrier to increase immunogenicity.

Also included within the scope of this document are novel products such as DNA vaccines and live genetically engineered micro-organisms used as vaccines themselves or 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.

A. PRECLINICAL EVALUATION OF VACCINES

GENERAL REMARKS

Preclinical evaluation of a vaccine is a prerequisite to the initiation of clinical trials. Laboratory evaluation should however be continued throughout both preclinical and clinical phases of vaccine development. This section on preclinical and laboratory testing is included as a preamble to the section on clinical trials. It discusses general principles for the preclinical evaluation of vaccines which should be taken into consideration before and during clinical trials. (A more detailed document which deals with the preclinical and laboratory evaluation of vaccines is under development by WHO).

The primary goal of preclinical testing of a new vaccine product, or a new combination vaccines 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 of a product, including the indicators of safety and immunogenicity in an appropriate animal model. In all cases, when preclinical animal testing is performed, there should be a clear rationale for doing so and the study should be performed in compliance with Good Laboratory Practice (GLP) guidelines (7) and with National Guidelines on animal experimentation. Preclinical and laboratory studies are necessary to establish the characteristics (physical, chemical and biological) of the candidate vaccine, and may also identify possible risks to the vaccinees and help to plan protocols for subsequent clinical studies in human subjects in which safety and efficacy of the candidate vaccine may be evaluated.

Close collaboration between the preclinical and the clinical investigators is particularly important in assessing the first results of administration of vaccines in humans. The clinician and his appropriate advisers have, however, the responsibility of satisfying themselves that the preclinical experiments are adequate in scope and they should request a full account of all relevant data.
2 PRODUCTION, CHARACTERIZATION AND QUALITY ASSURANCE OF CANDIDATE VACCINES

The basic principles for the production and control of vaccines can be found in WHO Technical Report Series (TRS) which cover general requirements (8-13). Specific guidelines and recommendations for particular vaccines are also available (6) and should be consulted as appropriate. WHO guidelines and recommendations are often adopted by NRAs as definitive national requirements. Other useful guidance may be obtained from the documents produced by other bodies (14). The characterization, standardization and control of vaccine preparations with respect to components, safety and potency are key issues during development. The amount of data needed to support clinical studies should increase throughout phase I and II, and product characterization should be completed by the beginning of phase III stage of development. In process testing should be performed to ensure 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 tests used to characterize the vaccine initially. A lot 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 under conditions of Good Manufacturing Practices. The general manufacturing recommendations contained in Good Manufacturing Practices for Pharmaceutical and Biological Products (2, 3, 81) should be applied to 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 change in the formulation of a vaccine should be considered carefully both by manufacturers and NRAs. Some changes in the formulation may have extremely high impact on the quality, safety and efficacy of vaccines and will require clinical trials.

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

In accordance with GCP 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 NCL for possible subsequent re-testing and investigation. Storage should be under safe and stable conditions for at least the duration of anticipated or approved shelf life and preferably longer.

3 TOXICITY AND SAFETY TESTING

Toxicity studies in animals may be considered to assess the potential toxic effects of a vaccine in target organs, including the hematopoietic and immune systems as well as to assess systemic toxicity. Toxicity studies may help to identify potential toxicity problems requiring further clinical monitoring. Detailed guidance on toxicological and
pharmacological testing may be found in EMEA Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (15). However, it should be recognized that a suitable animal model may not be available for undertaking toxicological evaluation of candidate vaccines and that such models are not necessarily predictive of human responses. Interpretation of data could be difficult. Furthermore, classical repeated dose toxicity test as applied to medicines may or may not be applicable for vaccines, depending on the vaccine dose regimen/composition, since usually there is no chronic exposure of the subject to a vaccine through repeated administration.

The design and value of repeated dose toxicity testing should therefore be considered on a case by case basis, as should be the selection of 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/foetal and perinatal toxicity should be considered on a case by case basis. Reproductive toxicity studies will need to be undertaken in any case before licencing.

Toxicity tests should include:

a) an evaluation of the initial safe dose and subsequent dose escalation schemes relevant to the clinical dose
b) an evaluation of single and repeat dose as appropriate
c) a determination of a relevant set of safety parameters for clinical monitoring
d) a demonstration of potential reversibility of virulence of attenuated vaccine strains
e) a demonstration of completeness of inactivation of inactivated vaccine strains
f) a demonstration of completeness of inactivation as well as reversibility to toxicity of toxoids
g) local tolerability studies, and
h) an evaluation of the potential of the vaccine antigen(s) to induce antibodies cross-reactive with human tissues, where appropriate (e.g. Streptococcal vaccine).

Where different routes of administration are proposed, multiple safety/toxicity studies should be considered in a suitable animal model addressing specific safety concerns associated with vaccine administration via each routes. Caution should be used when extrapolating safety data obtained using one route of administration to other routes.

4 POTENCY AND IMMUNOGENICITY

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 intracerebral mouse test for pertussis and rabies vaccines, and evaluations of infectious units of live attenuated organisms for viral vaccines and BCG. Ideally the potency assay should mimic the clinically expected function of the vaccine in humans (e.g. rabies vaccine). However, in many cases, this may not be possible and the assay is based on artificial challenge procedures that assess clinical protection (e.g. whole cell pertussis vaccine). For polysaccharide vaccines chemical characterization may be sufficient. For products where little is known about the pathogenic mechanism and or protective factors, animal testing with
subsequent serologic 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.

**Immunogenicity**

Immunization of animals with candidate vaccine preparations should be undertaken since the data obtained will provide valuable information to support a clinical indication. This may include testing in non-human primates but only if an appropriate disease model is available. Immunogenicity data derived from animal models can help select 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, the response to each antigen should be evaluated (e.g., acellular pertussis vaccine). 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 human disease and human immune responses. For example, the demonstration of humoral antibody responses in an animal model to a mucosal (oral or nasal) delivered vaccine may be irrelevant to the evaluation of the clinically expected secretory and cell mediated immune response.

Whilst immunogenicity testing in animals maybe necessary during vaccine development to demonstrate an ability to induce an appropriate immune response, an animal immunogenicity test may not necessarily be needed for routine lot release (e.g. *Haemophilus influenzae* type b conjugate vaccine) (16).

**5 SPECIAL CONSIDERATIONS**

**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 antigenic components present in the vaccine should be demonstrated. Where relevant, adsorption of all 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 (15, 17). 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 evaluate the safety profile of the adjuvant/vaccine combination should also be undertaken.

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

**Additives (excipients and preservatives)**

Where a new additive such as a preservative or excipient is to be used, the safety of the material needs to be investigated and documented. If a new preservative is used, it is necessary to provide documentation concerning its safety as well as efficacy or appropriateness for the use for that particular product. 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 antigen/additive combination in animal models.

**Other types of products needing special considerations**

Additionally, there are some data and testing which are specific for different 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.

**Combination vaccines**

New combinations of antigens or serotypes should be studied for appropriate immunogenicity in an animal model, if available, before initiation of human clinical trials (18,19). The response to each of the antigens in the vaccine should be assessed, including the quality of response. 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 testing.

**DNA vaccines**

Special considerations concerning production and control of DNA vaccines as well as preclinical evaluation of DNA vaccines are available in WHO guidelines for assuring the quality of DNA vaccines (9).

**Recombinant vaccines**

WHO Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology are already in place and should be consulted (10).
**Synthetic Peptide Vaccines**

Detailed information concerning production and control, including preclinical safety evaluation are described in Guidelines for the production and quality control of synthetic peptide vaccines (11, 21).

