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Guidelines on the quality, safety and efficacy of group B streptococcal conjugate vaccines

(Proposed new guidelines)

NOTE:

The distribution of this draft document is intended to provide information on the proposed document- *Guidelines on the quality, safety and efficacy of group B Streptococcal conjugate vaccines,* to a broad audience and to ensure the transparency of the consultation process.

Written comments proposing modifications to this text MUST be received by 30 January 2026 in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Medicines and Health Products Policies and Standards. Comments may also be submitted electronically to the Responsible Officer: **Dr Tiequn Zhou at email: zhout@who.int**.

The final agreed formulation of the document will be edited to be in conformity with the second edition of the *WHO style guide* (KMS/WHP/13.1).

Guidelines on the quality, safety and efficacy of group B streptococcal conjugate vaccines

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Contents

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

Abbreviations

ADH	adipic acid dihydrazide
CRM197	cross-reactive material 197
CTAB	hexadecyltrimethylammonium bromide
DT	diphtheria toxoid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (also abbreviated to EDAC)
EOD	early onset disease
GBS	group B streptococcus (*Streptococcus agalactiae*)
HIV	human immunodeficiency virus
HPAEC-PAD	high-performance anion exchange chromatography with pulsed amperometric detection
HPLC	high-performance liquid chromatography
HPSEC	high-performance size-exclusion chromatography
IAP	intrapartum antibiotic prophylaxis
IgG	immunoglobulin G
LOD	late onset disease
NCL	national control laboratory
NMR	nuclear magnetic resonance
NRA	national regulatory authority
OPA	opsonophagocytic antibody
TT	tetanus toxoid

Introduction

Group B streptococcal (GBS) disease, caused by the bacterium *Streptococcus agalactiae*, occurs most commonly as neonatal sepsis and meningitis, with a disproportionate burden of morbidity and mortality in low- and middle-income countries (LMICs) (1). Antibiotic prophylaxis given to women in labour (intra-partum antibiotic prophylaxis [IAP]) can be effective in preventing early onset GBS disease in neonates but its use has implications for antimicrobial resistance and IAP is poorly implemented in many LMICs (2). Maternal vaccination has long been proposed as an alternative to IAP and GBS vaccines are amongst the priority vaccines identified by WHO Product Development for Vaccines Advisory Committee (3,4). In addition, GBS disease is identified as a key target for vaccine development in the WHO *Defeating Meningitis by 2030* roadmap (5).

Multivalent capsular polysaccharide–protein conjugate vaccines are at an advanced stage of clinical development. Evidence indicates that they induce vaccine serotype-specific immune responses in pregnant women (6-8). Recognizing the challenges of conducting sufficiently powered pre-licensure efficacy trials for maternal GBS vaccines, efforts have been made to identify serotype-specific immune correlates of protection (8) to support initial regulatory approvals based on safety and immunogenicity data using standardized immunoassays and harmonized reference materials (8). Several protein-based GBS vaccines, including candidates targeting conserved proteins, are also under evaluation but these are at earlier stages of development (7).

Acknowledging the global impact of GBS disease, the WHO Expert Committee on Biological Standardization (ECBS) has expressed its support for the production of written standards to facilitate GBS vaccine development. At its 81st meeting in October 2025, the ECBS agreed to prioritize the development of WHO Guidelines on the quality, safety and efficacy of GBS conjugate vaccines (9).

These recommendations provide guidance for the production and control of GBS conjugate vaccines in Part A and for their nonclinical evaluation in Part B. Part C covers the clinical development programme for vaccines intended to prevent invasive GBS infections in neonates and infants by means of vaccinating pregnant women. These recommendations reflect technological advances including the removal of animal testing from quality control schemes (10,11).

Purpose and scope

These WHO Guidelines provide guidance for national regulatory authorities (NRAs) and vaccine manufacturers on the quality, nonclinical and clinical aspects of human group B streptococcal (GBS) polysaccharide-protein conjugate vaccines to assure their quality, safety and efficacy. The scope of the present document encompasses prophylactic GBS polysaccharide-protein conjugate vaccines intended for immunization of women in the third trimester of pregnancy with the primary aim of preventing early onset GBS disease (EOD) in neonates and late onset GBS disease (LOD) in neonates and infants.

This document should be read in conjunction with other relevant WHO guidance, especially on the nonclinical (12,13) and clinical (14) evaluation of vaccines, as well as the minimum requirements for an effective National Pharmacovigilance System (15). Other WHO guidance should also be consulted as appropriate.

It should be noted that there remain knowledge gaps in the scientific understanding of GBS vaccines which are being addressed by ongoing research and development. This document has been developed in the light of the available knowledge.

Terminology

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

Activated polysaccharide: purified polysaccharide that has been modified by a chemical reaction or a physical process in preparation for conjugation to the activated carrier protein.

Carrier protein: the protein to which the polysaccharide is covalently linked for the purpose of eliciting a T-cell-dependent immune response to the GBS polysaccharide.

