Recommendations to assure the quality, safety and efficacy of

DT-based combined vaccines

Replacement of TRS 800 Annex 2

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Adopted by the 63rd meeting of the WHO Expert Committee on Biological Standardization, 15 to 19 October 2012. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.
Recommendations and guidelines 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 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 for additional guidance intended for manufacturers and NRAs, which may benefit from those details.
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Introduction

A combined vaccine may be defined as a vaccine that consists of two or more antigens (preformulated or mixed immediately before administration) that is intended to protect against more than one infectious disease and/or against an infectious disease that is caused by different types/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) and/or whole-cell pertussis vaccines (DTwP). These were followed by combinations with various acellular pertussis antigens (DTaP) as an alternative to DTwP and with the addition of one or more of *Haemophilus influenzae* type b conjugates (Hib<sub>conj</sub>) and hepatitis B (HepB) surface antigen (HBsAg).

Currently there are many DTwP-based and DTaP-based combined vaccines available worldwide that vary in the amounts of each antigen and the total range of antigens according to the intended age range for use (i.e. infants, toddlers, older children, adolescents and 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 currently approved in some countries comprise all of DTaP, IPV, HBsAg and Hib<sub>conj</sub> but it is quite possible that even more extensive combined vaccines may be developed in future (e.g. containing conjugated meningococcal polysaccharides).

WHO’s *Requirements for diphtheria, tetanus, pertussis and combined vaccines* (1) incorporated guidance on the individual components (e.g. diphtheria vaccine, tetanus vaccine and pertussis vaccine, whole-cell). This is followed by a separate section on the manufacture of vaccines containing more than one of the individual components (e.g. DT and DTwP) that commences at the stage of the final bulk vaccine and considers the specifics of manufacture of the final combined product. However, the section on “Requirements for combined vaccines (Adsorbed)” stated that no attempt was “made to include other combinations, including those with *Haemophilus influenzae* and *Neisseria meningitidis* polysaccharides and poliomyelitis vaccines”.

In addition, it is current WHO policy to include in new recommendation documents 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 any such guidance.

Since the publication of the first *Requirements for diphtheria, tetanus, pertussis and combined vaccines* (1) new and revised WHO recommendations that are directly relevant to DT-based combined vaccines have been established or are under development. These documents all concern single vaccines as follows:

- Recommendations to assure the quality, safety and efficacy of diphtheria vaccines (2);
- Recommendations to assure the quality, safety and efficacy of tetanus vaccines (3);
Revised 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);
Amendment to potency testing of diphtheria and tetanus vaccines (9);
Manual for quality control of diphtheria, tetanus and pertussis vaccines (revised) (10).

WHO convened two meetings on the revision of 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, on 21–22 June 2011 and the second was held in Beijing, People’s Republic of China on 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 product 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 their clinical evaluation 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, with a revised version in order to take into account all developments that have occurred since 1990. The main changes that have been introduced in this revision are:

- a change of title from “requirements” to “recommendations”;
- abbreviations for DT-based combined vaccines and their components;
- replacement of self-standing sections on the components of DT-based combined vaccines with references to the individual vaccine recommendations, wherever relevant;
- inclusion of considerations for all DT-based combined vaccines that had been developed at the time of drafting of this revision (e.g. including those with all of HBsAg, IPV and Hibconj);
- inclusion of new sections on the clinical and nonclinical evaluation of DT-based combined vaccines;
- revision of the summary protocol for the information to be provided for the purpose of lot release of DT-based combined vaccines.
Scope

The scope of this document is to provide guidance on the data requirements for 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 the WHO recommendations for the individual component vaccines of a combined vaccine, the following considerations are considered to be 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, including national control laboratories.

Important issues for 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 that is appropriate for the intended use;
- the applicability of testing methodology originally established for mono-component vaccines;
- the suitability of mono-component reference material for use with combined vaccines;
- the corresponding release and stability criteria.

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

The nonclinical programme for development of a new DT-based combined vaccine should follow the general guidance (11), with particular attention to the choice of animal models 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 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 to separate
administration or administration in less complex vaccines. For example, inclusion of a conjugated polysaccharide in a combined vaccine has sometimes been associated with lower antibody levels compared with separate injections that are co-administered or separated in time. In addition, immunological interference resulting in 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 co-administering a conjugate-containing combined vaccine 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 concomitant vaccinations with other vaccines (including those of the schedule of the Expanded Programme on Immunization) has to be taken in consideration.

On the other hand, extensive experience with licensed and WHO-prequalified DT-based combined vaccines is now available which demonstrates that the above concerns can be overcome and that combined vaccines can be developed that have proved to be safe and effective.

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

**Terminology**

The definitions given below apply to the terms used throughout this document. They may have different meanings in other contexts.

*Adverse event:* Any untoward medical occurrence in a clinical trial subject to which a vaccine has been administrated. The occurrence does not necessarily have a causal relationship with the vaccine/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 drug or a vaccine (12).

*Booster vaccination:* Vaccination given at a certain time interval (at least six months) after primary vaccination in order to induce long-term protection (12).
**Bulk**: The processed purified material, prepared from either a single harvest or a pool of a number 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, and 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/effectiveness, or with traceability to a vaccine with established efficacy/effectiveness, that is tested in parallel with an experimental vaccine and serves as an active control in nonclinical or clinical testing (5). Examples of comparator vaccines that can be used in studies of combination vaccines can be found in Table 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 and further processed (e.g. freeze-dried) from a single final bulk container in one continuous working session. Different final lots may be filled and/or further processed from the same final bulk in different working sessions. These related final lots are sometimes referred to as sublots, 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, whole-cell agglutination) that can be demonstrated in laboratory tests.

**Immunogenicity**: The capacity of a vaccine to induce antibody-mediated and/or cell-mediated immunity and/or immunological memory (12).

**Non-inferiority margin or limit**: A prespecified limit based on an appropriate confidence interval. Meeting this criterion can exclude a prespecified difference in immune response believed to be clinically meaningful.

**Non-inferiority trial**: A trial with the primary objective of showing that the response to the product under investigation is not clinically inferior to the comparator vaccine (12)

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

**Primary vaccination**: 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 the vaccination (12).
Secondary endpoints: Prespecified endpoints that are considered in addition to the primary endpoints for evaluating the outcomes of a clinical trial.

Seroconversion: A predefined increase in antibody concentration, considered to correlate with the transition from seronegative to seropositive, providing information on the immunogenicity of a vaccine. If there are preexisting antibodies, seroconversion is defined by a transition from a predefined low level to a significantly higher defined level such as a fourfold increase in geometric mean antibody concentration \((12)\).

Vaccine effectiveness: The protection rate conferred by vaccination in a specified population. Vaccine effectiveness measures both direct and indirect protection (i.e. protection of nonvaccinated persons by the vaccinated population). Vaccine effectiveness is also determined by vaccination coverage, correlation of vaccine strains with circulating strains, and incidence of disease due to strains not included in the vaccine following introduction of the vaccine in that population \((12)\).

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 sample) \((12)\).
Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International names, proper names and abbreviations

The international names and abbreviations should follow the examples in Appendix 3. Other combined vaccines exist or may be developed in the future and the international names should follow the structure of the examples – e.g. combinations with meningitis antigens added. 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. Abbreviations are proposed for practical purposes and for use in this document only.