**Live attenuated vaccines**

The major concern related to live attenuated vaccines is reversion to virulence, 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 is the poliomyelitis vaccine, oral (20).
B. CLINICAL EVALUATION OF VACCINES

GENERAL REMARKS

Before clinical trials start (specially phase III trials) there needs to be a sound understanding of the epidemiology of the pathogen or disease of interest in the intended study population. This requires population-based or outbreak evaluations of individuals exposed to, at high risk from, or suffering from the disease in question. Such studies define disease incidence, the proportion of infected persons who develop clinical disease, as well as risk of transmission. The understanding of the full clinical spectrum of illness, optimization of diagnostic criteria as well as definition of the high risk groups frequently defined by age, gender, ethnic or population group membership, geography, social characteristics or seasonality, is essential for accurate vaccine evaluation. Consideration should also be given to defining laboratory values (platelet counts, white blood cell counts etc) in the intended study population. Use of inappropriate laboratory values very often results in too many people failing to meet “criteria for inclusion”. Laboratory values in the protocol should reflect “normal” values in the testing population. In some developing country settings these can be quite different from those taken as “normal” in industrialized countries due to widespread concurrent infections, such as with helminths. Sero-prevalence studies should also be undertaken, where appropriate, to assess at-risk populations and to evaluate potential protective mechanisms, such as persistence of maternal antibody. This is particularly important for the evaluation of live attenuated vaccines in infants because pre-existing maternal antibody can prevent infection with attenuated vaccine strains. The determination of study population sample size as well as the duration of the trial to achieve a statistically meaningful result with respect to efficacy and safety requires a clear understanding of background disease incidence as well as an understanding of the background incidence of various adverse reactions, including those that are specific to the wild type pathogen.

All clinical trials should adhere to standards described in good clinical practice. WHO Guidelines for Good Clinical Practice is already in place for trials of pharmaceutical products and these general principles also apply to vaccine studies. However, vaccines have special aspects, which demand special consideration such as:

- Vaccines are given to healthy individuals, mostly in the paediatric population
- Vaccines are given to prevent disease, which thus limits tolerance to adverse events
- Vaccines are biological products which are highly complex substances derived from living materials, and sometimes comprising of living organisms, requiring specialized assays and testing to assure their quality and safety on a lot to lot basis.

Manufacturing consistency for vaccine lots used in the clinical trials should be demonstrated and well documented. These lots should be adequately representative for the formulation intended for marketing. Clinical data may be required to help demonstrating manufacturing consistency.
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 very careful consideration should be given to this aspect during the trial design stage. Methods used should be clearly delineated in all trial protocols. Existing effective preventive measures (e.g. bed nets for malaria, counselling for HIV) should be continued for trial participants. (1, 40).

Study population

At least the initial Phase I study is usually conducted in healthy, immunocompetent adults who are at low risk for the vaccine relevant infection or complication. Generally, in phases II and III the trial population 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. If a vaccine is intended for children or other vulnerable populations, the vaccine should be tested in small number of intended population, usually following at least one Phase I study in healthy adults, but before proceeding to a larger number of the intended population. Definative criteria for inclusion or exclusion of subjects for enrolment in the clinical trial should be established.

Inclusion and exclusion criteria for the enrolment in the trial

Specific inclusion and exclusion criteria should be defined for a trial in any phase. Subjects included in the trial should be in the required age group, with a residence within the defined study area(s) during selection, examined by the study physician and with a signed informed consent (in a case of children parental or guardian consent). Previous vaccine and antigen exposure should be recorded for all participants.

Exclusion from the trial should be made for subjects who do not meet the medical or other eligibility criteria for entry into the trial such as chronic illness with signs of cardiac or renal failure, suspected progressive neurological disease, uncontrolled epilepsy/infantile spasms or other vaccinations within one or two weeks of administration of the test vaccine, and long term treatment with antibiotics. Immune status needs to be considered in the decision for inclusion or exclusion from study participation (e.g. immunodeficiency, immunosuppression and/or prematurity), (see table 1 Intrinsic and Extrinsic Factors influencing the immune response in vaccinees). Also, criteria for exclusion from a study might include planned moving 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, exclusion should be kept to minimum.

Criteria should also be established for contraindications to administration of a subsequent dose (2nd or 3rd) of vaccine, if applicable. These might include serious reaction after the first/second dose (e.g. neurological reaction), fever equal/greater 40°C within 48 hours, generalized allergic reaction within 48 hours.
Outcome measurement

The primary endpoint should be the most relevant for the disease in the target population.

Safety

When safety is the primary endpoint 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 and predictive for the target population for which the vaccine is to be used in practice (see also Monitoring and reporting Adverse Events, page 16).

Immunogenicity

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

Efficacy

In phase II and III clinical protection outcomes may be measured. Studies using clinical endpoints of 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 low 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 protective antibody level is well established (see correlates of protection). The absence of disease protection endpoints in such studies should be justified.

Factors influencing the choice of outcome measurement

The choice of outcome measurement in a specific trial may be constrained by scientific, logistical, economic and ethical considerations. When a randomised-controlled trial using clinical endpoints is not feasible, alternative strategies need to be considered (41). The feasibility and validity of such strategies should be considered in the protocol. Evaluation of the feasibility of a serological correlate of protection should address the relationship between surrogate endpoint and clinical endpoint, bearing in mind that this may not necessary be linear or direct.

Vaccine efficacy

Vaccine efficacy is the outcome of clinical protection and/or immunological surrogate endpoints in clinical trials. The definition of clinical cases should be described in the protocol (see case definition and case ascertainment) and those without confirmation (e.g., microbiological) should be justified in the protocol.

When relevant, both clinical and serological endpoints should be studied and data presented. The formula by which vaccine efficacy is calculated should be defined and validated (see
Glossary), (32, 31).

**Vaccine Effectiveness**

Population-level effects of vaccination depend on coverage and distribution of the vaccine, as well as on its efficacy in preventing disease and preventing colonization (32). In addition to intrinsic efficacy, effectiveness depends on the heterogeneity in susceptibility, rates of exposure to infectious agents and protection conferred by the vaccination (42). Vaccine effectiveness may also be influenced by time-related changes in protection caused by intrinsic properties of the vaccine (waning of efficacy and boosting) (32, 43, 44), changes in vaccination coverage, and population characteristics (such as age distribution).

**Case detection/ case ascertainment/ case definition**

The outcome of vaccine clinical protection trials 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 the predictive value for practice of the efficacy and/or safety estimate (31).

It is essential that the case definitions for the trial endpoints be clearly defined at the outset. They should be justified and described in the study protocol, taking into consideration case definitions and methods of case detection. The protocol should substantiate and provide a full discussion of the consequences of the anticipated sensitivity and specificity of the case definition. Consistent use of defined and validated methods should be applied for the duration of the study, at all study sites.

**Case detection**

The methods for detecting cases among vaccinated and unvaccinated populations should be the same.
- 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 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 for precise estimation of efficacy. If this is not possible, other surveillance data may be used to gain additional cases and increase precision of the estimate.

In cohort studies all cases from the vaccinated and non-vaccinated groups should be included in the analysis. This practise is consistent with the philosophy of “intent-to-treat” (55). 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 studying the cluster.

Case control studies use the same case detection methods as applied in other study designs, but not all cases need be detected.
**Case ascertainment / case definition**

The case definitions should be developed, defined and clearly documented in the study protocol before an efficacy study commences. This ordinarily involves using the efficacy case definition(s) in an earlier phase of clinical development. The validity of diagnosis is most important for an adequate evaluation of 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 through laboratory methods, antigen detection and the clinical picture is necessary to support a clinical case definition.

