Annex 9

Guidelines on clinical evaluation of vaccines: regulatory expectations

Replacement of Annex 1 of WHO Technical Report Series, No. 924

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Guidelines published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these WHO Guidelines are made only on condition that such modifications ensure that a vaccine is at least as safe and efficacious as one evaluated in accordance with the guidance set out below.

Abbreviations

AE	adverse event
AEFI	adverse event following immunization
AESI	adverse event of special interest
AR	attack rate
ARU	attack rate in unvaccinated (control group)
ARV	attack rate in vaccinated group
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
GCP	good clinical practice
GMC	geometric mean concentration
GMP	good manufacturing practice
GMT	geometric mean titre
HPV	human papillomavirus
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP	immune correlate of protection
IgG	immunoglobulin G
LLOD	lower limit of detection
LLOQ	lower limit of quantification
NRA	national regulatory authority
OPA	opsonophagocytic antibody
RNA	ribonucleic acid
RR	relative risk
SAE	serious adverse event
SBA	serum bactericidal antibody

1. Introduction

These WHO Guidelines are intended to replace the WHO *Guidelines on clinical evaluation of vaccines: regulatory expectations*, which were adopted by the Expert Committee on Biological Standardization in 2001 (*1*). The document of 2001 provided guidance on the clinical evaluation of vaccines as well as on WHO vaccine prequalification.

Since 2001, more than 20 vaccine-specific documents (each including a section on clinical evaluation) have been adopted by the Committee. Originally intended to be read in conjunction with the 2001 document, these documents provide guidance on both oral and inactivated polio vaccines, whole cell pertussis and acellular pertussis vaccines, meningococcal conjugate vaccines for serotypes A and C, and pneumococcal conjugate vaccines, as well as on vaccines intended to prevent diseases caused by rotaviruses, dengue viruses, human papillomaviruses (HPVs) and malaria parasites.

These revised WHO Guidelines have been prepared to reflect the scientific and regulatory experience that has been gained from vaccine clinical development programmes since the adoption of the 2001 version. They are intended for use by national regulatory authorities (NRAs), companies developing and holding licences for vaccines, clinical researchers and investigators. The document takes into account the content of clinical development programmes, clinical trial designs, the interpretation of trial results and post-licensing activities.

The main content changes (modification or expansion of previous text and additional issues covered) include, but are not limited to, the following:

Immunogenicity

- general principles for comparative immunogenicity studies, including selection of the comparators, end-points and acceptance criteria for concluding non-inferiority or superiority of immune responses;
- situations in which age de-escalation studies are not necessary;
- assessment of the need for and timing of post-primary doses;
- use of different vaccines for priming and boosting;
- assessment of the ability of vaccines to elicit immune memory or to cause hyporesponsiveness;
- use of immunogenicity data to predict vaccine efficacy, with or without bridging to efficacy data;
- the derivation and uses of immune correlates of protection (ICPs);
- vaccination of pregnant women to protect them and/or their infants.

Efficacy and effectiveness

- the need for, and feasibility of, conducting vaccine efficacy studies;
- selection of appropriate control groups in different circumstances;
- comparison of new and licensed vaccines containing antigens from different numbers of types or subtypes of the same organism;
- prediction of vaccine efficacy when there is no ICP and vaccine efficacy studies are not feasible;
- preliminary and pivotal vaccine efficacy studies and their design;
- vaccines with modest efficacy and/or that provide a short duration of protection;
- extrapolation of data between geographically or genetically diverse populations;
- the role and potential value of human challenge studies;
- the role of sponsors and public health authorities in generating vaccine-effectiveness data.

Safety

- detailed consideration of the collection and analysis of safety data from clinical trials;
- consideration of size of the pre-licensure database by type of vaccine and its novelty;
- consideration of the safety database by population subgroup;
- special safety considerations by vaccine construct;
- circumstances of limited pre-licensure safety data;
- use of registries;
- issues regarding vaccine pharmacovigilance activities.

Because a separate document on the nonclinical evaluation of vaccines was established in 2003 (*2*), the corresponding section in the 2001 Guidelines has been removed. Furthermore, the structure of the document has changed, with a number of methodological considerations now incorporated into the relevant sections and subsections rather than being described in a separate section. In line with all the changes made in the document, the terminology and references have been updated.

WHO has also made available several guidelines, manuals and reports relevant to vaccine clinical development programmes. These should be consulted as appropriate, and include:

- *Guidelines for good clinical practice (GCP) for trials on pharmaceutical products* (*3*);
- *WHO good manufacturing practices for pharmaceutical products: main principles* (*4*);
- *WHO good manufacturing practices for biological products* (*5*);
- *Guidelines on nonclinical evaluation of vaccines* (*2*);
- *Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines* (*6*);
- *Guidelines on procedures and data requirements for changes to approved vaccines* (*7*);
- *Guidelines for independent lot release of vaccines by regulatory authorities* (*8*);
- *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks* (*9*);
- *Clinical considerations for evaluation of vaccines for prequalification* (*10*);
- *The WHO manual Immunization in practice: a practical guide for health staff* (*11*);
- *Expert consultation on the use of placebos in vaccine trials* (*12*).

Furthermore, guidance on various aspects of pre-licensure clinical development programmes for vaccines and on post-licensure assessment is also available from several other bodies, such as the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), the European Medicines Agency (EMA), the United States Food and Drug Administration and the United Kingdom Medical Research Council. These WHO Guidelines are intended to complement these other documents.

2. Purpose and scope

These WHO Guidelines consider clinical development programmes for vaccines that are intended to prevent clinical disease in humans by eliciting protective immune responses. The protective immune response to vaccination may be directed against one or more specific antigenic components of microorganisms or against substances produced and secreted by them (for example, toxins) that are responsible for clinical disease. The clinical disease prevented by vaccination

may be an acute infectious disease and/or a disease that results from chronic infection with an infectious agent.

These Guidelines are applicable to the clinical development of:

- new candidate vaccines;
- licensed vaccines;
- vaccines that are given by any route of administration;
- vaccines that may be given before exposure or shortly after known or presumed exposure to an infectious agent to prevent the onset of clinical disease.

The Guidelines are further applicable to vaccines that contain one or more of the following:

- microorganisms that have been inactivated by chemical and/or physical means;
- live microorganisms that are not virulent in humans as a result of attenuation processes or specific genetic modification;
- antigenic substances that have been derived from microorganisms (these may be purified from microorganisms and used in their natural state, or they may be modified, for example, detoxified by chemical or physical means, aggregated or polymerized);
- antigens that have been manufactured by synthetic processes or produced by live organisms using recombinant RNA or DNA technology;
- antigens (however manufactured) that have been chemically conjugated to a carrier molecule to modify the interaction of the antigen with the host immune system;
- antigens that are expressed by another microorganism which itself does not cause clinical disease but acts as a live vector (for example, live viral vectored vaccines and live-attenuated chimeric vaccines).

In addition, although naked DNA vaccines are not specifically discussed, the principles and development programmes outlined are broadly applicable.

These Guidelines do not apply to:

- therapeutic vaccines (that is, those intended for treatment of disease);
- vaccines intended for any purpose other than the prevention of clinical disease caused by infectious agents.

3. Terminology

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

Adverse event (AE): any untoward medical occurrence in a participant in a clinical trial. An AE does not necessarily have a causal relationship with the vaccine.

Adverse event following immunization (AEFI): any untoward medical occurrence that follows immunization and which does not necessarily have a causal relationship with the use of the vaccine. The AEFI may be any unfavourable or unintended sign, abnormal laboratory finding, symptom or disease. In clinical trial documentation AEFI may often be shortened to AE.

Adverse event of special interest (AESI): a clinically important untoward medical occurrence that is either known to occur following administration of the type of vaccine under study (for example, hypotonic-hyporesponsive episodes or febrile convulsions) or is considered to be a possible risk on the basis of knowledge of the content of the vaccine and/or its interaction with the host immune system (for example, autoimmune disease or antibody-dependent enhanced clinical disease).

Attack rate (AR): the proportion of the population exposed to an infectious agent that goes on to develop clinically manifest disease.

Blinding: a procedure by which one or more parties involved in a clinical trial are kept unaware of the randomized intervention.

Booster dose: a dose that is given at a certain interval after completion of the primary series that is intended to boost immunity to, and therefore prolong protection against, the disease that is to be prevented.

Case ascertainment: the method adopted for detecting cases of the disease targeted for prevention by vaccination in a vaccine efficacy trial or in a study of vaccine effectiveness.

Case definition: the predefined clinical and/or laboratory criteria that must be fulfilled to confirm a case of a clinically manifest disease in a vaccine efficacy trial or in a study of vaccine effectiveness.

Cluster randomization: randomization of subjects by group (for example, by household or by community) as opposed to randomization of individual subjects within a clinical trial.

Geometric mean concentration (GMC): the average antibody concentration for a group of subjects calculated by multiplying all values and taking the nth root of this number, where n is the number of subjects with available data.

Geometric mean titre (GMT): the average antibody titre for a group of subjects calculated by multiplying all values and taking the nth root of this number, where n is the number of subjects with available data.

Good clinical practice (GCP): GCP is a process that incorporates established ethical and scientific quality standards for the design, conduct, recording and reporting of clinical research that involves the participation of human subjects. Compliance with GCP provides public assurance that the rights, safety and well-being of research subjects are protected and respected, consistent with the principles enunciated in the Declaration of Helsinki and other internationally recognized ethical guidelines, and also ensures the integrity of clinical research data.

Good manufacturing practice (GMP): GMP is the aspect of quality assurance that ensures that medicinal products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the product specification.

Immune correlate of protection (ICP): an ICP is most commonly defined as a type and amount of immunological response that correlates with vaccine-induced protection against an infectious disease and that is considered predictive of clinical efficacy (*13*).

Immune memory: an immunological phenomenon in which the primary contact between the host immune system and an antigen results in a T-cell-dependent immune response, often referred to as priming of the immune system. Effective priming results in the development of antigen-specific memory B-cells and an anamnestic (memory) immune response to post-primary doses, which are commonly referred to as booster doses.

Immunogenicity: the capacity of a vaccine to elicit a measurable immune response.

New candidate vaccine: a new candidate vaccine is a vaccine that is regarded in national regulations as separate and distinct from other candidate and licensed vaccines. Examples of new candidate vaccines include but are not limited to:

- a vaccine that contains a new antigenic component (that is, one not previously used in a licensed vaccine);
- a vaccine that contains a new adjuvant;
- a vaccine that contains antigen(s) ± adjuvant(s) not previously combined together in a vaccine;
- a vaccine with the same antigenic component(s) ± adjuvant as a licensed vaccine that is produced by a different manufacturer (including situations in which seed lots or bulk antigenic components used to make a licensed vaccine are supplied to other manufacturers for their own vaccine production).

Non-inferiority trial: non-inferiority trials aim to demonstrate that the test intervention is not worse than the reference intervention by more than

a small pre-specified amount known as the non-inferiority margin. In non-inferiority trials it is assumed that the reference intervention has been established to have a significant clinical effect (against placebo).

Pharmacovigilance: pharmacovigilance encompasses the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems (*14*).

Pivotal trials: pivotal clinical trials provide the major evidence in support of licensure.

Posology: the vaccine posology for a specific route of administration and target population includes:

- the dose content and volume delivered per dose;
- the dose regimen (that is, the number of doses to be given in the primary series and, if applicable, after the primary series);
- the dose schedule (that is, the dose intervals to be adhered to within the primary series and between the primary series and any further doses).

Post-licensure safety surveillance: a system for monitoring AEFIs in the post-licensure period.

Post-primary doses: additional doses of vaccine given after a time interval following the primary series of vaccination.

Preliminary trial: a clinical trial that is not intended to serve as a pivotal trial. Preliminary trials are usually conducted to obtain information on the safety and immunogenicity of candidate vaccine formulations and to select the formulation(s) and regimen(s) for evaluation in pivotal trials. Preliminary trials may also serve to inform the design of pivotal trials (for example, by identifying the most appropriate populations and end-points for further study). On occasion, a preliminary trial may provide an initial evaluation of vaccine efficacy.

Primary vaccination: the first vaccination or the initial series of vaccinations intended to establish clinical protection.

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

Randomization: in its simplest form, randomization is a process by which n individuals are assigned to test (n_T) or control (n_C) treatment(s) so that all possible groups of size $n = n_T + n_C$ have equal probability of occurring. Thus, randomization avoids systematic bias in the assignment of treatment.

Responder: a trial subject who develops an immune response (humoral or cellular) that meets or exceeds a predefined threshold value using a specific

assay. This term may be applied whether or not there is an established ICP and when the clinical relevance of achieving or exceeding the predefined response is unknown.

Responder rate: the responder rate is the percentage of subjects in a treatment group with immune responses that meet (or exceed) a predefined immune response.

Serious adverse event (SAE): an AE is serious when it results in: (a) death, admission to hospital, prolongation of a hospital stay, persistent or significant disability or incapacity; (b) is otherwise life-threatening; or (c) results in a congenital abnormality or birth defect. Some NRAs may have additional or alternative criteria for defining SAEs.

Seroconversion: a predefined increase in serum antibody concentration or titre. In subjects with no detectable antibody – below the lower limit of detection (LLOD) – or no quantifiable antibody – below the lower limit of quantification (LLOQ) – prior to vaccination, seroconversion is usually defined as achieving a quantifiable antibody level post-vaccination. In subjects with quantifiable antibody prior to vaccination, seroconversion is commonly defined by a predefined fold-increase from pre- to post-vaccination.

Sponsor: the individual, company, institution or organization that takes responsibility for the initiation, management and conduct of a clinical trial. The sponsor of a clinical trial may not be the entity that applies for a licence to place the same product on the market or the entity that holds the licence (that is, is responsible for post-licensing safety reporting) in any one jurisdiction.

Superiority trial: a trial with the primary objective of demonstrating that a test group is superior to a reference group on the basis of the primary end-point. In the context of vaccine development the primary end-point may be a safety parameter (for example, occurrence of a specific type of AE), a clinical condition (for example, occurrence of a specific infectious disease) or an immunological parameter (for example, a measure of the immune response to one or more antigenic components of the vaccine).

Vaccine efficacy: vaccine efficacy measures direct protection (that is, protection induced by vaccination in the vaccinated population sample). Vaccine efficacy is most commonly a measure of the proportionate reduction in disease attack rate (AR) between the control group that did not receive vaccination against the infectious disease under study (ARU) and the vaccinated (ARV) group(s). Vaccine efficacy can be calculated from the relative risk (RR) of disease among the vaccinated group as (ARU − ARV/ARU) × 100 and (1 − RR) × 100. This estimate may be referred to as absolute vaccine efficacy. Alternatively, vaccine efficacy may be defined as a measure of the proportionate reduction in disease AR between a control group that is vaccinated against the infectious disease under study and the group vaccinated with the candidate vaccine. This estimate may be referred to as relative vaccine efficacy.