Final bulk: The blend of monovalent conjugates present in a single container from which the final containers are filled, either directly or through one or more intermediate containers derived from the initial single container.

Final lot: a number of sealed, final containers that are equivalent with respect to the risk of contamination that may have occurred during filling and, when it is performed, freeze-drying. A final lot must therefore have been filled from a single container and if freeze-dried this should be completed in one continuous working session.

Master seed lot: bacterial suspensions for the production of serotype specific GBS polysaccharide or the carrier protein should be derived from a strain that has been processed as a single lot and is of uniform composition. The master seed lot is used to prepare the **working seed lots**. Master seed lots should be maintained in the freeze-dried form or be frozen at or below −45 °C.

Monovalent bulk conjugate: a conjugate prepared from a single lot or pool of lots of polysaccharide and a single lot or a pool of lots of protein by the covalent bonding of activated polysaccharide to the carrier protein. This is the parent material from which the **final bulk** is prepared.

Purified polysaccharide: the material obtained after final purification of polysaccharide. The lot of purified polysaccharide may be derived from a **single harvest** or a pool of single harvests that have been processed together.

Single harvest: the material obtained from one batch of culture that has been inoculated with the **working seed lot** (or with the inoculum derived from it), harvested and processed during one production run.

Working seed lot: a quantity of bacterial suspension for the production of GBS polysaccharide or the carrier protein that is of uniform composition and that has been derived from the **master seed lot** by growing the organisms and maintaining them in freeze-dried

aliquots or frozen at or below −45 °C. The working seed lot is used to inoculate the production medium.

General considerations

Group B streptococcus (Streptococcus agalactiae)

Streptococcus agalactiae is the most common human pathogen among the streptococci belonging to Lancefield group B. However, in most people it is a harmless commensal bacterium that commonly colonises the gastrointestinal and genitourinary tracts (16).

Historically, *Streptococcus agalactiae* was classified into nine serotypes according to capsular polysaccharides (Ia, Ib, II, III, IV, V, VI, VII, VIII) but a tenth (IX) was identified in 2007 (17, 18). The capsular polysaccharides enable GBS to evade the host's immune system while their heterogeneity contributes to serotype-associated variability in disease severity (17, 18). A review of studies that reported GBS serotypes isolated from cases of invasive GBS disease in infants indicated that 97% were serotypes Ia, Ib, II, III and V. Serotype III dominated (61.5%). No or few data were available from some geographical regions (19).

Invasive GBS disease

Invasive GBS is the leading cause of neonatal sepsis and meningitis worldwide and an important cause of meningitis in early infancy (19-22). Invasive GBS disease in neonates and infants is described according to its time of onset. Early-onset disease (EOD) occurs within the first six days of life and usually occurs due to vertical transmission from GBS-colonised mothers. Late onset disease (LOD) occurs between 7 and 89 days after birth and GBS may be acquired from the mother or may result from nosocomial or community infections.

It was estimated that in 2020 approximately 20 million pregnant women globally were colonised with GBS, leading to more than 230,000 cases of EOD and more than 160,000 cases of LOD annually (2). These cases were estimated to be associated with approximately 90,000 deaths resulting from deliveries without a skilled birth attendant. Infants who survive may develop sequelae such as neurodevelopmental impairment (23, 24).

GBS is also associated with adverse outcomes of pregnancy (e.g. stillbirth and preterm birth), maternal systemic infections during pregnancy or labour and invasive infections in older adults, especially in those with diabetes or cancer (25-27).

Prevention strategies

The primary prevention strategy for GBS EOD is intrapartum antibiotic prophylaxis (IAP). This is administered to women known to be colonised in late pregnancy through microbiological screening, which requires adequate laboratory facilities, or to women identified for receipt of IAP via other risk-based approaches (28, 29). Where used, IAP has reduced the incidence of EOD, but it has not eliminated EOD, and it has no effect on LOD.

Furthermore, IAP raises concerns about increasing antimicrobial resistance in GBS and disruption of the normal flora in neonates (30).

GBS vaccine development

An association between placental transfer of maternal anti-capsular IgG antibodies and protection from invasive GBS was first reported in the 1970s (31). These and later reports pointed to the possibility of preventing GBS EOD and LOD by maternal immunization during the last trimester of pregnancy when placental transfer of IgG is maximal (32, 33). While the focus of GBS vaccine development is on the prevention of EOD and LOD in neonates and infants, it is also possible that maternal vaccination may reduce the risk of maternal complications, stillbirth and preterm birth (30).

It has been estimated that a GBS polysaccharide conjugate vaccine that includes serotypes Ia, Ib, II, III and V could potentially prevent invasive disease caused by serotypes responsible for over 98% of global cases of GBS EOD and LOD (34). However, vaccine efficacy will depend not only on vaccine content but also on levels and persistence of serotype-specific anti-capsular antibodies in neonates and infants and on the levels needed to prevent invasive EOD or LOD due to each serotype. Furthermore, the initial effect of vaccination on GBS EOD rates will vary according to whether IAP is also available where routine maternal immunization is introduced.