Specific validated and sensitive methods for case ascertainment and consistent use of a reliable and valid case definition are vital to the outcome of study (56). Highly specific methods may be needed in certain cases, however, they 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 micro-organism will be performed after unblinding, or as part of pre-planned interim analysis, including where possible:

- evaluation of clustering of disease cases in the population with serological and/or microbiological confirmation
- 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

**Monitoring and reporting Adverse Events**

An adverse event is any untoward medical occurrence in a clinical trial subject administered a vaccine; it does not necessarily have a causal relationship with the vaccine/vaccination.

It is critically important, especially in vaccine trials, that Adverse Events are actively monitored and reported timely. The NRA may require the sponsor and/or the investigator to report certain types of adverse events or reactions (e.g. serious, previously unknown) to the NRA and the Independent Ethics Committee.

Investigators should report all serious adverse events (SAEs) to the sponsor immediately except for those SAEs that protocol identifies as not needing immediate reporting. They should also comply with the applicable regulatory requirements related to the reporting of unexpected serious adverse reactions to the NRA and the IEC. 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. Adverse event reporting should be part of the protocol design.

Standardized methods should be used for reporting and evaluating local and systemic adverse events to vaccination. All safety information should be recorded and the method should be described in the protocols. These should detail the methods of adverse events reporting with respect to the following (see GCP):
• who is going to report (trials, subjects, parents / guardian)
• how the reporting is planned (questionnaires, diary cards, etc.)
• duration of follow-up
• the intervals of reporting.

Adverse events to vaccination should be well documented including evaluation of injection site reactions (pain, induration, erythema) and systemic events (fever, nausea, malaise, headache, anaphylaxis), at base line and at pre-specified post-vaccination times. Any differences in safety profile related to injection site or route of administration should be recorded. In the case of children and infants, recording of reactions should be undertaken both by parents and by the study investigator/nurse in a structured manner. Parents should be contacted by the study investigator/nurse at defined intervals after vaccination to check for any residual or other reactions. Before the second/ third dose (if applicable) parents of infants/ children, or the vaccine recipient, should be asked by the study investigator/nurse about reactions to the previous dose, as well as consult the recorded data base on the individual in question.

Recording should be defined and carried out at appropriate intervals and for sufficient duration. Every effort should be made to improve adverse events reporting, such as the use of diaries and/or defining criteria for adverse events including qualitative and quantitative parameters. Prior instructions for the use of diary cards and follow-up visits or contacts by clinical study staff should be given. Temperature should be measured and the site specified. Open recording as well as recording of specified reactions or events should be provided for. All model forms to be used for monitoring (e.g., subject diaries) 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 (40). DSMBs need to be independent and preferably linked to the IEC (see GCP). If necessary, a DSMB may initiate a further study to investigate possible links with the vaccine under study whilst the trial continues. In the case of serious adverse events an Institutional Review Board should be able to unblind a study and, if necessary, stop a trial and report its findings to the appropriate National Regulatory Authority. 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 published literature. Issues that pertain to the publication of study data should be considered in the design of study protocols.

3 Statistical considerations

General principles

Statistical analysis should be based on recommendations in relevant WHO documents, where available, and other guidelines. Early phase trials are often exploratory and may lack statistical power for definitive inferences. However, if a study is aimed at providing conclusive information, e.g., the final determination of the optimal dose for use in a pivotal, phase III trial,
then such a 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:

- Procedures for randomisation and blinding should be indicated in the study protocol.
- The primary and secondary study objectives should be clearly stated.
- The protocol should be explicit regarding the outcome variables to be analysed, the null and alternative hypothesis to be tested, the significance level, and the anticipated power. The protocol should describe the statistical methods to be used for assessing each endpoint.
- For efficacy evaluation, 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 randomisation (55). The reason 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 along with appropriate significance level adjustments to be implemented.
- Statistical estimates should be presented with confidence intervals included (52).

**Trial objectives: efficacy, safety**

**Establishing efficacy**

Efficacy of a new vaccine is most convincingly demonstrated in a randomised, double-blind, placebo-controlled trial based on a clinical disease endpoint. The placebo may be an inactive product or a vaccine for a different disease indication, believed to be ineffective in preventing the disease of interest. This type of trial is called a superiority trial, for the vaccine must be sufficiently superior in efficacy to the placebo to be acceptable (see section on superiority trials below). High specificity of case definition is sought, since it is well known that low specificity has a deleterious effect on a study's ability to estimate vaccine efficacy accurately (56). The aim in 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%). Sample size for these trials depends on disease incidence rates in the study population, as well as the anticipated level of efficacy of the vaccine considered to be clinically relevant.

There are, however, situations when vaccine efficacy cannot be determined from cases of disease. For example, disease incidence in a population may have been reduced to very low levels by widespread immunization with a previously licensed vaccine. In such cases, evaluation of a new vaccine for the same disease indication is based on measures of the vaccine’s immunogenicity, when the serological parameters are known to correlate with clinical protection. 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 on non-inferiority trials below). Statistical inference of non-inferiority is based on the appropriate confidence limit excluding a pre-specified difference in immune response believed to be clinically meaningful. Sample size for establishing non-inferiority of immune response depends upon the variability in the immunogenicity measurements and the level of efficacy of the comparator vaccine.
Evaluating safety

Most vaccine trials are not aimed at testing specific hypotheses regarding adverse events. Consequently, safety assessment is typically 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 detection of a few prospectively specified serious adverse events is the primary focus of a large pre-licensure safety trial, then 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 sample size determination. Otherwise, if there are no a priori hypotheses regarding specific adverse events, so that an undetermined number of safety analyses will be performed, then adjustment for multiplicity is not customarily performed during initial inspections of the clinical trial data. Signals in the data suggesting possible vaccine-related adverse events may be investigated further for determination of potential causal association. However, the effect of multiple testing should be considered prior to final decisions 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 statistical evaluation of safety can be found in the E9 ICH document (76).

Study designs: superiority, non-inferiority, two-sided equivalence trials

Superiority trials

Superiority trials of vaccines are typically based on cases of disease. The control is either a placebo or a vaccine that is ineffective regarding the disease of interest. The purpose of these trials is to estimate the percentage reduction in the incidence rate of disease due to 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 Annex 1, Glossary). There are also a number of statistical methods for obtaining the confidence interval on vaccine efficacy (52).

Non-inferiority (one-sided equivalence) trials

A non-inferiority trial of vaccine efficacy is typically designed to show that the relative risk (or relative incidence rate, or relative hazard rate) of disease, infection, etc., with the new vaccine, 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 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 responding to the new vaccine is not less than that with the control by as much as a pre-specified quantity (often .10). For evaluation of titres, the trial may be designed to demonstrate that the ratio of the geometric mean titre/concentration of the new vaccine relative to the control is not less than some pre-specified ratio (for example, .50 or .67).

A non-inferiority trial for an adverse event can have as its comparative outcome measure either a difference or a ratio of risks. For a ratio, the trial is designed to show that the
relative risk of the adverse event with the new vaccine relative to the control is not greater than a pre-specified ratio (for example, 1.5). For a difference in rates, the trial is designed to show that the risk of the adverse event with the new vaccine is not greater than the risk with the control by as much as a pre-specified quantity.

Since 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 treatment difference is greater than the lower or upper equivalence margin. Alternatively, inference may be based on the corresponding one-sided confidence limit.