Vaccine effectiveness: vaccine effectiveness is an estimate of the protection conferred by vaccination. It is usually obtained by monitoring the disease to be prevented by the vaccine during routine use in a specific population. Vaccine effectiveness measures both direct and indirect protection (that is, the estimate may in part reflect protection of unvaccinated people secondary to the effect of use of the vaccine in the vaccinated population).

Vaccine vector: a vaccine vector is a genetically engineered microorganism (which may be replication competent or incompetent) that expresses one or more foreign antigen(s) (for example, antigens derived from a different microorganism).

4. Vaccine clinical development programmes

4.1 General considerations

4.1.1 Consultation with national regulatory authorities

It is strongly recommended that dialogue with the appropriate NRAs occurs at regular intervals during the pre-licensure clinical development programme to allow for agreement to be reached on the content and extent of the application dossier. This is especially important in the following cases:

- The clinical programme proposes a novel approach to any aspect of development for which there is no precedent or guidance available.
- The proposed programme conflicts with existing guidance to which the NRAs involved would usually refer when considering programme suitability.
- Particular difficulties are foreseen in providing evidence to support an expectation of vaccine efficacy (that is, there is no ICP and a vaccine efficacy study is not feasible).
- There are other special considerations for the total content of the pre-licensure programme (for example, when different vaccine constructs are to be used for priming and boosting).

Appropriate NRAs should also be consulted when planning clinical trials that are intended to support a revision of the prescribing information. In addition, changes to the manufacturing process of a vaccine before or after licensure should be discussed with NRAs to establish whether or not clinical trials are required. When issues of vaccine safety or effectiveness arise in the post-licensure period, consultation with NRAs is essential to determine any actions that are needed.

4.1.2 Use of independent monitoring committees

The members of an independent monitoring committee should not include persons who are employed by the sponsor of the clinical trial. The responsibilities of an independent monitoring committee may include one or more of the following:

- ongoing review of safety data;
- oversight of planned interim analyses of safety and/or efficacy, and recommending to the sponsor that a trial is terminated early in accordance with predefined stopping rules;
- determination of the eligibility of individual subjects for inclusion in the primary analysis population or other analysis population(s), as defined in the protocol;
- adjudication to determine whether cases of clinically apparent infections meet the predefined case definition for inclusion in analyses of efficacy;
- adjudication to determine whether reports of AEs meet the criteria for specified types of AEs and AESIs and/or to determine causality.

The same or different independent monitoring committees may be appointed to oversee one or more aspects of a clinical trial. Depending on their role(s), independent monitoring committees may be referred to by specific terms (for example, Data Monitoring Committee, Safety Data Monitoring Committee and Independent Data Adjudication Committee).

4.1.3 Registering and reporting clinical trials

Before any clinical trial is initiated (that is, before the first subject receives the first medical intervention in the trial) the details of the trial must be registered in a clinical trial registry so that the information is publicly available, free to access and can be searched. The registry should comply with individual NRA requirements and, as a minimum, should comply with the WHO internationally agreed standards.

The entry into the clinical trial registry site should be updated as necessary to include final enrolment numbers achieved and the date of actual study completion. A definition of study completion for this purpose should be included in the protocol. For example, this may be defined as the point in time when data analyses have been completed to address the major study objectives. If a clinical trial is terminated prematurely the entry should be updated to reflect this with a report of the numbers enrolled up to the point of termination.

The key outcomes of a clinical trial should be posted in the results section of the entry in the clinical trial registry and/or posted on a publicly available, free to access and searchable website (for example, that of the trial sponsor or principal investigator). It is recommended that posting of these results should usually occur within 12 months of completion or termination of the study, or in accordance with the relevant NRA requirements.

Depending on individual NRA requirements, some or all regulatory submissions may need to include a listing of all completed and ongoing trials conducted with the vaccine by the sponsor. It is recommended that any trials that are known to the sponsor (for example, from searching registries or from publications) that were initiated by entities other than the sponsor (for example, by a public health body, academic institution or another company that used the product as a comparator) should be included.

4.2 Pre-licensure clinical development programmes

The main objective of the pre-licensure clinical development programme is to accumulate adequate data to support licensure. The main elements of the programme are:

- to describe the interaction between the vaccine and the host immune response (see section 5 below);
- to identify safe and effective dose regimens and schedules (see sections 5 and 6);
- to estimate vaccine efficacy by directly measuring efficacy and/or to provide evidence of vaccine efficacy based on immune responses (see sections 5 and 6);
- to describe the safety profile (see section 7);
- to assess co-administration with other vaccines if this is relevant (see section 5.6.3).

Consideration of the content of pre-licensure clinical development programmes is undertaken on a product-specific basis. Requirements may differ depending on the type of vaccine, its manufacturing process, its mechanism of action, the disease to be prevented and the target population.

4.2.1 Preliminary trials

The clinical programme for new candidate vaccines usually commences with an exploration of the safety of different amounts of the antigen(s) in each dose of candidate vaccine formulations, with or without an adjuvant. It is usual that immune responses to the antigenic components are also explored. These are commonly referred to as Phase I trials. In most cases the first clinical trials

are conducted in healthy adults. It may be appropriate, if feasible, that the first trials are confined to subjects who have no history of previous exposure to the organism(s) against which the candidate vaccine is intended to protect.

Further safety and immunogenicity trials that are conducted to build on the Phase I trial results are commonly referred to as Phase II trials. In most cases these trials are conducted in subjects who are representative of the intended target population for the vaccine at the time of licensure. For vaccines intended for a broad age range it may not be necessary in all instances to apply an age de-escalation approach (for example, to move from adults to adolescents, then to children aged 6–12 years, followed by younger children, toddlers and finally infants) to sequential trials or to groups within trials. For example, if a vaccine has negligible potential benefit for older children it may be acceptable in some cases to proceed directly from trials in adults to trials in younger children, including infants and toddlers.

These trials are usually designed to provide sufficient safety and immunogenicity data to support the selection of one or more candidate formulations for evaluation in pivotal trials (that is, to select the amount(s) of antigenic component(s) and, where applicable, adjuvant in each dose).

4.2.2 Pivotal trials

Pivotal trials are intended to provide robust clinical evidence in support of licensure. They are commonly referred to as Phase III trials. There may be exceptional cases in which licensure is based on a Phase II trial that has been designed to provide robust statistical conclusions. It is usual that the investigational formulations used in pivotal trials are manufactured using validated processes and undergo lot release in the same way as intended for the commercial product.

Pivotal trials may be designed to provide an estimate of vaccine efficacy or to provide an indication of the ability of the vaccine to prevent clinical disease on the basis of immunogenicity data (see section 6.1 below). On occasion, an assessment of a specific safety aspect may be the primary (or a co-primary) objective in a pivotal trial (see section 7.2.1 below).

4.3 Post-licensure clinical evaluations

After licensure:

- It is essential to monitor vaccine safety in routine use (see section 7 below).
- Studies designed to address specific safety issues that were identified as potential concerns from pre-licensure trials may need to be conducted.

- It may be appropriate to conduct studies specifically intended to estimate vaccine effectiveness (see section 6.4 below).

Sponsors may choose to conduct additional trials that are intended to extend or to otherwise modify the use of the vaccine through revision of the prescribing information. In some jurisdictions, conducting one or more trials after licensure to address specific issues may be a formal requirement.

5. Immunogenicity

5.1 General considerations

Immunogenicity trials are conducted at all stages of pre-licensure vaccine development and additional trials may be conducted in the post-licensure period. The evaluation of immune responses relies upon the collection of adequate specimens at appropriate time intervals and the measurement of immune parameters most relevant to the vaccine.

Pre-licensure and post-licensure clinical trials commonly evaluate and compare immune responses between trial groups to address a range of objectives. In trials that are primarily intended to estimate vaccine efficacy and/or safety, assessment of the immune response is usually a secondary objective but it is important that data on immune responses are collected to support analyses of the relationship between immunogenicity and efficacy, which may lead to the identification of ICPs.

5.2 Characterization of the immune response

The appropriate range of investigations to be conducted should be discussed with NRAs. As a general rule, for vaccines that contain microorganisms and antigens that have not been used previously in human vaccines a thorough investigation of their interaction with the human immune system should usually be conducted as part of the overall clinical development programme. For microorganisms and antigens that are already in licensed vaccines, it is not usually necessary to repeat these types of investigations but consideration should be given to conducting at least some trials in certain circumstances (for example, when a new adjuvant is to be added to known antigens, a different method of attenuation is used, a different carrier protein is used for antigen conjugation or an antigen previously obtained by purification from cultures is to be manufactured using recombinant technology).

In general the clinical development programme should include a description of the magnitude of the immune response, including an assessment of functional antibody (for example, antibody that neutralizes viruses or toxins, or antibody that mediates bactericidal activity or opsonophagocytosis) if this can

be measured. Decisions on the range of additional investigations that may be appropriate should take into account what is known about the immune response resulting from natural exposure and whether or not this provides partial or complete protection and, if so, whether it is temporary or lifelong. The range of investigations chosen should also reflect the characteristics of the infecting microorganism (for example, whether there are multiple subtypes that cause human disease) and the content of the vaccine (*15*).

On a case-by-case basis, other investigations of the immune response could possibly include some of the following:

- assessment of the ability of the vaccine to elicit a T-cell-dependent primary immune response, with induction of immune memory (that is, priming of the immune system) giving rise to anamnestic responses to: (a) natural exposure following vaccination; (b) further doses of the same vaccine; and/or (c) further doses of a vaccine that contains closely related but non-identical microorganisms or antigens (that is, cross-priming);
- assessment of the specificity and cross-reactivity of the immune response;
- assessment of changes in antibody avidity with sequential doses, which may be useful when investigating priming;
- evaluation of factors that could influence the immune responses, such as the effect of maternal antibody on the infant immune response to some antigens, pre-existing immunity to the same or very similar organisms, and natural or vaccine-elicited antibody against a live viral vector.

5.3 Measuring the immune response

5.3.1 Collection of specimens

Immune responses to vaccination are routinely measured in serum (humoral immune responses) and blood (cellular immune responses). For some vaccines it may be of interest to explore immune responses in other body fluids relevant to the site at which the target microorganism infects and/or replicates (for example, in nasal washes or cervical mucus), especially if it is known or suspected that the systemic immune response does not show a strong correlation with protective efficacy for the type of vaccine under trial (for example, intranasal vaccination against influenza). Nevertheless, specimens other than sera have not to date provided data that have been pivotal in regulatory decision-making processes and have not resulted in the identification of ICPs. Therefore the rest of this section focuses on the collection of blood samples.

Pre-vaccination samples should be collected from all subjects in early preliminary immunogenicity trials, after which it may be justifiable to omit these samples or to obtain them from subsets (for example, if antibody is rarely detectable or quantifiable prior to vaccination in the target population). Pre-vaccination sampling remains essential if it is expected that the target population will have some degree of pre-existing immunity due to natural exposure and/or vaccination history, since the assessment of the immune response will need to take into account seroconversion rates and increments in geometric mean titres (GMTs) or geometric mean concentrations (GMCs) from pre- to post-vaccination. Pre-vaccination sampling is also necessary if it is known or suspected that pre-existing immune status may have an impact on the magnitude of the immune response to vaccination that is positive (for example, because pre-existing antibody reflects past priming) or negative (for example, due to maternal antibody interfering with primary vaccination with certain antigens in infants).

The timing of post-vaccination sampling should be based on what is already known about the peak immune response after the first and, if applicable, sequential doses (for example, for vaccines that elicit priming, the rise in antibody after a booster dose is usually much more rapid than the rise after earlier doses). For antigens not previously used in human vaccines, sampling times may be based on nonclinical data and then adjusted when data that are specific to the antigen(s) under trial have been generated. As information is accumulated, the number and volume of samples taken from individual subjects may be reduced to the minimum considered necessary to meet the trial objectives.

5.3.2 Immunological parameters

Immunological parameters are measures that describe the humoral immune response (for example, antibody concentrations or antibody titres, depending on the assay output) or the cell-mediated immune response (for example, percentages of sensitized T-cells). To date, immunological parameters other than those that measure the humoral immune response have not played a pivotal or major role in vaccine licensure, so the focus is usually on determination of antibody levels.

- For known microorganisms or antigens in a candidate vaccine the range of parameters to be measured in clinical trials is usually selected on the basis of prior experience and whether or not there is an established ICP.
- For microorganisms or antigens not previously included in human vaccines the selection of parameters to be measured should take into account what is known about natural immunity. For some infectious diseases the nature of the immune response to infection in animal models may also be useful for parameter selection.

5.3.2.1 Humoral immune response

The humoral immune response is assessed from the post-vaccination appearance of, or increase after vaccination in, antibody directed at specific microorganisms or antigens in the vaccine.

- If data are available, most weight is usually placed on functional antibody responses – for example, serum bactericidal antibody (SBA), toxin- or virus-neutralizing antibody or opsonophagocytic antibody (OPA). In some cases an appropriate assay for functional antibody may not be available (for example, for typhoid vaccines based on the Vi polysaccharide) or the only available assay may have low feasibility for application to large numbers of samples (for example, because it is very labour-intensive or requires high-level biocontainment facilities).
- Alternatively, or in addition to the determination of functional antibody, the immune response may be assessed by measuring total antibody – for example, total immunoglobulin G (IgG) measured by enzyme-linked immunosorbent assay (ELISA) that binds to selected antigens (or, on occasion, to specific epitopes). Only a proportion of the total antibody detected may be functional.

The following should be taken into consideration when deciding how to measure the humoral immune response:

- If a correlation has already been established between total and functional antibody responses to a specific microorganism or antigen it may be acceptable to measure only total IgG in further trials (for example, antibody to tetanus toxin). However, determination of functional immune responses might be important for specific age groups or target populations where it is known or suspected that the binding and functional capacity of the antibodies elicited differs (for example, pneumococcal conjugate vaccines in older people).
- For antigens for which there is an established ICP it may suffice to measure only the relevant functional antibody (for example, SBA for meningococcal vaccines) or total IgG (for example, for antibody to tetanus toxin) response.
- If the ICP is based on total IgG there may be instances where there is still merit in measuring functional antibody (for example, for antibody to diphtheria toxin for which a microneutralization assay is available).

- If there is no ICP the functional antibody response should be measured if this is feasible.
- Occasionally there may be more than one immunological parameter that can measure functional antibody but one is considered to be a more definitive measure than the other (for example, neutralizing antibody to influenza virus versus antibody that inhibits haemagglutination). In this case the more definitive parameter may be determined, at least in a subset.
- For some vaccines against certain viruses there is a possibility that some of the total antibody detected has no protective effect (for example, is non-neutralizing) but could enhance cellular infection by wild-type virus and result in an increased risk of severe disease after vaccination (for example, this may apply to dengue vaccines). To assess this possibility, the routine measurement of total antibody to assess the humoral immune response to vaccination should be supported by other detailed investigations.

5.3.2.2 Cell-mediated immune response

For some types of infectious disease (such as tuberculosis) assessment of the cell-mediated immune response may have a role to play in the assessment of the interaction between the vaccine and the human immune system. In other cases, evaluation of the cellular immune response may serve to support findings based on the humoral immune response (for example, when assessing the benefit of adding an adjuvant or when evaluating the degree of cross-priming elicited by a vaccine).