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 vaccine components. For instance:

- DTwP-HepB is a combined vaccine composed of diphtheria toxoid, tetanus toxoid, whole-cell pertussis suspension and purified hepatitis B surface antigen and presented with a suitable adjuvant (e.g. aluminium salts).
- DTaP-HepB-IPV-HibX or DTaP-HepB-IPV+HibX 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 and 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 is currently no International Standard or International Reference Preparation specifically designed for combined vaccines. For WHO reference materials that may be used in laboratory or clinical evaluations of combined vaccines, reference is made to section A.1.3 of the relevant WHO recommendations for the individual vaccine components. 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 mono-component international, regional and national reference materials which 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 as it has been shown that, in some cases, the qualitative differences in antigen and/or excipient composition between a monovalent reference preparation and a combined vaccine under test may result in invalid test

results (e.g. deviations from parallelism of the dose-response lines) and/or excessive variability within and between assays and between laboratories (17–21). The latter is of particular significance for the NRA lot release. Therefore, in some laboratories, combined vaccines with a composition that is close to that of the combined vaccine under test 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 was evident in 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 procedure or otherwise) to give official status to such process-specific or product-specific reference materials. This can be done by assessing in collaborative studies the suitability and behaviour of such reference materials by both manufacturers and national control laboratories. Where calibration of such references in International Units (IU) is not possible, the specifications stating the acceptable limits of 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).

A.2 General manufacturing recommendations

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

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

A.3 Production control

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

A.3.1 Control of final bulk

It should be noted that, in general, formulation conditions that have been established as optimal for mono-component vaccines may not be optimal for some combined vaccines. Important considerations include the choice and concentration of any preservative agent of adjuvant and its optimal ratio to antigen(s), pH and ionic strength. Formulation conditions should be validated to ensure optimal clinical immunogenicity, reactogenicity and stability of the vaccine.
For combined vaccines with a Hib component, two types of formulation have been developed: vaccines with all components in the same container (fully liquid or all-in-one) and those with the Hib component in a separate container (lyo-liquid). The specific testing conditions and issues for these two types differ, as described in the relevant test sections.

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 vaccine component bulk materials may be adsorbed to/mixed with adjuvant at an acceptable concentration prior to blending into the final vaccine bulk (these intermediates have been called pre-adsorbed 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 WHO’s Guidelines on stability evaluation of vaccines (25).

A.3.1.2 Preservative
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 components and to cause no unexpected adverse reactions in humans. The preservative and its concentration should be approved by the NRA. Certain antimicrobial preservatives, particularly those of the phenolic type, were shown to adversely affect the antigenic activity of the tetanus and diphtheria components and are not recommended for use in combined vaccines that are within the scope of these recommendations. Similarly, thiomersal is known to affect adversely the antigenic activity of the poliomyelitis (inactivated) component (26). 2-phenoxy-ethanol has been shown to be a suitable alternative for some vaccines but its compatibility with the antigens in the combined vaccine should be evaluated on a case-by-case basis. For prequalification of multidose presentations, programmatic issues such as the compatibility of the formulation with 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 as to its effect on the safety and immunogenicity/efficacy of the combined vaccine. If adjuvants are used, their concentration and quality characteristics, demonstrating their suitability as an adjuvant and compatibility with the components of the combined vaccine under consideration, should be approved by the NRA.

Aluminium compounds are generally used as mineral carriers. The quality characteristics for aluminium hydroxide, hydrated, for adsorption have been described in the European Pharmacopoeia monograph 1664 (28).

It should be noted that the concentration of aluminium may be higher in combined vaccines than in mono-component vaccines due to the contribution of the individual pre-adsorbed component bulks during blending. The final bulk may also contain a mixture of adjuvants coming from the individual component pre-adsorbed 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 a parameter for consistency, release and stability.
In some countries, upper limits for the concentration of mineral carriers are set at lower (less than half) amounts 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 D₁, D₂, D₃; T₁, T₂, T₃; w/aP₁, w/aP₂, w/aP₃; and HepB₁, HepB₂, HepB₃. At the level of the formulated final bulk the combination would be D₁T₁w/aP₁HepB₁, D₂T₂w/aP₂HepB₂, D₃T₃w/aP₃HepB₃.

In the case of the addition of a new component (e.g. IPV) to an established (licensed) combined vaccine (e.g. DTw/aP-HepB), and after demonstration of the consistency of the new component at the level of the bulk antigen (if new to the particular manufacturer), consistency is demonstrated as: D₁T₁w/aP₁IPV₁, D₁T₁w/aP₁IPV₂, and D₁T₁w/aP₁IPV₃.

A.3.2 Control tests of final bulk

Each final bulk of the combined vaccine should be tested for sterility, potency of each component and specific toxicity, in accordance with the individual recommendations for each of its components. In general, the testing described in the corresponding sections of the WHO recommendations for the individual vaccine components is applicable to combined vaccines. Reference is also made to the WHO manual for quality control testing 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. Further development and use of simplified in vivo models (e.g. single-dilution models), and particularly those that would allow the concurrent serological testing of multiple components (e.g. purified pertussis antigens and diphtheria and tetanus toxoids (30–34)), would result in a significant reduction of laboratory animal use. A laboratory that intends to introduce an alternative method should perform adequately validation studies in comparison 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 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 without a whole-cell pertussis component. Indeed, 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 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 per SHD is not useful for monitoring consistency as the observed potency estimates are always higher. Therefore, in addition to setting a minimum specification for the potency estimate of 30 IU per SHD for vaccine used for the immunization of children, and as approved by the NRA, manufacturers should set lower and upper consistency limits that reflect the potency values that are found in practice for the combined vaccine under consideration and that have been demonstrated to be safe and effective in the clinic. The close monitoring of such limits and the trending of all key consistency data, both by manufacturers and NRA is of particular importance (35).

A.3.2.2 Tetanus potency testing
In analogy with diphtheria toxoid, potency values determined by the tests described in the Recommendations to assure the quality, safety and efficacy of tetanus vaccines (3) are significantly higher in the presence of a whole-cell pertussis 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 (60 IU for vaccines containing wP when assayed in mice) per SHD is not useful for monitoring consistency since the observed potency estimates are always much higher. Therefore, in addition to setting a minimum specification for the potency estimate of 40 IU (60 IU for vaccines containing wP when assayed in mice) per SHD for vaccine used for the primary immunization of children, and as approved by the NRA, manufacturers should set lower and upper consistency limits that reflect the potency values that are found in practice for the combined vaccine under consideration and that have been demonstrated to be safe and effective in the clinic. The close monitoring of such limits and the trending of all key consistency data, both by manufacturers and the NRA, is of particular importance (35).

