Annex 6

Recommendations to assure the quality, safety and efficacy of DT-based combined vaccines

Replacement of Annex 2 of WHO Technical Report Series, No. 800

Recommendations published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Recommendations be made only on condition that modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the Recommendations set out below. The parts of each section printed in small type are comments or examples intended to provide additional guidance to manufacturers and NRAs.

Introduction

A combined vaccine may be defined as a vaccine that consists of two or more antigens, either combined by the manufacturer or mixed immediately before administration, that is intended to protect either against more than one infectious disease or against an infectious disease caused by different types or serotypes of the same organism.

Combined vaccines that allow simultaneous administration of diphtheria (D) and tetanus (T) toxoids with several other antigens have been in use since the middle of the 20th century. Some of the earliest DT-based combined vaccines included inactivated poliomyelitis vaccines (IPV) or whole-cell pertussis vaccine (wP), or both. These were followed by combinations with various acellular pertussis antigens (aP), which were used as an alternative to DTwP, and with combinations that included the addition of one or more of the *Haemophilus influenzae* type b (Hib) conjugates – Hib(conj) – and hepatitis B (HepB) surface antigen (HBsAg).

There are many DTwP-based and DTaP-based combined vaccines available worldwide that vary in the amounts of each antigen and the range of antigens according to the intended age range for use (i.e. infants, toddlers, older children, adolescents or adults). There are also DT-based vaccines available without pertussis components, some of which contain other antigens such as IPV. Hence, DT-based combined vaccines commonly include antigens derived from both bacteria and viruses. The most complex vaccines approved in some countries include DTaP, IPV, HBsAg and Hib(conj), but it is quite possible that more extensive combined vaccines may be developed in future (e.g. containing conjugated meningococcal polysaccharides).

The WHO Requirements for diphtheria, tetanus, pertussis and combined vaccines (*1*) incorporated guidance on the individual components of these vaccines (e.g. diphtheria vaccine, tetanus vaccine and whole-cell pertussis vaccine). In addition, a separate section on the manufacture of combined vaccines containing more than one of the individual vaccines (e.g. DT and DTwP) was included; this guidance commences at the stage of the final bulk vaccine and considers the specifics of manufacture of the final combined product. However, the section on the requirements for combined vaccines (adsorbed) stated that no attempt had been "made to include other combinations, including those with *Haemophilus influenzae* and *Neisseria meningitidis* polysaccharides and poliomyelitis vaccines". In addition, it is now WHO policy to include in new recommendations guidance on the nonclinical and clinical aspects of vaccine development. The 1990 WHO Requirements for diphtheria, tetanus, pertussis and combined vaccines (*1*) do not contain such guidance.

Since the publication of the first Requirements for diphtheria, tetanus, pertussis and combined vaccines (*1*), new and revised WHO recommendations

on single vaccines that are directly relevant to DT-based combined vaccines have been established or are under development. Published documents include:

- Recommendations to assure the quality, safety and efficacy of diphtheria vaccines (*2*);
- Recommendations to assure the quality, safety and efficacy of tetanus vaccines (*3*);
- Recommendations for whole-cell pertussis vaccine (*4*);
- Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines (*5*);
- Recommendations to assure the quality, safety and efficacy of recombinant hepatitis B vaccines (*6*);
- Recommendations for the production and control of poliomyelitis vaccine (inactivated) (*7*);
- Recommendations for the production and control of *Haemophilus influenzae* type b conjugate vaccines (*8*);
- Recommendations for diphtheria, tetanus, pertussis and combined vaccines (Amendments 2003) (*9*);
- the *WHO Manual for quality control of diphtheria, tetanus and pertussis vaccines* (*10*).

WHO convened two meetings on the revision of its Recommendations to assure the quality, safety and efficacy of diphtheria vaccines, tetanus vaccines and DT-based combined vaccines. The first meeting was held in Geneva, Switzerland, from 21–22 June 2011, and the second was held in Beijing, China, from 7–11 November 2011. At these meetings, scientific experts, regulatory professionals and other stakeholders met to develop the revisions. The recommendations in this document are intended to provide background and guidance to national regulatory authorities (NRAs) and vaccine manufacturers on the production, quality control and evaluation of the safety and efficacy of diphtheria vaccines, tetanus vaccines and DT-based combined vaccines. Part A of this document sets out the guidance on the manufacture and quality assessment of DT-based combined vaccines. Guidance specific to the nonclinical evaluation of DT-based combined vaccines is provided in Part B; and guidance on the clinical evaluation of these vaccines is contained in Part C. This document should be read in conjunction with all relevant WHO guidelines, including those on the nonclinical (*11*) and clinical evaluation (*12*) of vaccines. This guidance is based on experience with the products developed so far, as described below, and may need to be updated in response to future developments.

Taking into account the history of guideline development, it was considered appropriate to replace Annex 2 of WHO Technical Report Series, No. 800 (*1*) with a revised version in order to take into account the developments that have occurred since 1990. The main changes introduced in this revision are:

- a change of title from Requirements to Recommendations;
- the abbreviations for DT-based combined vaccines and their components;
- the replacement of self-standing sections on the components of DT-based combined vaccines with references to the recommendations for individual vaccines wherever relevant;
- the inclusion of considerations for all DT-based combined vaccines that had been developed at the time the revision was drafted (e.g. combined vaccines that include HBsAg, IPV and Hib(conj);
- the inclusion of new sections on the clinical and nonclinical evaluation of DT-based combined vaccines;
- a revision of the model protocol for the information to be provided for the lot release of DT-based combined vaccines.

Scope of the Recommendations

The scope of this document is to provide guidance on the data needed to ensure the quality, safety and efficacy of DT-based combined vaccines. These Recommendations mention a number of combined vaccines as examples. The principles expressed in this document may also apply to combined vaccines that are not explicitly mentioned.

General considerations

In addition to WHO recommendations for an individual component vaccine of a combined vaccine, the following considerations are specific to combined vaccines.

Vaccines that can offer protection against several infectious diseases allow for the simplification of vaccination programmes, improved acceptance by parents and vaccinees, and increased vaccine coverage. However, the development, production, control and use of combined vaccines present a number of challenges to both manufacturers and NRAs, as well as national control laboratories (NCLs).

Important issues for ensuring the quality of DT-based combined vaccines include:

- the development of optimal formulations (including the choice of compatible adjuvants) and formulation conditions that lead to vaccines of adequate immunogenicity, acceptable reactogenicity, and stability, and that are appropriate for the intended use;
- the applicability of testing methods originally established for monocomponent vaccines;
- the suitability of using monocomponent reference materials in evaluating combined vaccines;
- the corresponding release and stability criteria.

Specific issues regarding the suitability of combined vaccines intended for prequalification – and therefore critical to the WHO Programmatic Suitability for Prequalification Standing Committee (*13*) and the Immunization Practices Advisory Committee (*14*) – include the use of the appropriate vaccine-vial monitor, the choice of effective antimicrobial preservatives for multiple-dose presentations in relation to the open-vial policy, and the need to demonstrate adequate in-use stability.

The nonclinical programme for the development of a new DT-based combined vaccine should follow the general guidance (*11*), but particular attention should be given to the choice of animal models used for the assessment of the clinical immunogenicity, efficacy and reactogenicity of the final product.

Important features of the clinical development programme include the assessment of the reactogenicity that results from the administration of multiple antigens simultaneously and via a single injection site, and the potential for a clinically important reduction in the immune response to one or more antigens when delivered in the combined product compared with the delivery of separate vaccines or administration in less complex vaccines. For example, the inclusion of a conjugated polysaccharide in a combined vaccine has sometimes been associated with lower antibody levels when compared with separate injections that are co-administered or separated in time. In addition, immunological interference resulting in a lower antibody response to a conjugate antigen may arise when more than one conjugate is included in the same DT-based combined vaccine or when conjugate-containing combined vaccine is co-administered with other conjugate vaccines (*15*, *16*).

In addition, established vaccination schedules may have to be adapted to the simultaneous administration of several antigens, and the potential effect of delivering concomitant vaccinations on other vaccines (including those in the schedule for the Expanded Programme on Immunization) has to be taken into consideration.

On the other hand, extensive experience with licensed and WHO-prequalified DT-based combined vaccines has demonstrated that the above

concerns can be overcome, and that safe and effective combined vaccines can be developed.

In the process of drafting this document it was considered that, at least up to the stage of the production of purified bulk antigens, the quality aspects would be identical for monocomponent and combined vaccines. In addition, it was felt that many issues regarding the production of the formulated final bulk and the final lot, and some of the issues for the nonclinical and clinical programmes, would be similar for monocomponent and combined vaccines. Therefore, these Recommendations are – wherever possible and relevant – limited to the production and development of DT-based combined vaccines, with appropriate reference made to the corresponding WHO recommendations for the individual component vaccines.

Terminology

Definitions for some common terms used throughout this document are given below. They may have different meanings in other contexts.

Adverse event: any untoward medical occurrence affecting a participant in a clinical trial to whom a vaccine has been administered. The occurrence may not necessarily have a causal relationship with the vaccine or vaccination (*12*).

Adverse reaction: a response to a vaccine that is noxious and unintended, and that occurs at doses tested in humans for prophylaxis or during subsequent clinical use following licensure. The term "adverse reaction" is usually reserved for a true causal association with a medicine or a vaccine (*12*).

Booster vaccination: a vaccination given at a certain time interval (at least six months) after primary vaccination in order to induce long-term protection (*12*).

Bulk: processed purified material, prepared from either a single harvest or a pool of single harvests. It is the parent material from which the final bulk is prepared.

Combined vaccine: a vaccine that consists of two or more antigens, either combined by the manufacturer or mixed immediately before administration, that is intended to protect either against more than one disease or against one disease caused by different strains or serotypes of the same organism (*11*).

Comparator vaccine: an approved vaccine with established efficacy or effectiveness, or with traceability to a vaccine with established efficacy or effectiveness, that is tested in parallel with an experimental vaccine to serve as an active control during nonclinical or clinical testing (*5*). Examples of comparator vaccines that can be used in studies of combination vaccines can be found in Table 6.1 in section C.2.2.

Final bulk: the homogeneous final vaccine present in a single container from which the final containers are filled either directly or through one or more intermediate containers.

Final lot: a collection of sealed final containers that is homogeneous in all respects. In principle, a final lot must have been filled from a single final bulk container and processed further (e.g. freeze-dried) in one continuous working session. Different final lots may be filled or processed from the same final bulk in different working sessions. These related final lots are sometimes referred to as sub-lots, filling lots or freeze-drying lots, and should be identifiable by a distinctive final lot number.

Functional antibody: an antibody that binds to an antigen and has a biological effect (e.g. toxin neutralization, viral inactivation, opsonic or bactericidal activity, or whole-cell agglutination) that can be demonstrated by laboratory testing.

Immunogenicity: the capacity of a vaccine to induce antibody-mediated or cell-mediated immunity, or immunological memory, or some combination of these (*12*).

Noninferiority margin or limit: a prespecified limit based on an appropriate confidence interval. Meeting this criterion may exclude a prespecified difference in immune response believed to be clinically meaningful.

Noninferiority trial: a trial that has the primary objective of showing that the response to a vaccine being investigated is not clinically inferior to the response to the comparator vaccine (*12*).

Primary end-points: the prespecified end-points that are considered most relevant for evaluating the outcome of a clinical trial (e.g. safety, efficacy or immunogenicity).

Primary vaccination: the first vaccination, or series of vaccinations, given within a predefined period, with an interval of less than six months between doses, to induce clinical protection (*12*).

Reactogenicity: reactions, either local or systemic, that are considered to have a causal relationship to vaccination (*12*).

Secondary end-points: prespecified end-points that are considered in addition to the primary end-points when evaluating the outcomes of a clinical trial.

Seroconversion: a predefined increase in antibody concentration that is considered to correlate with the transition from seronegative to seropositive and that provides information about the immunogenicity of a vaccine. If there are pre-existing antibodies, seroconversion is defined by a transition from a predefined low level to a significantly higher level, such as a four-fold increase in geometric mean antibody concentration (*12*).

Vaccine effectiveness: the protection rate conferred by vaccination in a specified population. Vaccine effectiveness measures both direct protection and indirect protection (i.e. protection of unvaccinated persons by the vaccinated

population). Vaccine effectiveness is also determined following the introduction of a vaccine in a population by measuring vaccination coverage, how well the vaccine strains correlate with circulating strains, and the incidence of disease caused by strains not included in the vaccine (*12*).