The cell-mediated immune response is most commonly assessed by detecting and quantifying sensitized T-cells in blood from trial subjects. These investigations may also serve to characterize the predominant cytokines released and to detect differences in sensitization between T-cell subpopulations. Several methods may be used. These are typically based on measuring the production of a range of cytokines following in vitro stimulation of T-cells with individual or pooled antigens.

The results may provide useful comparisons between treatment groups within any one study (for example, they could describe the effect, if any, of an adjuvant). If there are marked discrepancies in the patterns of responses observed between cell-mediated and humoral responses (for example, if adding an adjuvant has a major effect on antibody levels but does not increase the percentages of sensitized cells in one or more T-cell subsets) the findings should be carefully considered and discussed.

5.3.3 Assays

Assays of functional or total antibody that are used to report immune responses to vaccination (whether to the candidate vaccine or to co-administered vaccines) in trials intended to support licensure (that is, in pivotal trials) should be acceptable to the relevant NRAs. They may be:

- commercially available assays specifically designed and intended for quantification of antibody (that is, assays that have undergone a robust regulatory review);
- assays that are not commercially available but have been validated according to principles similar to those recommended for quantitative lot release assays in the ICH Q2 (R1) document *Validation of analytical procedures: text and methodology* (*16*);
- assays that are not commercially available but have been shown to be comparable to a reference assay (for example, to an assay established in a WHO reference laboratory or to an assay that is established in a recognized public health laboratory and has been used previously to support clinical trials that were pivotal for licensure).

It is expected that, if these exist, WHO International Standards and Reference Reagents will be used in assay runs. Any omission of their use should be adequately justified.

Clinical trial protocols should specify which assays will be used. Clinical trial reports should include a summary of the assay methodology and its commercial or other validation status. For assays that are not commercially available any available validation reports should be provided.

The same assays should preferably be used in the same laboratories throughout the clinical development programme (including pre- and post-licensure trials) for an individual vaccine. It is also preferable that each assay (whether it measures the response to the candidate vaccine or to a concomitant vaccine) is run by one central laboratory. If this is not possible (for example, because different laboratories have to be used, assays change over time, or a switch is made to an improved and/or more suitable assay) the new and original assays should be shown to give the same result or interpretation, or the impact of any differences should be evaluated and the use of a new assay justified. It is recommended that, as a minimum, a selection of stored sera (for example, covering a range of low to high results when using the previous assay) should be re-run using the previous and new assays in parallel. The number of sera retested should be sufficient to support a statistical assessment of assay comparability and/or reproducibility.

The microorganisms (for example, in assays of SBA, OPA and virus neutralization) and antigens (for example, in ELISAs and for in vitro stimulation of sensitized T-cells) used in the assay may affect both the result and the interpretation of the result. For example:

- It is important to use purified antigen to avoid the possibility that the assay detects and measures antibody to any extraneous antigenic substances that may be in the vaccine.
- For vaccines that contain antigens from multiple strains of the same pathogen (for example, multiple bacterial capsular types) the assays selected (whether separate or multiplex) should determine the immune response to each antigen.
- Although it is usually acceptable to conduct routine testing using the same microorganisms or antigens as those present in the vaccine, it may be very informative to perform additional testing, at least in subsets of samples, using circulating wild-type organisms or antigens derived from them in the assay. It is not expected that these additional assays will necessarily be validated since they are exploratory in nature. The results of additional testing can provide an indication as to whether the results of routine testing could represent an overestimate of the immune response to circulating strains. This additional testing can also provide an assessment of the cross-reactivity of the immune responses elicited by the vaccine to other organisms of the same genus or species (for example, to different flaviviruses, different clades of influenza virus or different HPV types) and can guide decisions on the need to replace or add strains or antigens in a vaccine to improve or maintain its protective effect.

5.4 Identification and use of immune correlates of protection

5.4.1 Immune correlates of protection and their uses

All established ICPs are based on humoral immune response parameters that measure functional or total IgG antibody. Some examples of well-established ICPs include those for antibody to diphtheria and tetanus toxoids, polioviruses, hepatitis B virus and *Haemophilus influenzae* type b capsular polysaccharide (*17*). In most cases established ICPs have been shown to correlate with prevention of clinically apparent infectious disease, but for some pathogens the ICP \ correlates with prevention of documented infection (for example, hepatitis A and hepatitis B).

Sections 5.5.2 and 5.5.3 below consider trial end-points and the approach to analysis and interpretation of immunogenicity data in the presence or absence of an ICP.

5.4.2 Establishing an ICP

Documentation of the immune response to natural infection, the duration of protection after clinically apparent infection (that is, whether natural protection is lifelong (solid immunity), temporary or absent) and the specificity of protection (that is, whether the individual is protected only against specific subtypes of a microorganism) should be taken into account when attempting to establish an ICP from clinical data. For example, to date, widely accepted clinical ICPs have been established on the basis of one or more of the following:

- serosurveillance and disease prevalence in specific populations;
- passive protection using antibody derived from immune humans or manufactured using recombinant technology;
- efficacy trials;
- effectiveness trials;
- investigation of vaccine failure in immunosuppressed populations.

In the majority of cases clinical ICPs have been determined from vaccine efficacy trials that were initiated pre-licensure, often with long-term follow-up of subjects that extended into the post-licensure period. Efficacy trial protocols should plan to collect sufficient information to allow for analyses of the relationship between immune parameters and protection against clinically apparent disease. At the minimum this requires the collection of post-vaccination samples from all, or from a substantial subset of, the vaccinated and control groups. Serial collection of samples over the longer term, along with follow-up surveillance for vaccine breakthrough cases, has also served to support identification of ICPs.

To investigate the predictive capacity of a putative ICP, protocols should predefine the assessments to be applied to all cases of the disease to be prevented that occur in the vaccinated and control groups. These assessments should include investigation of the immune status of subjects as well as microbiological studies with the infecting microorganisms whenever these have been recovered. For breakthrough cases from which both post-vaccination sera and organisms have been recovered it is recommended that, whenever feasible, functional antibody (or, if not possible, total antibody) should be determined for individuals against their own pathogen. An exploration of vaccine-elicited cell-mediated responses in individuals against their own pathogen may also be useful and, for some types of infectious disease (such as tuberculosis), may be very important for further understanding vaccine-associated protection. These data may be very important for investigating the broad applicability of the ICP, depending on host and organism factors.

A single clinical ICP identified from a vaccine efficacy trial in a defined population may not necessarily be applicable to other vaccine constructs

intended to prevent the same infectious disease. In addition, an ICP may not be applicable to other populations and disease settings. For example, putative ICPs have sometimes differed between populations of different ethnicities with variable natural exposure histories for subtypes of a single microorganism. Thus, the reliance that is placed on a clinical ICP, even if regarded as well supported by the evidence, should take into account details of the efficacy trials from which it was derived.

Clinical ICPs have also been derived from, or further supported by, data collected during use of a vaccine to control an outbreak and from analyses of effectiveness data. The methods used to derive ICPs from these types of data have been very variable. The estimates may in part reflect the type of immunization programme put in place and the extent to which the protection of individuals relies on herd immunity rather than the initial and persisting immune response in the individual. Therefore the wider applicability of ICPs derived from interventional or routine use should be viewed in the light of how and in what setting the estimates were obtained.

If it is not possible to derive a clinical ICP the interpretation of the human immune response data may take into account what is known about immunological parameters that correlate with protection in relevant animal models and any nonclinical ICPs that have been identified (for example, from trials that assess passive protection and active immunization). This approach may be the only option available for interpreting immune responses to some new candidate vaccines. Nevertheless, ICPs derived wholly from nonclinical data should be viewed with caution and attempts should be made to obtain a clinical ICP whenever the opportunity arises (for example, when the vaccine is used in the context of an outbreak).

If conducted, human challenge trials may also provide preliminary evidence supporting an ICP. If a human challenge trial suggests a correlation between a specific immunological parameter and protection, this may be further investigated during the clinical development programme.

5.5 Immunogenicity trials

5.5.1 Objectives

The objectives of immunogenicity trials include, but are not limited to, the following:

- to select vaccine formulations and posologies (including primary and booster doses) (see section 5.6.1 below);
- to compare immune responses documented in a specific population and, using one vaccine formulation and posology, to immune responses to the same vaccine when used in other settings (for example, different populations) or with alternative posologies, or to

a different vaccine intended to protect against the same infectious disease(s) (see section 5.6.2);
- to support co-administration with other vaccines (see section 5.6.3);
- to support maternal immunization (see section 5.6.4);
- to support major changes to the manufacturing process (see section 5.6.5);
- to assess lot-to-lot consistency (*7*) (see section 5.6.6).

5.5.2 General considerations for trial designs

Immunogenicity trials are almost without exception comparative trials. For candidate vaccines containing antigens for which there are well-established ICPs that can be applied to interpret the results sponsors may sometimes question the value of including a comparative arm. Nevertheless, there is great value in conducting a randomized controlled trial. For example, the inclusion of a control group that receives a licensed vaccine provides assurance of the adequacy of the trial procedures and methods, including the assays, and facilitates interpretation of data in circumstances in which unexpected results (for example, low immune response to one or more antigens, high rates of specific AEs or unexpected AEs) are observed.

Comparative trials include those in which all subjects receive the same vaccine formulation but there are differences between groups in terms of how or to whom the vaccine is administered (for example, using a different dose or dose interval, or administering the vaccine to different age groups) as well as trials in which one or more group(s) receive an alternative treatment, which may be placebo and/or another licensed vaccine.

The design of comparative immunogenicity trials is driven by the characteristics of the vaccine, the trial objectives, the stage of clinical development, the trial population, the availability and acceptability of suitable comparators, and what is known about immune parameters that correlate with protection (including whether or not there is an established ICP).

In comparative immunogenicity trials, subjects should be randomized to one of the trial groups at enrolment. This also applies to trials that enrol sequential cohorts of subjects (as in ascending dose trials in which at least some subjects are assigned to receive placebo or another vaccine). In some cases it may be appropriate that subjects who meet certain criteria (for example, completed all assigned doses in the initial part of the trial) are re-randomized at a later stage of the trial to receive a further dose of a test or control treatment.

In all comparative trials the assays should be performed by laboratory staff unaware of the treatment assignment. Whenever possible, comparative immunogenicity trials should be of double-blind design. If the vaccines to be compared are visually distinguishable it is preferable that designated individuals

at each trial site who are not otherwise involved in the trial should administer the products. If this is not feasible, or if the vaccines to be compared are given by different routes or according to different schedules, attempts should be made to maintain blinding of the trial site staff conducting the study visits and assessments.

In trials intended to provide only descriptive analyses of the immunogenicity data the trial sample size is usually based on considerations of feasibility and collection of sufficient safety data to support the design of sequential trials. Trials that aim to assess superiority or non-inferiority between vaccine groups should be sized according to the intended power and the predefined margins. It is recommended that protocols and statistical analysis plans for each trial should be developed in conjunction with an appropriately experienced statistician.

5.5.2.1 End-points

The choice of the primary trial end-point and the range of other end-points for immunogenicity trials should take into account sections 5.2, 5.3 and 5.4 above. Protocols should predefine the primary, co-primary, secondary and any other end-points (which may be designated tertiary or exploratory). Co-primary end-points may be appropriate in some cases, namely:

- The vaccine is intended to protect against multiple subtypes of the same microorganism (for example, HPV vaccines or pneumococcal conjugate vaccines).
- The vaccine contains multiple microorganisms (such as measles, mumps and rubella vaccine) or multiple antigens (such as combination vaccines used for the primary immunization series in infants).

The following should be taken into consideration when selecting the primary end-point(s) following primary vaccination:

- When an ICP has been established the primary end-point is usually the percentage of subjects that achieves an antibody level at or above the ICP, which is sometimes referred to as the seroprotection rate.
- When there is no established ICP the primary end-point or the co-primary end-points is/are usually based on a measure of the humoral immune response.
  - (a) In some instances there may be evidence to support the application of a threshold value (that is, the primary end-point may be the percentage of subjects that achieves antibody levels at or above the threshold value).

(b) If there is no threshold value that can be applied it may be appropriate to base the primary end-point on the seroconversion rate or on some other definition of the magnitude of the immune response that differentiates responders from non-responders. Comparisons of post-vaccination seropositivity rates may also be informative if pre-vaccination rates are very low.

An anamnestic (memory) immune response is anticipated following administration of a vaccine to subjects who are already primed (by natural exposure or prior vaccination) against one or more microorganisms or antigens in the vaccine. Thus the seroprotection, seroconversion (fold-rise from pre-boost to post-boost) and seropositivity rates after the booster dose are likely to be very high. In these cases, and in other situations in which post-vaccination seroprotection and/or seroconversion rates are expected to be very high (that is, the vaccine is very immunogenic) the most sensitive immunological parameter for detecting differences between groups may be the GMC or GMT.

After primary vaccination and after any additional doses the results for all measured immunological parameters should be presented in the clinical trial report.

5.5.2.2 Trials designed to demonstrate superiority

Trials may assess whether a specific candidate vaccine formulation elicits superior immune responses compared to no vaccination against the disease to be prevented. In some cases trials may also assess whether immune responses elicited by a specific formulation of a candidate vaccine are superior to those elicited by other formulations.

An assessment of superiority may also be applicable when an adjuvant is proposed for inclusion in the vaccine (for example, to demonstrate that the immune response to at least one of the antigenic components in an adjuvanted formulation is superior to the response in the absence of the adjuvant).

Protocols should predefine the magnitude of the difference between vaccine groups or between vaccine and control groups that will be regarded as evidence of superiority. This difference should be defined in such a way that it provides some evidence of a potential clinical advantage.

5.5.2.3 Trials designed to demonstrate non-inferiority

Most comparative immunogenicity trials are intended to show that the test vaccinated groups achieve comparable immune responses to the selected reference groups. If these trials are intended to be pivotal they should be designed and powered to demonstrate non-inferiority using a predefined and justifiable non-inferiority margin.

Factors to consider with regard to the stringency of the non-inferiority margin include the clinical relevance of the end-point, seriousness of the disease to be prevented, vulnerability of the target population, availability of a well-established ICP and the performance characteristics of the assay(s). A more stringent margin may be appropriate when the vaccine is intended to prevent severe or life-threatening diseases and/or will be used in particularly vulnerable populations (for example, infants and pregnant women). A more stringent margin could also be considered when there is potential for a downward drift in immunogenicity such as that which could occur when a new candidate vaccine can be compared only with vaccines that were approved on the basis of non-inferiority trials. In contrast, if a new candidate vaccine is known to offer substantial benefits in terms of safety or improved coverage then margins that are less stringent may be considered. As a result of such considerations it is possible that different non-inferiority margins may be considered appropriate in different settings.