A.3.2.3 Hepatitis B potency testing
In principle, in vitro assays can be used for combined vaccines, as outlined in 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 in 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) as compared to vaccines containing only the HepB component. Specifications should be set accordingly and manufacturers should set lower and upper consistency limits, approved by the NRA, that reflect the potency values that are found in practice for the combined vaccine under consideration and that have been demonstrated to be safe and effective in the clinic. The close monitoring of such limits and the trending of all key consistency data, both by manufacturers and NRA, is of particular importance (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 the potency-related and stability-indicating testing of the Hib component (total saccharide, molecular weight distribution, free saccharide, free carrier protein). Manufacturers are encouraged to develop a methodology that allows such tests to be performed on the formulated vaccine (including at the stage of the final lot). If
justified, and with the approval of the NRA, performing such tests at the bulk conjugate stage may be considered acceptable. Animal models (e.g. mice, rats, rabbits or guinea pigs), although less used for routine lot release, are useful to characterize the protective potency or immunogenicity and its consistency and, if needed, to monitor stability.

For combined vaccines with a separate freeze-dried Hib component, the testing required by the WHO recommendations for the individual components may be performed on the separate containers, as appropriate to the combined vaccine under consideration (see further under section A5 on Control of final product).

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 CHO cell-based assay may not be applicable for testing 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 PT (e.g. aluminium-based adjuvants, IPV). Proper standardization of the in vivo test and the development and introduction of alternative test methods are strongly encouraged. Section A.3.4.2.5 on residual activity of pertussis toxin in Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines provides further information (5).

A.3.2.6 wP Potency tests
For some vaccines, 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. estimated potency not less than 4.0 IU in the volume recommended for an SHD, and the lower fiducial limit \[ P = 0.95 \] of the estimated potency not less than 2.0 IU).

A.3.2.7 Endotoxin
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 such vaccines, the wP component is by far the major contributor to the final endotoxin content. In general, for each component of combined vaccines, 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 per SHD.

A.4 Filling and containers
The requirements concerning filling and containers given in WHO’s Good manufacturing practices: main principles for pharmaceutical products (23) and Good manufacturing practices for biological products (24) should 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 for identity of each component, sterility, pyrogenicity or endotoxin content, adjuvant content, preservative content, potency of each component, and innocuity in accordance with the individual recommendations for each component. In general, the testing methodology described in the corresponding sections of WHO’s recommendations for the individual vaccine components is applicable to combined vaccines. A number of issues that are specific to combined vaccines are described in section A.3.

It should be noted that, when a combined vaccine is composed of two separate preparations which should be reconstituted with each other at the time of administration (lyo-liquid formulations), full approved release testing carried out on each of the two preparations separately is deemed sufficient for routine release testing purposes. 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 compatibility of the two components following reconstitution have been shown by the manufacturer to be satisfactory, with due consideration of batch consistency, batch size and frequency of production. These studies will show that the components and the final reconstituted combination have sufficient comparability of quality, innocuity and immunogenicity to meet the release specifications and that any systematic effect of the reconstitution is consistent between batches and compatible with 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’s Good manufacturing practices: main principles for pharmaceutical products (23) and Good manufacturing practices for biological products (24) should apply.

A generic model of a suitable summary protocol to be used for combined vaccines is provided in Appendix 1.

A.7 Labelling

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

- the word "combined" or “combination”;
- the word "adsorbed", 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 carton, or the leaflet accompanying the container should contain the following:

– 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.8 Retained samples

The requirements given in WHO’s *Good manufacturing practices: main principles for pharmaceutical products* (23) and *Good manufacturing practices for biological products* (24) should apply.

A.9 Distribution and transport

The recommendations given in WHO’s *Good manufacturing practices: main principles for pharmaceutical products* (23) and *Good manufacturing practices for biological products* (24) should 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 *Guidelines on stability evaluation of vaccines* and in particular with the section of those guidelines on combined vaccines (25).

A.10.1 Stability

Stability evaluation is an important part of the quality assessment. The purpose of stability studies is to ensure that, at the end of its shelf-life, storage period or period of use, each of the component of the vaccine still has the required characteristics to support quality, safety and efficacy. If applicable, the desorption of antigens from the adjuvant, which may occur over time, should be investigated and, where possible, limits should be agreed with the NRA.
The real-time stability of the vaccine in its final containers, maintained at the recommended storage temperature, should be demonstrated to the satisfaction of the NRA. In general, stability for licensure, stability at different stages of the manufacturing process, and stability for clinical trial approval of a combined vaccine should be studied by the manufacturer in accordance with WHO’s *Guidelines on stability evaluation of vaccines* (25).

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 in the production procedure, the stability of the vaccine produced by the new method should be demonstrated.

### A.10.2 Storage conditions

Recommended storage conditions and defined maximum duration of storage should be based on stability studies, as described in section A.10.1 above, and should be approved by the NRA. For DT-based combined vaccines, a temperature of 2–8 °C is generally considered to be satisfactory. This 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 shelf-life if the conditions under which the vaccine is stored are in accordance with what is stated on the label.

The manufacturer should recommend conditions of storage and transport that should ensure that 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 on the basis of a shelf-life justified by the stability studies, as described above (section A.10.1), and should be approved by the NRA.
Part B. Nonclinical evaluation of 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 (proof of concept) and safety testing in animals. The extent to which nonclinical studies will be required depends on the type of antigen, the complexity of the formulation, and the clinical experience that already exists with the different vaccine components, both alone and in combination. More extensive nonclinical testing is likely to be required when the combined vaccine includes novel antigens or 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 support that (i) the individual vaccine antigens and final product are well-defined and 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, on the basis of animal immunogenicity and/or protection data, to provide an acceptable level of protection against the diseases targeted by each of the vaccine components 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 characterization of the vaccine. The goal is to collect data that can be submitted in support of a submission to the NRA. The purpose of the submissions will vary during the product development process. In some cases, the nonclinical data will be submitted to support the initiation of a specific clinical study; in other cases, the nonclinical data will be included in a marketing authorization application. The goal of preclinical testing, defined as the nonclinical testing done prior to initiation of any clinical investigations, 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 assessment in immunogenicity and/or challenge studies in animal models (proof of concept) and safety testing in animals. However, extensive nonclinical testing may not be required for vaccines that use component antigens that are the same (i.e. from the same manufacturer and produced by the same methods) as those in vaccines that have already been approved. New combined vaccines that require nonclinical testing could result from any of the following:

- combination of two or more already approved products;
- addition of a new (and not currently licensed) vaccine antigen to an existing vaccine;
- a replacement of one antigen in a combination with another antigen for the same indication;
- removal of an antigen from an approved combination;
– a change in manufacturing of one or more of the individual vaccine components;
– a change in the amount of one or more of the vaccine antigens or excipients;
– a change in the adjuvant, preservative, or other excipient.

The specific questions to be addressed in nonclinical testing depend on the nature of the changes. However, the primary concerns relate to the compatibility of each of the vaccine components, 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 combined, and the potential for increased reactogenicity. Some of these evaluations will include testing in relevant animal models, as discussed below. Comprehensive toxicology studies (section B.6) will not necessarily be required for all new combined vaccines. Prior to 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 the nonclinical studies with respect to manufacturing, immunological activity/potency, purity, stability and other characteristics of quality.

B.2 Characterization of the individual vaccine components prior to formulation

For vaccines based on novel vaccine antigens or on formulations for which one or more of the components are produced using a new manufacturing process that is different from the established one, nonclinical testing should include detailed characterization and evaluation of the individual vaccine components 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 the individual vaccine components in the combination

Creation of a new combined vaccine by any of the scenarios described above leads to a change in environment for each of the component vaccine antigens. 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 such as the degree of adsorption to adjuvant, physicochemical or immunochemical integrity, or stability.