Vaccine efficacy: the reduction in the chance or odds of developing clinical disease after vaccination relative to the chance or odds when unvaccinated. Vaccine efficacy measures direct protection (i.e. protection induced by vaccination in the vaccinated population) (*12*).

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International names, proper names and abbreviations

The international names and abbreviations of combined vaccines should follow the examples in Appendix 3. Other combined vaccines exist or may be developed in the future – e.g. combinations with meningitis antigens added – and their international names should follow the structure of the examples. The proper name should be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the recommendations formulated below.

A.1.2 Descriptive definition

The descriptive definition of a combined vaccine should be based on the definitions stated in section A.1.2 of the recommendations for the individual vaccines; for example:

- DTwP-HepB is a combined vaccine composed of diphtheria toxoid, tetanus toxoid, whole-cell pertussis suspension and purified hepatitis B surface antigen presented with a suitable adjuvant (e.g. aluminium salts);
- DTaP-HepB-IPV-Hib$_X$ or DTaP-HepB-IPV+Hib$_X$ is a combined vaccine composed of diphtheria toxoid, tetanus toxoid, acellular pertussis components, purified hepatitis B surface antigen, inactivated poliomyelitis antigens and *Haemophilus influenzae* type b (X-) conjugate presented with a suitable adjuvant (e.g. aluminium salts). The product may be a mixture of all components or may be presented with the *Haemophilus influenzae* component in a separate container, the contents of which are mixed with the other components immediately before use.

A.1.3 International reference materials

There are no international standards or international reference preparations specifically designed for combined vaccines. For reference materials from WHO that may be used in laboratory or clinical evaluations of combined vaccines, refer to section A.1.3 of WHO recommendations for the individual vaccine. The WHO catalogue of international reference preparations[1] should be consulted for the latest list of appropriate standards and reference materials. The use of stable, monocomponent international, regional and national reference materials that have been calibrated against the international standard for assaying the potency of combined vaccines serves as the primary consideration; it has practical advantages and should be used whenever possible. The suitability of this approach should be carefully evaluated on a case by case basis for combined vaccines since it has been shown that, in some cases, qualitative differences in antigen or excipient composition, or both, between a monovalent reference preparation and a combined vaccine that is being tested may result in invalid test results (e.g. deviations from parallelism of the dose–response curves) or excessive variability within and between assays and between laboratories (*17–21*), or a combination of these. The suitability of using monovalent reference preparations is of particular significance for the NRA in terms of lot release. Therefore, in some laboratories, combined vaccines that have a composition that is close to that of the combined vaccine being tested have been used successfully as in-house reference material after suitable calibration of the components to the international standard, where this exists. Also, in some cases the need for a product-specific reference vaccine has been evident during specific toxicity monitoring of acellular pertussis vaccines in mice. Such in-house or homologous reference materials should be stable and should preferably have shown satisfactory performance in clinical trials, or should have the same composition and production process as a vaccine lot previously shown to have satisfactory performance in clinical trials. Appropriate procedures should be operational during the licensing process or to give official status to such process-specific or product-specific reference materials. This can be done through collaborative studies carried out by manufacturers and NCLs to assess the suitability and behaviour of such reference materials. Where calibration of such references in International Units (IUs) is not possible, the specifications stating the acceptable limits for the relevant tests and the conditions for their validity should be determined and validated by the individual manufacturers and approved by the NRA. Reference is made to the *WHO manual for the establishment of national and other secondary standards for vaccines* (*22*).

[1] See: http://www.who.int/bloodproducts/catalogue/en/index.html

A.2 General manufacturing recommendations

The general manufacturing requirements described in WHO good manufacturing practices for pharmaceutical products: main principles (*23*) and Good manufacturing practices for biological products (*24*) apply to the establishment of facilities that manufacture combined vaccines. Reference is made to the recommendations for any individual vaccine-specific additions.

A written description of procedures for preparing and testing a combined vaccine, together with appropriate evidence that each production step has been validated, should be submitted to the NRA for approval. Proposals for modifying the manufacturing process or quality control methods should also be submitted to the NRA for approval before such modifications are implemented.

A.3 Control of vaccine production

For all production stages, production control should be carried out in accordance with the corresponding sections and subsections of WHO recommendations for the individual vaccines. In addition, the following considerations apply for combined vaccines.

A.3.1 Control of the final bulk

It should be noted that, in general, formulation conditions that have been established as optimal for monocomponent vaccines may not be optimal for some combined vaccines. Important considerations include the choice and concentration of any preservative agent or adjuvant and their optimal ratio to the antigen(s), pH and ionic strength. Formulation conditions should be validated to ensure optimal clinical immunogenicity, reactogenicity and stability of the vaccine.

For vaccines containing a Hib vaccine, two types of formulation have been developed: vaccines with all components in the same container (known as fully liquid or all in one) and those with the Hib component in a separate container (known as a "lyo-liquid"). The specific testing conditions and issues for these two types differ, as described in the relevant sections on testing provided in this annex.

A.3.1.1 Preparation

The final bulk is prepared by blending all components of the combined vaccine. Suitable antimicrobial preservatives may be added. With the approval of the NRA, one or several component vaccine bulk materials may be adsorbed to or mixed with an adjuvant at an acceptable concentration prior to blending into the final vaccine bulk (these intermediates have been called preadsorbed bulks).

Such intermediates may be kept at validated storage temperatures for validated storage times. For the stability aspects of such intermediates, reference is made to the WHO Guidelines on stability evaluation of vaccines (*25*).

A.3.1.2 Preservatives

If the vaccine is to be dispensed into multidose containers, a suitable antimicrobial preservative should be added. The amount of preservative in the final bulk should have been shown to have no deleterious effect on any of the vaccine's components, and to cause no unexpected adverse reactions in humans. The preservative and the concentration used should be approved by the NRA. Certain antimicrobial preservatives, particularly those of the phenolic type, have been shown to adversely affect the antigenic activity of tetanus and diphtheria vaccines, and are not recommended for use in combined vaccines that fall within the scope of these Recommendations. Similarly, thiomersal is known to adversely affect the antigenic activity of IPV (*26*). In some vaccines, 2-phenoxyethanol has been shown to be a suitable alternative, but its compatibility with the antigens in the combined vaccine should be evaluated on a case by case basis. For the prequalification of multidose presentations, programmatic issues, such as the compatibility of the formulation with an open-vial policy, and the need for in-use stability data, should be taken into account (*25*, *27*).

A.3.1.3 Adjuvants

The use of an adjuvant should be carefully evaluated to determine its effect on the safety, immunogenicity and efficacy of the combined vaccine. If adjuvants are used, their concentration and quality characteristics, demonstrating their suitability as an adjuvant and their compatibility with the component vaccines in the combined vaccine being considered, should be approved by the NRA.

> Aluminium compounds are generally used as mineral carriers. The quality characteristics of aluminium hydroxide, hydrated, for adsorption have been described in the European Pharmacopoeia (*28*).

It should be noted that the concentration of aluminium may be higher in combined vaccines than in monocomponent vaccines due to the contribution of the individual preadsorbed component bulks during blending. The final bulk may also contain a mixture of adjuvants from the individual preadsorbed bulks. When aluminium compounds are used as adjuvants, the concentration of aluminium should not exceed 1.25 mg per single human dose (SHD). For combined vaccines, it is important to determine the degree of adsorption of each of the antigens as parameters for consistency, release and stability.

> In some countries, upper limits for the concentration of mineral carriers are set at lower amounts (i.e. less than half) than that given above.

The formulation should be such that the vaccine remains suspended after shaking for a time that is considered adequate to allow a representative sample to be withdrawn from the container.

A.3.1.4 Consistency

The consistency of a combined vaccine should be evaluated at several stages (*29*). At the bulk antigen stage there should be at least three consecutive batches of each component – e.g. a new DTw/aP-HepB combination should be from D_1, D_2, D_3 plus T_1, T_2, T_3 plus w/aP_1, w/aP_2, w/aP_3 plus $HepB_1$, $HepB_2$ and $HepB_3$. At the level of the formulated final bulk, the combination would be D_1T_1w/aP_1HepB_1, D_2T_2w/aP_2HepB_2, and D_3T_3w/aP_3HepB_3.

When a new vaccine is added (e.g. IPV) to an established, licensed combined vaccine (e.g. DTw/aP-HepB), and after demonstration of the consistency of the new vaccine at the level of the bulk antigen (if its production is new to the particular manufacturer), consistency is demonstrated as: D_1T_1w/aP_1IPV_1, D_1T_1w/aP_1IPV_2 and D_1T_1w/aP_1IPV_3.

A.3.2 Control tests on the final bulk

Each final bulk of the combined vaccine should be tested for sterility, the potency of each component vaccine and specific toxicity in accordance with the individual recommendations for each of the component vaccines. In general, the testing described in the corresponding sections of WHO recommendations for the individual vaccines is applicable to combined vaccines. Reference is also made to the WHO *Manual for quality control of diphtheria, tetanus and pertussis vaccines* (*10*). A number of issues that are specific to combined vaccines are described below.

Multiple-dilution in vivo potency testing of combined vaccines requires a considerable number of laboratory animals. A significant reduction in the use of laboratory animals could be achieved through the development and use of simplified in vivo models (e.g. single-dilution models) and particularly through those that would allow for the concurrent serological testing of multiple components (e.g. concurrent testing of purified pertussis antigens and diphtheria and tetanus toxoids) (*30–34*). A laboratory that intends to introduce an alternative method should perform adequate validation studies to enable comparisons to be made with the multiple-dilution in vivo model (*32–34*).

A.3.2.1 Diphtheria potency testing

In general, potency values determined by a test in guinea-pigs, as described in the WHO Recommendations to assure the quality, safety and efficacy of diphtheria vaccines (*2*), are significantly lower in the absence of a whole-cell pertussis

component than the values found in vaccines containing this component. This may hamper the implementation of a single-dilution assay for combined vaccines that do not contain a whole-cell pertussis component. In general, if the true potency of a vaccine antigen is close to the minimum required specification, a single-dilution model often gives inconclusive results.

In contrast, diphtheria potency values for all-in-one liquid vaccine or reconstituted combined vaccines with a Hib component produced with CRM197 as a carrier tend to be higher than the values observed for vaccines with a Hib component produced with a different carrier, and a single-dilution model may successfully replace the multiple-dilution model. In this case, a minimum specification of 30 IU/SHD is not useful for monitoring consistency since the observed potency estimates are always higher. Therefore, in addition to setting a minimum potency specification of 30 IU/SHD for vaccines used to immunize children, manufacturers, with NRA approval, should also set lower consistency limits and upper consistency limits that reflect the potency values found in practice for combined vaccines that have been demonstrated to be safe and effective in the clinical setting. Manufacturers and the NRA must closely monitor such limits and the trends in key consistency data (*35*).

A.3.2.2 Tetanus potency testing

Similar to diphtheria toxoid, potency values for tetanus toxoid determined by the tests described in the WHO Recommendations to assure the quality, safety and efficacy of tetanus vaccines (adsorbed) (*3*) are significantly higher in the presence of a wP component and in the presence of a Hib component produced with a tetanus toxoid carrier than the values found in the absence of such components, particularly when assayed in mice. In such cases, a minimum specification of 40 IU/SHD, or 60 IU/SHD for vaccines containing wP when assayed in mice, are not useful for monitoring consistency since the observed potency estimates are always much higher. Therefore, in addition to setting a minimum potency specification of 40 IU/SHD for vaccines used for the primary immunization of children (or 60 IU for vaccines containing wP when assayed in mice), manufacturers, with NRA approval, should also set lower consistency limits and upper consistency limits that reflect the potency values found in practice for the combined vaccines that have been demonstrated to be safe and effective in the clinical setting. Manufacturers and the NRA must closely monitor such limits and the trends in key consistency data (*35*).

A.3.2.3 Hepatitis B potency testing

In principle, in vitro assays can be used for combined vaccines as outlined in the WHO Recommendations to assure the quality, safety and efficacy of recombinant

hepatitis B vaccines (*6*). However, some in vitro assays have been shown to work less well for combined vaccines with a wP component. If that is the case, an in vivo assay may have to be used. Furthermore, in vivo potency estimates for the HepB component have been shown to be significantly higher in some combined vaccines (e.g. those containing wP) than in vaccines containing only the HepB component. Specifications should be set accordingly, and manufacturers should set lower consistency limits and upper consistency limits, all of which should be approved by the NRA, that reflect the potency values found in practice for the combined vaccine and that have been demonstrated to be safe and effective in the clinical setting. Manufacturers and the NRA must closely monitor such limits and the trends in key consistency data (*35*).