When it is proposed to demonstrate non-inferiority between vaccine groups based on GMT or GMC ratios for antibody titres or concentrations it is suggested that the lower bound of the 95% confidence interval around the ratio (test versus reference vaccine) should not fall below 0.67. Under certain circumstances NRAs may consider allowing a lower bound (for example, 0.5) or alternative criteria. The selection of a criterion should take into account whether or not an ICP has been identified. In addition, any marked separations between the reverse cumulative distributions of antibody titres or concentrations should be discussed in terms of potential clinical implications, including those which occur at the lower or upper ends of the curves.

5.5.3 Analysis and interpretation

A statistical analysis plan should be finalized before closing the trial database and unblinding treatment assignments (if these were blinded). This should include any planned interim analyses, which should be adequately addressed in terms of purpose, timing and any statistical adjustments required.

The immunogenicity data from all subjects with at least one result for any immunological parameter measured in the trial should be included in the clinical trial report. The analysis of the immune response based on any one parameter is commonly restricted to all subjects with a pre-vaccination measurement (if this is to be obtained from all subjects) and at least one post-vaccination measurement. Protocols may also restrict the primary analysis population to subjects with pre- and post-vaccination results, or to those with post-vaccination results who received all the assigned doses within predefined windows of the intended schedule and had no other major protocol violations. Other analysis populations of interest may be predefined in accordance with the primary or secondary objectives (for example, age subgroups or pre-vaccination serostatus

subgroups). Whatever the predefined primary analysis population, all available immunogenicity data should be presented in the clinical trial report.

If a trial fails to meet the predefined criteria for superiority and/or non-inferiority with respect to any of the antigenic components, the possible reasons for the result and the clinical implications of it should be carefully considered before proceeding with clinical development or licensure. The considerations may take into account: (a) the basis for setting the predefined criteria (for example, does failure to meet the criteria strongly imply that lower efficacy may result?); (b) the comparisons made for all other immune parameters measured (for example, were criteria not met for only one or several of many antigenic components of the vaccine?); (c) any differences in composition between the test and comparator vaccines that could explain the result; (d) the severity of the disease(s) to be prevented; and (e) the overall anticipated benefits of the vaccine, including its safety profile (*17*). Section 5.6 below provides further examples and issues for consideration.

If additional analyses of the data that were not pre-specified in the protocol and/or the statistical analysis plan (that is, post hoc analyses) are conducted, they should usually be viewed with caution.

5.6 Specific uses of immunogenicity trials

5.6.1 Selection of formulation and posology

The vaccine formulation is determined by the numbers of microorganisms or amounts of antigens and, if applicable, the amount of adjuvant that is to be delivered in each dose, as well as by the route of administration.

The vaccine posology for a specific route of administration includes:

- the antigen content (as for formulation) and volume delivered per dose;
- the dose regimen (number of doses to be given in the primary series and, if applicable, after the primary series);
- the dose schedule (dose intervals within the primary series and between the primary series and any further doses).

The posology for any one vaccine may vary between target populations (for example, between age groups or according to prior vaccination history) in one or more aspects (content, regimen or schedule).

The following sections outline the immunogenicity data that are usually generated to support vaccine formulation and posology, and to assess the need for, and immune response to, additional doses of the vaccine after completion of the primary series. Section 7 below then addresses the importance of the safety profile when selecting vaccine formulations and posologies.

5.6.1.1 Selecting the formulation and posology

The vaccine formulation and posology should be supported by safety and immunogenicity data, with or without efficacy data, collected throughout the pre-licensure clinical development programme. At the time of licensure the data should at least support the formulation and posology for the primary series, which may consist of one or more doses.

Depending on the intended formulation of the new candidate vaccine, the following considerations may apply:

1. When a new candidate vaccine contains any microorganisms or antigens not previously used in human vaccines, with or without others already used in human vaccines, the preliminary trials may explore the immune responses to different amounts of each of the new microorganisms or antigens when given alone to non-immune healthy adult subjects. These trials can be used to describe the dose–response curve and may indicate a plateau for the immune responses above a certain dose level. The next trials usually evaluate immune responses to further doses at various dose intervals in order to evaluate the kinetics of the immune response and any increment in immune response achieved by further doses. The transition from trials in healthy adults to trials in subjects in the target age range at the time of licensure should occur as soon as this can be supported, taking into account the safety profile.

   However, evaluating the immune response to each of the new microorganisms or antigens alone may not be a feasible undertaking. For example, if the vaccine construct is manufactured in such a way that production of individual antigens is not feasible then evaluation of the appropriate vaccine dose may be based solely on studies with the entire construct. Another example concerns vaccines intended to protect against multiple subtypes of an organism. In this case, the use of microorganisms or antigens that could be regarded as broadly representative in the first trials may provide some idea of the likely response to other subtypes. Further trials may then explore formulations that contain increasing numbers of the subtypes with the objective of assessing the effect on the immune response of combining them into a single product.
2. For new candidate vaccines that contain known antigenic components not previously combined in a single vaccine, the preliminary trials are usually conducted in subjects within the age ranges approved for licensed vaccines that contain some or all of the same antigenic components. The aim is to demonstrate non-

inferiority of immune responses to each of the intended antigenic components when combined in a candidate formulation compared with co-administration of licensed vaccines that together provide all of the same antigenic components. The same approach applies whenever the antigenic components are not combined into a single formulation but the contents of more than one product have to be mixed immediately before administration to avoid a detrimental physicochemical interaction.

3. For new candidate vaccines that contain both known and one or more new antigenic components the preliminary trials may aim to demonstrate non-inferiority of immune responses to each of the known antigenic components when combined into a candidate formulation compared with the separate administrations of known and new antigenic components. It may also be informative to include a control group that receives co-administration of the known and new antigenic components. The exact trial design will depend upon the availability of a single licensed vaccine that contains the known antigenic components and whether more than one licensed vaccine has to be given.
4. For vaccine formulations to which an adjuvant is to be added there should be adequate data already available (known adjuvants) or data should be generated (new adjuvants or when using any adjuvant with a new antigenic component) to describe the effect of the adjuvant on the immune responses. Some, or a major part, of the evidence supporting the addition of an adjuvant may come from nonclinical studies. The addition of an adjuvant, which may or may not elicit superior immune responses to one or more antigens, should not have a potentially detrimental effect on the responses to any antigenic components. Addition of an adjuvant may allow for the use of a much lower dose of an antigenic component to achieve the desired level of immune response, and it may also broaden the immune response (for example, it may result in higher immune responses to antigens closely related to those in the vaccine). Trials should evaluate a sufficient range of combinations of antigenic components and adjuvants to support the final selected formulation (that is, the ratio of adjuvant to antigenic components).
5. The total data generated should be explored to identify the criteria that should be applied to the release and stability specifications, and to the determination of an appropriate shelf-life for the vaccine. This is usually of particular importance for vaccines that contain

live microorganisms. Depending on data already generated, it may be necessary to conduct additional trials with formulations known to contain a range of microorganism numbers or antigen doses in order to identify appropriate limits at the end of the shelf-life.

6. Comparative immunogenicity trials may be needed to determine schedules that are appropriate for specific target populations, taking into account the urgency to achieve protective immunity (that is, trials based on diseases to be prevented and their epidemiology). The data generated across all the trials should determine the minimum period that should elapse between doses, as well as the effects of delaying doses to support acceptable windows around scheduled doses. Additionally, for some vaccines it may be useful to explore the shortest time frame within which doses may be completed without a detrimental effect on the final immune response (for example, for vaccines for travellers who may need to depart at short notice or for vaccines intended to provide post-exposure prophylaxis).

   Assessment of the effects of dose interval and the total time taken to complete the primary series is a particular issue for vaccines intended for use in infants as there is a very wide range of schedules in use in different countries (for example, 3-dose schedules include 6–10–14 weeks and 2–4–6 months). In general, experience indicates that the magnitude of the post-primary series immune responses broadly correlates with the age of infants at the time of the final dose.

7. All data generated in accordance with points 1–6 above should be taken into account when selecting the final formulation and posology or posologies. The selection process is more straightforward if there are established ICPs that can be applied to the interpretation of the results for at least some of the antigenic components. In the absence of an ICP the posology may be selected on the basis of consideration of any plateau effects that are observed and on the safety profile of various doses and regimens.

   It is not unusual for the final selected formulation and posology to represent, at least to some extent, a compromise between immunogenicity and safety or, for combination vaccines, a compromise between the potential benefits of a vaccine that can protect against multiple types of infectious disease and some negative effects on immune response that may occur. These negative effects may result from a physicochemical interaction between vaccine components and/or a negative immune interference

effect of some antigenic components. Such negative effects may be accompanied by enhanced immune responses to other vaccine components. The rationale for the final selection should be carefully discussed in the application dossier.

5.6.1.2 Amending or adding posologies

Clinical trials may be considered necessary to address one or more of the following situations:

- *Change to the number of doses or dose intervals* – in this case the control group could be vaccinated using the licensed posology and the trial could be conducted in a population for which the vaccine is already licensed.
- *Use of the licensed posology in a new population* (for example, in subjects who are younger or older than the currently licensed age group, or in subjects with specific underlying conditions, such as immunosuppression) – in this case the trial could compare use of the licensed posology in the new target population with use in the population for which the vaccine is already licensed.
- *Use of an alternative to the licensed posology in a new population* – in this case the trial could compare the alternative posology administered to the new population with the licensed posology in the population for which the vaccine is already licensed.
- *Support for alternative routes of administration for the licensed formulation* (for example, adding subcutaneous or intradermal injection to intramuscular use).

Post-licensure clinical trials may also be conducted to support changes in formulation. Formulation changes other than adding or removing a preservative or removing thiomersal from the manufacturing process may or may not result in a modified product that is considered to be a new candidate vaccine from a regulatory standpoint (that is, would require a new application dossier and adequate trials to support separate licensure).

5.6.1.3 Post-primary doses

5.6.1.3.1 *Need for post-primary doses*

The need to administer additional doses, and the timing of these doses, may be determined before and/or after first licensure.

There may be experience with other similar vaccines indicating that additional doses of a new candidate vaccine will be needed after completion of the primary series (for example, after infant immunization with *H. influenzae*

type b and *Neisseria meningitidis* group C vaccines). In such cases the clinical development programme should usually incorporate an assessment of immune responses to a post-primary dose.

If it is not known whether post-primary doses of a new candidate vaccine will be needed to maintain protection, it is preferable that this should be determined from long-term follow-up of subjects who were enrolled in efficacy trials and/or from post-licensure effectiveness studies. Although the long-term monitoring of antibody persistence is important, these data alone cannot determine if another dose is needed unless there is evidence, or a strong reason to expect, that failure to maintain circulating antibody above a certain level (for example, above the ICP if there is one) is associated with a risk of breakthrough disease.

If it is unclear whether additional doses are needed it is prudent to plan to obtain data on the immune response to doses administered at different intervals after the last dose of the primary series so that such data are available should it become clear that a further dose is required.

5.6.1.3.2 *Assessment of prior priming*

It is not always necessary to assess whether or not a vaccine elicits a T-cell-dependent immune response that results in priming of the immune system and an anamnestic (memory) response to further doses. However, for some new candidate vaccines (for example, polysaccharide-protein conjugate vaccines in which the polysaccharide and/or conjugate protein have not previously been included in a licensed vaccine) there may be considerable interest in understanding the ability of the vaccine to prime the immune system.

When assessing the immune response to additional doses and determining whether or not the primary series elicited immune memory, the following should be taken into account:

- Trials in which additional doses are administered may be extension phases of primary series trials or new trials in subjects with documented vaccine histories.
- When assessing whether the primary series elicited immune memory the optimal design is to compare subjects who previously completed a full primary series of the candidate vaccine with a control group consisting of subjects not previously vaccinated. Control subjects should be matched for age and for any host or demographic factors that might have an impact on their immune response (for example, they should be resident in similar areas so that any natural exposure is likely to be similar).
- If the new candidate vaccine elicited immune memory in the primary series the immune response to the additional (that is, booster) dose

should usually be superior (on the basis of comparisons of the GMCs or GMTs of antibody) to that observed in individuals who have not been vaccinated against the disease to be prevented. The percentages that achieve seropositivity or seroprotection (as defined) may not differ between the two groups if a single dose of the vaccine is highly immunogenic even in unprimed individuals.

- The immune response to the additional dose in primed and unprimed subjects may also be differentiated on the basis of the rapidity of the rise in antibody levels (faster in primed) and in terms of antibody avidity (greater in primed). Note that not all primed individuals (whether priming results from natural exposure or from previous vaccination) have detectable humoral immunity against the relevant organism or the toxin that causes clinical disease.
- If the immune response as measured by geometric mean antibody concentrations or titres in the vaccine-primed group is not superior to that in controls this does not always mean that the primary series did not elicit immune memory. For example, the immune response in the vaccinated group may not be superior to the immune response in the control group when natural priming has occurred in a substantial proportion of subjects not previously vaccinated against the disease to be prevented – in which case the rapidity of response and measurements of avidity may also not be distinguishable between groups. If natural priming has occurred it may or may not be detectable from pre-vaccination antibody levels in the control group.
- If an immune memory response is elicited in the primary series it may be possible to achieve a robust anamnestic response using a much lower dose of an antigenic component compared to the primary series. A lower boosting dose may also provide a better safety profile (for example, as occurs with diphtheria toxoid).
- For polysaccharide-protein conjugate vaccines that elicit immune memory it may be informative to compare boosting with the same type of conjugate used for priming with an alternative conjugate (for example, to prime with a tetanus toxoid conjugate and boost with a CRM197 conjugate and vice versa).
- It may also be informative to assess the ability of a candidate vaccine to achieve cross-priming by using heterologous antigenic components for priming and boosting. This may be assessed by comparing boosting with the same vaccine used to prime with administration of a formulation (which may be a licensed vaccine

or an unlicensed product manufactured specifically for the trial) containing a different microorganism or antigen that is known to be closely related but not identical to that in the vaccine (for example, material derived from an influenza virus of a different clade).

- Elicitation of an immune memory response to a vector for an antigen after the first dose(s) may sometimes interfere with or wholly prevent the immune response to the antigen after subsequent doses (for example, this may be observed when using certain adenoviruses capable of infecting humans as live viral vectors). It is essential to understand whether or not this occurs since it may necessitate the use of a different vector for the antigen or an entirely different vaccine construct to deliver subsequent doses.
- Some antigens elicit immune hyporesponsiveness to further doses. The best known examples are some of the unconjugated meningococcal and pneumococcal polysaccharides (*18*, *19*). In the past these were sometimes administered to assess whether corresponding conjugated polysaccharides had elicited immune memory in the primary series, based on the premise that this would better mimic the immune response to natural exposure compared to administration of a further dose of the conjugate. This practice is not recommended since it is possible that a dose of unconjugated polysaccharide could result in blunted immune responses to further doses of the conjugate.
- Studies of cell-mediated immunity may provide supportive evidence that the primary series elicited immune memory and may be particularly useful for assessing cross-priming.

5.6.2 Using immunogenicity data to predict efficacy

5.6.2.1 Bridging to efficacy data

Immunogenicity data may be used to provide evidence of efficacy when:

- there is a well-established ICP that can be used to interpret the immune responses to a specific antigenic component;
- it is possible to use immune responses to bridge to estimates of vaccine efficacy obtained from prior well-designed clinical trials (that is, to conduct bridging trials).