Thus, the combined antigens should be examined by appropriate means to evaluate possible changes to antigen properties in the combination. Compatibility of all the antigenic components of the vaccine should be demonstrated in nonclinical studies. Where relevant, adsorption of all the antigenic components present in the vaccine should be shown to be consistent on a lot-to-lot basis. Possible desorption of antigen during the shelf-life of the product should be evaluated 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 adjuvant and antigen, as formulated for clinical use. Whenever possible, the properties of the individual antigens should be evaluated through a comparison with the properties of the same antigens in uncombined licensed vaccine. In some situations, the licensed comparator(s) may be lower-order combined vaccines (e.g. DTP as comparator for DTP-Hepatitis B vaccine combinations).

B.4 Immunogenicity evaluation in animal models

Before 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 between combined antigens. When possible, it is preferable to study a new combination in comparison with the individual antigens (or 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 vaccine components can be evaluated is encouraged for such investigations.

Immunogenicity studies in animal models can provide important information with respect to optimization of adjuvant formulations and the evaluation of immunological characteristics of the antigen including, for some antigens, the ability to induce functional antibodies and/or protection from challenge. However, experience has shown that extrapolation of data from animal models to human disease has to be approached with caution. The following 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 to each of the component antigens should be directly compared between the candidate vaccine and at least one licensed comparator – preferably one for which there has been extensive clinical use and data generation that supports its effectiveness in routine use. If testing is performed due to a significant change in manufacturing, 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, the evaluation of functional antibody responses and/or cellular immunity.
- If a new candidate vaccine contains a new adjuvant, its inclusion should be supported by adequate immunogenicity data that, in addition to measuring humoral antibody, 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 the 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 and/or a group receiving the antigen adsorbed to an aluminium compound.

**B.5 Nonclinical safety studies**

Preclinical animal studies to determine the safety profile of the combination of adjuvant and vaccine should also be undertaken. The safety of the new combination should be evaluated in an animal model on a case-by-case basis, and especially if there is a concern that combining antigens and/or adjuvants may lead to toxicity problems (e.g. a novel adjuvant). For vaccines that contain one or more chemically inactivated toxins (e.g. diphtheria, tetanus, 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 and efficacy/appropriateness for use in a particular product must be documented. The safety of new additives can be evaluated by the use of vaccine formulations without antigen. However, the compatibility of a new additive with all vaccine antigens should be documented, in addition to the toxicological profile of the particular combination of antigen(s) and additive in animal models.

**B.6 Toxicology studies**

Toxicology studies on the final formulation (including antigens and adjuvant) 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 is formulated with a novel adjuvant, nonclinical toxicology studies should be conducted as appropriate for the final vaccine formulation, including adjuvant. Repeated dose toxicity study 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, toxicity studies of the adjuvant alone, in some situations, 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 been 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 evaluation of immunogenicity of vaccine together with adequate animal safety/toxicological studies, taking into account existing guidelines.
Part C. Clinical evaluation of combined vaccines

C.1 Introduction and scope

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 is proposed. Clinical trials should adhere to the general principles described in international guidelines on good clinical practice (36) and to WHO’s *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 under consideration and according to previous clinical experience with the vaccine components and similar vaccines. The vaccine-specific requirements for clinical studies should be discussed with the appropriate NRAs.

These recommendations specifically address the clinical evaluation of combined vaccines that contain diphtheria and tetanus toxoids. Currently approved DT-based combined vaccines include one or more of the following additional components: pertussis (whole-cell or acellular), Hib-conjugate, inactivated polio, and/or Hepatitis B. Although this document focuses on combinations in current 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 as this is the most effective prevention strategy for many diseases. However, catch-up and booster strategies, adult vaccination, and vaccination of special populations are common. These recommendations address issues that are relevant to the diverse indications and usage of DT-based combined vaccines.

The main goals of the clinical development programme for a DT-based combined vaccine are to evaluate the safety of the combined vaccine and the immunogenicity of each component within the vaccine. Generally, the clinical development programme should include comparative clinical trials. Section C.2 of these recommendations discusses the overall design of comparative clinical trials and the choice of comparator vaccine(s). Unless an alternate 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 those antigens that are 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 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 in 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. Both immunogenicity and safety
effects 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 vaccines administered separately and to obtain an adequate safety database that is needed for risk–benefit considerations prior to licensure. With respect to immunogenicity, the primary concern is typically 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 for providing clinical benefit. The existence of established serological correlates of protection for some components of DT-based combined vaccines facilitates the selection of immunological endpoints and the interpretation of immunogenicity data. The use of immunogenicity studies to infer clinical benefit for vaccines requires the careful selection, proper design, and adequate validation of the assays (see section C.3 for additional discussion). The NRA should be consulted when immunoassays are being selected and evaluated for 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 is essential in order to make recommendations regarding concomitant use (12). Concomitant administration may give rise to lower immune responses to one or more of the co-administered antigens (i.e. immune interference) (15), although the clinical significance of the observed phenomena 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 this diversity of possible interactions, initial assessment of the effects of concomitant vaccine administration is advisable at an early stage of clinical development. Nevertheless, data on the effects of co-administration will be accumulated throughout the clinical development programme and in 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’s Guidelines on clinical evaluation of vaccines: regulatory expectations (12). A clinical development programme for a new vaccine will typically begin with smaller safety and immunogenicity studies and then progress to larger studies. For paediatric vaccines containing novel antigens and formulations, it may be appropriate to obtain preliminary evaluation of safety and immunogenicity in adults and then progress in a stepwise fashion from older to younger age groups. When evaluating such studies, it should be noted that safety and immunogenicity may be dependent on age, prior infection and/or prior immunization.

Prior to initiation of any clinical study, the manufacturer should provide justification for the choice of the 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 individual vaccine components, as well as nonclinical studies, and formal dose-ranging clinical studies. In all cases, clinical studies should be initiated only for products with adequate information on nonclinical testing and manufacturing.

Consistency of manufacture 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 with respect to the manufacturing consistency of the product). Nevertheless, multiple lots of the combined vaccine formulation intended for marketing, using different bulk lots for each of the immunogens, should be used during the late 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 late 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 for which there is considerable clinical experience. In late-phase clinical development, the most appropriate study design is usually a randomized, controlled trial in the target age group. The selection of the comparator(s) 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(s). 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 (into separate injection sites) or whether there should be staggered administrations (i.e. on different days).

Table 1 describes the most common scenarios that are likely to be encountered in the clinical evaluation of a new combined vaccine. New combinations could result from different types of changes to existing combined vaccines, including addition of a new antigen, replacement of one antigen with another antigen for the same indication, removal of an antigen, or significant change in manufacturing or formulation. Additionally, a new manufacturer may wish to begin the production of 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 these new situations. For each trial, manufacturers should justify the choice of comparator vaccine, the trial design, and the safety and immunogenicity endpoints.