A.3.2.4 Potency-related tests on combined vaccines with a Hib component (full liquid or all-in-one formulations) or reconstituted lyo-liquid formulations

For some vaccines it has proved difficult to perform potency-related and stability-indicating testing of the Hib component (i.e. measuring total saccharide content, molecular weight distribution, free saccharide content and free carrier protein). Manufacturers are encouraged to develop a method that allows such tests to be performed on the formulated vaccine, including at the final-lot stage. If justified, performing such tests at the bulk conjugate stage may, with NRA approval, be considered acceptable. Animal models (e.g. mice, rats, rabbits or guinea-pigs) – although used less often for routine lot release – may be useful in characterizing the protective potency or immunogenicity, the consistency and, if needed, for monitoring stability.

For combined vaccines with a separate freeze-dried Hib component, the testing required by WHO recommendations for the individual vaccines may be performed on the separate containers as described for the combined vaccine (see section A.5).

A.3.2.5 Safety-related testing of aP components (residual activity of pertussis toxin and reversion to toxicity)

In the presence of aluminium-based adjuvants, the in vitro Chinese hamster ovary (CHO) cell-based assay may not be applicable for testing the formulated product and for some chemically detoxified antigens. In addition, the in vivo test may be sensitive to other components in the formulation rather than to any residual native pertussis toxin (PT) (e.g. aluminium-based adjuvants or IPV). Proper standardization of the in vivo test, and the development and introduction of alternative test methods, are strongly encouraged. Further information can be found in section A.3.4.2.5 of the WHO Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines (*5*).

A.3.2.6 wP potency tests

For some vaccines, the suppression of in vivo wP potency has been observed in the presence of an IPV component. However, for such vaccines, the minimum specifications stated in the Recommendations for whole-cell pertussis vaccine (*4*) are maintained (i.e. an estimated potency of at least 4.0 IU in the volume recommended for an SHD, and the lower fiducial limit – $P = 0.95$ – of the estimated potency of at least 2.0 IU).

A.3.2.7 Endotoxins

For monovalent wP vaccines, the Recommendations for whole-cell pertussis vaccine (*4*) state that since "there is no agreement as to what constitutes an acceptable level of endotoxin in whole-cell pertussis vaccines, monitoring of endotoxin level on a lot-to-lot basis is encouraged as a monitor of consistency of production". This statement also holds true for combined vaccines containing a wP component. For combined vaccines, the wP component is by far the major contributor to the final endotoxin content. In general, for each component in a combined vaccine, the content of bacterial endotoxins should be less than the limit approved for the particular vaccine and, in any case, for combined vaccines that do not contain a wP component, the contents should be such that the final vaccine as administered contains less than 100 IU/SHD.

A.4 Filling and containers

The requirements concerning filling and containers given in WHO good manufacturing practices for pharmaceutical products: main principles (*23*) and Good manufacturing practices for biological products (*24*) apply to vaccine filled in the final form.

Single-dose and multiple-dose containers may be used. Vaccine in multiple-dose containers should contain a suitable antimicrobial preservative.

A.5 Control of final product

Each final lot of the combined vaccine should be tested to assess the identity of each component, and the sterility, pyrogenicity or endotoxin content, adjuvant content, preservative content, the potency of each component and innocuity in accordance with the recommendations for each individual vaccine. In general, the methods described in the corresponding sections of WHO recommendations for each individual vaccine are applicable to combined vaccines. A number of issues that are specific to combined vaccines are described in section A.3.

For routine release testing purposes it should be noted that when a combined vaccine is composed of two separate preparations that need to be reconstituted with each other at the time of administration (i.e. lyo-liquid

formulations) the full approval of release testing carried out on each of the two preparations separately is deemed to be sufficient. Repeating the tests – particularly those such as potency testing, which involve animals – on the reconstituted combined vaccine is not required provided that during development duly validated studies demonstrating the compatibility of the two components following reconstitution have been conducted by the manufacturer, and that due consideration has been given to issues of batch consistency, batch size and the frequency of production. These studies must show that the component vaccines and the final reconstituted combination are sufficiently comparable in terms of quality, innocuity and immunogenicity to meet the release specifications, and that any systematic effect associated with reconstitution is consistent between batches and compatible with the vaccine's clinical safety and effectiveness. Please refer to the note on potency-related tests in section A.3.

A.6 Records

The recommendations given in WHO good manufacturing practices for pharmaceutical products: main principles (*23*) and Good manufacturing practices for biological products (*24*) apply.

A model protocol to be used for DT-based combined vaccines is provided in Appendix 1.

A.7 Retained samples

Vaccine samples should be retained, as recommended in WHO good manufacturing practices for pharmaceutical products: main principles (*23*) and Good manufacturing practices for biological products (*24*).

A.8 Labelling

The recommendations given in WHO good manufacturing practices for pharmaceutical products: main principles (*23*) and Good manufacturing practices for biological products (*24*) apply with the addition of the following:

- the word "combined" or "combination" to be added
- the word "adsorbed" to be added, if applicable
- the name and address of the manufacturer
- the recommended storage temperature and the expiry date if kept at that temperature
- the recommended SHD and route of administration.

In addition, the label printed on or affixed to the container, or the label on the cartons, or the leaflet accompanying the container should contain the following information:

- a statement that the vaccine satisfies the recommendations of this document;
- the nature and amount of any preservative present in the vaccine (if there is no preservative in single-dose containers, this should be stated);
- the nature and amount of the adsorbing agent, if applicable;
- the nature and amount of any substances added to the vaccine;
- the recommended conditions for storage and transport;
- a warning that the vaccine should not be frozen;
- a warning that the vaccine should be shaken before use;
- instructions for the use of the vaccine, and information on contraindications and reactions that may follow vaccination.

A. 9 Distribution and transport

The recommendations given in WHO good manufacturing practices for pharmaceutical products: main principles (*23*) and Good manufacturing practices for biological products (*24*) apply.

A.10 Stability, storage and expiry date

The stability-indicating parameters are those selected for the individual component vaccines. Stability studies should be performed in accordance with WHO Guidelines on stability evaluation of vaccines and in particular with the section of those Guidelines relevant to combined vaccines (*25*).

A.10.1 Stability testing

Stability evaluations are an important part of quality assessment. The purpose of stability studies is to ensure that at the end of the combined vaccine's shelf-life, and during the storage period or period of use, each of the component vaccines retains the characteristics necessary to support the combined vaccine's quality, safety and efficacy. If applicable, the desorption of antigens from the adjuvant, which may occur over time, should be investigated and limits should be agreed with the NRA.

The real-time stability of the vaccine in final containers maintained at the recommended storage temperature, should be demonstrated to the satisfaction of the NRA. In general, manufacturers should follow WHO Guidelines on stability evaluation of vaccines (*25*) when assessing a combined vaccine's stability for licensure, at different stages of the manufacturing process, and to gain approval for a clinical trial.

Accelerated stability studies may provide additional evidence of product stability, but cannot replace real-time studies.

When any changes that may affect the stability of the product are made to the production process, the stability of the vaccine produced by the new method should be demonstrated.

A.10.2 Storage conditions

The recommended storage conditions and the defined maximum duration of storage should be based on stability studies, as described in section A.10.1, and should be approved by the NRA. For DT-based combined vaccines, storage at a temperature of 2–8 °C is generally considered to be satisfactory. Storage at this temperature range should ensure that the minimum potency specified on the label of the container or package will be maintained after release and until the end of the product's shelf-life if the conditions under which the vaccine is stored are in accordance with the instructions on the label.

The manufacturer should recommend conditions of storage and transport that will ensure the vaccine satisfies the potency requirements until the expiry date stated on the label.

The vaccine must not be frozen.

A.10.3 Expiry date

The expiry date should be defined based on a shelf-life that has been justified by stability studies as described in section A.10.1, and should be approved by the NRA.

Part B. Nonclinical evaluation of DT-based combined vaccines

B.1 Introduction

Nonclinical testing is a prerequisite for the initiation of clinical studies in humans, and includes extensive product characterization, immunogenicity studies (known as proof-of-concept studies) and safety testing in animals. The extent to which nonclinical studies will be required depends on the type of antigen used, the complexity of the formulation, and clinical experience with the different individual vaccines, used alone and in combination. More extensive nonclinical testing is likely to be required when the combined vaccine includes novel antigens or novel adjuvant systems. Details of the design, conduct, analysis and evaluation of nonclinical studies are available in WHO guidelines on nonclinical evaluation of vaccines (*11*). The nonclinical studies performed should provide proof that: (i) the individual vaccine antigens and final product are well defined

and have been thoroughly characterized; (ii) the combined vaccine administered to humans is expected to be well tolerated and is unlikely to introduce new safety concerns; and (iii) the vaccine is reasonably likely, based on data from animal immunogenicity studies or protection studies, to provide an acceptable level of protection against the diseases targeted by each of the individual vaccines present in the final combined vaccine. These issues are discussed in detail below.

The following sections describe the types of nonclinical information that should be considered in the context of the development of a new combined vaccine, or when significant changes to the manufacturing process require re-evaluation and re-characterization of the vaccine. The goal is to collect data that can be submitted to the NRA. The purpose of the submissions will vary during the product-development process. In some cases, nonclinical data will be submitted to support the initiation of a specific clinical study; in other cases, the nonclinical data will be included in an application for marketing authorization. The goal of preclinical testing, defined as the nonclinical testing done prior to the initiation of a clinical investigation, is to develop a package of supporting data and product information that justifies the move to clinical studies.

Many considerations influence the extent of the nonclinical testing required. New vaccine formulations that have not been evaluated previously for safety and efficacy require extensive characterization, including immunogenicity studies or challenge studies in animal models (known as proof-of-concept studies), and safety testing in animals. However, extensive nonclinical testing may not be required for vaccines that use antigens that are the same as those in vaccines that have already been approved (i.e. from the same manufacturer and produced by the same methods). New combined vaccines may require nonclinical testing if:

- they include a combination of two or more already approved products; or
- a new and not currently licensed vaccine antigen has been added to an existing vaccine; or
- one antigen in a combination vaccine has been replaced with an antigen used for the same indication; or
- an antigen has been removed from an approved combination; or
- changes have been made to the manufacturing process for one or more of the individual component vaccines; or
- changes have been made to the amount of one or more of the antigens or excipients; or
- changes have been made to the adjuvant, preservative or another excipient.

The specific questions to be addressed by nonclinical testing depend on the nature of the changes. However, the primary concerns relate to the compatibility of each of the vaccines with one another, the physicochemical and immunochemical integrity of each of the antigens in the combination, the stability of the individual components, the potential for immunological interactions when the individual vaccines are combined, and the potential for increased reactogenicity. Some of these evaluations will include testing in animal models, which is discussed below. Comprehensive toxicology studies (see section B.6) will not necessarily be required for all new combined vaccines. Prior to the initiation of toxicology studies, it is recommended that the NRA should be consulted regarding the need for and the design of toxicology studies for a new combined vaccine.

The vaccine lots used in nonclinical studies should be adequately representative of the formulation intended for clinical investigation, and, ideally, should be the same lots as those used in clinical studies. If this is not feasible, then the lots used clinically should be comparable to those used in nonclinical studies with respect to the manufacturing process, immunological activity or potency, purity, stability and other aspects of quality.

B.2 Characterization of individual vaccines prior to formulation

For vaccines based on novel antigens or on formulations for which one or more of the components have been produced using a new manufacturing process that is different from the established one, nonclinical testing should include detailed characterization and evaluation of the individual vaccines prior to formulation. A detailed discussion of this characterization is beyond the scope of this document; instead, one should refer to the product-specific WHO document dealing with that component as well as to the general guidance provided in the WHO guidelines on nonclinical evaluation of vaccines (*11*).

B.3 Characterization of individual vaccines in the combined vaccine

Creating a new combined vaccine using any of the scenarios described above leads to a change in environment for the antigens in each of the individual vaccines. For example, there could be a change in pH, diluent composition, adjuvant nature or concentration, or protein concentration. Any of these could lead to changes in the degree of adsorption on to the adjuvant, physicochemical or immunochemical integrity, or stability.