The following two main situations should be considered when using immunogenicity data to bridge to estimates of vaccine efficacy obtained in prior clinical trials. In both cases comparative immunogenicity trials designed to

demonstrate non-inferiority are recommended. The choice of comparator is a critical factor in the interpretation of the results.

5.6.2.1.1 *Modifying the use of the vaccine for which efficacy has been estimated*

As described in section 6 below, vaccine efficacy trials are usually conducted in specific target populations – characterized by factors such as age, region (which may define the endemicity of some infectious diseases) and health status – using the intended final vaccine posology. Before or after licensure, trials may be conducted with the aim of extending the use of the vaccine to other populations and/or to support alternative posologies.

When a different age group or posology is proposed it is usually very clear that a bridging trial is necessary. A bridging trial may be required if there are compelling scientific reasons to expect that the immune response to the vaccine, and therefore its efficacy, could be significantly different to that documented in a prior efficacy trial because of host factors (such as common underlying conditions that may affect immune responses) and/or geographical factors (such as distribution of subtypes of organisms and levels of natural exposure). In infants there is also the possibility that very different levels of maternal antibody could occur in different regions, resulting in variable interference with infant immune responses to the primary series.

The trial design may involve a direct comparison between: (a) the new posology and that used in the efficacy trial; or (b) the new intended population and a control group consisting of subjects who are representative of the prior efficacy trial population. It may also be acceptable to make an indirect (cross-trial) comparison with the immunogenicity data that were obtained during the efficacy trial.

The vaccine formulation and assay used should be the same as those used in the efficacy trial whenever possible:

- If the exact vaccine used in the efficacy trial is no longer available the comparator should be as similar as possible to the original vaccine that was evaluated. Over time, it may be that the only bridge back to the efficacy data is via a comparison with a licensed vaccine that was itself licensed on the basis of a bridging efficacy trial. As the number of bridging steps that have occurred between the original efficacy data and the licensed comparator vaccine increases, the reliance that may be placed on a demonstration of non-inferiority to predict efficacy is weakened. This consideration also applies when the vaccine for which efficacy was estimated contained a certain number of subtypes but was later replaced by a vaccine containing a larger number of subtypes on the basis of comparing immune responses to the shared subtypes.

- If the assay has changed and has not been, or cannot be, directly compared to the original assay used during the efficacy trial it may be possible to re-assay stored sera collected during the prior efficacy trial in parallel with the sera from the new trial population.

If it remains unknown which immunological parameter best correlates with efficacy it is preferable that the primary comparison between vaccines is based on functional antibody whenever this is feasible.

5.6.2.1.2 *Inferring the efficacy of a new candidate vaccine*

In this case the main evidence of efficacy for licensure comes from one or more bridging efficacy trials. The same considerations described above regarding primary comparison, choice of comparative vaccine and assay apply.

If the new candidate vaccine contains additional subtypes of an organism compared to licensed products and/or it contains subtypes of an organism that have not previously been included in any licensed vaccine then interpretation of the immune responses to the added or new subtypes is not straightforward. Approaches that could be considered include comparing immune responses to each added or new subtype with the mean immune response to all subtypes or with the lowest immune response to any individual subtype included in a vaccine for which efficacy was demonstrated. Although these approaches may provide a route to licensure, the limitations of these comparisons in predicting efficacy should be taken into account when considering the overall risk–benefit relationship for the new vaccine.

5.6.2.2 Other approaches

When there is no ICP and it is not possible to bridge to a prior demonstration of efficacy the evidence that may be provided to support likely vaccine efficacy must be considered and discussed with NRAs on a case-by-case basis. In each case the strength of evidence that may be provided should be weighed against the advantages of having a licensed vaccine – one that has been subjected to a full review of quality and nonclinical data, and for which it is considered that there are adequate clinical safety and immunogenicity data – available for use when needed.

Potential approaches may include establishing a nonclinical model of efficacy that is thought to be relevant to the human infection and identifying which immunological parameter best correlates with protection (and, if possible, a putative ICP). Data on immune responses that occur in response to natural infection and the resulting protection against further disease may be useful, as may any passive protection data that are available from nonclinical or clinical trials.

5.6.3 Co-administration trials

Comparative immunogenicity trials that are intended to support co-administration of a vaccine with one or more other vaccines should demonstrate non-inferiority for immune responses to each of the co-administered antigenic components in the group that receives co-administered vaccines compared with the groups that receive each vaccine given alone.

When multiple licensed products contain the same antigenic components that could be co-administered with the vaccine under trial (for example, combination vaccines intended for the routine infant primary immunization series) it is not feasible, nor is it usually necessary, to assess co-administration with each licensed product.

A particular issue arises when there are several different types of polysaccharide-protein conjugate vaccines available that may be co-administered with the vaccine under trial. When the vaccine under trial contains protein that is the same as, or similar to, that in available conjugate vaccines it is important to appreciate that the results obtained with any one conjugate may not be applicable to other types of conjugate (for example, lack of immune interference with a tetanus toxoid conjugate does not rule out the possibility that this could occur when a different protein is used in the conjugate). Therefore, if co-administration with several different conjugate vaccines is foreseen the effects of representative vaccines that contain different conjugative proteins should be evaluated.

If multiple doses of the co-administered vaccines are needed then it is usual to make the comparison between groups only after completion of all doses. The schedule at which the vaccines are co-administered may also be a consideration if there are several possibilities (for example, as in the case of vaccines for the primary immunization series in infants or for vaccines against hepatitis A and B). Consideration may be given to using a schedule that is most likely to detect an effect of co-administration on immune responses if there is one.

Trials that assess the effects of co-administration may randomize subjects to receive only one or all of the vaccines proposed for co-administration. Alternatively, all subjects may receive all vaccines proposed for co-administration but are randomized to staggered administration or co-administration. Staggered administration is necessary when it is not possible to withhold any antigenic components to be co-administered (for example, during the infant primary schedule). In staggered administration trials the final dose and post-dose sampling occur later compared to the co-administration group, which in infants could have some impact on the magnitude of the immune response.

5.6.4 Immunization of pregnant women

Whenever the target population for a vaccine includes women of childbearing age there is a need to consider the importance of generating data in pregnant women. These considerations should take into account the nature of the vaccine

construct (for example, whether the vaccine contains a live organism that is replication competent), whether pregnant women can reasonably avoid exposure to an infectious agent (for example, by not travelling) and whether they may have the same risk of exposure but a greater risk of experiencing severe disease compared to non-pregnant women of the same age.

Not all vaccines are, or need to be, evaluated in trials in pregnant women. If there is no or very limited experience of the use of a vaccine in pregnant women, NRAs may consider whether nonclinical data and any data available from the clinical use of the vaccine and very similar vaccines could be provided in the prescribing information.

5.6.4.1 Aims of immunization during pregnancy

The immunization of women during pregnancy may benefit the mother and, in some cases, may also result in benefit to the infant for a limited postnatal period by means of placental transfer of maternal antibody (for example, influenza, acellular pertussis and tetanus vaccines). In other cases the immunization of women during pregnancy may provide some benefit to the infant with no or negligible benefit to the mother (for example, respiratory syncytial virus vaccine).

It is also possible that immunization during pregnancy could prevent an infection occurring in the mother and so protect the fetus from the consequences of infection in utero.

5.6.4.2 Safety and immunogenicity in pregnancy

Before conducting trials in pregnant women, safety and immunogenicity data should be available from clinical trials conducted in non-pregnant women of childbearing age (*20*). Once there are adequate relevant nonclinical data with satisfactory findings and some clinical data on safety and immune responses in non-pregnant women, data may be obtained from pregnant women covering a representative age range, so that the effects of pregnancy on the immune response can be evaluated. The doses tested in pregnant women should be based on the non-pregnant adult data but may need to be adjusted (in terms of antigen dose or dose regimen) if the results indicate an effect of pregnancy on the immune response.

In all trials conducted in pregnant women adequate mechanisms should be in place to document the outcome of the pregnancy, including the duration of gestation at time of delivery, the condition of the infant at birth and the presence of any congenital conditions (see section 7.4 below).

5.6.4.3 Passive protection of infants

If there is already evidence of humoral immunity in a substantial proportion of pregnant women against the infectious disease to be prevented, such that that

the aim of vaccination during pregnancy is to increase the amount of antibody transferred to the fetus, then the trials in pregnant women may need to include exploration of maternal immune responses to vaccination in both seropositive and seronegative subjects.

Dose-finding trials in pregnant women should include measurement of antibody levels in cord blood samples taken at delivery. The number of samples obtained should be sufficient to provide an estimate of inter-individual variability. Additional investigations may include the collection of cord blood covering a range of times between maternal vaccination and delivery. Cord blood antibody levels in infants born to vaccinated mothers who received the final selected vaccine posology should be superior to those in infants born to mothers who were not vaccinated. Secondary analyses could examine whether this finding also applies within subsets of mothers who were seronegative or seropositive prior to vaccination.

To avoid multiple bleeds in individual infants when evaluating the duration of detectable maternal antibody, mothers may be randomized so that their infants are sampled once or a few times at defined intervals. The total data collected can be used to describe the antibody decay curve. These data are particularly important when it is planned that passive protection via maternal antibody will be followed by active vaccination of infants against the same antigen(s) because of the possibility that high levels of maternal antibody may interfere with the infant immune response.

If an ICP is established for the infectious disease to be prevented then the aim of the immunogenicity trials should be to identify a maternal vaccination regimen that results in cord blood antibody levels that exceed the ICP in a high proportion of newborn infants. If no ICP exists there should be discussion with NRAs regarding whether vaccine efficacy should be estimated in a pre-licensure efficacy trial or whether an evaluation of vaccine effectiveness may suffice.

5.6.5 Changes to the manufacturing process

Changes made to product composition (for example, adding, removing or changing a preservative) or to product manufacture (such as changes to process, site or scale of manufacture) during the pre-licensure clinical development programme or after licensure do not always need to be supported by comparative clinical immunogenicity trials between the prior and newer products.

For example, although it is common for the scale of manufacture to change during the pre-licensure development programme, this step alone may not necessarily have a clinically significant effect in the absence of other changes. To avoid the need for additional clinical trials to address manufacturing changes the pivotal trials should whenever possible be conducted using vaccine made according to the final process. If this is not the case, and for all changes that are

made post-licensure, consideration must be given to whether a clinical trial is required to compare vaccines manufactured using the prior and new processes. This decision must be taken on a case-by-case basis after a full evaluation of the in vitro data, and of any nonclinical in vivo data describing and supporting the change. Although a single lot of vaccine made using each process may typically be sufficient for the comparison, data may on occasion be required from multiple lots.

In the post-licensure period there may be many changes to the manufacturing process over time. Whereas each one of these changes may be considered too minor to merit the conducting of a clinical trial, the product that results from multiple minor changes could be substantially different from that which was first licensed. Therefore, when considering the merit of a clinical trial, it may be important to consider the full history of changes that have been allowed without clinical data and whether the sum total of these changes could have a clinical impact. In this situation, when many years have passed, a clinical trial of the current vaccine compared to the original licensed vaccine will not be possible. However, if disease surveillance suggests that there could be a problem with vaccine effectiveness, a clinical trial that compares the current vaccine against another licensed vaccine may be considered useful.

5.6.6 Clinical lot-to-lot consistency trials

Clinical lot-to-lot consistency trials are conducted to provide an assessment of manufacturing consistency in addition to the information provided on the manufacturing process. Clinical lot-to-lot consistency trials may or may not be considered necessary. Such trials may be considered particularly useful for certain types of vaccines where there is inherent variability in the manufacture of the product or when manufacturing consistency cannot be characterized adequately by bio-physicochemical methods.

If a clinical lot-to-lot consistency trial is conducted then the usual expectation is that the 95% confidence interval around each pair-wise comparison of the post-vaccination geometric mean antibody concentrations/titres falls within predefined limits. The clinical implications of results that show that one or more comparisons do not meet the predefined criteria set around the ratios should be considered in light of all available clinical immune response data and relevant product-characterization data.

Whether or not a clinical lot-to-lot consistency trial is conducted, the consistency of manufacturing for the vaccine lots used in clinical trials should be both demonstrated and well documented. The lots used in clinical trials should also be adequately representative of the formulation intended for marketing.

6. Efficacy and effectiveness

6.1 General considerations for efficacy trials

The need for, and feasibility of, evaluating the protective efficacy of a candidate vaccine should be considered at an early stage of vaccine development because the decision made will determine the overall content of the pre-licensure clinical programme and will impact on its duration. In all application dossiers that do not include an evaluation of vaccine efficacy the sponsor should provide sound justification for the lack of such data, taking into account the points raised in the following sections 6.1.1–6.1.3.

6.1.1 Efficacy data are not required

Vaccine efficacy trials are not necessary if it is established that clinical immunological data can be used to predict protection against disease. For example, if there is an established ICP against a specific disease (for example, antitoxin levels against diphtheria and tetanus toxins, or antibody against hepatitis B surface antigen) the candidate vaccine should be shown to elicit satisfactory responses based on the relevant correlate(s).

6.1.2 Efficacy data are usually required

Vaccine efficacy trials are usually required whenever a new candidate vaccine is developed with intent to protect against an infectious disease and one or more of the following apply:

- There is no established ICP that could be used to predict the efficacy of the new candidate vaccine.
- There is no existing licensed vaccine with documented efficacy against a specific infectious disease to allow for bridging to a new candidate vaccine.
- Use of immune responses to bridge the documented efficacy of a licensed vaccine to a new candidate vaccine is not considered to be possible. For example, because there is no known relationship between specific immune response parameters and efficacy or because the new candidate vaccine does not elicit immune responses to the same antigen(s) as the licensed vaccine.
- There are sound scientific reasons to expect that the efficacy of a vaccine cannot be assumed to be similar between the population(s) included in the prior efficacy trial(s) and one or more other populations.

- It cannot be assumed that the vaccine efficacy demonstrated against disease due to specific strains of a pathogen (for example, serotypes or subtypes) would apply to other strains.

6.1.3 Efficacy data cannot be provided

It may not be feasible to conduct efficacy trials. For example, if the new candidate vaccine is intended to prevent an infectious disease that:

- does not currently occur (for example, smallpox);
- occurs in unpredictable and short-lived outbreaks that do not allow enough time for the conducting of appropriately designed trials to provide a robust estimation of vaccine efficacy (for example, some viral haemorrhagic fevers);
- occurs at a rate that is too low for vaccine efficacy to be evaluated in a reasonably sized trial population and period of time. This situation may apply:
  (a) because of natural rarity of the infectious disease (for example, plague, anthrax and meningitis due to *N. meningitidis* group B);
  (b) because of rarity of the disease resulting from the widespread use of effective vaccines.