**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 lot consistency evaluation is inherently two-sided is that there would be concern if an outcome measure for one lot were either too high or too low compared to another lot. Such a finding might suggest that the two lots are not similar enough to be considered consistently manufactured. The lots are considered equivalent, or consistently manufactured, when a two-sided CI 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.

**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 small so that, if the new vaccine meets the criterion, it is clear that it will provide an acceptable level of protection from disease. Feasibility of attaining the resulting sample size may also be a factor in the choice of the criterion, since the calculated sample size can be very large when the criterion is small or the variability of the outcome measure is large.

**Sample size**

The number of subjects in a clinical trial must be sufficient to provide a reliable answer to the questions posed. The sample size in a vaccine efficacy trial should be large enough to allow precise interval estimation of efficacy. This is usually determined by the primary endpoint chosen. Generally, the sample size should be large enough 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 efficacy.

The protocol should describe sample size calculations for each primary endpoint (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.
Sample size in non-inferiority/equivalence trials

Sample size should be such that, if a new vaccine is truly non-inferior, there is a high probability that the appropriate confidence limit 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 upper and lower confidence limits 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 sample sizes than placebo-controlled superiority trials or non-inferiority trials based on immunogenicity measurements (46).

Undersized superiority trials with 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 can be found in the ICH guidance E9 (76).

Considerations underlying sample size determination in efficacy evaluations

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

Sample size considerations in immunogenicity evaluations

Immunogenicity evaluation, when part of a clinical endpoint efficacy trial, should ideally comprise a randomly selected subsample from the population initially enrolled. When immunogenicity is the only primary endpoint, it should be studied in the 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 it may be necessary to evaluate a subset of the population by additional methodologies. Aspects such as the appropriate choice of control and expected protection rates should always be taken into account.

Sample size considerations in safety evaluations

Prior to licensure, comparative studies of common adverse events (e.g., injection site reactions with DTPw) require large numbers for 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 group. However, depending on the type of vaccine, the disease indication, and the target population, enrolment of more than 5000 may be appropriate in order to provide reasonable assurance of safety pre-licensure in randomized, controlled settings. These numbers are based on a one-sided confidence interval when no events are observed. They increase if one event is observed.
Investigation of uncommon or rare events already occurring in the population requires long-term prospective population-based surveillance studies. These are often not feasible in pre-marketing trials and such data are obtained from post-marketing surveillance studies. In practice, such events are studied either in retrospective closed cohorts and/or case control studies. Valuable sources of information for such purposes are large patient-linked databases. These databases may include several hundreds of thousands of subjects for evaluation.

**Duration of study**

The impact of a particular vaccination schedule is evaluated by the primary outcome measure of the clinical trial. In principle, for all vaccine development there is a need for 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 (clinical endpoint, immunogenicity, and safety), the vaccination strategy and the novelty and/or type of the vaccine. Long term follow up may be in the total study population or in a relevant subset.

For vaccines intended for use in immunization programmes, follow-up should be at least 1 year following the last vaccination to determine serological and clinical information on persistence of protection and the need for 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 follow-up information should be obtained on as many individuals as possible enrolled in the trial until all final outcomes are recorded.

**4 ETHICAL CONSIDERATIONS**

WHO GCP guidelines, which describe the clinical standards and ethical issues to be considered in the design and conduct of vaccine trials, should be followed. Compliance with GCP 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 (23). For any study, review by an Independent Ethics Committee, functioning in accordance with GCP standards, are mandatory (24).

To assure protection of the rights of research subjects the appropriate Independent Ethics Committee has to approve the trial prior to its start. No subject may be included in a clinical trial without proper informed consent. In the case of children, informed consent should be obtained from their parent or guardian. Written informed consent should be obtained.

The specific roles and responsibilities of the Ethical Review Boards and Regulatory Authorities are country specific.

Special attention should be given to ethical considerations underlying testing 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 for only selected diseases without serious complications or long-term sequellae and where successful treatment is available. Such studies can provide valuable information regarding the pathophysiology, clinical manifestations, diagnosis, immunology, treatment response and importantly protective efficacy of vaccines.
Subjects in vaccine trials should not be exposed to unreasonable and important risks of illness or injury and measures should be in place to ensure that all are given the full benefits of scientific innovation. This also implies no harm to the benefits of existing national vaccination programmes. It is important to ensure that economically and socially deprived communities, which are often at the greatest risk of disease, are not exploited in research that will be of no benefit to them. Detailed information is available in the ethical guidance documents issued by WHO, CIOMS, UNAIDS and other bodies (24, 25, 26, 27) and these should be consulted as appropriate. Other relevant national or international requirements must be considered (such as from the US OHRP).

5 PHASE I STUDIES

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

Phase I studies should be undertaken to define acceptable safety and reactogenicity of a vaccine candidate as well as preliminary information on its immunogenicity (28). The dose and method of administration should also be assessed with respect to these parameters. Generally phase I studies are small-scale studies and the primary focus is the determination of clinical tolerance and safety.

All Phase I studies should be very carefully monitored and conducted in research environments with adequate laboratory support. These studies are usually open label studies and not randomized with placebo control groups. However, the need for controlled trials, even in phase I is recognised in order to have at least some comparison of intercurrent common non-vaccine induced adverse experiences. When possible, the concomitant use of other vaccines or therapeutic agents should be avoided in order to optimize the safety evaluations. Phase I studies might be conducted in 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. CBC, LFTs) should be undertaken to establish baseline database. A short period of evaluation in a clinical research centre or extended observation in a clinic, day care 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 require daily return visits to the clinic.

Live attenuated vaccines (viral or bacterial) can potentially cause clinically significant infections in the recipient or in contacts. Major concerns in the evaluation of a live attenuated vaccine is the possible shedding of the agent, transmission to contacts, genetic stability and reversion to a more virulent state. Therefore, they require intensive investigations in closely monitored clinical settings. Initial studies of candidate attenuated vaccines should be undertaken to evaluate in a preliminary manner 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 study may provide data to design further clinical phase studies.

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 phases 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 are, however, 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 in the target population.

Ultimately, the Phase II studies should define the optimal dose, initial schedule and safety profile of a candidate vaccine before work can proceed to phase III trials.

Phase II studies should be undertaken to evaluate multiple variables associated with the host immune response such as age, ethnicity, gender, presence of maternal or pre-existing antibodies. In future trials, genotype may need to be considered. Other factors to be evaluated for their influence on immune response include: 1) dose of vaccine, 2) sequence or interval between vaccine doses; 3) number of vaccine doses, and 4) 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 multiple studies are often required for definitive evaluations. If the answer to the scientific question under study will be final, e.g., 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 so that conclusive information can be obtained from the study.

For a live attenuated vaccine, continued specific active monitoring 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, including the degree of shedding, transmission and potential reversion characteristics, which may have been identified in the phase I studies.

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 provided by a particular immunogen thought to be relevant to protection, such as level, class, sub-class and function of specific antibodies produced, as well as appearance and duration of adequate antibody titres. Other relevant information such as neutralizing antibodies, cross-reactive antibodies, immune complex formation, cell mediated immunity and any interaction which might affect the immune system (e.g. pre-existing antibodies, concomitant administration of vaccine or drugs) should be recorded.

The percentage of responders should be defined and described based on predefined criteria for immune response (e.g., antibodies and/or cell mediated immunity). For vaccines where immunological correlates of protection are not known, the immunological profile should be studied in details.