Thus, the combined antigens should be examined by appropriate means to evaluate possible changes in antigen properties that arise as a result of combining them. The compatibility of all of the antigenic components of the vaccine with one another should be demonstrated in nonclinical studies. Where

relevant, adsorption of all of the antigenic components present in the vaccine should be shown to be consistent from lot to lot. The possible desorption of an antigen during the shelf-life of the product should be evaluated and reported, and specifications should be set. More extensive studies will be required if a new adjuvant is proposed for use in a vaccine formulation. Preclinical studies should evaluate the combination of adjuvants and antigens as formulated for clinical use. Whenever possible, the properties of the individual antigens should be evaluated by comparison with the properties of the same antigens when used in uncombined licensed vaccines. In some situations, the licensed comparator vaccine or vaccines may be lower-order combined vaccines (e.g. DTP may be used as a comparator for a DTP-HepB combination).

B.4 Immunogenicity evaluation in animal models

Before the initiation of human clinical trials, new combinations produced either by formulation or by reconstitution should be studied for adequate immunogenicity in an appropriate animal model if available. The immune response to each of the antigens in the vaccine should be assessed including the quality of the response, the potential interference, and incompatibilities among combined antigens. When possible, it is preferable to study a new combination in comparison with the individual antigens (or an approved lower-order combined vaccine) in animals to determine whether augmentation or diminution of response occurs. The use of an animal model in which more than one of the individual vaccines can be evaluated is encouraged for such investigations.

Immunogenicity studies in animal models can provide important information with respect to optimizing adjuvant formulations and evaluating the immunological characteristics of the antigen including the ability to induce functional antibodies or protection from challenge. However, experience has shown that extrapolating data from animal models to human disease has to be approached with caution. The following issues should be considered when evaluating immunogenicity as part of a nonclinical programme.

- Preclinical studies should evaluate the combination of adjuvant and antigen as formulated for clinical use.
- The quantity of antibody directed towards each of the component antigens should be directly compared between the candidate vaccine and at least one licensed comparator, preferably a comparator that has been used extensively and for which data support its effectiveness in routine use. If testing is performed as a result of a significant change in the manufacturing process, the candidate vaccine should be compared with the corresponding licensed vaccine. Depending on the nature of the changes, the comparator could be the licensed individual component vaccine, a lower-order combined vaccine, or a

licensed vaccine with the same composition. For some combinations, more than one comparator will be required to allow for the assessment of each of the component antigens.

- The potential need to characterize the immune response in more depth should be assessed including, when possible, evaluating functional antibody responses or cellular immunity, or both.
- If a new candidate vaccine contains a new adjuvant, its inclusion should be supported by adequate immunogenicity data that in addition to measuring humoral antibodies, may include an assessment of the cellular immune response. Studies should compare the adjuvanted candidate vaccine with appropriate comparator vaccines. In the case of new adjuvants intended to replace well established aluminium adsorbants in a vaccine already in use, the selection of appropriate control groups of animals should be considered carefully. These groups may include one group receiving the antigen alone or a group receiving the antigen adsorbed to an aluminium compound, or both.

B.5 Nonclinical safety studies

Preclinical animal studies should be undertaken to determine the safety profile of the combination of adjuvant and vaccine. The safety of a new combination should be evaluated in an animal model on a case by case basis, especially if there is a concern that combining antigens or adjuvants may lead to toxicity problems (e.g. in the case of a novel adjuvant). For vaccines that contain one or more chemically inactivated toxins (e.g. diphtheria, tetanus and acellular pertussis), studies should specifically evaluate the presence of residual active toxin and the potential for reversion to toxicity in the final combination.

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

B.6 Toxicology studies

Toxicology studies on the final formulation, which includes the antigens and adjuvants, should be undertaken in accordance with the WHO guidelines on nonclinical evaluation of vaccines (*11*). When toxicology studies are needed, the design should take into consideration the intended clinical use of the vaccine.

This is of particular concern for vaccines that will be used in certain target populations, such as infants, young children, pregnant women or women of childbearing potential. As noted in section B.1, it is recommended that the NRA should be consulted prior to the initiation of toxicology studies.

If the vaccine has been formulated with a novel adjuvant, appropriate nonclinical toxicology studies should be conducted on the final vaccine formulation, which should include the adjuvant. Repeated-dose toxicity studies may be used to compare the safety profile of the novel adjuvant with the safety profile of an established vaccine formulation, taking into account existing guidelines. If no toxicological data exist for a new adjuvant, in some situations toxicity studies of the adjuvant alone may provide information that is useful for interpretation; however, the NRA should be consulted for guidance.

If a novel cell substrate (i.e. a substrate that has not been previously licensed or used in humans) is used for the production of one of the component antigens, safety aspects, such as potential immune responses elicited by residual host-cell proteins, should be investigated in a suitable animal model.

Variations to the route of administration may require re-evaluation of the immunogenicity of the vaccine as well as adequate studies of animal safety and toxicology, taking into account existing guidelines.

Part C. Clinical evaluation of DT-based combined vaccines

C.1 Introduction

Part C provides guidance on issues related to the design and evaluation of clinical studies for new combined vaccines and for existing vaccines for which a significant change to the manufacturing process has been proposed. Clinical trials should adhere to the general principles described in international guidelines on good clinical practice (*36*) and to the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*12*). The clinical programme should be preceded by adequate nonclinical studies as discussed in Part B. The content and extent of the clinical programme will vary according to the specific combined vaccine being tested and according to previous clinical experience with the individual vaccines and similar vaccines. The vaccine-specific requirements for clinical studies should be discussed with the appropriate NRA.

These Recommendations specifically address the clinical evaluation of combined vaccines that contain diphtheria and tetanus toxoids. As of 2012, approved DT-based combined vaccines included one or more of the following additional components: pertussis (wP or aP); Hib(conj); inactivated poliovirus; and HepB. Although this document focuses on combinations currently in use, the general principles and procedures apply to new antigens that may be included in future DT-based combined vaccines. Many of the vaccines considered here are intended for infant immunization because immunizing infants is the most

effective prevention strategy for many diseases. However, catch-up and booster strategies, the vaccination of adults, and the vaccination of special populations are common. These Recommendations address issues that are relevant to the diverse indications and use of DT-based combined vaccines.

The main goals of a clinical development programme for a DT-based combined vaccine are to evaluate the safety of the combined vaccine and the immunogenicity of each individual vaccine in the combined vaccine. Generally, a clinical development programme should include comparative clinical trials. Section C.2 discusses the overall design of comparative clinical trials and how to choose a comparator vaccine or vaccines. Unless an alternative approach can be justified adequately, the safety and immunogenicity of a new combination should be compared in a randomized, controlled trial with the safety and immunogenicity of one or more approved vaccines that contain the antigens in the new combination. The value of randomized, controlled trials cannot be overemphasized. The inclusion of a control group receiving approved vaccines provides assurance of the adequacy of the trial's procedures and methods, including the immunoassays, and facilitates the interpretation of data in circumstances in which unexpected results (e.g. low immune response to one or more antigens, high rates of specific adverse events, or unexpected adverse events) are observed following immunization with the new combined vaccine.

The specific questions to be addressed during clinical testing depend on the nature of the new combined vaccine; however, the primary concerns usually relate to the potential for immunological interference and increased reactogenicity. Effects on both immunogenicity and safety have been observed as a result of combining antigens. Generally, safety studies should be designed to determine whether the combined vaccine is more reactogenic than the individual vaccines administered separately, and to obtain an adequate safety database, which is needed to assess risks and benefits prior to licensure. With respect to immunogenicity, the primary concern is typically to evaluate whether the presence of an antigen in a combination interferes with, or in some way influences, the response to any of the other antigens in the vaccine. For the antigens included in currently approved DT-based combined vaccines, direct measurement of clinical efficacy is, with rare exceptions, impractical or impossible. Thus, the evaluation of immunogenicity has been accepted as an appropriate approach for evaluating the adequacy of a DT-based combined vaccine in providing clinical benefit. The existence of established serological correlates of protection for some individual vaccines used in DT-based combined vaccines facilitates the selection of immunological end-points and the interpretation of immunogenicity data. The use of immunogenicity studies to infer clinical benefit for vaccines requires careful selection, proper design, and adequate validation of the assays (see section C.3). The NRA should be consulted when immunoassays are being selected and evaluated for use in clinical studies.

Although not unique to combined vaccines, data on the safety and immunogenicity of new combined vaccines when co-administered with other routinely used vaccines are essential in order to make recommendations regarding concomitant use (*12*). Concomitant administration may cause lower immune responses to one or more of the co-administered antigens (i.e. immune interference) (*15*), although the clinical significance of this phenomenon is not always clear. An exaggerated immune response has been observed in some situations in which the carrier protein used in a co-administered conjugate vaccine is related to one of the antigens in the combined vaccine (*16*). Due to the diversity of possible interactions, the initial assessment of the effects of concomitant vaccine administration should be evaluated at an early stage of clinical development. Nevertheless, data on the effects of co-administration will be accumulated throughout the duration of the clinical development programme and during post-approval studies.

C.2 Scenarios and clinical trial designs

C.2.1 Considerations for the clinical development programme

The clinical development programme should be developed in consultation with the NRA, and should follow available general guidance, including WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*12*). A clinical development programme for a new vaccine typically begins with small safety and immunogenicity studies, and then progresses to larger studies. For paediatric vaccines containing novel antigens and formulations, it may be appropriate to conduct preliminary evaluations of safety and immunogenicity in adults and then progress in a stepwise fashion from older age groups to younger age groups. When evaluating such studies, it should be noted that safety and immunogenicity may be dependent on age, prior infection or prior immunization, or a combination of these.

Prior to the initiation of any clinical study, the manufacturer should provide justification for the choice of vaccine formulation and the design of the study. The amount of each antigen in each dose of a combined vaccine requires justification, which may be based on previous experience with each individual vaccine, as well as on nonclinical studies and formal dose-ranging clinical studies. In all cases, clinical studies should be initiated only for products for which there is adequate information on nonclinical testing and on manufacturing.

Consistency in manufacturing should be demonstrated and well documented for the vaccine lots used in clinical trials. Although a formal clinical trial to evaluate lot consistency may not always be needed, in some instances clinical data may be required to provide evidence to support manufacturing consistency (e.g. if there is a particular concern about the consistency of the product). Nevertheless, multiple lots of the combined vaccine formulation that

are intended for marketing and that have been manufactured using different bulk lots for each of the immunogens, should be used during the later stages of the clinical development programme. For components that are already licensed vaccines, fewer lots in combination may be needed than for components that are not licensed. Guidance should be sought from the NRA when determining the composition of lots to be used during the later stages of clinical development.

C.2.2 Overview of potential scenarios that may be encountered with new combined vaccines

New combined vaccines should be compared directly with one or more licensed vaccines with which there has been considerable clinical experience. During late-phase clinical development, the most appropriate study design is usually a randomized, controlled trial with participants from the target age group. The selection of the comparator vaccine or vaccines should be discussed with the NRA, and should take into account the study population, the proposed immunization schedule, the total antigen composition of the candidate vaccine, and previous clinical experience with the comparator vaccine. For some products, more than one comparator vaccine, administered concomitantly, may be required for adequate clinical evaluation of all component antigens. In this case, it is necessary to consider whether these licensed vaccines are recommended for co-administration at separate injection sites or whether there should be staggered administrations (i.e. occurring on different days).

Table 6.1 describes the most common scenarios that are likely to be encountered during the clinical evaluation of a new combined vaccine. New combinations could result from making changes to existing combined vaccines, including adding a new antigen, replacing one antigen with another antigen for the same indication, removing an antigen, or making a significant change to the manufacturing process or formulation. Additionally, a new manufacturer may wish to begin producing a vaccine that is similar in composition to an already approved combination. Although scenarios not specifically addressed here may be encountered, the general principles outlined here should be adaptable to other situations. For each trial, manufacturers should justify the choice of the comparator vaccine, the trial design, and the safety and immunogenicity end-points.

The comparative clinical trial should be designed to enable adequate evaluation of safety and immunogenicity, and should prespecify appropriate end-points related to the rates of adverse events and immune responses to each of the antigens in the vaccine. Issues related to the immunogenicity end-point are discussed in section C.3; issues related to safety are discussed in section C.4). Although the trial designs outlined below apply both to safety and immunogenicity assessments, Table 6.1 provides more detail on evaluating immune responses owing to their increased complexity.