Table 1 provides more detail for immune response evaluations due to their increased complexity.
Table 1. Overview of potential scenarios that may be encountered with new combination vaccines $^a$

<table>
<thead>
<tr>
<th>Category</th>
<th>Scenario</th>
<th>Example</th>
<th>Suggested design $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen addition</td>
<td>Combination of two or more already approved products (e.g. AB + C $\rightarrow$ ABC).</td>
<td>Licensed IPV is added to a licensed DTwP-HepB.</td>
<td>Immune responses to ABC are compared with immune responses to the separately administered licensed vaccines, AB and C.</td>
</tr>
<tr>
<td></td>
<td>Combination of one already approved product (AB) and a new (and not currently licensed) vaccine antigen (C) (e.g. AB + C $\rightarrow$ ABC).</td>
<td>A new and unlicensed antigen is added to a licensed DTaP-HepB.</td>
<td>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 the licensed product.</td>
</tr>
<tr>
<td>Antigen replacement</td>
<td>One of the antigens in a combination is replaced by an already approved antigen (for the same vaccine component) (e.g. ABC $\rightarrow$ ABC*).</td>
<td>The wP component of licensed DTwP is replaced by a licensed aP component.</td>
<td>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 a licensed product containing C*.</td>
</tr>
<tr>
<td></td>
<td>One of the antigens in a combination is replaced by a novel (unlicensed) antigen (for the same vaccine component) (e.g. ABC $\rightarrow$ ABC*).</td>
<td>The aP component of licensed DTaP is replaced by a new aP component containing genetically modified aP antigen(s).</td>
<td>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 the licensed product.</td>
</tr>
<tr>
<td>Change in manufacturing and/or</td>
<td>Increase or decrease in the amount of one or more of the vaccine</td>
<td>The diphtheria toxoid content is reduced.</td>
<td>Immune responses to ABc are compared with immune responses to a licensed</td>
</tr>
<tr>
<td>Antigen removal</td>
<td>Change in nature and/or content of adjuvant, preservative or other excipient.</td>
<td>Introduction of a new adjuvant.</td>
<td>Immune responses to the investigational vaccine are compared with immune responses to the licensed product manufactured by the approved process.</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------------------------------------------------------</td>
<td>-------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Significant change in the manufacture of one or more of the individual vaccine components (e.g. ABC → ABC*).</td>
<td>Change from use of lyophilized Hib-conjugate component to a fully liquid formulation.</td>
<td>Immune responses to the investigational vaccine are compared with the immune responses to the licensed product manufactured by the approved process.</td>
<td></td>
</tr>
<tr>
<td>Antigen removal</td>
<td>Removal of one or more antigens (e.g. ABC → AB).</td>
<td>Removal of HepB antigen from a DTwP-Hib-HepB.</td>
<td>Immune responses to antigens A and B of AB are compared with immune responses to the licensed vaccine ABC.</td>
</tr>
<tr>
<td>New manufacturer</td>
<td>A combination comparable to another licensed product is produced by new manufacturer (e.g. ABC → A<em>B</em>C*).</td>
<td>Production by new manufacturer of a DTwP-Hib-IPV.</td>
<td>Immune responses to ABC are compared with immune responses to a licensed product with similar composition.</td>
</tr>
</tbody>
</table>

Notes:

a) Table 1 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 the clinical evaluations.

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

c) When reducing antigen content, clinical studies should be designed to verify that antigen reduction does not lead to a clinically important reduction in immunogenicity.

d) A rationale for the change in adjuvant, preservative or other excipient should be provided. In particular, clinical studies evaluating a change in 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 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(s) that are already approved for similar vaccines. However, a formal evaluation of 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 and/or an adjuvant that is considerably different than that 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 very large range of geographical regions. For instance, within a specific population, immune responses and/or rates of some adverse events following immunization with vaccine with 6-, 10- and 14-week schedule may differ from those following administration of the same vaccine on a 2-, 4- and 6-month schedule, or on a 3-, 5- 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 extrapolation of the findings. When a vaccine is anticipated for use according to different schedules, the recommendation of the 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 for which the least immune response is expected). However, there is still a need to collect some safety data with schedules that are proposed for approval because the local and systemic reactogenicity associated with a vaccine may also differ between schedules within a specific population due to age-related prevalence of specific adverse events. For all clinical trials, the study population should be carefully defined and justified by the manufacturer and 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 recommended according to the schedule of the country and region 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 and control vaccines, 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 WHO guidance (12). In some settings, there may be multiple licensed vaccines directed against the same diseases and that are 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(s) to be used in clinical studies 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 indicate that immune responses are lower to one or more of the antigens on co-administration with a new combined vaccine compared to the separately administered licensed vaccine(s), 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 on co-administration will need to be weighed against the convenience of administering multiple vaccines at 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. prematurity, immunodeficiency, or severe pulmonary disorders, including
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 these populations at increased risk. In many instances, these studies may be performed after initial licensure.

C.3 Assessment of immune responses

C.3.1 Design and scope of the immunogenicity studies

The specific questions to be addressed in immunogenicity studies depend on the nature of the new combined vaccine; however, the primary concerns usually relate to the potential for immunological interference between antigens. This document applies to a wide range of combined vaccines with potentially a large number of antigens for which immunogenicity evaluation is required. The sections below provide guidance related to the selection of assays and endpoints 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 immunization of older children, adolescents and adults is 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 in booster immunization. This section therefore also includes information related to the evaluation of vaccines used in booster immunization.

C.3.2 Assays to assess antibody responses

For many of the antigens used in DT-based combined vaccines that are currently approved, WHO guidelines or recommendations have been written and are available to provide guidance on the most appropriate assays and endpoints for 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. These documents, when available, should be consulted. However, such guidelines are not available for some of the individual antigens used in many combined vaccines. Table 2, which lists antigens, commonly used assays and suggested endpoints, is provided as a reference. However, available WHO guidelines should be considered the primary source of information.

For some antigens, the endpoints used for the evaluation of primary immunization studies are not optimal for the evaluation of booster immunization studies. This may occur, for example, if a significant proportion of a study population has a concentration of antibody that exceeds a protective threshold prior to immunization. In such cases, an evaluation of the proportion of subjects who show a significant increase in antibody concentration may provide a more sensitive assessment of the response to immunization. To reflect these differences, suggested endpoints for primary and booster immunization studies are provided in separate columns in Table 2.

The assessment of the immune response should be based on measurement of the antibody concentration for each component antigen in serum using a validated and standardized assay (12). To improve the comparability and acceptability of the serological data across trials, results of immunogenicity outcomes should be expressed in IU per ml of human serum whenever an
international reference is available. The selection of assays for evaluation of 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. The 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 to each other (e.g., whether the criteria for evaluation are based on percentages with 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 the processing and storage of samples, reference standards and reagents. The assay validation data should be reviewed and should be 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 vaccine components. For some vaccine antigens, a functional assay is the recommended assay for immunogenicity evaluation (Table 2). In other cases, a nonfunctional assay has been accepted for primary evaluations; however, in such cases, the functional assay (if available) 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 yet been identified for some commonly-used antigens included in some acellular pertussis vaccines (5).