If it is not feasible to perform vaccine efficacy trials and there is no ICP it may be possible to obtain evidence in support of vaccine efficacy and/or to derive an immunological marker of protection from one or more of the following:

- Nonclinical efficacy trials.
- Passive protection trials – that is, nonclinical or clinical trials which assess the effects of administering normal or hyperimmune human gamma globulin or convalescent sera. The results may point to the sufficiency of humoral immunity for the prevention of clinical disease and may suggest a minimum protective antibody level that could be used to interpret data obtained in clinical trials with candidate vaccines.
- Comparison of immunological responses with those seen in past trials of similar vaccines with proven protective efficacy even if the relationship between immune responses to one or more antigenic components and efficacy remains unknown.
- Human challenge trials.

6.2 Types of efficacy trials

6.2.1 Human challenge trials

Human challenge trials, in which subjects are deliberately exposed to an infectious agent in a controlled setting, are not always feasible or appropriate. However, in some settings it may be useful and appropriate to obtain an assessment of vaccine efficacy from human challenge trials. If conducted, human challenge trials may be of particular use:

- when there is no appropriate nonclinical model (for example, when a candidate vaccine is intended to protect against an infectious disease that is confined to humans);
- when there is no known ICP;
- when vaccine efficacy trials are not feasible.

6.2.2 Preliminary efficacy trials

If conducted, preliminary vaccine efficacy trials may provide an estimate of the magnitude of protection that can be achieved by the new candidate vaccine. However, preliminary efficacy trials are not usually designed and powered to provide robust estimates of vaccine efficacy. These trials may be used to inform the design of pivotal trials. For example:

- by evaluating the efficacy of different doses and dose regimens;
- by estimating efficacy on the basis of a range of efficacy variables;
- by analysing efficacy on the basis of various case definitions in order to identify or refine the most appropriate case definition;
- by exploring efficacy in specific subgroups in order to decide if there is a need to design pivotal trials specifically to further evaluate efficacy in such subgroups;
- by assessing the method of case ascertainment for feasibility in larger and more geographically diverse trials;
- by using immunogenicity and efficacy data to support a provisional assessment of potential ICPs.

If the candidate vaccine is intended to prevent a severe and/or life-threatening infectious disease for which there is no vaccine, or no satisfactory vaccine, already available then individual NRAs may agree to accept an application for licensure based on one or more preliminary efficacy trial(s). In these cases it is essential that sponsors and NRAs should discuss and agree

upon the main features of the design of the trials before initiation (including the sample size) so that, subject to promising results, the data may be considered robust and sufficient.

The availability of a licensed vaccine has potentially important implications for the acceptability and feasibility of initiating or completing additional efficacy trials that include a control group that does not receive active vaccination. These issues should be discussed between NRAs and sponsors so that expectations for the completion of additional efficacy trials are agreed upon prior to the start of trials that could potentially support licensure.

6.2.3 Pivotal efficacy trials

Pivotal vaccine efficacy trials are designed and powered to provide statistically robust estimates of vaccine efficacy to support licensure. Pivotal efficacy trials may evaluate one or more vaccination regimen(s), and may or may not include evaluations of efficacy before and after booster doses.

6.3 Design and conduct of efficacy trials

The protective efficacy of a vaccine against a specific infectious disease is usually determined in randomized trials that compare the incidence of disease after vaccination relative to the incidence of disease in the control group that has not been vaccinated. Less frequently, vaccine efficacy may be determined in a prospective randomized trial which compares the incidence of disease after vaccination between the group that received the new candidate vaccine and a control group that received a licensed vaccine intended to prevent the same infectious disease.

The following sections (6.3.1–6.3.9) are applicable to both types of trial. As the details of statistical methodologies are beyond the scope of these WHO Guidelines only broad principles are described. It is recommended that an appropriately experienced statistician should be consulted.

6.3.1 Selection of trial sites

Vaccine efficacy trials require the presence of a sufficient burden of clinical disease to enable estimates to be obtained from feasible numbers of subjects within a reasonable time frame. The infectious disease to be prevented may occur at sufficiently high rates to enable efficacy trials to be conducted only in certain geographical areas. Even when the disease to be prevented is more widespread it may be necessary to confine efficacy trials to specific areas for reasons that may include feasibility, the need to ensure adequacy of monitoring, and a desire to accumulate representative numbers of cases due to specific serotypes or subtypes of the relevant pathogen.

If adequate data are not already available from public health authorities then sponsors may have to conduct feasibility assessments in order to accurately ascertain the clinical disease rates in various age subgroups of populations before selecting trial sites. Any nationally recommended non-vaccine-related preventive measures that are in place (for example, prophylactic drug therapy in high-risk settings or in individuals at high risk, or the use of insect repellents and bednets) should be identified. Trials are usually conducted against a background of such measures.

Trial sites need to be sufficiently accessible to allow regular visits for monitoring. Prior to initiation of the trial, sponsors may have to engage in site capacity-building exercises, including training of study personnel, and may need to provide essential infrastructure to support the trial (for example, adequate blood-collection and processing facilities, refrigeration facilities suitable for the vaccine and/or sera, access to competent laboratories, data-handling capacity and communication methods to allow for electronic randomization schemes, rapid reporting of safety data and other trial issues to the sponsor).

6.3.2 Candidate (test) vaccine group(s)

If previous data do not support selection of a single dose level or regimen of the candidate vaccine for assessment of efficacy then trials may include one or more groups in which subjects receive the candidate vaccine (for example, more than one dose or schedule may be evaluated). In some cases one or more placebo doses may need to be interspersed with candidate vaccine doses to enable the matching of all regimens under trial in a double-blind design (for example, if two or three doses of the candidate vaccine are to be compared with the control group).

6.3.3 Control (reference) group(s)

Control groups comprise all subjects who do not receive the candidate vaccine. Usually only one control group is enrolled in any one trial. Sometimes it may be important to include more than one of the possible types of control groups discussed below.

6.3.3.1 Control groups not vaccinated against the infectious disease to be prevented

Following consultation between the sponsor, NRA, ethics committees, local public health authorities and investigators it may be appropriate to use a control group that is not vaccinated against the disease to be prevented by the new candidate vaccine. For example, this may be the case when the trial is to be conducted in countries in which:

- no vaccine is yet licensed for prevention of the disease in question; and/or

- no such vaccine is included in the routine immunization schedule; and/or
- there are sound reasons to believe that no licensed vaccine is likely to provide useful efficacy (for example, because the licensed vaccine does not cover, or is known/expected to have poor efficacy against, the pathogen types that are most prevalent in a specific region).

In these cases the control group may receive:

- A true placebo (that is, material without any pharmacological activity, such as normal saline). This has the advantage of providing safety data against a control that has no pharmacologically active components. The use of an injectable placebo may not be acceptable to all NRAs, ethics committees, investigators, trial subjects or their caregivers in some age groups (for example, particular objections may be raised against true placebo injections in infants). In contrast, there is usually no objection to the use of a true placebo when the candidate vaccine is administered orally or by nasal instillation.
- A licensed vaccine that does not prevent the infectious disease under study but may have some benefit for recipients. In some cases both licensed vaccine and placebo doses may have to be administered to the control group to match the candidate vaccine regimen in order to maintain blinding.

If there are major objections to the use of placebo injections but no potentially beneficial licensed vaccine would be suitable for the target age group, the control group may be randomized to receive no injection. This is an undesirable situation and should be regarded as a last resort since it precludes the blinding of trial personnel or subjects/caregivers.

6.3.3.2 Control groups vaccinated against the infectious disease to be prevented

In this case the control group receives a vaccine that is already licensed to prevent the same infectious disease as the candidate vaccine.

In some instances the control group may receive a licensed vaccine that prevents infectious disease due to some, but not all, types of the pathogen responsible for the disease that is to be prevented – in which case the group that receives the licensed vaccine may be regarded as an unvaccinated control group for the types found only in the candidate vaccine.

It is important that selection of the control vaccine takes into account the available evidence supporting its efficacy and, if relevant, whether it appears to have similar efficacy against all types of the pathogen involved. When there is more than one available licensed control vaccine, or the selected control vaccine

is unlicensed or is not the product in routine use in a particular jurisdiction(s), sponsors are advised to discuss selection of the comparator with the relevant NRA(s). If it is not possible to reach agreement on the use of the same control vaccine in all regions where efficacy is to be evaluated, consideration should be given to conducting more than one efficacy trial with a different vaccine used in the control group in each trial.

6.3.4 Trial designs

6.3.4.1 Randomization

The unit of randomization is most usually the individual. Alternatives include the household or the cluster under trial (for example, a school population or a local community). Randomization of groups or clusters, rather than individuals, may be preferred when it is logistically much easier to administer the vaccine to groups than to individuals and when estimates of the indirect effects of vaccination (for example, herd immunity) are of interest. When the trial aims to vaccinate pregnant women to protect the infant during the early months of life then the unit of randomization is the mother.

6.3.4.2 Types of trial design

The simplest design involves randomization of equal numbers of subjects to the candidate vaccine and control groups (that is, 1:1). In trials that employ a control group that is not vaccinated against the disease to be prevented, but some clinical data are available to support the likely efficacy of the candidate vaccine, it may be appropriate (subject to statistical considerations and an assessment of the impact on the total trial sample size) to use unbalanced randomization (for example, 2:1 or 3:1) to reduce the chance that individual subjects will be randomized to the control group, thus ensuring that the majority of trial subjects receive the candidate vaccine.

Trials may be planned to follow trial subjects for a fixed period after the last dose of the primary series. The time at which the primary analysis is conducted should take into account the anticipated rates of the disease under study in each treatment group, including the unvaccinated control group if applicable. Other considerations regarding the timing of the primary analysis may include the possible importance of having some information on the duration of protection before licensure occurs, the feasibility of following up subjects for prolonged periods, and whether or not the vaccine could address a pressing unmet need (for example, in an outbreak situation where there is no approved vaccine to prevent the disease).

Alternatively, a case-driven approach may be taken based on the anticipated rates of the primary efficacy end-point in the control group and the expected or minimum desirable level of efficacy of the candidate vaccine.

In this design the primary analysis is conducted once a pre-specified total number of cases has been detected – based, in a double-blind setting, on the anticipated numbers in test and control groups required to demonstrate the projected vaccine effect.

Alternative designs that allow for comparison with a control group that is not vaccinated against the disease to be prevented may, at least in the short term, include the following:

- In a randomized stepped wedge trial, the candidate vaccine is administered to predefined groups in a sequential fashion. Each predefined group is a unit of randomization. These may be geographical groups or groups defined by host factors (for example, age) or other factors (for example, attendance at a specific school or residence within a specific health-care facility catchment area). Such a design may be chosen when there is good evidence to indicate that the vaccine will do more good than harm (affecting the equipoise associated with randomization to a control group that is not vaccinated against the disease to be prevented) and/or when it is impossible to deliver the intervention to all trial participants within a short time frame.
- In a ring vaccination trial, the direct contacts (and sometimes secondary contacts) of a case may be randomized to vaccine or control or may be randomized to receive immediate vaccination or vaccination after a period of delay (*21*). This type of post-exposure cohort trial usually requires smaller sample sizes than prospective randomized controlled trials.

  Ring vaccination trials may be particularly applicable when the infectious disease to be prevented is associated with a relatively high incidence of secondary cases in susceptible populations. Therefore the use of this trial design requires prior knowledge of the infectivity of the infectious agent and of the proportion of infections that are clinically apparent, as well as of the general susceptibility of the trial population.

  Ring vaccination trials may not be appropriate if the vaccination regimen requires multiple doses over an extended period to induce a protective immune response.

  The follow-up period for subjects after contact with the index case should extend to the upper limit of the incubation period, taking into account both the period during which the index cases were infectious and the contact period. The inclusion period for new cases and controls and their contacts following the detection of the first

case should be stated in the protocol. The duration of the inclusion period should take into account the potential for introducing bias if the disease incidence changes over time.

6.3.5 Clinical end-points

6.3.5.1 Primary end-points

The primary end-point(s) in preliminary trials may be different from the primary end-point(s) used in the pivotal trial(s).

In most cases the focus of vaccine efficacy trials is the prevention of clinically apparent infections that fit the primary case definition based on clinical and laboratory criteria.

If an organism causes a range of disease manifestations (for example, from life-threatening invasive disease to disease that is not serious if adequately treated or is self-limiting) the primary end-point in any one trial should be carefully selected in accordance with the proposed indication(s) for use.

A candidate vaccine may contain antigens derived from one or several types (serotypes, subtypes or genotypes) of the same organism. There may also be some potential for cross-protection against types not included in the vaccine (for example, as observed with rotavirus vaccines and HPV vaccines). In such cases it is usual for the primary end-point to comprise cases due to any of the types included in the vaccine, and the trial is powered for this composite end-point. It is not usually possible to power the trial to assess efficacy against individual types in the vaccine or to assess cross-protection against types not in the vaccine.

Alternative primary end-points may include:

- clinical manifestations of reactivated latent infection (for example, herpes zoster);
- established chronic infections that may be asymptomatic but predispose to infection-related disease later in life (for example, chronic hepatitis B infection or persistent infection with HPV);
- other markers that predict progression to clinically apparent disease (for example, histological changes associated with HPV infection that are established precursors of malignant neoplasia).

6.3.5.2 Secondary end-points

As applicable to the individual candidate vaccine, other important end-points may include:

- cases that occur after each dose, when the vaccine schedule includes multiple doses and/or a booster;

- cases due to each of the individual types of the organism included in the vaccine;
- cases due to the organism, regardless of whether the cases are caused by types that are or are not included in the candidate vaccine;
- cases due to non-vaccine types;
- cases occurring in groups with host factors of interest (for example, age or region);
- cases meeting criteria for disease severity – if available, validated measures of criteria for severity should be used to facilitate interpretation of the results;
- duration and/or severity of the illness, which may include clinical measurements (for example, duration of fever or rash) and laboratory measurements (for example, duration of shedding).

Eradication of carriage and/or reduction in disease transmission that is not directly linked to, and/or accompanied by, a clinical benefit of vaccination to the individual are not usually considered to be sufficient to support licensure. Sponsors contemplating trials with these as primary end-points are advised to consult widely with NRAs.

6.3.6 Case definition

As part of the predefined primary efficacy end-point, the protocol should describe the clinical and laboratory criteria that must be met to define a case.

- If an end-point is defined as the occurrence of an acute infectious disease then the case definition should include the core clinical features as well as laboratory confirmation of the presence of the target pathogen.
- If the end-point is defined as a consequence of a persistent infection then details of sampling (frequency and method) and grading (if applicable) should be described.

All laboratory assays used to define a case should be validated to the satisfaction of relevant NRAs prior to initiating pivotal clinical trials.

Adequate case definitions should also be provided for secondary end-points.

6.3.7 Case ascertainment

It is critical that the same methodology for case detection should be applied consistently at all clinical sites throughout the duration of the trial. Active case ascertainment usually requires frequent monitoring and contact with trial

subjects/caregivers. Passive case ascertainment is usually based on trial subjects/caregivers presenting to or otherwise contacting a local health-care facility due to the onset of specific symptoms. In this case, contact is commonly triggered by one or more of a list of signs or symptoms given to trial subjects/caregivers at the time of randomization, when they may also have been instructed to contact a specific health-care facility. Alternatively, or in parallel, cases may be detected by monitoring all local clinics and hospitals.