Table 6.1
Overview of potential scenarios that may be encountered during the clinical evaluation of new combination vaccines[a]

Category	Scenario	Example	Suggested design[b]
Antigen addition	Combination of two or more already approved products (e.g. AB + C → ABC)	Licensed IPV is added to a licensed DTwP-HepB combination	Immune responses to ABC are compared with immune responses to the separately administered licensed vaccines, AB and C
	Combination of one already approved product (AB) and a new (and not currently licensed) vaccine antigen (C) (e.g. AB + C → ABC)	A new and unlicensed antigen is added to a licensed DTaP-HepB combination	Immune responses to antigens A and B of ABC are compared with immune responses to the separately administered licensed vaccine, AB; responses to the new antigen C are based on criteria appropriate for C; if a vaccine comparable to C is already licensed, responses to C should be compared with responses to the licensed product
Antigen replacement	One of the antigens in a combination is replaced by an already approved antigen (for the same vaccine component) (e.g. ABC → ABC*)	The wP component of a licensed DTwP combination is replaced by a licensed aP component	Immune responses to antigens A and B of ABC* are compared with immune responses to a separately administered licensed vaccine containing A and B; responses to new antigen C* are based on comparison with responses to a licensed product containing C*
	One of the antigens in a combination is replaced by a novel (unlicensed) antigen (for the same vaccine component) (e.g. ABC → ABC*)	The aP component of a licensed DTaP combination is replaced by a new aP component containing a genetically modified aP antigen or antigens	Immune responses to antigens A and B of ABC* are compared with immune responses to the separately administered licensed vaccine, AB or ABC; responses to new antigen C* are based on criteria appropriate for C*; if a vaccine comparable to C* is already licensed, responses to C* should be compared with responses to the licensed product

Table 6.1 *continued*

Category	Scenario	Example	Suggested design[b]
Change in manufacturing or formulation	Increase or decrease in the amount of one or more of the antigens (e.g. ABC → ABc)	The diphtheria toxoid content of a combination vaccine is reduced	Immune responses to ABc are compared with immune responses to a licensed product with a matching composition or to the product with the most similar composition[c]
	Change in the nature or content of an adjuvant, preservative or another excipient[a]	Introduction of a new adjuvant[d]	Immune responses to the investigational vaccine are compared with immune responses to the licensed product manufactured by the approved process
	Significant change in the manufacture of one or more of the individual vaccine components (e.g. ABC → ABC*)	Change from the use of lyophilized Hib-conjugate component to a fully liquid formulation	Immune responses to the investigational vaccine are compared with immune responses to the licensed product manufactured by the approved process
Antigen removal	Removal of one or more antigens (e.g. ABC → AB).	Removal of HepB antigen from a DTwP-Hib-HepB combination	Immune responses to antigens A and B of AB are compared with immune responses to the licensed vaccine ABC
New manufacturer	A combination comparable to another licensed product is produced by a new manufacturer (e.g. ABC → A*B*C*)	Production by a new manufacturer of a DTwP-Hib-IPV combination	Immune responses to ABC are compared with immune responses to a licensed product with a similar composition[e]

IPV = inactivated poliomyelitis vaccine; DTwP = diphtheria–tetanus–whole-cell pertussis vaccine; HepB = hepatitis B virus vaccine; DTaP = diphtheria–tetanus–acellular pertussis vaccine; Hib = *Haemophilus influenzae* type b.

[a] This table does not specifically address the case in which a manufacturer formulates the final vaccine using one or more components purchased from another manufacturer. However, the source of the component is not expected to influence the overall design of clinical evaluations.

[b] Trial designs other than the suggested ones, including the choice of a comparator vaccine (or vaccines) may be used if justified, and if approved by the NRA.

[c] When the antigen content is being reduced, clinical studies should be designed to verify that the reduction does not lead to a clinically important reduction in immunogenicity.

[d] A rationale for changing the adjuvant, preservative or another excipient should be provided. In particular, clinical studies evaluating a change in the adjuvant may need to take into consideration additional safety and immunogenicity parameters.

[e] Due to the limitations of immunogenicity assessments, the selection of an appropriate comparator is particularly complex when one of the components of a new combination is an acellular pertussis vaccine. The WHO Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines (*5*) should be consulted for additional guidance.

C.2.3 Schedules and populations

In most cases, a new combined vaccine will be tested following the primary series schedule for vaccinations that have been approved for similar vaccines. However, a formal evaluation of the schedule may be necessary in certain circumstances, such as when a different schedule is required for programmatic reasons or if a candidate vaccine contains an antigen dose or an adjuvant that is considerably different from that used in licensed vaccines.

Safety and immunogenicity have been shown to vary for many vaccines according to the schedule used, the population studied, the antigen composition and the nature of the vaccines that are administered concomitantly. Whenever possible, the combined vaccine should be evaluated in the target population following the intended schedule. However, it may not be feasible to study new vaccines at every possible schedule in current use or in a wide range of geographical regions. For instance, within a specific population, immune responses or rates of some adverse events following immunization with a vaccine that has a 6-week, 10-week and 14-week schedule may differ from those following administration of the same vaccine on a 2-week, 4-week and 6-month schedule, or on a 3-month, 5-month and 12-month schedule. Manufacturers should justify the relevance of the clinical data provided to each country in which approval is sought, and should discuss the basis for extrapolating their findings. When it is anticipated that a vaccine will be used according to different schedules, the recommendation of WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*12*) is that the primary evaluation should be conducted using the schedule expected to be the most restrictive (i.e. the schedule from which the least immune response is expected). However, there is still a need to collect some safety data using schedules that are proposed for approval because the local and systemic reactogenicity associated with a vaccine may vary when different schedules are used in a specific population due to the age-related prevalence of specific adverse events. For all clinical trials, the study population should be carefully defined and justified by the manufacturer, and the population studied should be approved by the NRA.

C.2.4 Co-administered vaccines

Vaccinees enrolled into the types of comparative studies described above will also receive other licensed vaccines according to the schedule of the country in which they reside, and experience has indicated that unexpected interactions can occur when vaccines are administered concomitantly. Due to the possible effects of these additional vaccines on the safety and immunogenicity of the test vaccine and control vaccine, as well as the possible effects of the test vaccine on other routinely administered vaccines, manufacturers should conduct studies that evaluate the effects of co-administration as described in guidance from

WHO (*12*). In some settings, multiple vaccines may be licensed for the same disease, and these may be given on the same schedule as the investigational vaccine. Whenever there is more than one licensed vaccine of a certain type that could be co-administered, the choice of the specific vaccine to be used in a clinical study should take into account the recommended routine immunization schedule as well as the likelihood of co-administration. The choice should be justified, and should be discussed with the NRA. If the results of the clinical trial indicate that immune responses to one or more of the antigens administered routinely are lower when they are co-administered with a new combined vaccine compared with the separately administered licensed vaccine, the NRA will need to consider the potential clinical consequences on a case by case basis. Any incremental increase in adverse reactions that is observed during co-administration will need to be weighed against the convenience of administering multiple vaccines at the time of a single health-care contact.

C.2.5 Studies in special populations

There may be underlying diseases and conditions that predispose an individual to a particular disease (e.g. conditions and diseases such as prematurity, immunodeficiency, or severe pulmonary disorders, including cystic fibrosis (mucoviscidosis) or that may be associated with a poor response to specific vaccines. Clinical studies may be conducted specifically to assess the safety and immunogenicity of new combined vaccines in populations that are at an increased risk for particular diseases. In many instances, these studies may be performed after initial licensure.

C.3 Assessment of immunogenicity in humans

C.3.1 Design and scope of immunogenicity studies

The specific questions to be addressed by immunogenicity studies depend on the nature of the new combined vaccine; however, the primary concerns usually relate to the potential for immunological interference among antigens. This document applies to a wide range of combined vaccines that potentially have a large number of antigens for which immunogenicity evaluation is required. The sections below provide guidance related to the selection of assays and end-points for these assessments. Many combined vaccines are developed for the purpose of primary immunization so this document discusses in detail the evaluation of vaccines used for primary immunization. However, booster immunizations for older children, adolescents and adults are also important for the control of several diseases. In some cases, the vaccine developed for primary immunization is also used for booster immunization, while in other cases vaccines have been developed solely for use as booster doses. Therefore, this section also includes information related to the evaluation of vaccines used for booster immunization.

C.3.2 Assays to assess antibody responses

For many of the antigens used in the DT-based combined vaccines that have been approved, WHO guidelines or recommendations are available that provide guidance on the most appropriate assays and end-points for the clinical evaluation of the component antigens (*2*–*6*, *8*, *37*). In addition, some NRAs or regional regulatory authorities have provided guidance that will assist in the selection and establishment of immunoassays. When available, all such guidance should be consulted. However, guidelines are not available for some of the individual antigens used in many combined vaccines. Table 6.2 lists antigens, commonly used assays, and suggested end-points. However, WHO guidelines should be considered the primary source of information.

For some antigens, the end-points used for primary immunization studies are not optimal for the evaluation of booster immunization. For example, this may occur if prior to immunization a significant proportion of a study population has a concentration of antibodies that exceeds a protective threshold. In such cases, an evaluation of the proportion of participants who show a significant increase in antibody concentration may provide a more sensitive assessment of the response to immunization. To reflect these differences, suggested end-points for studies of primary and booster immunizations are provided in separate columns in Table 6.2.

The assessment of the immune response should use a validated and standardized assay to measure the antibody concentration for each component antigen in serum (*12*). To improve the comparability and acceptability of serological data across trials, the results of immunogenicity outcomes should be expressed in IU/ml of human serum whenever an international reference is available. The selection of assays for evaluating the human immune response to the vaccine should be justified by the vaccine manufacturer. For many vaccines, suitable assays are unlikely to be commercially available. The use of validated quantitative assays is critical, and testing should be conducted by laboratories that implement quality assurance of testing procedures. Validation studies should be designed to demonstrate that the assay is suitable for the clinical study, and should consider the way in which the vaccines are to be compared with one another (e.g. whether the criteria for evaluation are based on percentages of post-primary series titres above a threshold, seroconversion rates, or geometric mean antibody concentrations). The validation report should include a detailed description of the calibration of any in-house references, and information on the processing and storage of samples, reference standards and reagents. The assay validation data should be reviewed and approved by the NRA.

When developing the clinical programme, emphasis should be given to the role of assays that measure the functional activity of antibodies induced by the individual vaccines. For some vaccine antigens, a functional assay is

recommended for immunogenicity evaluation (Table 6.2). In other cases, a nonfunctional assay has been accepted for primary evaluations; however, in such cases, if a functional assay is available, it should be used in validation studies to verify that the nonfunctional assay provides a meaningful assessment of the immune response. It is important to note that no functional assay has been identified for some commonly used antigens included in some aP vaccines (*5*).

Cell-mediated immunity (CMI) responses may play a role in developing immunity to some infections. However, the standardization of immunological assays to evaluate CMI responses following immunization has been challenging, and such assays have not been used to support licensure. Nevertheless, when appropriate, an exploratory assessment of CMI should be encouraged in order to enlarge the body of knowledge regarding all aspects of the immune response to vaccine antigens.

C.3.3 Immunogenicity end-points for immunization studies

For antigens contained in licensed DT-based combined vaccines, Table 6.2 provides a summary of the recommended assays and suggested primary end-points for the clinical evaluation of vaccines intended for primary or booster immunization. References to documents developed by WHO, to national or regional guidelines, or to other publications are provided. These should be consulted for more complete information.

C.3.4 Primary analyses

The primary analyses should be based on the antibody response following completion of the defined immunization series. In the case of vaccines used for booster indications, this typically will consist of only a single immunization. Responses to antigens shared between a new vaccine and the licensed comparator, and to antigens found only in a new vaccine, should be regarded as coprimary end-points.

The definition of the appropriate time intervals for assessing immune responses should take into account the study's objectives. In most cases, clinical studies for new vaccines are designed to determine the antibody response to the vaccine's components at approximately four weeks following the final dose. However, the timing of serum sampling should be justified, and should be approved by the NRA. In studies evaluating booster doses, blood samples are generally obtained at four weeks after the booster dose but in persons whose immune systems are already primed, the peak response may be achieved in a shorter time – i.e. within two weeks of the booster dose. Thus, some exploration of immune responses at less than four weeks after the booster dose in randomized subsets of the study population could be informative, and may provide insight into the rapidity of the response to antigen challenge.