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

**C.3.3 Immunogenicity endpoints for immunization studies**

For antigens contained in currently licensed DT-based combined vaccines, Table 2 provides a summary of the recommended assays and suggested primary endpoints for clinical evaluation of vaccines intended for primary and/or booster immunization. References to available WHO documents, national or regional guidelines, or literature publications are provided. These should be consulted for more complete information.
### Table 2. Immunogenicity assays and endpoints for primary and booster immunization studies\textsuperscript{a}

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Assay</th>
<th>References</th>
<th>Suggested primary endpoint(s)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria toxoid</td>
<td>Micro-neutralization assay (Vero cells)</td>
<td>(2, 31, 38–41)</td>
<td>Proportion ≥ 0.01 IU/ml or ≥ 0.1 IU/ml (see comment)</td>
<td>Proportion with significant increase \textsuperscript{d}                                                                                     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 per ml may be acceptable if toxin neutralization assay is used and if a booster dose is administered in the second year of life. Otherwise a threshold of 0.1 IU per ml should be used. For booster immunization, the decision to use a threshold level or significant increase should take into account the proportion expected to exceed threshold prior to vaccination. An antigen-binding assay that has been shown to correlate with the mouse neutralization assay is most commonly used.</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td>ELISA</td>
<td>(3, 30)</td>
<td>Proportion ≥ 0.1 IU per ml</td>
<td>Proportion with significant increase \textsuperscript{d}</td>
</tr>
<tr>
<td>Whole-cell pertussis \textsuperscript{b}</td>
<td>1) Agglutination assay  2) ELISA for PT  3) ELISA for other antigens</td>
<td>(4, 42–45)</td>
<td>a) GMT/GMC  b) Proportion with 4-fold rise</td>
<td>a) GMT/GMC  b) Proportion with 4-fold rise \textsuperscript{d}</td>
</tr>
<tr>
<td>Acellular pertussis</td>
<td>ELISA for all pertussis antigens in vaccine \textsuperscript{c}</td>
<td>(5, 42–45)</td>
<td>a) GMT/GMC  b) Proportion with 4-fold rise</td>
<td>a) GMT/GMC  b) Proportion with 4-fold rise \textsuperscript{d}</td>
</tr>
</tbody>
</table>
Inactivated polio vaccine | Virus neutralization assay for each of the three serotypes | (46) | Proportion with neutralization titre ≥1:8 | a) GMT/GMC b) Proportion with neutralization titre ≥1:8 | Presence of neutralizing antibody (titre ≥1:8) is considered protective against poliovirus types 1, 2 and 3.

**Haemophilus influenzae type b conjugate vaccine** | ELISA (Haemophilus type b capsular polysaccharide; PRP) | (8, 47–49) | a) Proportion ≥ 0.15 µg per ml b) Proportion ≥ 1.0 µg per ml | Proportion ≥ 1.0 µg/mL | A post-vaccination anti-PRP level of 0.15 µg per ml is considered the minimal protective level; a post-vaccination level of 1.0 µg per ml indicates protection through a subsequent one-year period. For primary immunization, both a) and b) are recommended as co-primary endpoints.

Hepatitis B vaccine | ELISA for Hepatitis B surface antigen antibodies | (6) | Proportion ≥ 10 mIU per ml | Proportion ≥ 10 mIU per ml | Notes:

a) Abbreviations used in table – ELISA: enzyme-linked immunosorbtant assay; GMT: geometric mean titre; GMC: geometric mean concentration; PT: pertussis toxin; PRP: *Haemophilus influenzae* type b capsular polysaccharide, polyribosyl-ribitol-phosphate; IU: international unit.
b) There is substantial heterogeneity in the antibody response to highly effective whole-cell pertussis vaccines. However, the assays listed could be used in the evaluation of a comparative immunogenicity trial.
c) Additional supportive data using the pertussis toxin neutralization assay and whole-cell agglutination assay is encouraged.
d) The magnitude of increase (e.g. 4-fold) in antibody concentration from pre- to post-vaccination should be predefined and justified. A lower magnitude of increase may be justified for persons with specified high antibody concentration prior to vaccination (2, 3).

### 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 co-primary.

Definition of the appropriate time intervals for assessing the immune response should take into account the study objectives. In most cases, clinical studies for new vaccines are designed to determine the antibody response to the vaccine 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 who 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 could be informative and may provide insight into the rapidity of the response to antigen challenge.

The selection of the primary parameters for the assessment of non-inferiority, the predefined margins of non-inferiority, and hence the total sample size for a comparative study will need careful justification. Factors to consider regarding the stringency of the non-inferiority criteria include the clinical relevance of the endpoint, 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 endpoint is known to correlate well with protection against 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 non-inferiority criteria will influence sample size for the study, 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 setting non-inferiority margins, consideration also should be given to the potential for downward drift in immunogenicity over time with sequential comparative studies (50). The consequence of such drift, if it occurs, is that a new vaccine could be considerably less immunogenic than the vaccine originally licensed. 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 candidate and licensed vaccines are generally required, comparisons to historical data generated during previous protective efficacy studies using similar assays may, in some cases, provide supportive evidence.

For the majority of the antigens contained in currently-approved DT-based combined vaccines, the primary assessment will be the proportion of subjects who respond to the vaccine (as defined in Table 2). Typically, this will be the proportion of subjects reaching a prespecified threshold. However, for some vaccines and some indications, a response is defined as the proportion of vaccinees with significant increase (e.g. greater than fourfold) in immune response above pre-immunization levels. Alternative definitions for responders could be considered if they are well justified. The groups should be compared using an appropriate predefined non-inferiority limit; generally the upper bound of the two-sided 95% confidence interval of the observed difference (comparator minus new combined vaccine) should be less than the criterion agreed with the NRA – most commonly 0.05 or 0.10.

For some antigens and for some indications, co-primary analyses should compare the magnitude of the response to the vaccine antigens induced by the new vaccine and the licensed comparator. Such endpoints are recommended, for instance, in the evaluation of whole-cell and acellular pertussis vaccines because no threshold protective response has been widely accepted, and 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 for each vaccine component is compared on the basis of the ratio of the geometric mean concentrations (GMCs) or geometric mean titres (GMTs) using a pre-defined margin of non-inferiority. Specifically, the upper bound of the two-sided 95% confidence interval of 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 – most commonly 1.5 or 2.0.

Measurement of pre- and post-immunization antibody concentrations involves the collection of an extra blood sample and may not be necessary in all studies. Pre-immunization samples will be required when endpoints are based on the proportion of subjects with a rise in antibody, but these samples may not be needed from all subjects when the endpoint is based on the proportion of subjects that reach a specified threshold. However, even when a pre-immunization sample is not required to evaluate a study endpoint, 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 the post-vaccination antibody values.

Due to limitations of sera volumes it is commonly necessary to perform an additional randomization step to select sera for use in the different antibody assays and/or prioritize so that the most relevant questions for that combination can be addressed.