Subjects who fulfil immunogenicity (very often seroconversion) criteria are regarded as responders (having sero-converted) and the result of an immunogenicity study includes the proportion of those responders. For the validation of an immune response, sera should be collected from all participants during the entire study period at regular, pre-defined intervals.
For certain vaccines (e.g. nasal vaccines) it should be considered whether samples from other body fluids should be collected in addition. Immunological data from Phase II should be documented, including Geometric Mean Titre (GMT), Median, Standard Deviation (SD), and the range of antibodies in pre and post-vaccination sera (47). In the case of vaccines in 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 to arbitrary (or, if known, protective) antibody levels, e.g. 0.01, 0.1 and 1 IU / ml for diphtheria and tetanus antibodies. In addition, presenting reverse cumulative distribution (RCD) curves may provide additional insight (29,48). Validated standardized assay methodologies should be utilized when available and may be found in WHO recommendations, European Pharmacopoeia or FDA documents. Each assay should be fully documented and consistent use of a validated assay is essential.

7 PHASE III STUDIES

The Phase III trials 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 such large-scale efficacy studies, that may enrol many thousands of subjects, serological data are usually collected from at least a subset of the immunised population at pre-defined intervals and from any persons classifiable as vaccine failures.

However, 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 these instances, the Phase III trials, although involving larger numbers of persons, will be confined to the evaluation of immune responses and comparison with any recognised correlates of protection. However, sometimes there will be 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. In either case, 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 type of vaccine and other relevant factors (disease incidence, immunological marker value and safety) will determine the duration of follow-up.

The ultimate acceptance of a prophylactic vaccine as a general public health measure depends upon clear and definitive evidence that the vaccine is safe and actually prevents the infectious disease in question or significantly alters the natural history of the disease.

7.1 Considerations for formal trials of protective efficacy

Vaccine efficacy is the percentage reduction in the incidence rate of disease in vaccinated compared to unvaccinated individuals. Vaccine efficacy measures direct protection (e.g. protection induced by vaccination in the vaccinated population sample).
**Trial design**

There are basically two general approaches to efficacy studies: 1) experimental studies, and 2) observational studies. The gold standard for assessment of prevention of disease/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 endpoints. Thus the design maximises 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 in designing a vaccine trial so as to maximise efficiency and to eliminate bias. Observational studies of efficacy/effectiveness are usually part of phase IV post licensure studies.

**Randomized double-blind controlled trial**

Efficacy trials should be double-blind, randomised and controlled. This is the most definitive design for evaluating efficacy, because other variables, which might affect disease risk, are controlled by prospectively randomising study groups. Double blinding is necessary to avoid bias in the assessment of endpoints. The choice and feasibility of blinded, randomized-controlled trials (RCTs) depend on the vaccination strategy and demographic and epidemiological characteristics of the study population.

There are several possible approaches:

- Prospective cohort studies for population-based vaccination strategies.
- Pre-exposure cohort studies in-groups at risk of the target infection (e.g., traveller vaccination).

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

Randomization is necessary to avoid bias in assignment 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-randomised designs such as the use of historical controls or case control studies allow detection of only larger differences. If possible, these non-randomised approaches should be avoided in trials.

The unit of randomisation is usually the individual included in the trial and it is ideally the unit of statistical analysis. In some situations, however, it may be necessary to randomise on the basis of clusters or groups, e.g., school, geographic or political region (54). It is important to adhere to adequate pre-specified randomisation procedures. Failure to do so may lead to biased results.
Every effort should be undertaken to use randomised well controlled designs for phase III trials. However, it is understood that randomized-controlled trials can be technically difficult. Decision to undertake such studies should be made on a case by case basis.

Other approaches for obtaining efficacy data

Several alternative types of studies can be considered, depending upon the incidence and epidemiology of disease, the characteristics of the population and the expected efficacy of the vaccine or prophylactic agent. However, the use of designs other than double-blind randomised-well controlled trials to provide efficacy data is only allowed when fully justified.

Possible alternative approaches may include:

- Secondary attack rate study, or household contact study (which can be randomised)
- Uncontrolled, open studies; these are used only for additional information on serological responsiveness and tolerance.
- Observational cohort studies
- Case-control studies

The secondary attack rate study is a specific type of pre-exposure cohort trial. Secondary attack rate trials usually require smaller sample sizes than other randomized-controlled trials.

This may be the method of choice in infections with a relatively high secondary attack rate in closed communities and/or susceptible populations (30, 31). The unit to which the intervention is applied may be the individual, family (household) or community (environment). The unit of randomisation will correspond with this. Randomisation of groups or clusters rather than of individuals may be preferred for several possible reasons:

- When the vaccination is due to be applied to a geographical area or community
- When it is logistically easier to administer the vaccine to groups rather than to individuals
- When the purpose of the vaccination is to reduce transmission of 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 (or clusters), the population and geographic area under investigation should all be defined in the protocol. Attack rates from different infecting pathogen is essential. The follow-up period of the subjects after contact with the index case may be short, covering at minimum 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 since the detection of the first case. Inclusion over a longer period may cause bias in favour of vaccine efficacy, since the exposure to the infecting pathogen and thus infection risk will be reduced in the vaccinated groups or clusters compared with non-vaccinated groups or clusters (32).

Supportive evidence may be obtained from observational studies, where randomised-controlled trials or secondary attack rate trials are not ethically justified or not feasible due to
low incidence of the disease, or requirement for long-term follow-up for 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 where a double-blind RCT is not ethically justified or where the clinical endpoint requires long-term follow up (e.g., hepatitis B vaccination in neonates, see observational cohort studies, page 20-21), or where the number of individuals is too large to follow up (34).

However, the absence of randomization is a major limitation (33). Where the results of these studies are the principal or only evidence of efficacy careful assessment of quality of study and strength of results is needed. The advice of persons with expertise in the conduct and evaluation of such studies is recommended. In all cases, the use of supportive studies should be justified and the relevance for the situation considered.

Case-control studies may be useful when prospective controlled trials are not feasible due to low incidence of disease (see case control studies, page 37).

**7.2 General considerations for efficacy trials**

**Size of trial**

A vaccine efficacy trial may be based on clinical endpoints, incidence of the infection (as in the case of HIV) or, if they exist, immunological correlates of protection. Efficacy trials based on clinical endpoints often require large sample sizes, possibly thousands of subjects in each arm. Large numbers of subjects are needed for precise estimation of vaccine efficacy if the incidence rate of disease in the study population is expected to be low. If disease incidence is greater, e.g., as with influenza, smaller sample sizes will often suffice. When an immunological endpoint that correlates with clinical protection is used as primary efficacy endpoint, the numbers of subjects per arm to provide a statistically adequate evaluation may be considerably smaller e.g. several hundreds per group (see correlate of protection). In the case of large trials (e.g., 10000-50000 subjects) it may take many months to recruit the subjects who then might need to be followed 2 or 3 additional years. Large field trials of this type may simulate conditions in clinical public health practice and evaluate a heterogeneous population in large numbers. However, trials of this size and duration may become logistically difficult. In all cases, the applicant should provide adequate justification of the size and duration of the trial.

**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 but allows for maintenance of blinding without the use of a true placebo. In the case that the antigen of interest is incorporated into a combination vaccine, the "control" arm may consist either of a licensed vaccine that contains all the same antigens except that in question, or again it may be a vaccine that is indicated for prevention of a completely different infectious disease(s).
A control may thus also be a vaccine (usually already marketed) indicated for a different infectious disease(s). 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 depends on an appropriate control.

- For monovalent vaccines, an inert placebo or a vaccine which protects against another disease but gives no protection against the target disease may serve as control.
- Combination vaccines involving a component for a new infectious disease indication require omission of the new component of the vaccine from 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, the control arm may include the new component, separately administered.