Table 6.2
Immunogenicity assays and end-points for studies of primary and booster immunizations

Antigen	Assay	References	Suggested primary end-point(s)		Comments
			Primary immunization	Booster immunization	
Diphtheria toxoid	Microneutralization assay (Vero cells)	*2*, *31*, *38–41*	Proportion with ≥ 0.01 IU/ml or ≥ 0.1 IU/ml (see comments)	Proportion with significant increase[a]	The toxin neutralization assay is generally preferred. An antigen-binding assay that has been shown to correlate with the neutralization assay may be acceptable; for primary immunization, a threshold of 0.01 IU/ml may be acceptable if the toxin neutralization assay is used and if a booster dose is administered during the second year of life; otherwise a threshold of 0.1 IU/ml should be used; for booster immunization, the decision to use a threshold level or significant increase should take into account the proportion of persons with antibody concentrations expected to exceed the threshold prior to vaccination
Tetanus toxoid	ELISA	*3*, *30*	Proportion with ≥ 0.1 IU/ml	Proportion with significant increase[a]	An antigen-binding assay that has been shown to correlate with the mouse neutralization assay is most commonly used

Table 6.2 *continued*

Antigen	Assay	References	Suggested primary end-point(s)		Comments
			Primary immunization	Booster immunization	
Whole-cell pertussis (wP)[b]	1) Agglutination assay 2) ELISA for pertussis toxin 3) ELISA for other antigens	*4, 42–45*	a) GMT and/or GMC b) Proportion with four-fold rise	a) GMT and/or GMC b) Proportion with four-fold rise[a]	No value that correlates with protection has been defined; the goal is to compare the responses of the test and control groups for each assay; both a) and b) are recommended as coprimary end-points
Acellular pertussis (aP)	ELISA for all pertussis antigens in vaccine[c]	*5, 42–45*	a) GMT and/or GMC b) Proportion with four-fold rise	a) GMT and/or GMC b) Proportion with four-fold rise[a]	No value that correlates with protection has been defined; the goal is to compare the responses of the test and control groups for each assay; both a) and b) are recommended as coprimary end-points
Inactivated poliovirus vaccine	Virus neutralization assay for each of the three serotypes	*46*	Proportion with neutralization titre ≥ 1:8	a) GMT and/or GMC b) Proportion with neutralization titre ≥ 1:8	The presence of neutralizing antibody (titre ≥ 1:8) is considered protective against poliovirus types 1, 2 and 3

Table 6.2 *continued*

Antigen	Assay	References	Suggested primary end-point(s)		Comments
			Primary immunization	Booster immunization	
Haemophilus influenzae type b conjugate vaccine	ELISA (*Haemophilus influenzae* type b capsular polysaccharide; PRP	*8*, *47–49*	a) Proportion with ≥ 0.15 µg/ml b) Proportion with ≥ 1.0 µg/ml	Proportion with ≥ 1.0 µg/ml	A post-vaccination anti-PRP level of 0.15 µg/ml is considered the minimum protective level; a post-vaccination level of 1.0 µg/ml indicates protection through a subsequent 1-year period; for primary immunization, both a) and b) are recommended as coprimary end-points
Hepatitis B vaccine	ELISA for hepatitis B surface antigen antibodies	*6*	Proportion with ≥ 10 mIU/ml	Proportion ≥ 10 mIU/ml	

IU = International Unit; ELISA = enzyme-linked immunosorbent assay; GMT = geometric mean titre; GMC = geometric mean concentration; PRP = polyribosylribitol phosphate.

[a] The magnitude of the increase (e.g. four-fold) in antibody concentration from pre-vaccination to post-vaccination should be predefined and justified. A lower magnitude of increase may be justified for subjects with a specified high antibody concentration prior to vaccination (*2*, *3*).

[b] There is substantial heterogeneity in the antibody response to highly effective wP vaccines. However, the assays listed could be used in the evaluation of a comparative immunogenicity trial.

[c] The collection of additional supporting data using the pertussis toxin neutralization assay and the whole-cell agglutination assay is encouraged.

The selection of the primary parameters for the assessment of noninferiority, the predefined margins of noninferiority, and hence the total sample size for the comparative study will need careful justification. Factors to consider regarding the stringency of the noninferiority criteria include the clinical relevance of the end-point, the seriousness of the disease being prevented, and the vulnerability of the target population. More stringent margins may be justified for severe or debilitating diseases, for populations that are particularly vulnerable, or when the serological end-point is known to correlate well with protection against the disease. If a new vaccine is known to offer substantial benefits in terms of safety or improved coverage, less stringent margins may be considered. The noninferiority criteria will influence the study's sample size, and feasibility considerations may need to be taken into account. Thus, there may be situations in which different limits for the same antigen may be appropriate in different settings. In determining noninferiority margins, consideration also should be given to the potential for a downward drift in immunogenicity over time occurring with sequential comparative studies (*50*). The consequence of such drift, if it happens, is that a new vaccine could be considerably less immunogenic than the originally licensed vaccine. It should be noted, however, that there may be other explanations for a downward drift in immunogenicity, such as the absence of natural boosting following the reduction in pathogen circulation in the community.

Although studies that compare immune responses between a candidate and licensed vaccines are generally required, comparisons with historical data generated during previous protective efficacy studies using similar assays may, in some cases, provide supporting evidence.

For the majority of the antigens contained in currently approved DT-based combined vaccines, the primary assessment will be the proportion of participants who respond to the vaccine as defined in Table 6.2. Typically, this will be the proportion of participants reaching a prespecified threshold. However, for some vaccines and some indications, a response is defined as the proportion of vaccinees with a significant increase (e.g. greater than four-fold) in immune response above preimmunization levels. Alternative definitions for responders may be considered if they have been well justified. The groups should be compared using an appropriate predefined noninferiority limit; generally the upper bound of the two-sided 95% confidence interval of the observed difference (i.e. the comparator vaccine minus the new combined vaccine) should be less than the criterion agreed with the NRA, which is most commonly 0.05 or 0.10.

For some antigens and for some indications, coprimary analyses should compare the magnitude of the response to the vaccine antigens induced by the new vaccine and the licensed comparator. Such end-points are recommended, for instance, in the evaluation of whole-cell and acellular pertussis vaccines because no threshold of protective response has been widely accepted, and they

are also recommended in the evaluation of the response to booster doses in situations in which a substantial proportion of the study population exceeds the protective threshold prior to immunization. When used, the magnitude of the response to each component vaccine is compared on the basis of the ratio of the geometric mean concentrations (GMCs) or geometric mean titres (GMTs) of the comparator vaccine to the new vaccine using a predefined margin of noninferiority. Specifically, the upper bound of the two-sided 95% confidence interval of the observed ratio of the GMC or GMT of the comparator vaccine relative to the new vaccine should be less than the criterion agreed with the NRA, which is most commonly 1.5 or 2.0.

Measurement of preimmunization and postimmunization antibody concentrations involves the collection of an extra blood sample, and may not be necessary in all studies. Preimmunization samples will be required when end-points are based on the proportion of participants having a rise in antibody concentration, but these samples may not be needed from all participants when the end-point is based on the proportion of participants that reaches a specified threshold. However, even when a preimmunization sample is not required to evaluate a study's end-point, it is recommended that at least some information on pre-vaccination antibody values should be generated during the clinical development programme in order to aid in interpreting post-vaccination antibody values.

Due to limitations on the volumes of serum that can be collected, it is commonly necessary to perform an additional randomization step to select serum samples for use in different antibody assays or to prioritize samples, or both, so that the most relevant questions for the combination vaccine can be addressed.

For complex combined vaccines, immunogenicity evaluations may include a substantial number of coprimary end-points. If any immune interference is observed with respect to any of the combined antigens, the possible clinical implications and the reasons for not meeting the predefined noninferiority criteria should be carefully considered before proceeding with clinical development or pursuing product approval. The NRA may take into consideration the results from the antibody responses to each of the antigens, any differences in composition between the test vaccine and the comparator vaccine, the severity of the disease, the likelihood that the measured immune parameters predict clinical protection, and the potential benefits of the combination in terms of improving coverage or safety.

C.3.5 Secondary analyses

For most studies, one or more secondary analyses should be defined to provide for a more complete assessment of immune responses. If not included among the primary end-points, comparisons of the magnitude of the response to the

vaccine antigens induced by the new vaccine and the licensed comparator should be considered. As described above, the magnitude of the response for each vaccine component is compared by using the ratio of the GMC or GMT of the comparator vaccine to the test vaccine using a predefined margin of noninferiority. The noninferiority margins should be justified, and should be agreed with the NRA.

C.3.6 Assessment of functional antibody responses

When available, assays that measure the functional activity of antibodies against the individual vaccines used in the combined vaccine may play an important part in the evaluation, even when antigen-binding assays are used in the evaluation of the primary end-points. For example, the measurement of functional antibodies should be considered in at least a subpopulation of the comparator group and the test vaccine group, particularly when there is limited experience with an antigen or formulation. Additionally, as noted in section C.3.2, functional assays play an important role in validation studies by verifying that the nonfunctional assay provides a meaningful assessment of the immune response.

C.3.7 Additional information from reverse cumulative distribution curves

The use of reverse cumulative distribution (RCD) curves, which display the accumulated proportion of individuals who have an antibody concentration greater than or equal to a given level, has been shown to be particularly useful when comparing the response to the test vaccine with the response to the licensed comparator vaccine, and when monitoring changes in antibody levels over time (*51*). As one example, the RCDs may reveal the proportion of the population that has values at or below the protective threshold, and provide data that can inform decisions on the timing of booster doses. When using RCDs, comparisons among the study groups are generally qualitative and exploratory in nature because RCD curves do not lend themselves readily to comparative statistical analyses.

C.3.8 Immune responses to carrier proteins

The carrier proteins used in licensed polysaccharide conjugate vaccines have included a nontoxic genetically modified diphtheria toxin molecule (CRM197), diphtheria toxoid, tetanus toxoid, protein D from *Haemophilus influenzae*, and an outer membrane protein complex (OMPC) from *Neisseria meningitidis* serogroup B. Monitoring the immune response to these carrier proteins may be appropriate in some circumstances. Administration of a conjugate vaccine that employs diphtheria toxoid or tetanus toxoid or CRM197 as the carrier has been found to enhance the relevant antitoxin antibody levels. However, this has not been accepted yet as a replacement for routine immunization with

vaccines containing diphtheria toxoid or tetanus toxoid. The co-administration of a new conjugate vaccine with routine infant and toddler vaccines (i.e. vaccines containing diphtheria toxoid and tetanus toxoid) may result in high antitoxin levels (*52*). Careful attention should be paid to the reactogenicity observed under these circumstances since increased rates of some reactions could be associated with high antitoxin levels. As noted in section C.1.2, diminished responses to a combined vaccine antigen conjugated to a carrier protein may occur with concomitant administration of another conjugate vaccine that uses the same carrier protein.

C.3.9 Immune memory

For some antigens in a combined vaccine (e.g. polysaccharide conjugate vaccines) it may be appropriate for the clinical development programme to generate data to demonstrate that the vaccine induces an immune memory response during the infant immunization series. These data can be obtained as part of the assessment of immune responses to booster doses of the new vaccine.

C.3.10 Persistence of antibody concentrations and timing of booster doses

The waning of antibody concentrations over time is inevitable, and longer-term follow-up to assess the persistence of immunity should occur at various time points following the primary vaccination series. The total duration of serological follow-up should be discussed and planned in advance with the NRA. In some situations, these data may be provided after first approval. The waning of antibody concentrations over time should not be interpreted per se as a loss of immunity or an indication of the need for a booster dose. Longer-term antibody concentrations should be viewed in conjunction with effectiveness data to assess the potential need for additional doses later in life in order to maintain protection. A determination of the need for, and timing of, booster doses should be based on epidemiological investigations and long-term surveillance (see section C.5). A detailed discussion of these issues is outside the scope of this document.

C.4 Safety evaluation

The prelicensure assessment of vaccine safety is a critically important part of the clinical programme, and should be developed to meet the general principles described in WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*12*). The assessment of safety, with appropriately defined objectives, should be part of the comparative studies mentioned in section C.2. Such studies should be designed to monitor actively for common adverse events as well as less common adverse events, including serious adverse events and specific adverse events that have been associated with vaccines of similar

composition (e.g. excessive limb swelling, hypotonic–hyporesponsive episodes and febrile seizures).

The minimum acceptable size of the safety database at the time of approval should take into account the vaccine's composition including all antigens and adjuvants, the presence of novel antigens, past experiences with vaccines having the same or similar composition, the severity of the diseases being prevented, and the size of the target population. For new vaccines, a total safety database comprising all trials in the targeted age group and approximately 3000–5000 participants who received the new vaccine is commonly expected because this allows for the detection of uncommon adverse events – i.e. those that occur at a rate of approximately 1 in 1000 (*53*). However, depending on the composition of the investigational vaccine and the relevant safety data about it, the NRA may accept a smaller number or may request a larger database prior to first approval.