For complex combined vaccines, immunogenicity evaluations may include a substantial number of co-primary endpoints. If there is any immune interference observed with respect to any of the combined antigens, the possible clinical implications and the reasons for not meeting the predefined non-inferiority 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 and the comparator vaccines, the severity of the disease, the likelihood that the measured immune parameter(s) predict clinical protection, and the potential benefits of the combination in terms of improved 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 the immune responses. If not included among the primary endpoints, comparisons of the magnitude of the response to the vaccine antigens induced by the new vaccine and the licensed comparator should be considered. As above, the magnitude of the response for each vaccine component is compared on the basis of the ratio of the GMCs or GMTs using a predefined margin of non-inferiority. The non-inferiority 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 vaccine components may play an important role in the evaluation of combined vaccines, even when antigen-binding assays are used in the evaluation of primary endpoints. For example, the measurement of functional antibodies should be considered in at least a subpopulation of the comparator and the test vaccine groups, 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 to verify 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 with 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 and licensed comparator vaccines and when monitoring changes over time in the antibody levels (51). As one example, the RCDs reveal the proportion of the population at or below protective threshold values and provide data that 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

To date, the carrier proteins used in licensed polysaccharide conjugate vaccines have included a non-toxic genetically-modified diphtheria toxin molecule (CRM197), diphtheria toxoid, tetanus toxoid, protein D from *Haemophilus influenzae*, and an outer membrane protein complex (OMPC) of *Neisseria meningitidis* serogroup B. Monitoring of the immune response to these carrier proteins may be appropriate in some circumstances. Administration of a conjugate vaccine that employs diphtheria or tetanus toxoid or CRM197 as the carrier(s) has been found to enhance the relevant anti-toxin antibody levels. However, this has not been accepted to date as a replacement for routine immunization with vaccines containing diphtheria or tetanus toxoid. Co-administration of a new conjugate vaccine with routine infant and toddler vaccines (i.e. vaccine containing diphtheria and tetanus toxoids) could result in high antitoxin levels (52). Careful attention should be paid to the reactogenicity observed under these circumstances of use 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 induced 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 and timing of booster doses

Waning of antibody concentrations over time is inevitable and longer-term follow-up of persistence of immunity should be evaluated at various time-points following the primary vaccination series. The total duration of serological follow-up should be discussed and planned in advance in conjunction with the NRA. In some situations, these data may be provided after first approval. The waning of antibody over time should not be interpreted *per se* as 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 will be based on epidemiological investigations and long-term surveillance (see section C.5). A detailed discussion is outside the scope of this document.

C.4 Safety evaluation

The pre-licensure 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’s 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 above (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 composition (including all antigens and adjuvant), the presence of novel antigens, any past experience with vaccines with 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 (combined from all trials in the targeted age group) of approximately 3000–5000 subjects who receive 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 available for it, the NRA may accept a smaller number or may request a larger database prior to first approval.

Additionally, the safety evaluations should include high-risk individuals (e.g. preterm infants, the chronically ill or immunocompromised subjects) that may benefit from the vaccination. Safety in these groups is often assessed in 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 safety and effectiveness following initial approval of a new vaccine. NRAs should ensure that adequate pharmacovigilance plans are in place regarding these activities at the time of first licensure. 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 post-licensure studies and continued oversight of vaccines after licensure are provided in WHO’s Guidelines on clinical evaluation of vaccines: regulatory expectations (12).

Through active post-marketing surveillance, every effort should be made to improve current scientific understanding of the protection in humans afforded by vaccines. Vaccine effectiveness in the population should be reported wherever 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 for longer-term protection and for evidence of any changes in vaccine effectiveness. Post-marketing assessments designed to monitor effectiveness are particularly important in the specific case when 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 pre-licensure 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 for any safety concerns identified in pre-approval trials, as well as for 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. Pre- and post-approval discussions between vaccine manufacturers responsible for placing the product on the market and national and international public health bodies are essential for ensuring that reliable safety and effectiveness data are collected in the post-marketing period in selected countries and regions.
Part D. Recommendations for national regulatory authorities

D.1 General

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

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of DT-based combined vaccine, should be discussed with and approved by the NRA. For control purposes, the International Standards currently in force should be obtained for calibration of the national/regional/working standards (22). The NRA may obtain the product-specific/working reference from the manufacturer to be used for lot release until international/national standard preparation is established.

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

D.2 Official release and certification

A vaccine should be released only if it fulfills national requirements and/or satisfies Part A of these recommendations (35).

A protocol based on the model 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 release of 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 vaccine in question meets all national requirements as well as Part A of the present recommendations. The certificate should provide sufficient information on 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.

Authors and acknowledgements

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of comments from national regulators, the vaccine industry and the general public during public consultation on WHO web site.

References


Appendix 1

Model generic summary protocol for manufacturing and control of a DT-based combined vaccine.

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by the manufacturer to the NRA. Information and tests may be added or deleted as required by the NRA, if applicable.

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 on 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 accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a lot release certificate from the NRA of the country in which the vaccine was produced stating that the product meets national requirements as well as the recommendations in Part A of this document published by WHO.

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

A summary protocol for the Hib component was drafted (Appendix 1a) as a separate appendix to this document. This cross-referral is for the purpose of simplifying the layout of this guideline only. The information provided by the manufacturer in individual protocols should not cross-refer between different products.

Summary information on the 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 package lot: __________________________
Final container lot number: __________________________
Number of filled containers in this final lot: ____________________
Date of manufacture: ____________________
Nature 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 per human dose, including any adjuvant used and other excipients)
______________________________________________________________________________
______________________________________________________________________________

Shelf-life approved (months): ____________________
Expiry date: ____________________
Storage condition: ____________________

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 of 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 of each production stage of each component: ____________________
Date(s) of manufacture: ____________________

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

**Detailed information on manufacture and control**

**Starting/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” of WHO’s *Recommendations to assure the quality, safety and efficacy of diphtheria vaccines* (1).
For a T component: refer to the sections on “Production strain and seed lots”, “Single harvests” and “Bulk purified toxoid” of WHO’s Recommendations to assure the quality, safety and efficacy of tetanus vaccines (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” of WHO’s Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines (3).

For a wP component: refer to the sections on “Strains” and “Control of single harvests” of WHO’s Recommendations for whole-cell pertussis vaccine (4).

For an IPV component: refer to the sections on “Control of source materials” (virus seed lots, cell cultures, serum for cell cultures) and “Control of vaccine production” (single harvests and monovalent pools) of WHO’s Recommendations for the production and control of poliomyelitis vaccine (inactivated) (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)” of WHO’s Recommendations to assure the quality, safety and efficacy of recombinant hepatitis B vaccines (6).

For a Hib component: refer to the relevant sections of Appendix 1a on the Hib component of this generic summary protocol for 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 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 approved storage period: _______________________

Information on composition of the final bulk: Specify relevant (adsorption, blending) production dates, reference number(s), volume(s) and concentrations (in Lf per ml for each of Diphtheria and Tetanus, in µg per ml for each of aP component UD per ml of D antigen of IPV component, in µg per ml of HBsAg of HepB component).

Blending

<table>
<thead>
<tr>
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<th>Identification</th>
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<td>Diphtheria toxoid (Lf):</td>
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<td>__________</td>
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</tr>
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<td>aP (µg):</td>
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<tr>
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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: _________________________________________
Result: _______________________________________

**Residual BSA**
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 number, strain and sex of animal; 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 dates of beginning and end of incubation, number, strain and sex of animals; this test is not necessary for a product obtained by genetic modification.