**Active control**

Vaccines containing a new antigen, or an established antigen with a changed formulation (e.g., liquid versus lyophilised; changed adjuvant, excipient or preservative; changed dose of antigen) or which involve a new method of administration (e.g., aerosol vs. intramuscular administration of an influenza vaccine) may be investigated in a comparative study versus an antigenically similar active control. As for the active vaccine, there must be adequate information about the control vaccine, 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, demographic, epidemiological, socio-economic and other characteristics of the population.

**Correlates of protection**

In clinical trials where prevention of disease is used as an endpoint, considerable effort should be made to establish immunological correlate of protection, in addition. Such correlates are also useful, even necessary, for the situations where the conduct of clinical trials using prevention of disease as an endpoint cannot be practically or ethically justified. Nevertheless, it needs to be recognized 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 (82). 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 the majority of a protected group (ie vaccinated) and not achieved by the majority of a susceptible group (ie unvaccinated). The level of protection correlated with that antibody level is the vaccine efficacy measured in the
Phase 3 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 3 cohort.

The individual-based correlate of protection involves the measurement of pre-immunization and at least 1 post-immunization antibody levels in all study subjects and relating this to whether they subsequently develop 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 3 cohort. An alternative approach is for those who have a defined exposure during the course of the trial, may be to measure early post exposure antibody levels before boosting has occurred.

Immune responses should always be evaluated as a part of a phase III clinical protection study with the aim of identifying immune correlates of protection. For such 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 from 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 (page 25) should be also applied in a clinical protection trial.

7.3 Duration of protection and need for booster vaccinations

Randomised controlled trials may provide early indication of likely long-term protection and the need for booster vaccination. 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. That is, in addition to the quality and dynamics of the antibody response, information should be obtained on the relative importance of antibody titre, the extent of seroconversion and the induction of memory.

When efficacy trials have been done, controlled follow-up of the entire, or a subset of, the study population, which may be into the post-licensure period, provides the best opportunity to define with confidence the serological correlate(s) of protection and the need for and timing of booster vaccination(s). In those cases where efficacy studies have not been possible, subsets of recipients may be followed over time for 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 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.

7.4 Safety evaluations in Phase III trials

Safety evaluation during clinical development 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.

Comparative data with antigenically similar active controls (vaccines used to prevent the same infectious disease) should be provided, if available. Safety issues emerging from pre-clinical testing should be specifically addressed in phase I, II and III clinical trials. These include special considerations on preclinical safety issues and environmental aspects of vaccines based on genetically modified organisms (35).

Frequent adverse events must be thoroughly investigated and special features of the product explored (e.g., clinically relevant interference with other vaccines or drugs, factors leading to differences in effect, such as age or epidemiological characteristics). Obtaining such evidence is often the most difficult task of clinical research, requiring large-scale randomised trials that employ clinical, epidemiologic, biostatistical and laboratory methods. It is important that there be a prospective definition and prioritization of adverse outcomes. The difficulty of conducting such trials is usually determined by the incidence of infection and disease and the ability to establish specific clinical or laboratory diagnosis for the disease in question. This, as well as 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 events, but not necessarily very rare (<1/10000) adverse events (66). Safety evaluation should include all subjects enrolled in the trial who receive at least one dose of vaccine, and safety surveillance should begin from the start of enrolment.

For the earlier study phases, a specific monitoring plan with a timetable and methods should be specified in the protocol for all subjects (see methodological considerations). When adequate phase I and II safety data are available, it may be acceptable in the phase III study to monitor actively 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 (hospitalisation, death).

**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. The following information about SAEs should be captured: patients study number or identification number, study identification, type of adverse event, time of onset of SAE in relation to vaccination, patient characteristics, including any underlying diseases, concomitant vaccinations or drugs, actions taken, e.g. therapy administered, course including duration, outcome and investigator’s assessment of causality. The possibility of biological plausibility and/or a causal relation 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 SIDS). Active monitoring of reported serious adverse events following the last immunization is of major importance, since serious adverse events should be evaluated with regard to a specific pattern.

Prior to licensure, both the applicant and the regulatory authority need to consider whether ADR reports raise sufficient concern that either there should be a suspension (perhaps only temporary) of product development, or whether additional clinical safety studies may be needed to confirm the relationship between the vaccine and the event, and more rigorously to establish incidence.

The duration of monitoring following an SAE depends upon the characteristics. Standards case report forms should be developed and used to record information for SAEs. Such forms should be used from phase I onwards.

However, some serious adverse events following vaccination may be too uncommon to be observed in clinical trial programmes for marketing approval (see Glossary for definition). Therefore, to obtain more precise insight into the risk-benefit balance of the vaccine, a post-marketing surveillance programme should be implemented. In addition, specific post-marketing studies are often performed.

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, dose regimen to another. The need for performing bridging studies should be considered carefully and justified in the protocol. The endpoints for clinical bridging studies are usually the relevant immune responses and clinical safety parameters.

Various methodology may be performed, depending of the purpose of the study. These are considered under the design of a clinical bridging study.

8.1 Design and extent of a clinical bridging study

Clinical bridging studies (to support comparability of a manufacturing, product composition change, or a new immunization dose, route or schedule) should ordinarily be randomized-controlled trials. At minimum, these studies should have adequate power to establish comparability of the relevant immune responses (see “non-inferiority”, page 19) and to evaluate 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 randomised. However, for the validity of the outcomes it is important to minimise relevant confounding variables. The vaccine composition and manufacturing procedure should be close as possible for the groups, even using the same lot 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. They 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 physico-chemical 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 in support of different schedules should show which schedule is the more protective, given the incidence of the disease to be prevented (36).

Controlled bridging trials using clinical endpoints are necessary when:

- A change in manufacturing process or site has happened and extrapolation of pharmaceutical and preclinical efficacy and safety of the original product to the new product cannot be made, and a serological correlate for protection is not established.

In the target region:

- 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 randomised controlled trials carried out elsewhere
- the vaccine is antigenically new in the region of the target population

To minimize confounding assay-related factors, 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 randomised or 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. Through this blinding any identification, that distinguishes a separate group and sequential testing by group may be avoided.

8.2. Different circumstances where bridging studies could be required

The situations where the need for such studies can arise are discussed below.

Bridging studies for manufacturing change

Changes made to the product composition (adjuvants, 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 change in the production of a vaccine places responsibility on the manufacturer to show that the product is equivalent to that used in preclinical or earlier clinical testing.

Such changes should be evaluated on a case by case basis to determine what supporting data are needed to show comparability of the “new” product to the previous version. An additional clinical study that compares the new to the previous version may or may not be required.

Bridging studies for new dosing schedules

A concern regarding comparability also exists when changes have been made in the immunization schedule, dose and/or route of administration (e.g., subcutaneous to intramuscular). In most cases, these changes should be supported by a clinical bridging
study. 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.

**Bridging studies for new population**

In vaccine development there are many situations 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 endpoints.

Population bridging addresses the concern that the safety and/or efficacy profiles of a vaccine in a particular target population may differ from those observed in the original efficacy trial population. Efficacy may be addressed by showing that the relevant vaccine-elicited immune response in the new population is similar to that in the original efficacy trial population. Thus, retaining sera or other relevant samples from the original efficacy trial for such comparisons is important, and this 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 they do not unnecessarily duplicate clinical studies or delay the supply of important vaccines to populations requiring them for their benefit. Ethnic factors may be genetic, physiological (intrinsic) or epidemiological, cultural and environmental (extrinsic). Cultural characteristics include the nature of the health infrastructure and available resources (57).