Additionally, safety evaluations should include high-risk individuals (e.g. preterm infants, people with chronic illnesses, or people who are immunocompromised) who may benefit from vaccination. Safety in these groups is often assessed during post-marketing studies (see section C.5), but a prespecified plan for such studies may be requested at the time of application for marketing authorization.

C.5 Post-marketing studies

The manufacturer has a responsibility to assess the safety and effectiveness of the new vaccine following initial approval. At the time of first licensure, NRAs should ensure that adequate pharmacovigilance plans are in place regarding these activities. There should be specific commitments made by manufacturers to provide data to NRAs on a regular basis and in accordance with national regulations. The data that are collected and submitted to the responsible NRA should be assessed rapidly so that action can be taken if there are implications for the marketing authorization. The basic principles for the conduct of postlicensure studies and continued oversight of vaccines after licensure are provided in WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*12*).

Through active post-marketing surveillance, every effort should be made to improve the scientific understanding of the protection in humans afforded by vaccines. The effectiveness of the vaccine in the population should be reported whenever possible. However, reliable estimates of effectiveness can be obtained only in geographical locations where there is a suitable infrastructure in place to identify cases of disease. Ongoing surveillance programmes should be in place to monitor longer-term protection and to collect evidence of any changes in the vaccine's effectiveness. Post-marketing assessments designed to monitor effectiveness are particularly important in the specific case in which there has

been a transition to acellular pertussis vaccines that have not been evaluated in efficacy trials. The reason for this emphasis is that there are no immune responses that can be measured in preapproval studies that have been shown to predict clinical efficacy (*5*).

Because prelicensure studies may not be large enough to detect certain rare adverse events, safety should be monitored as part of post-marketing surveillance programmes. These programmes should specifically monitor any safety concerns identified in preapproval trials, as well as collect data on new and rare adverse events not detected prior to licensure.

The collection of reliable and comprehensive post-marketing data on safety and effectiveness requires close cooperation between manufacturers and public-health authorities. Preapproval and postapproval discussions between the vaccine manufacturers responsible for placing the product on the market and national and international public-health bodies are essential for ensuring that reliable data on safety and effectiveness are collected during the post-marketing period.

Part D. Recommendations for NRAs

D.1 General

The general recommendations for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (*54*) and the WHO Guidelines for independent lot release of vaccines by regulatory authorities (*35*) apply.

The details of production and quality control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of DT-based combined vaccines, should be discussed with and approved by the NRA. For control purposes, the international standards currently in use should be obtained to calibrate the national, regional and working standards (*22*). The NRA may obtain the product-specific or working references from the manufacturer to be used for lot release until an international or national standard preparation has been established.

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

D.2 Release and certification by the NRA

A vaccine should be released only if it fulfils all national requirements or satisfies Part A of these Recommendations, or both (*35*).

A protocol based on the models given in Appendix 1 and Appendix 1a, signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for the release of a vaccine for use.

A statement signed by the appropriate official of the NRA should be provided to the manufacturing establishment to certify that the lot of the vaccine in question meets all national requirements as well as Part A of the present Recommendations. The certificate should provide sufficient information about the vaccine lot. A model certificate is given in Appendix 2. The official national release certificate should be provided to importers of the vaccines. The purpose of the certificate is to facilitate the exchange of vaccines between countries.

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Appendix 1

Model protocol for the manufacturing and control of DT-based combined vaccines

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

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

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

The following summary protocol is given as an example for a combined vaccine that consists of a freeze-dried Hib component to be reconstituted with a liquid D, T, aP or wP, IPV and HepB component.

A summary protocol for the Hib component has also been provided below as a separate appendix (Appendix 1a). This is done solely for the purpose of simplifying the layout of the guideline. The information provided by the manufacturer in individual protocols should not use cross-references between different products.

1. Summary information on finished product (final lot)

International name: _____
Trade name/commercial name: _____
Product licence (marketing authorization) number: _____
Country: _____
Name and address of manufacturer: _____

_____

Name and address of licence holder,
if different: _____
Final packaging lot number: _____
Type of container: _____

Number of containers in this packaging: _____
Final container lot number: _____
Number of filled containers in this final lot: _____
Date of manufacture: _____
Description of final product (adsorbed): _____
Preservative, and nominal concentration: _____
Volume of each single human dose: _____
Number of doses per final container: _____

Summary of the composition (include a summary of the qualitative and quantitative composition of the vaccine in each human dose, including any adjuvant used and other excipients):

_____

_____

Shelf-life approved (months): _____
Expiry date: _____
Storage conditions: _____

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

Production information

Site(s) of manufacture for each production stage of each component: _____
Date(s) of manufacture: _____

Summary information on lot-specific production data, including dates of different production stages, identification numbers and blending scheme.

2. Detailed information on manufacture and control

Starting materials or source materials, and bulk antigens

- For a D component: refer to the sections on "Production strain and seed lots", "Single harvests" and "Bulk purified toxoid" in the corresponding WHO Recommendations (*1*).
- For a T component: refer to the sections on "Production strain and seed lots", "Single harvests" and "Bulk purified toxoid" in the corresponding WHO Recommendations (*2*).

- For an aP component: refer to the sections on "Strain", "Culture media for production", "Control of antigen purification", "Test on purified antigens", "Detoxification" and "Control of bulk materials" in the corresponding WHO Recommendations (*3*).
- For a wP component: refer to the sections on "Strains" and "Control of single harvests" in WHO Recommendations for whole-cell pertussis vaccine (*4*).
- For an IPV component: for information on virus-seed lots, cell cultures and serum for cell cultures refer to the section on "Control of source materials"; and for information on single harvests and monovalent pools refer to the section on "Control of vaccine production" in the corresponding WHO Recommendations (*5*).
- For a HepB component: refer to the sections on "Cell substrate for antigen production", "Fermentation", "Single harvests (or pools)" and "Control of aqueous bulk (purified antigen)" in the corresponding WHO Recommendations (*6*).
- For a Hib component: refer to the relevant sections in Appendix 1a below on the Hib component of the generic summary protocol for the production and testing of a combined vaccine.

Adsorbed bulk concentrates (individual or combined components as applicable)

Lot number(s): _____
Date(s) of adsorption: _____
Volume(s), storage temperature, storage time and duration of approved storage period: _____

_____

Report results of tests for each adsorbed bulk concentrate

Completeness of adsorption

Method: _____
Specification: _____
Date: _____
Result: _____

Final bulk vaccine (D, T, aP, IPV, HepB)

Lot number: _____
Date(s) of manufacture: _____
Volume(s), storage temperature, storage time and duration of approved storage period: _____

_____

Information on composition of the final bulk: specify the relevant (adsorption, blending) production dates, reference number(s), volume(s) and concentrations (in Lf/ml for each of diphtheria and tetanus; in µg/ml for the aP component; in DU/ml of D antigen of the IPV component; and in µg/ml of HBsAg for the HepB component).

Blending	Identification	Prescription (SHD)	Added
Tetanus toxoid (Lf):			
Diphtheria toxoid (Lf):			
aP (µg/ml):			
IPV D antigen (DU/ml):			
HBsAg (µg/ml):			
Adjuvant (mg):			
Preservative (specify):			
Others (salt):			
Final volume (ml):			

Appearance

Method: _____
Specification: _____
Date of test: _____
Result: _____

pH

Method: _____
Specification: _____
Date of test: _____
Result: _____

Aluminium content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Osmolality

Method: _____
Specification: _____
Date of test: _____
Result: _____

Antimicrobial preservative

Method: _____
Specification: _____
Date of test: _____
Result: _____

Residual bovine serum albumin

Method: _____
Specification: _____
Date of test: _____
Result: _____

Free formaldehyde

Method: _____
Specification: _____
Date of test: _____
Result: _____

Test for sterility

Method: _____
Media: _____
Volume inoculated: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Absence of residual activity of pertussis toxin

Specify the number, strain and sex of animals used – this test is not necessary for a product obtained by genetic modification:

_____

_____

Method: _____
Dose: _____
Specification: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Reversion to toxicity of pertussis toxin
Specify the dates of the beginning and end of incubation, and the number, strain and sex of animals used – this test is not necessary for a product obtained by genetic modification:

_____

_____

Method: _____
Dose: _____
Specification: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

In vivo assay for D, T, aP and, depending on the licence dossier, for IPV and HepB components
Specify the strain, sex, weight range and number of animals used; the dates, volumes, route and doses used for immunization and challenge or bleeding; the nature, lot number and potency of the reference vaccine; and the responses at each dose. Express results in International Units (IUs) where applicable; and specify the confidence interval, slope of the parallel line model and the outcome of tests for the absence of linearity and parallelism:

_____

_____

Method: _____
Specification: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

For the IPV in vivo assay (where applicable)
Species, strain, sex, and weight range: _____
Date of vaccination: _____
Lot number of reference vaccine: _____
Vaccine doses: _____
Date of bleeding: _____
Date of assay: _____
Number of animals responding at each dose: _____
ED_{50} of reference and test vaccines: _____
Potency of test vaccine: _____
Validity criteria (linearity, parallelism, precision, ED_{50} between highest and lowest responses): _____
Results: _____

In vitro assay, depending on the licence dossier, for IPV and HepB components

Method: _____
Reference preparation: _____
Specification: _____
Validity criteria (linearity, parallelism): _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Final bulk vaccine (Hib)

Refer to the section on "Final bulk" in Appendix 1a below for the Hib component of this model protocol for the production and testing of a combined vaccine.

Final lot

For the D, T, aP, IPV, HepB vaccine
Lot number: _____
Date of filling: _____
Type of container: _____
Number of containers remaining after inspection: _____
Filling volume: _____

Appearance

Method: _____
Specification: _____
Date of test: _____
Result: _____

Identity of each component

Method: _____
Specification: _____
Date of test: _____
Result: _____

Degree of adsorption for each component

Method: _____
Specification: _____
Date of test: _____
Result: _____

Extractable volume

Method: _____
Specification: _____

Date of test: _____
Result: _____

pH

Method: _____
Specification: _____
Date of test: _____
Result: _____

Aluminium content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Test for sterility

Method: _____
Media: _____
Volume inoculated: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Bacterial endotoxins

Method: _____
Specification: _____
Date of test: _____
Result: _____

Preservative content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Osmolality

Method: _____
Specification: _____
Date of test: _____
Result: _____

If performed at the final lot stage: in vivo assay for D, T, aP and, depending on the marketing authorization, for IPV and HepB components

Specify the strain, sex, weight range and number of animals used; the dates, volumes, route and doses used for immunization and challenge or bleeding; the nature, lot number and potency of the reference vaccine; and the responses at each dose. Express results in International Units (IUs) where applicable; and specify the confidence interval, slope of the parallel line model and the outcome of tests for the absence of linearity and parallelism:

_____

_____

_____

In vivo assay for IPV (where applicable) (if not performed on the final bulk)

Species, strain, sex, and weight range: _____
Date of vaccination: _____
Lot number of reference vaccine: _____
Vaccine doses: _____
Date of bleeding: _____
Date of assay: _____
Number of animals responding at each dose: _____
ED_{50} of reference and test vaccines: _____
Potency of test vaccine: _____
Validity criteria (linearity, parallelism, precision, ED_{50} between highest and lowest responses): _____
Results: _____

In vitro assay, depending on the marketing authorization, for IPV and HepB components

Method: _____
Reference preparation: _____
Specification: _____
Validity criteria (linearity, parallelism): _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Date of start of period of validity: _____

For Hib component

Lot number: _____
Date of filling: _____

Appearance

Method: _____
Specification: _____
Date of test: _____
Result: _____

Identity

Method: _____
Specification: _____
Date of test: _____
Result: _____

Extractable volume

Method: _____
Specification: _____
Date of test: _____
Result: _____

pH

Method: _____
Specification: _____
Date of test: _____
Result: _____

PRP content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Aluminium

Method: _____
Specification: _____
Date of test: _____
Result: _____

Antimicrobial preservative

Method: _____
Specification: _____
Date of test: _____
Result: _____

Residual moisture (for freeze-dried preparations)

Method: _____
Specification: _____
Date of test: _____
Result: _____

Test for sterility

Method: _____
Media: _____
Volume inoculated: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Pyrogenicity or bacterial endotoxins

Method: _____
Specification: _____
Date of test: _____
Result: _____

Free purified polysaccharide

Method: _____
Specification: _____
Date of test: _____
Result: _____

Stabilizer

Method: _____
Specification: _____
Date of test: _____
Result: _____

Date of start of period of validity: _____

Additional tests that may be performed on the final mixture (D, T, aP, IPV, HepB and Hib) if applicable

Bacterial endotoxins

Method: _____
Specification: _____
Date of test: _____
Result: _____

Osmolality

Method: _____
Specification: _____
Date of test: _____
Result: _____

pH

Method: _____
Specification: _____
Date of test: _____
Result: _____

Appearance

Method: _____
Specification: _____
Date of test: _____
Result: _____

Inspection of final containers

Date of inspection: _____
Organoleptic characteristics: _____
Number of containers inspected: _____
% of containers rejected: _____

3. Certification by the manufacturer

Name of the manufacturer _____

Name of head of production (typed) _____

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

I certify that lot no. _____ of DT-based combined vaccine, whose number appears on the label of the final container, meets all national requirements and/or satisfies Part A[1] of the WHO Recommendations to assure the quality, safety and efficacy of DT-based combined vaccines (2014).[2]

Signature _____
Name (typed) _____
Date _____

[1] With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

[2] WHO Technical Report Series, No. 980, Annex 6.