Method: ______________________
Dose: ______________________
Specification: ______________________
Date test on: ______________________
Date test off: ______________________
Result: ______________________

In vivo assay for D, T, aP and, depending on the licence dossier, for IPV and HepB components

Specify strain, sex, weight and number of animals; dates, volumes, route and doses of immunization and challenge or bleeding; nature, lot number and potency of reference vaccine; and responses at each dose level. Express results in International Units where applicable, specify confidence interval, slope of parallel line model and outcome of tests for 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 specifications: ______________________
Date of vaccination: ______________________
Lot number of reference vaccine: ______________________
Vaccine doses: _______ _________
Date of bleeding: ______________________
Date of assay: ______________________
Number of animals responding at each dose: ______________________
ED50 of reference and test vaccine: ______________________
Potency of test vaccine: ______________________
Validity criteria (linearity, parallelism, precision,
ED$_{50}$ between highest and lowest response) :

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 on the Hib component of this generic summary protocol for 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 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 strain, sex, weight and number of animals; dates, volumes, route and doses of immunization and challenge or bleeding; nature, lot number and potency of reference vaccine; and responses at each dose level. Express results in International Units where applicable, specify confidence interval, slope of parallel line model and outcome of tests for absence of linearity and parallelism.

In vivo assay for IPV (where applicable) (if not performed on the final bulk)
Species, strain, sex, and weight specifications: ________________________________
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 vaccine: ________________________________
Potency of test vaccine: ________________________________

Validity criteria (linearity, parallelism, precision,
ED$_{50}$ between highest and lowest response): ________________________________
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 PRP
Method: ______________________________
Specification: _________________________
Date of test: _________________________
Result: ______________________________

Stabiliser
Method: ______________________________
Specification: _________________________
Date of test: _________________________
Result: ______________________________

Date of start of period of validity: ________________________________

Additional tests which can be performed on the final mixture (D,T, aP, IPV, HepB 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 rejected containers: ______________________

Certification by the manufacturer

Name of head and production (typed) ______________________

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

I certify that lot number ... of ...combined vaccine, whose number appears on the label of the final containers, meets all national requirements and satisfies Part A of the Recommendations to assure the quality, safety and efficacy of DT-based combined vaccines (if applicable)
Name (typed)  __________________________
Signature  __________________________
Date  __________________________
Appendix 1a

Model summary protocol for Hib component

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

Preparation date and reference number of seed-lot(s). Date of approval of protocol indicating compliance with the 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 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 approved storage period: ____________________

For diphtheria or tetanus toxoid used as carrier protein

Certification of production in compliance with the WHO Recommendations to assure the quality, safety and efficacy of diphtheria vaccines (1) and Recommendations to assure the quality, safety and efficacy of tetanus vaccines (2) (except, if applicable, the differing requirements for the antigenic purity for tetanus toxoid).

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 or tetanus toxin**

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

**Reversion to toxicity**

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

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

**Antigenic purity**

- **Method:**
- **Specification:**
- **Date of test:**
- **Result (Lf per mg protein N):**

**For diphtheria protein CRM197**

**Identity**

- **Method:**
- **Specification:**
### Test for Sterility

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<tr>
<td>Media</td>
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</tr>
<tr>
<td>Volume inoculated</td>
<td></td>
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<tr>
<td>Date of start of test</td>
<td></td>
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<tr>
<td>Date of end of test</td>
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<td>Result</td>
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### Purity

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### Toxicity

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</tr>
<tr>
<td>Date of test</td>
<td></td>
</tr>
<tr>
<td>Result (Lf per mg protein N)</td>
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</table>

### For OMP (meningococcal group B outer membrane protein complex)

### Identity

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<tbody>
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<td>Result</td>
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**Test for sterility**

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<tr>
<td>Volume inoculated:</td>
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<tr>
<td>Date of start of test:</td>
<td>__________________________</td>
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<tr>
<td>Date of end of test:</td>
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**Lipopolysaccharide content**

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**Pyrogenicity**

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<tr>
<td>Result:</td>
<td>__________________________</td>
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**Bulk conjugate**

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<th>Lot number(s) :</th>
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<tbody>
<tr>
<td>Date(s) of manufacture:</td>
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</tr>
<tr>
<td>Volume(s), storage temperature, storage time and approved storage period:</td>
<td>__________________________</td>
</tr>
</tbody>
</table>

**PRP content**

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<tbody>
<tr>
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<tr>
<td>Date of test:</td>
<td>__________________________</td>
</tr>
<tr>
<td>Result:</td>
<td>__________________________</td>
</tr>
</tbody>
</table>
**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 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

Appendix 2

Model certificate for the release of combined vaccines by national regulatory authorities

Lot release certificate

Certificate no. ________________

The following lot(s) of xxx combined vaccine produced by ____________________________ in ______________ whose numbers appear on the labels of the final containers, meet all national requirements and comply with Part A of Recommendations to assure the quality, safety and efficacy of DT-based combined vaccines (2012) and the recommendations for the vaccine’s individual components, and also comply with Good manufacturing practices for pharmaceutical products, Good manufacturing practices for biological products and Guidelines for independent lot release of vaccines by regulatory authorities.

The release decision is based on ____________________________________________.

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
• Lot number(s) (including subplot numbers, packaging lot numbers if necessary)
• Type of container
• Number of doses per container
• Number of containers/lot size
• Date of start of period of validity (e.g. manufacturing date) and/or expiry date
• Storage condition
• Signature and function of the authorized person and authorized agent to issue the certificate

• Date of issue of certificate
• Certificate number.

The director of the National Regulatory Authority (or authority as appropriate):
Name (typed) ________________________________________________________________
Signature _________________________________________________________________
Date __________________________________________________________________

1. Name of manufacturer.
2. Country of origin.
3. If any national requirements are not met, specify which one(s) and indicate why 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.
9. Evaluation of summary protocol, independent laboratory testing, and/or specific procedures laid down in defined document etc. as appropriate.
Appendix 3

Examples of international names, proper names and abbreviations (Abbreviations are proposed for practical purposes and for use in this document only)

**DT-based combined vaccines**
- 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 or DTwP+HibX
- Diphtheria, Tetanus, Pertussis, Polyomielitis (inactivated) and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed), (all-in-one or with separate freeze-dried Hib), Abbreviation: DTwP-IPV-HibX or DTwP-IPV+HibX
- 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-HibX or DTwP-HepB+HibX
- Diphtheria, Tetanus, Pertussis, Hepatitis B, Polyomielitis (inactivated) and *Haemophilus influenzae* type b (X-) conjugate vaccine (adsorbed), all-in-one or with separate freeze-dried Hib, Abbreviation: DTwP-HepB-IPV-HibX or DTwP-HepB-IPV+HibX

**DTaP-based combined vaccines**

---

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 the WHO Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines, the international name for this component is “acellular Pertussis vaccine” for both.

2 Where X stands for the carrier protein, e.g. Tetanus Toxoid-, CRM197-...

3 Where X stands for the carrier protein, e.g. T, CRM197-...
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-HibX or DTaP+HibX
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-HibX or DTaP-IPV+HibX
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-HibX or DTaP-HepB+HibX
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-HibX or DTaP-HepB-IPV+HibX