**Bridging studies for safety**

A bridging safety study 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 address 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/are:
  - an index case (the individual in whom the event was first reported) or cases of a serious adverse event in foreign clinical data
  - differences in reporting adverse events elsewhere
  - insufficient safety data in the target population from an efficacy bridging study
  - the safety profile cannot be extrapolated from foreign data to the target population
  - immunization schedules and/or antigenic doses differ from those used in foreign trials
POST LICENSURE STUDIES AND SURVEILLANCE

Following licensure, when a vaccine is in use, monitoring of its efficacy, safety and quality is referred to as Post-Marketing Surveillance (PMS) or Post-Marketing studies (phase IV studies). The purpose of post-marketing surveillance and studies is to monitor the performance of a vaccine in the large target population under conditions of routine use. The objectives are to detect adverse reactions and to monitor efficacy/effectiveness. More accurate estimates of adverse events and effectiveness can be obtained by active surveillance and phase IV studies using carefully designed surveys. Resource constraints usually limit these to a subgroup of the population, although for rare diseases it may be necessary to survey the entire population for statistically valid data.

Postmarketing studies are preplanned in study protocols, and are referred to as Phase IV studies. Although occasionally these studies may use designs as used in prelicensure trials, in most cases phase IV studies are set up as observational cohort or case control studies. Whereas phase I-III studies make every attempt to standardize subjects, immunizations, evaluations and laboratory studies, it is usually impossible and not the intent to do so in phase IV studies.

Post marketing surveillance and studies may be conducted to investigate:

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

To ensure an adequate postmarketing surveillance Marketing Authorization Holders (MAHs) 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. Outcomes of surveillance (effectiveness, adverse events and quality) should be reported to these 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 be defined before initiating a post- marketing surveillance programme, without exception. The design and/or planning of a PMS programme requires assessment of:

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

Ideally, a Post-Marketing programme is based on criteria for quality, safety and efficacy set for a particular vaccine for marketing approval. The essential standards for these should always be defined. To ensure that an intervention is conducted to acceptable standard, to identify areas where special attention is required, and to ascertain (in cases of vaccine or
programme failure) possible reasons for vaccine failure, each step should be carefully monitored and described in protocols. An important application of PMS is in the early stages of use of a novel vaccine, or in changing circumstances, such as emergence of new antigenic variants of a pathogen, when doubts are raised about the continuing efficacy of the current formulation.

Safety evaluation

Post-marketing surveillance may be the only means of detecting long-term or acute events that occur too infrequently to have been revealed in clinical trials. Under specific circumstances an active post-marketing surveillance of 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 be seen in the smaller Phase II/III studies because of limitation in statistical power. Rare events are often idiosyncratic. A causal relationship is difficult to establish and usually can not be done prior to licensure.

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

Targeted studies of a specific adverse event are usually case control or retrospective exposure cohorts linked to historical controls (37). In retrospective exposure cohorts the event of interest can be studied in a controlled setting using sampled historical data identified prospectively. Post-marketing surveillance safety evaluation should include information from all possible sources. Databases linked to large patient cohorts are a valuable source for investigating serious adverse events (38). Post-marketing surveillance safety data gathering may be set as a condition for marketing approval, as a structured, planned post-marketing surveillance study.

Vaccine effectiveness evaluation

Following efficacy evaluation in a randomised controlled Phase III clinical trial, effectiveness of a new vaccine in routine practice should be determined (39). Vaccine effectiveness measures direct and indirect protection (e.g. protection to non-vaccinated persons by the vaccinated population-herd immunity). Vaccine effectiveness is affected by number of factors, including:

- vaccination coverage of subpopulation,
- immune status of the population
- correlation of strains used in vaccine production with circulating strains and
- selection of strains not included in the vaccine following introduction of the vaccine in that population.
• If done consistently over a prolonged period, post-marketing surveillance makes possible longitudinal assessment of efficacy under a range of conditions, and it may disclose variations in vaccine quality. Duration of follow-up of subjects in the post-marketing programme should be described in a protocol. Implementation of an immunization programme in a certain population may necessitate the development of a structured post-marketing serosurveillance as appropriate 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 assessment of why disease is still occurring
- whether new immunization strategies are necessary
- possible harm caused by replacement disease following the intervention (e.g., other serotypes replacing serotypes in the vaccine).

A protocol for serosurveillance should be presented at the time of marketing authorization or implementation in 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).

**Study design**

Several study designs could be considered.

**Observational cohort studies**

Evaluation of the benefit of a community-based immunization programme requires large-scale surveillance. An observational cohort study, concentrated on 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 using a select data collection may minimise bias. In some cases randomisation from phase III trials may be continued concurrently.

Observational cohort studies may require community-wide sampling. Sample size will depend upon the characteristics of the intervention applied, such as risk group interventions, community intervention and traveller immunization.

**Case-control studies**

Case-control studies should be considered in diseases of low incidence or when studying adverse events to vaccines when they can be particularly useful (50).

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 page 29). The advantage of Case Control studies is that they can be small-scale and the follow-up period is short.
The main limitations are the potential for selection bias and other bias due to lack of randomisation 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.

**Stepped wedge design**

The stepped wedge design should be considered when previous studies have indicated that the intervention is likely to be beneficial (40). The public health need to introduce the intervention precludes withholding it from a population. The intervention is introduced by phases, group-by-group, until the entire target population is covered. The groups form the unit of randomisation.

**Outbreak interventions**

At the start of an outbreak (or epidemic), equal susceptibility of the individuals in the target population to the infecting pathogen is assumed. The methodological approach chosen to study effectiveness of the intervention should be based on 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 low-incidence disease or in small isolated outbreaks.
- Community-based cohort studies are unsuitable for short-term evaluation; however, they may be useful for post-hoc evaluation of performance of a vaccination programme or long-term follow-up of specific clinical outcomes or safety questions.

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 triggering the investigation, vaccination coverage in the community, and the extent of clustering of vaccination failures in the population.

**Monitoring of post marketing surveillance**

A post-marketing oversight policy should be established by a National Regulatory Authority for control of product release, periodic inspections, reporting mechanisms, recall of batches or, should it be necessary, for revoking marketing approvals, approval of manufacturing changes and evaluation and approval of new indications and/or dose regimens.

- General considerations for continued oversight of vaccines after licensure are described in WHO Technical Report Series 858, and should be followed (4).
- Guidance on the operation of a monitoring system for adverse events is provided in CIOMS reports. Standards for causality assessment are described in these and other regulatory documents. Targeted monitoring and special studies may be required for certain adverse events (38).
- Monitoring vaccines for use in the Expanded Programme of Immunization (EPI) should not only include efficacy and safety but also compatibility with current EPI antigens (51). Ideally, this should be considered prior to marketing approval. In addition, the
immunization programme and vaccine supply should be considered.

10 SPECIAL CONSIDERATIONS FOR COMBINATION VACCINES

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

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 simultaneous, but separate, administration of the individual vaccines.

Efficacy studies

When serological correlates of protection are 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 that, prospective controlled clinical studies or alternative approaches such as post-marketing surveillance are required.

Combination vaccine studies are usually designed and analysed (for efficacy or immunogenicity) as non-inferiority trials, the aim being to demonstrate that the combination is compared with the individual components. Each of the individual components needs to add materially to the prophylactic effect of the vaccine (53, 46).

Clinical studies should:

- have sufficient power to rule out pre-existing differences in response parameters between the study groups.
- take into account sample size considerations, as for monovalent vaccines (see methodological considerations).
- 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 multiple diseases (multidisease combination vaccine) 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 or simultaneous administration of the individual vaccines at different sites, it should be demonstrated that these findings are not clinically relevant. Any change in dose or dose scheme for individual components should be
justified.