For efficacy end-points based on clinically apparent disease the possible range of clinical presentations will determine the mode of case ascertainment. For example, this may be hospital based for cases of life-threatening infections, or community-based for less severe infections. If community-based, case detection may depend on family practitioners and on initial suspicion of infection by vaccinated subjects or their caregivers. It is critically important that the individuals who are most likely to initiate detection of a possible case should have clear instructions. These may need to cover issues such as the criteria for initiating contact with designated health-care professionals, telephone contacts, first investigations and further investigations once a case is confirmed.

For efficacy end-points other than clinically apparent disease it is essential for subjects to be monitored at regular intervals to detect clinically non-apparent infections or changes in other selected markers (for example, the appearance of histological changes). The frequency of these visits, and acceptable windows around the visits, should be stated in the trial protocol and carefully justified.

The appropriate period of case ascertainment during a trial should be determined mainly by the characteristics of the disease to be prevented and the claim of protection that is sought at the time of licensure. For infectious diseases that have marked seasonality, at least in some geographical locations (for example, influenza and respiratory syncytial virus), it is usual to follow subjects through one or more seasons to accumulate sufficient cases to conduct the primary analysis. In these settings it is usual to conduct an enrolment campaign over a short period just before the expected onset of each season.

6.3.8 Duration of follow-up

At the time of conducting the primary analysis for the purposes of obtaining licensure the duration of follow-up in vaccine efficacy trials may be relatively short (for example, 6–12 months) and may be insufficient to detect waning protection, if this occurs. If feasible, case ascertainment may continue in vaccine efficacy trials with maintenance of the randomized populations for a sufficient duration to assess waning protection over time. Alternatively, or in addition, waning protection may be assessed during the post-licensure period. These data may serve both to indicate the need for, and optimal timing of, booster doses and to estimate efficacy after booster doses.

6.3.9 Analysis of efficacy

Detailed plans for the analysis of efficacy, including any interim analyses and/or plans to adjust the sample size during the study on the basis of specific criteria, should be developed in conjunction with appropriately experienced statisticians, and should be discussed with the NRA(s) before the protocol is finalized (and/or during the conducting of the study, as necessary).

6.3.9.1 Sample size calculation

The trial sample size should be calculated on the basis of:

- the selected primary efficacy end-point, which could be a composite of cases due to any of the organism types included in the candidate vaccine;
- the primary analysis population (see below);
- the primary hypothesis (that is, superiority or non-inferiority and the predefined success criteria).

If the primary analysis population represents a subset of the total randomized population then the sample size calculation should include an adequate estimation of numbers likely to be excluded from the primary analysis for various reasons. In addition, a blinded review (for example, using an independent data adjudication committee) of total numbers of subjects enrolled who are eligible for the primary analysis population may be conducted after randomization of a predefined number so that the trial sample size can be adjusted accordingly.

6.3.9.2 Analysis populations

Clinical efficacy is usually assessed in the total randomized trial population (that is, those who are assigned to receive vaccine and/or control) and in predefined subsets of the randomized population.

The predefined trial populations should include as a minimum:

- all randomized subjects (that is, the full analysis set);
- all vaccinated subjects regardless of the numbers of assigned doses actually received and whether or not doses were administered within predefined windows;
- subjects who have generally complied with the protocol and have received all assigned doses within predefined windows.

Other populations may be appropriate for some predefined secondary or exploratory analyses. These may include, for example:

- those who completed specific numbers of assigned doses or received all doses within predefined windows around the scheduled trial visits (that is, analyses of efficacy according to adherence to the vaccination regimen);
- subsets of all vaccinated subjects separated according to baseline seropositivity versus seronegativity;
- subgroups defined by demographic factors known or postulated to have an impact on vaccine efficacy.

6.3.9.3 Primary analysis

The primary analysis may sometimes be based on estimating efficacy in the "per protocol" population and on rates of true vaccine failures. In this case, the calculation of efficacy takes into account only those cases with onset after a minimum time has elapsed following completion of the assigned doses. For example, depending on knowledge of the kinetics of the immune response, true vaccine failures may be limited to cases with onset more than a specified number of days or weeks after the final dose of the primary series. In addition, for a vaccine that contains antigens from only certain serotypes or subtypes the primary analysis may be based on cases due to vaccine types only. Alternative primary analysis populations that may be preferred by NRAs in some cases include the all-randomized or the all-treated populations.

In trials that compare a candidate vaccine group with a group that is not vaccinated against the disease to be prevented, the aim is to demonstrate that the lower bound of the 95% confidence interval around the estimate of vaccine efficacy is above a predefined percentage (which will always be above zero). The predefined percentage should be selected on the basis of the expectation of the point estimate of vaccine efficacy, taking into account what might be seen as the minimum level of efficacy that could be considered clinically important. The sample size calculation is based on this objective.

In trials that compare a candidate vaccine with an active control the aim is usually to demonstrate non-inferiority of the candidate vaccine against a control vaccine with demonstrated efficacy. This requires a predefined non-inferiority margin, which should be justified in accordance with prior estimates of vaccine efficacy for the disease to be prevented and the level of alpha on which the sample size calculation depends. If the sponsor also intends to assess superiority of the candidate vaccine over the active control the statistical analysis plan should predefine a hierarchical assessment so that superiority is assessed only after establishing that non-inferiority has been demonstrated.

6.3.9.4 Other analyses

The full range of secondary and exploratory analyses will depend on the predefined end-points. Some of these analyses may be conducted in specific predefined trial populations. For example, important sensitivity analyses for supporting the primary analysis include those based on all proven cases whenever they occurred after randomization and in each analysis population. If the schedule includes more than one dose, analyses should be conducted to count cases from the time of each dose or from a specified number of days after each dose for all subjects who were dosed up to that point.

Other analyses may be based on cases that meet some but not all of the case definition criteria, cases that are severe and cases that require a medical consultation or hospitalization.

6.3.9.5 Other issues

6.3.9.5.1 *Vaccines that contain antigens derived from several serotypes, subtypes or genotypes*

If the primary analysis was confined to cases due to organism types included in the vaccine then additional analyses should be conducted to evaluate efficacy on the basis of all cases, regardless of the organism type responsible. If there are sufficient numbers of cases due to organism types not included in the vaccine these analyses may provide some indication of cross-protection.

If the data suggest unusually low efficacy against one or more organism types in the vaccine it may be necessary to explore this issue in further trials.

6.3.9.5.2 *Magnitude of vaccine efficacy*

The point estimate of vaccine efficacy and 95% confidence intervals that are obtained may indicate that a relatively modest proportion of cases can be prevented. This fact alone does not preclude licensure provided that the sponsor can provide evidence that the vaccine efficacy observed represents an important clinical benefit (for example, if the vaccine prevents life-threatening infections for which there is no very effective specific therapy and for which no vaccine is available).

6.4 Approaches to determination of effectiveness

Vaccine effectiveness reflects direct (vaccine-induced) and indirect (population-related) protection during routine use. The information gained from assessments of vaccine effectiveness may be particularly important to further knowledge on the most appropriate mode of use of a vaccine (for example, the need for booster doses to maintain adequate protection over time). Vaccine effectiveness is influenced by a number of factors, including:

- vaccination coverage of the population;

- pre-existing immune status of the population;
- differences between organism types included in a vaccine and the predominant circulating types;
- changes in circulating predominant types over time;
- transmissibility of the pathogen and any effect that the introduction of routine vaccination may have had on transmission rates.

Vaccine effectiveness may be estimated in several ways, namely:

- In observational cohort studies that describe the occurrence of the disease to be prevented in the target population over time. However, there is no randomization step and there is a potential for considerable biases to be introduced.
- During phased introduction (for example, in sequential age or risk groups) of the vaccine into the target population in which the groups might form the units of randomization (that is, using a stepped wedge design).
- Using other designs such as a case test-negative study design. In this modification of a case control study, subjects with symptoms suggesting the infectious disease under trial and seeking medical care are tested for the infectious agent of interest. The cases are those who are positive and controls are those who are negative for the pathogen of interest. Bias may occur if vaccinated cases are less or more severely ill and seek care at different rates compared to cases that occur in individuals who are not vaccinated against the disease to be prevented (*22*).

It may not be possible or appropriate for sponsors to conduct studies to estimate vaccine effectiveness themselves. For reasons of feasibility it may be necessary to collect the data via regional or national networks. For some types of disease the use of data collected by means of national or international registries may be appropriate. In addition, in some jurisdictions the estimation of vaccine effectiveness in the post-licensure period is not considered to fall within the remit of the licence holder.

Whatever the local requirements and arrangements, sponsors should discuss arrangements for ongoing disease surveillance and the potential for estimating effectiveness with the public health authorities in countries where the vaccine is to be used and where appropriate surveillance systems are in place. The plans for estimation of effectiveness should also be agreed with NRAs at the time of licensure and the requirements for reporting effectiveness data to the NRA, either via the sponsor or directly from a public health authority, should be clarified.

It may be that reliable estimates of effectiveness can be obtained only in certain countries in which vaccination campaigns are initiated and where there is already a suitable infrastructure in place to identify cases. In addition, it would likely be inappropriate to extrapolate any estimates of effectiveness that are obtained to other modes of use (such as introducing the same vaccine to different or highly selected sectors of the population).

7. Safety

7.1 General considerations

All clinical trials that are conducted pre-licensure or post-licensure should include an exploration of safety.

The assessment of safety may be the primary objective, a co-primary objective or a secondary objective in a clinical trial. Since the methods for collection, analysis and interpretation of safety data during clinical trials contrast with those applicable to post-licensure routine safety surveillance they are considered separately below.

In principle, many of the approaches to documenting and reporting safety data during vaccine clinical trials and conducting vaccine pharmacovigilance activities are similar to those used for all medicinal products. The sections that follow should be read in conjunction with the extensive guidance that is available from numerous publications, and on the websites of WHO, the Council for International Organizations of Medical Sciences (CIOMS), ICH and individual regulatory bodies. The focus of the following sections is thus on a number of methods and practices that are different for vaccines compared to other medicinal products, and on issues that may need to be addressed because of vaccine composition.

7.2 Assessment of safety in clinical trials

7.2.1 Safety outcomes as primary or secondary end-points

7.2.1.1 Safety outcomes as primary end-points

When the assessment of safety is a primary objective of a clinical trial it is usual for the primary analysis to be based on a specific safety end-point (for example, rates of a certain AE or rates of AEs that may be part of a clinical syndrome of interest). The trial may or may not be powered to address the pre-specified hypothesis.

7.2.1.2 Safety outcomes as secondary end-points

When the assessment of safety or specific aspects of the safety profile is a secondary objective, trials are not usually powered a priori to support statistical

analyses of end-points such as rates of all, or of specific, AEs. Descriptive comparisons are commonly used to screen for any differences in AE rates between treatment groups. If statistical analyses of AE rates are conducted they should be pre-specified in the protocol and in the statistical analysis plan. If any findings indicate statistically significant differences in rates of AEs (overall or for specific AEs) between treatments then they should be interpreted with caution unless the trial was primarily designed to address pre-specified hypotheses regarding safety end-points. The biological plausibility that AEs that occur more frequently in the new candidate vaccine group may be related to vaccination should be taken into consideration when deciding on the need for further pre- or post-licensure clinical trials to investigate and quantify the potential risks.

7.2.2 Recording and reporting adverse events

7.2.2.1 Methods

AEs should be reported and recorded by investigators and sponsors according to detailed procedures described in the trial protocol. AEs should be classified according to a standardized terminology (such as ICH MedDRA) to enable their categorization by System Organ Class (SOC) and Preferred Term (PT). If the classification terminology is updated while the trial is being conducted then the clinical trial report should indicate how the changes affect the tabulations.

Expedited reporting of AEs that meet specific criteria should take place in accordance with the requirements of individual NRAs relevant to the location of the trial sites.

It is standard practice for vaccinees to be observed immediately after each dose (for example, for a defined period – commonly 20–60 minutes) for any severe immediate reactions (for example, severe hypersensitivity reactions requiring immediate medical attention).

It is usually expected that all AEs are collected from all randomized subjects for defined periods after each dose:

- Solicited signs and symptoms are usually recorded daily for at least 4–7 days after each dose (see section 7.2.2.2 below). Longer periods (for example, 10–14 days) may be appropriate for certain vaccines, such as those that replicate in recipients.
- Unsolicited AE reports are usually collected for the entire period between each dose or, for single doses or final doses of regimens, for approximately 4 weeks post-dose (see section 7.2.2.3 below).
- Reports of serious adverse events (SAEs) and any pre-specified AEs of special interest (AESIs) should be collected from all trial subjects for at least 6 months after the last dose of assigned treatment.

- For vaccines that contain new adjuvants it is recommended that there should be follow-up for at least 12 months after the last dose to allow for the documentation of any autoimmune diseases or other immune-mediated AEs.

In trials involving large numbers of subjects (for example, vaccine efficacy trials) it may be acceptable for reports of non-serious AEs to be collected from a representative (and preferably randomized) subset or, occasionally, not at all, taking into account the safety profile observed in the previous trials and the number of subjects from which detailed safety data have already been obtained, In this case, reports of all SAEs and any pre-specified AESIs should be collected from all randomized subjects. It may be acceptable that only SAE and AESI reports are collected during long-term safety follow-up.

7.2.2.2 Solicited signs and symptoms

In most trials it is common practice for certain local and systemic AEs to be documented for a predefined period after each dose of a vaccine or placebo. The recording of AEs may be facilitated by the use of diary cards or other methods to ensure that the information is captured. If diary cards are used they may be completed by vaccinees, caregivers or by study staff who have questioned the vaccinees or their caregivers. These AEs are commonly referred to as "solicited signs and symptoms" since information on their occurrence is actively sought and they should be listed in the trial protocol.

For injectable vaccines the local signs and symptoms to be documented usually include, as a minimum, pain, redness and swelling at the injection site in all age groups. Pain should be graded according to a scoring system and preferably one that has been validated. Measuring devices of various types may be used to record the extent of redness and swelling.

Consideration should be given to assessing whether reports of pain are associated with immediate pain during and just after the injection or whether the pain is of later onset. If there is frequent reporting of pain at or around the injection site during the hours or days following vaccination this may suggest that the overall tolerability of the vaccine could negatively impact on vaccine uptake in routine immunization programmes. In these circumstances it may be appropriate to consider whether an attempt should be made to reformulate the vaccine to improve local tolerability.

When two or more vaccines are given by injection at the same time, the diary card should ensure that separate data are recorded for the new candidate vaccine injection site.

The systemic signs and symptoms to be collected and documented are determined by the age range in the trial (for example, those appropriate for

infants will not be wholly applicable to toddlers and older subjects) and by the route of administration (for example, nausea and vomiting could be solicited symptoms for vaccines given orally). Fever should be documented using digital thermometers and should be determined at a specific site (for example, rectal or axillary in infants). Recordings of fever should be made at predefined times and for a specified number of days after each dose. For subjective symptoms (for example, fatigue and myalgia) a simple scoring system should be included in the diaries to allow for the grading of severity.