4. Certification by the NRA

If the vaccine is to be exported, attach a certificate from the NRA (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.

Appendix 1a

Model protocol for the manufacturing and control of the Hib component of DT-based combined vaccines

Identification and source of starting materials (particularly any materials of human or animal origin – e.g. strain of bacteria; master and working seeds; excipients and preservatives).

Preparation date and reference number of seed lot(s). Date of approval of protocol indicating compliance with national requirements and with the marketing authorization.

Tests on starting materials:

Production details, in-process controls and dates of tests

Intermediate stages

Purified polysaccharide (PRP)
Lot number(s):
Date(s) of manufacture:
Quantities, storage temperature, storage time and duration of approved storage period:

Identity
Method:
Specification:
Date of test:
Result:

Moisture content
Method:
Specification:
Date of test:
Result:

Molecular size distribution

Method: _____
Specification: _____
Date of test: _____
Result: _____

Degree of polymerization

Method: _____
Specification: _____
Date of test: _____
Result: _____

Ribose content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Phosphorus content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Protein content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Nucleic acid content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Pyrogenicity or bacterial endotoxins

Method: _____
Specification: _____

Date of test: _____
Result: _____

Residual reagents
Method: _____
Specification: _____
Date of test: _____
Result: _____

Carrier protein

Lot number(s): _____
Date(s) of manufacture: _____
Quantities, storage temperature, storage time and duration of approved storage period: _____

For diphtheria toxoid or tetanus toxoid used as a carrier protein

Certification of production in compliance with corresponding WHO Recommendations for diphtheria vaccines (*1*) and for tetanus vaccines (2) – unless different requirements are approved for the antigenic purity for tetanus toxoid for use as a carrier protein.

Identity
Method: _____
Specification: _____
Date of test: _____
Result: _____

Test for sterility
Method: _____
Media: _____
Volume inoculated: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Specific toxicity of diphtheria toxin or tetanus toxin
Method (specify Lf injected): _____
Specification: _____
Date of test: _____
Result: _____

Reversion to toxicity

Specify the dates of the beginning and end of incubation, the dates of the beginning and end of the test, the number of animals used, the volume inoculated into cell culture (for diphtheria only) or injected into animals, the number of animals used (if relevant), and the test results.

Method (specify Lf injected): _____
Specification: _____
Date of test: _____
Result: _____

Antigenic purity

Method: _____
Specification: _____
Date of test: _____
Result (Lf/mg protein (nondialysable) nitrogen): _____

For diphtheria protein CRM197

Identity

Method: _____
Specification: _____
Date of test: _____
Result: _____

Test for sterility

Method: _____
Media: _____
Volume inoculated: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Purity

Method: _____
Specification: _____
Date of test: _____
Result: _____

Toxicity

Method: _____
Specification: _____

Date of test: _____
Result (Lf/mg protein (nondialysable) nitrogen): _____

For meningococcal group B outer membrane protein complex

Identity

Method: _____
Specification: _____
Date of test: _____
Result: _____

Test for sterility

Method: _____
Media: _____
Volume inoculated: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Lipopolysaccharide content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Pyrogenicity

Method: _____
Specification: _____
Date of test: _____
Result: _____

Bulk conjugate

Lot number(s): _____
Date(s) of manufacture: _____
Volume(s), storage temperature, storage time and duration of approved storage period: _____

PRP content

Method: _____
Specification: _____
Date of test: _____
Result: _____

Protein content

Method: _____
Specification: _____
Date of test: _____
Result: _____

PRP to protein ratio

Method: _____
Specification: _____
Date of test: _____
Result: _____

Molecular size distribution

Method: _____
Specification: _____
Date of test: _____
Result: _____

Free PRP

Method: _____
Specification: _____
Date of test: _____
Result: _____

Free carrier protein

Method: _____
Specification: _____
Date of test: _____
Result: _____

Unreacted functional groups

Method: _____
Specification: _____
Date of test: _____
Result: _____

Residual reagents

Method: _____
Specification: _____
Date of test: _____
Result: _____

Test for sterility

Method: _____
Media: _____
Volume inoculated: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Final bulk vaccine

Lot number: _____
Date of manufacture: _____
Volume, storage temperature, storage time and duration of approved storage period: _____

Test for sterility

Method: _____
Media: _____
Volume inoculated: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Antimicrobial preservative

Method: _____
Specification: _____
Date of test: _____
Result: _____

References

1. Recommendations to assure the quality, safety and efficacy of diphtheria vaccines. In: *WHO Expert Committee on Biological Standardization. Sixty-third report*. Geneva, World Health Organization, 2014 (WHO Technical Report Series, No. 980), Annex 4.
2. Recommendations to assure the quality, safety and efficacy of tetanus vaccines (adsorbed). In: *WHO Expert Committee on Biological Standardization. Sixty-third report*. Geneva, World Health Organization, 2014 (WHO Technical Report Series, No. 980), Annex 5.
3. Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines. In: *WHO Expert Committee on Biological Standardization. Sixty-second report*. Geneva, World Health Organization, 2014 (WHO Technical Report Series, No. 979), Annex 4.
4. Recommendations for whole-cell pertussis vaccine. In: *WHO Expert Committee on Biological Standardization. Fifty-sixth report*. Geneva, World Health Organization, 2007 (WHO Technical Report Series, No. 941), Annex 6.

5. Recommendations for the production and control of poliomyelitis vaccine (inactivated). In: *WHO Expert Committee on Biological Standardization. Fifty-first report*. Geneva, World Health Organization, 2002 (WHO Technical Report Series, No. 910), Annex 2.
6. Recommendations to assure the quality, safety and efficacy of recombinant hepatitis B vaccines. In: *WHO Expert Committee on Biological Standardization. Sixty-first report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 978), Annex 4.

Appendix 2

Model certificate for the release of DT-based combined vaccines by NRAs

Lot-release certificate

Certificate no. _____

The following lot(s) of _____ combined vaccine produced by _____[1] in _____,[2] whose numbers appear on the labels of the final containers, complies with the relevant national specifications and provisions for the release of biological products[3] and Part A[4] of the WHO Recommendations to assure the quality, safety and efficacy of DT-based combined vaccines (2014),[5] and with corresponding WHO recommendations for each of the vaccine's individual components, as well as with WHO good manufacturing practices: main principles for pharmaceutical products;[6] Good manufacturing practices for biological products;[7] and Guidelines for independent lot release of vaccines by regulatory authorities.[8]

The release decision is based on _____[9]

The certificate may include the following information:

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

---

1 Name of manufacturer.

2 Country of origin.

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

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

5 WHO Technical Report Series, No. 980, Annex 6.

6 WHO Technical Report Series, No. 961, Annex 3.

7 WHO Technical Report Series, No. 822, Annex 1.

8 WHO Technical Report Series, No. 978, Annex 2.

9 Evaluation of the summary protocol, independent laboratory testing, or procedures specified in a defined document etc., as appropriate.

- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary)
- type of container used
- number of doses per container
- number of containers or lot size
- date of start of period of validity (e.g. manufacturing date) and/or expiry date
- storage conditions
- signature and function of the person authorized to issue the certificate
- date of issue of certificate
- certificate number.

The Director of the NRA (or other appropriate authority)

Name (typed) _____
Signature _____
Date _____

Appendix 3

Examples of international names, proper names and abbreviations of combined vaccines

DT-based combined vaccines[1]

- diphtheria and tetanus vaccine (adsorbed) – abbreviation: DT;
- diphtheria and tetanus vaccine (adsorbed, reduced diphtheria antigen content) – abbreviation: dT;
- diphtheria and tetanus vaccine (adsorbed, reduced diphtheria and tetanus antigen content) – abbreviation: dt;
- diphtheria, tetanus and whole cell pertussis vaccine (adsorbed) – abbreviation: DTwP;
- diphtheria, tetanus and acellular pertussis vaccine (adsorbed) – abbreviation: DTaP;
- diphtheria, tetanus and acellular pertussis vaccine (adsorbed, reduced diphtheria, tetanus and acellular pertussis antigen content) – abbreviation: dtap.

DTwP-based combined vaccines

- diphtheria, tetanus, whole-cell pertussis and poliomyelitis (inactivated) vaccine (adsorbed) – abbreviation: DTwP-IPV;
- diphtheria, tetanus, whole-cell pertussis and recombinant hepatitis B vaccine (adsorbed) – abbreviation: DTwP-HepB;
- diphtheria, tetanus, whole-cell pertussis, recombinant hepatitis B vaccine and poliomyelitis (inactivated) vaccine (adsorbed) – abbreviation: DTwP-HepB-IPV.

DTwP-based combined vaccines with Hib

- diphtheria, tetanus, pertussis and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed); fully liquid or lyo-liquid – abbreviation: DTwP-Hib_X or DTwP+Hib_X;[2]

[1] The acellular pertussis vaccine component of the combination vaccine may be produced by purification or co-purification of the acellular pertussis components. In accordance with section A.1.1 of WHO Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines, the international name for this component is "acellular pertussis vaccine" in both cases.

[2] Subscript "X" denotes the carrier protein – e.g. tetanus toxoid or CRM197.

- diphtheria, tetanus, pertussis, poliomyelitis (inactivated) and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed) (all-in-one or with separate freeze-dried Hib) – abbreviation: DTwP-IPV-Hib$_X$ or DTwP-IPV+Hib$_X$;
- diphtheria, tetanus, pertussis, hepatitis B and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed) (all-in-one or with separate freeze-dried Hib) – abbreviation: DTwP-HepB-Hib$_X$ or DTwP-HepB+Hib$_X$;
- diphtheria, tetanus, pertussis, hepatitis b, poliomyelitis (inactivated) and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed) (all-in-one or with separate freeze-dried Hib) – abbreviation: DTwP-HepB-IPV-Hib$_X$ or DTwP-HepB-IPV+Hib$_X$.

DTaP-based combined vaccines

- diphtheria, tetanus, acellular pertussis and poliomyelitis (inactivated) vaccine (adsorbed) – abbreviation: DTaP-IPV;
- diphtheria, tetanus, acellular pertussis and recombinant hepatitis B vaccine (adsorbed) – abbreviation: DTaP-HepB;
- diphtheria, tetanus, acellular pertussis, recombinant hepatitis b and poliomyelitis (inactivated) vaccine (adsorbed) – abbreviation: DTaP-HepB-IPV.

DTaP-based combined vaccines with Hib

- diphtheria, tetanus, acellular pertussis and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed) (all-in-one or with separate freeze-dried Hib) – abbreviation: DTaP-Hib$_X$ or DTaP+Hib$_X$;
- diphtheria, tetanus, acellular pertussis, poliomyelitis (inactivated) and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed) (all-in-one or with separate freeze-dried Hib) – abbreviation: DTaP-IPV-Hib$_X$ or DTaP-IPV+Hib$_X$;
- diphtheria, tetanus, acellular pertussis, recombinant hepatitis B and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed) (all-in-one or with separate freeze-dried Hib) – abbreviation: DTaP-HepB-Hib$_X$ or DTaP-HepB+Hib$_X$;
- diphtheria, tetanus, acellular pertussis, hepatitis b, poliomyelitis (inactivated) and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed) (all-in-one or with separate freeze-dried Hib) – abbreviation: DTaP-HepB-IPV-Hib$_X$ or DTaP-HepB-IPV+Hib$_X$.