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

The study should have sufficient power to make possible meaningful separate analysis of the prevalent strains or serotypes identified as being of major significance to public health in the target area. The appropriateness of the limited coverage provided by the individual vaccine components in the target population should be justified. The feasibility of extrapolation from limited numbers of strains or serotypes to other strains or serotypes should be substantiated.

Safety analysis of combination vaccines

For safety evaluation of combination vaccines, as much information as possible should be obtained from randomised, controlled trials. Such studies are usually designed and analyzed as non-inferiority trials the aim being to demonstrate that the combination is not inferior regarding safety compared with the individual components. Where applicable, controls should be the already marketed vaccines with the same antigen composition, if applicable. The size of 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 valid comparisons and accurate determination of rates of events causally related to vaccination. If blinding of a study is not feasible, the methods used to minimise bias should be described.

Dose reductions of some or all components of a combination vaccine necessitated by the volume of the combination of components being too large for safe administration; require demonstration of both efficacy and safety of the new formulation.

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 separate administration of the (new) vaccine component(s) at different times.
ANNEX 1

GLOSSARY

Terminology used within the context of the WHO guidelines on clinical evaluation of vaccines:

**Adverse event** Any untoward medical occurrence in a clinical trial subject administered a vaccine; 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 a subsequent clinical use, following licensure.

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

**Audit** A systematic examination, carried out independently of those 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 or more parties to the trial are kept unaware of the treatment assignment(s). Single blinding usually refers to the subject(s) being unaware, and double blinding usually 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** are studies intended to support the extrapolation of efficacy, safety, and immunogenicity data from one formulation, population, 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 this exposure is compared between individuals who experience an event (the disease, in vaccine studies), the cases, and individuals who do not, the controls.

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

**Case report forms** A document that is used to record data on a clinical trial subject during the course of the clinical 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** The occurrence of an unusual number of cases in person, place or time.

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

**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** Population based trial in predefined large segments of the population to investigate the impact of a treatment on a preventable infectious disease.

**Community surveillance** Surveillance where the starting point is a health event occurring in the community and reported by a community worker or actively sought by the investigators. This may be particularly useful during an outbreak and where syndromic case definition can be used.

**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 a case in a way that is considered as having caused significant exposure and therefore risking 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 with the primary objective of showing that the response to two or more treatments differs by an amount which 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** Study in which the conditions are under direct control of the investigator. Such studies may include randomisation of subjects to treatment or control groups and blinding of subject and investigator to the placement status.

**Exposure** Someone who has met with an infectious agent in a way that we from experience know may cause disease.

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

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

**Good Clinical Practice (GCP)** A standard for clinical studies which encompasses the design, conduct, monitoring, terminations, audit, analyses, reporting and documentation of the studies and which ensures that the studies 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 (GMP)** That part of the pharmaceutical quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and a required by the marketing authorization. In these guidelines, GMP refers to the current GMP guidelines published by WHO.

**Immunogenicity** 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 willingness to participate in a particular trial, and the documentation thereof. This consent should be sought after appropriate information has been given 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, 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 participating 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 credentials. Decisions relating to and the provision of, medical or dental care 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 in an individuals usual daily activity. Minimal risk would include 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 investigational product is not clinically inferior to a comparative agent.

**Observational studies** Observational studies focus on events, exposures and diseases occurring in the population during the course of routine living conditions, 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 monovalent vaccine studies this may imply an inert placebo (e.g., saline solution, vehicle of the
vaccine), or an antigenically different vaccine. In combined vaccines, this may imply a control arm in which the test vaccine is lacking.

**Post Marketing Surveillance (PMS)** is a system intended to monitor adverse events following licensure. Post-marketing surveillance can be passive or active. The objective of post-marketing surveillance include, but are not limited to the following:

i) to identify rare adverse reactions not detected during pre-licensure studies; and
ii) to identify 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** 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 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 organisation, including statistical considerations, and the conditions under which it is to be performed and managed. The protocol should be dated and signed by the investigator, the institution involved and the sponsor. It can also serve as a contract.

**Randomisation** In its simplest form, randomisation 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 randomisation avoids systematic bias in the assignment of treatment. It also promotes balance with respect to known and unknown prognostics 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 randomisation 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** Events that are considered to have occurred in causal relationship to the vaccination. These reactions may be either local or systemic.

**Reproductive rate** The average number of secondary cases of an infection arising from one single primary case. The measure is inherent to the potential (infectiousness, susceptibility, measures of protection) of a micro-organism 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 observational or experimental. The unit of randomisation may be the individual, a household or a cluster.
Sensitivity (statistical) The probability that a test turns out positive when it is used on an individual who truly has the disease. It is estimated in a study as the proportion of individuals classified as diseases by a “gold standard” who test positive for the disease.

Serious adverse event 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.

Seroconversion Predefined increase in antibody concentration, considered to correlate with the transition of seronegative to seropositive, providing information on the immunogenicity of a vaccine. If there are pre-existing antibodies, seroconversion is defined by a transition from a clinical unprotected to a protected state.

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 sub-population.

Specificity (statistical) The probability that a test turns out negative when it is used on an individual who truly does not have the disease. It is estimated in a study as the proportion on individuals classified as disease-free by a “golden-standard” that test negative for the disease.

Sponsor An individual, a company, an institution or an organisation, which 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 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 investigational product is superior to a comparative agent (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 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 and in a defined time period. Unlike surveillance it is not ongoing though it may be repeated. 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:

\[ VE = \left( 1 - \frac{I_v}{I_u} \right) \times 100\% \]

where:
- \( I_u \) = incidence in unvaccinated population
- \( I_v \) = incidence in vaccinated population

Vaccine efficacy = (1 - relative risk) x 100%
RR=relative risk (in case control studies or other studies when the incidence of target disease or adverse event is low, to be replaced by odds ratio’s (OR)).

**Vaccine effectiveness** The protection rate conferred by vaccination in a certain population. Vaccine effectiveness measures direct and indirect protection (i.e. protection to non-vaccinated persons by the vaccinated population). Vaccine effectiveness is also determined by vaccination coverage, correlation of vaccine strains with circulating strains and selection of 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 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 vector is an animal, most often an animal or arthropod, which picks up the pathogen from an infected person(s) or animal and transmit it to a susceptible.

**Annex 2**

**Summary protocol for vaccine evaluations**

1. Title and summary
2. Study sites – brief description of the site(s)
3. Investigators
4. Background and rationale
5. Preclinical and laboratory evaluation of vaccines
6. Summary of product characteristics (details of production and control of candidate vaccine)
7. Primary and secondary objectives
8. Study design
   - hypothesis
   - endpoints
   - study plan
   - trial size
   - duration of study
9. Study population
   - inclusion and exclusion criteria
10. Methods and procedures
    - recruitment
    - handling and allocation
    - vaccine delivery
    - follow-up
    - laboratory methods
    - statistical plan and analyses
11. Monitoring of the trial
    - data monitoring
    - quality assurance of data and laboratory methods
12. Timetable (Time schedule)
    - start and end of recruitment
    - end of follow-up
    - date of report
13. Ethical approval

Annex 3

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The first draft of these guidelines was prepared in October 1999 by Dr. Bettie Voordouw, Medicine Evaluation Board, Hague, The Netherlands and Dr. Mika Kawano, Scientist, Access to Technologies, World Health Organization, following the Informal Consultation held in WHO, Geneva, (9-11 June, 1999) attended by the following participants:

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