Any self-administered treatments used to address signs or symptoms (such as antipyretic and analgesic medicines) and any contact with – or treatment administered by – a health-care professional should be captured. Instructions on the use of antipyretics and analgesics should be stated in the clinical trial protocol. If at the time of each dose a supply of a specific antipyretic or analgesic was provided for use as needed, or as instructed in accordance with the protocol, the post-dose usage recorded should be checked against returned supplies. If prior safety data suggest that pre-vaccination antipyretic use is appropriate then this can be administered and recorded by trial staff at the vaccination visit.

At each trial visit, whether involving face-to-face or telephone contact between the trial subject/caregiver and site staff, all diary cards completed by vaccinees or caregivers should be checked for level of completion and further instructions given as needed to improve data recording after the next dose is given. At face-to-face visits the prior vaccination site(s) should be inspected for any remaining signs such as induration. Trial subjects or caregivers should also be asked about the maximum extent of signs (for example, to determine whether whole limb swelling occurred). Any unresolved local or systemic signs and symptoms should be recorded and action taken as appropriate.

7.2.2.3 Unsolicited adverse events

Trial subjects/caregivers should be questioned at each visit on the occurrence of any AEs since the last visit or for predefined periods following the last dose. For each AE, the timing of onset in relation to vaccination should be captured, as should any consultation with a health-care professional, whether hospitalization occurred and any treatment that was given (prescribed or non-prescribed). If the AE is not already resolved there should be further follow-up to document the outcome.

It may be useful to pose specific questions to trial subjects/caregivers at each visit to ensure that certain AEs or AESIs are captured in a systematic fashion – for example, to determine whether persistent inconsolable crying or hypotonic-hyporesponsive episodes occurred in infants. Where well-established and widely applied definitions of these and other AEs are available, they should be included in the protocol.

For all AEs that meet the criteria for classification as SAEs there should be careful documentation of dates of onset, underlying conditions and concomitant medications, and adequate follow-up to record the outcomes.

7.2.2.4 Other investigations

The collection of data on routine laboratory tests (haematology, chemistry and urine analysis) is not necessary in many clinical trials of vaccines. If the sponsor or NRA considers that there is a good rationale for obtaining such data then the protocol should specify the tests to be performed at certain time points. The tests should be conducted in appropriately certified laboratories and results reported using well-established grading scales for laboratory abnormalities.

For vaccines that contain live organisms (including attenuated wild-types, organisms that have been genetically engineered to render them non-virulent and/or non-replicative, and live viral vector vaccines) additional investigations related to safety may include the detection of viraemia and assessments of shedding (quantity and duration) unless the omission of such studies can be justified (for example, on the basis of prior experience with the same or very similar strains and/or nonclinical data). Organisms recovered from vaccinees may also be subject to genetic analyses to determine any instances of recombination with wild-types and reversion to virulence and/or replication competency.

The release specifications for vaccines should take into account the safety profile documented for the highest amount(s) of antigen(s) that have been administered in the clinical trials. It may be necessary to support the final proposed release specification by conducting a trial with the primary objective of comparing safety between formulations that contain different numbers of live organisms or amounts of antigen(s).

7.2.3 Categorization of adverse events

7.2.3.1 Causality

Section 8.5 of the WHO *Global manual on surveillance of adverse events following immunization* (*23*) recommends that in clinical trials the investigator should make a judgement on relatedness to vaccination for all solicited signs and symptoms, and unsolicited AEs. The sponsor may have access to additional information that is not available to investigators and should assess causality for all SAEs. The assessment of relatedness to vaccination should take into account factors such as:

- plausibility of relatedness, taking into account the vaccine construct (for example, live-attenuated vaccines may be associated with modified manifestations of natural infection, such as rashes);

- timing in relation to dosing (while most vaccine-related AEs occur within 1–2 weeks of the dose, there may reasons to suspect that illnesses with onset many months after the last dose could be related to prior vaccination);
- concurrent illnesses, vaccines or other medications;
- the frequency with which any one AE occurred in groups that received the candidate vaccine compared to groups that received another vaccine or placebo;
- any correlation between rates of any one AE and dose of antigenic components;
- changes in rates of any one AE with sequential doses;
- the results of medical investigations (for example, diagnostic tests for concurrent illnesses) and of autopsies (for example, in cases of sudden infant death).

7.2.3.2 Severity

Sufficient data should be collected for each solicited sign and symptom and unsolicited AE in order to assess severity. Wherever possible, widely used grading scales (including scales that may be age specific) should be used. The same scales should be applied throughout the clinical development programme.

7.2.3.3 Other categorization

The classification of AEs as serious and the categorization of frequencies (that is, very common, common, uncommon, rare and very rare) should follow internationally accepted conventions, as described in section 3.1.2 of the WHO *Global manual on surveillance of adverse events following immunization* (*23*). Frequencies of solicited signs and symptoms by subject and of AEs in each treatment group should be calculated on the basis of the denominator of all vaccinated subjects in that group. Calculation of the frequencies of solicited signs and symptoms after each dose should use as the denominator the number of subjects who received each dose.

7.2.4 Adverse event reporting rates within and between trials

During any clinical development programme the reporting rates in clinical trials for all AEs and/or for specific types of AEs, whether solicited or unsolicited, may demonstrate the following:

- Differences between candidate vaccines and control groups within a clinical trial. For example, differences in AE rates may be anticipated between a candidate vaccine group and a placebo group or a

group that receives a licensed vaccine that does not have a similar composition to the candidate vaccine. Any marked differences between a candidate vaccine and a licensed vaccine that has the same or very similar composition are generally not anticipated and may require further investigation.

- Differences between clinical trials that may be observed in one or both of the candidate vaccine and control groups for total or specific AE reporting rates. It is important to consider possible explanations, taking into account whether or not the same effect on the pattern of reporting rates was observed in groups that received candidate vaccines and licensed vaccines and whether the study was double-blind or open-label. There may be real and anticipated differences in vaccine reactogenicity between trial populations (for example, age-related differences for specific AEs, such as higher fever rates in trials conducted in infants and toddlers compared to trials in older children and adults). When there is no clear explanation for the differences observed, further investigation is merited. For example, there may have been incomplete reporting of AEs or data-entry errors, as well as cultural factors that lead to a greater reluctance to report side-effects in some regions.

7.3 Size of the pre-licensure safety database

The size of the pre-licensure safety database must be considered on a case-by-case basis and agreed with relevant NRAs. It is not possible to predefine a minimum number of exposed subjects (usually confined to the number exposed to the final dose and regimen appropriate for their age group and who received the final vaccine formulation) that can be generally applied across vaccine development programmes.

When considering the pre-licensure safety database the need for a sufficient sample size to estimate AE rates with precision is an important factor. For example, a total database of 3000 subjects across all trials and populations provides a 95% chance of observing one instance of an AE that occurs on average in 1 in 1000 subjects.[1] Nevertheless, this figure should not be assumed to be appropriate in all settings. In particular, this figure should not be applied to application dossiers for any type of new candidate vaccine without further consideration. When considering the size of the pre-licensure safety database, factors to take into account include, but are not limited to, the following:

[1] The number that would provide a 95% chance of observing one instance of an AE that occurs on average in 1 in 10 000 subjects is 30 000.

- Fewer than 3000 subjects may be acceptable if the new candidate vaccine consists only of antigenic components that are already licensed in other vaccines with which there is considerable experience in routine use. The method of manufacture should also be taken into account.
- For specific types of vaccines (for example, new constructs or new adjuvants) or specific modes of use (for example, in a population considered to be vulnerable or otherwise at high risk that could predispose it to certain AEs) individual NRAs may require that considerably more than 3000 subjects are exposed prior to licensure.
- Additional considerations may apply to vaccines that contain antigenic components not previously used in human vaccines but for which efficacy trials are not possible. For example, the safety profile documented in the preliminary trials may lead to reluctance to expose large numbers of subjects unnecessarily in the absence of an immediate threat and/or to expose large numbers in particular population subsets.
- The acceptable size of the pre-licensure safety database should take into account the actual safety profile observed in the clinical trials. If there is concern regarding the occurrence and/or severity of a particular AE and the available safety data do not allow for a clear assessment of risk then, depending on the perceived benefit of the vaccine, it may be appropriate to conduct further pre-licensure trials and/or to conduct a post-licensure safety study to better estimate the risk.

The total number of subjects exposed in clinical trials may cover many age subgroups, or a single age group may predominate. In general there should be adequate representation of all target age groups in the total safety database. In some cases, and depending on the actual safety profile, it may be acceptable for the majority of subjects included in the safety database to come from a specific age range.

7.4 Post-licensure safety surveillance

The main purpose of post-licensure safety surveillance is to detect AEs that occur too infrequently for detection in pre-licensure clinical trials.

The requirements of individual NRAs for reporting safety data collected from post-licensure safety surveillance activities should be consulted along with other guidance such as ICH E2E. NRAs should provide publicly available guidance regarding their requirements for the content and timing of periodic reports of safety data and for any expedited reporting considered necessary.

Licence holders should demonstrate that they have adequate capability and appropriate staff to collect, interpret and act upon the safety data received. It is important that efforts are made to accurately identify the vaccine(s) and lot number(s) associated with each AEFI report.

It has become routine at the time of licensure for detailed proposals to be in place for post-licensure safety surveillance activities, often in the form of risk-management plans. These documents and proposals are then routinely updated at intervals in line with additional data that become available. The plans usually outline the safety specification for the vaccine on the basis of all available safety data at the time of submitting each version of the plan, along with details of routine and proposed additional pharmacovigilance and risk-minimization activities.

When planning pharmacovigilance activities for a vaccine it is important to take into account that, in addition to routine pharmacovigilance (that is, passive surveillance), important information may come from other sources, namely:

- Data from active safety surveillance, which may be put in place by public health bodies when a vaccine is introduced into a national routine immunization programme, or when the use of a vaccine within a programme changes significantly (for example, an entirely different age group is vaccinated for the first time). Active surveillance seeks to ascertain completely the number of AEs in persons given a dose of a vaccine using a pre-organized process. It may involve reviewing medical records or interviewing patients and/or physicians in a sample of sentinel sites to ensure that complete and accurate data are collected on reported AEs from those sites.
- Large databases that link information on vaccination history in patient records with the occurrence of specific types of illness. These databases can be searched to explore links between specific vaccines and safety issues in the short and longer term.
- Various types of registries intended to capture details of vaccine use in specific populations. For example, some registries collect information on exposure of pregnant women to various types of vaccines and indicate the outcome of the pregnancy (including rates of spontaneous abortion, premature delivery and congenital malformations in infants).

The limitations of each of these approaches are well known. Furthermore, access to information from these other sources varies greatly between countries. These factors underline the need to consider safety data from all sources along with data that may come from post-licensure trials.

An additional consideration for vaccines is that when a safety signal is identified for any one vaccine it may or may not be possible to ascribe the AEFIs observed to any one antigenic component of the vaccine or to an adjuvant. Furthermore, if there was concomitant administration of vaccines in some or all cases generating the signal, it may not be possible to ascribe the AEFI to only one of the products co-administered. The same or very similar antigenic component(s) or adjuvant in the vaccine(s) from which the signal arose may be present in several other licensed products marketed worldwide. Ultimately, several different licence holders and NRAs without established data-sharing agreements may need to be involved. As a result, the actions taken, if any, and the speed at which action is taken are sometimes very variable between countries. Such issues underscore the need for the efficient use of electronic databases to facilitate rapid data sharing.

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The second draft was prepared by a WHO drafting group, taking into account comments received from: Dr B. Fritzell, BFL Conseils, France; Dr G. Chen, National Institutes of Health, the USA; Dr G. Coleman, Health Canada, Canada; Dr Z. Kusynová, The Hague, the Netherlands (*provided the consolidated comments of the International Pharmaceutical Federation*); Dr M. Nijs, GlaxoSmithKline Vaccines, Belgium; Clinical team of Novilia Sjafri and PT Bio Farma, Indonesia; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr I. Uhnoo, Uppsala Universitet, Sweden; Dr T. Yamaguchi, Pharmaceuticals and Medical Devices Agency, Japan; and Dr K. Zoon, National Institutes of Health, the USA.

The draft document was then posted on the WHO Biologicals website for a second round of public consultation from 1 February to 15 March 2016. Comments were received from: Dr B. Brock, Sanofi Pasteur, the USA (*provided the consolidated comments of the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA)*); Dr K. Farizo, United States Food and Drug Administration, Center for Biologics Evaluation and Research, the USA; Dr C. Meric, Lausanne University Hospital, Switzerland; Mr J.F. Modlin, Bill & Melinda Gates Foundation, the USA; Dr D. Pratt, United States Food and Drug Administration, Center for Biologics Evaluation and Research, the USA; Dr A. Rinfret, Health Canada, Canada; and Dr K. Sohn, Ministry of Food and Drug Safety, Republic of Korea.

A WHO meeting of the Working Group on clinical evaluation of vaccines was then held in Geneva, Switzerland, 3 May 2016 and was attended by the following participants: Dr G. Coleman, Health Canada, Canada; Dr M. Darko, Food and Drugs Authority, Ghana; Dr D. Etuko, National Drug Authority, Uganda; Dr E. Griffiths, Consultant, Kingston-upon-Thames, England; Dr S. Kennedy, University of Liberia, Liberia; Dr J. McEwen, Therapeutic Goods Administration, Australia; Dr M. Powell, Medicines and Healthcare products Regulatory Agency, England; Dr R. Sheets, Consultant, Silver Spring (MD), the USA; Dr J. Southern, Medicines Control Council, South Africa; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr K. Zoon, National Institutes of Health, the USA; and Dr I. Knezevic, World Health Organization, Switzerland.

Based on the comments received during the public consultation and on the discussions of the above Working Group meeting, the document WHO/BS/2016.2287 was prepared by the above-mentioned WHO drafting group.

The document was then posted on the WHO Biologicals website for a third round of public consultation from 27 July to 16 September 2016 and comments received from: Dr B. Brock, Sanofi Pasteur, the USA (provided the consolidated comments of the IFPMA); Dr M. Cavaleri, European Medicines Agency, England; Dr G. Coleman, Health Canada, Canada; Dr D. Kim and Dr M. Jin, Ministry of Food and Drug Safety, Republic of Korea; Dr A.W. Lee, Dr A. Sitlani and Dr W. Straus, Merck & Co., the USA; Dr T. Lu, Therapeutic Goods Administration, Australia; Dr S.A. Nishioka, Ministry of Health, Brazil; Office of International Affairs, Instituto Nacional de Vigilancia de Medicamento, Colombia; Dr S-C Shin, Green Cross Corporation, Republic of Korea; and Dr Y. Sun, Paul-Ehrlich-Institut, Germany. Dr Noni MacDonald, DalHousie University, Halifax, Canada and Dr Anna Taddio, University of Toronto, Toronto, Canada, provided comments on the safety evaluation, particularly on the pain of injection.

Further changes were subsequently made to document WHO/BS/2016.2287 by the WHO Expert Committee on Biological Standardization.

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