Initial clinical trials in the 1980s tested vaccines with purified native type Ia, II or III polysaccharides that had limited immunogenicity (35). To improve immune responses and to elicit immune memory, second-generation candidates used conjugation of capsular polysaccharides to tetanus toxoid or to CRM197 carrier proteins (36-39). For example, a trivalent CRM197-conjugated vaccine containing polysaccharides of serotypes Ia, Ib and III demonstrated an acceptable safety profile and good immunogenicity in Phase 1 and 2 trials that included pregnant women and those with HIV (39). More recently, a GBS polysaccharide-protein conjugate vaccine including six serotypes has been evaluated in clinical trials (6).

Immune response to GBS as a basis for licensure

Phase 3 placebo-controlled GBS vaccine efficacy studies are predicted to require very large sample sizes and may require several years to complete (40). For example, it has been estimated that such a trial with a primary endpoint of invasive GBS disease in neonates and infants would require at least 60,000 pregnant women to be enrolled in countries with a known incidence of >1:1000 live births. Even then, it may take approximately 10 years to accrue sufficient cases to conduct a primary analysis that provides a robust estimate of overall vaccine efficacy. In addition, even a very large trial may not provide reliable estimates of efficacy for each serotype or for each of EOD and LOD. Furthermore, to accrue sufficient cases in a reasonable timeframe such trials would probably have to be conducted in areas where IAP is not standard of care, such that the estimate of efficacy against EOD would not be applicable where IAP is routinely administered.

Given these constraints, initial licensure of GBS vaccines is expected to rely on safety data and on immunogenicity data obtained from cord blood of neonates born to women who were immunized in the third trimester (40). In the absence of a pre-licensure efficacy trial and when there is no well-established immune correlate of protection, it is usually preferred that inference of vaccine efficacy is based on levels of functional antibody (14). In the case of GBS this would require measurement of opsonophagocytic antibody (OPA) titres (41, 42). Although OPA titres would capture the total effect of naturally-acquired functional antibodies directed at GBS capsular and sub-capsular antigens, it has not been possible to establish OPA titre threshold values that strongly correlate with risk of invasive GBS. Therefore, sero-epidemiological studies have focused on identifying threshold values for cord blood anti-capsular IgG that seem to be strongly associated with protection against invasive GBS EOD or LOD caused by specific serotypes (43-45).

Various studies have pointed to possible IgG threshold values that might be applied to infer vaccine efficacy from cord blood anti-capsular antibody levels. For example, a sero-epidemiological study conducted as part of a South African vaccine trial reported a 75% risk reduction for invasive GBS due to serotype III when cord blood IgG against this serotype was at least 0.15 µg/mL with an estimate across serotypes at 0.184 µg/mL (44). Subsequently, a study in Finland suggested that cord blood IgG values of 0.12 µg/mL for serotype III and 0.168 µg/mL across all serotypes were associated with a 75% risk reduction for invasive GBS (46). More recently, a large sero-epidemiological study conducted by the United States Centre for Diseases Control has reported IgG levels associated 75% risk reduction for EOD and LOD due to certain serotypes, including 0.27 µg/mL for serotype III EOD and 0.03 µg/mL for serotype III LOD (47). Further sero-epidemiological studies are ongoing or are still to report.

It will be for individual NRAs to determine the acceptability of initial licensure of a GBS polysaccharide-protein conjugate vaccine based on safety and immunogenicity data. Attention should be paid to the methodologies applied to collect and interpret sero-epidemiological data (48) such that the selected threshold values of IgG derived from reported studies are those that appear most reliable and relevant to individual NRA jurisdictions.

In situations in which initial approval of a vaccine is based on safety and immunogenicity data and inference of vaccine efficacy rests on the application of threshold values to the immune responses to vaccination, it is especially important that there are adequate plans in place at the time of licensure for the conduct of vaccine effectiveness studies (49). These studies should aim to estimate vaccine-associated protection against individual serotypes as well as against each of EOD and LOD. Wherever possible, these studies should include collection of cord blood samples to allow further estimates of IgG concentrations that correlate with protection as well as investigation of the possible relationship between OPA titres and invasive GBS. These data may serve to support existing and/or point to new threshold values that are serotype-specific and/or relevant to EOD and LOD.

International reference materials

When these WHO Recommendations were adopted, no formally established international reference materials were available to support the standardization of immune responses to GBS polysaccharide conjugate vaccines.

The following reagents are available from reference laboratories:

- GBS strains of serotypes Ia, Ib, II, III and V could be obtained from the National Collection of Type Cultures (NCTC; UK Health Security Agency, Colindale, London, United Kingdom) with the identification numbers (14094, 14092, 14093, 14091, 14095, respectively).
- HL-60 cells, available from the American Type Culture Collection (ATCC; Manassas, VA, USA) or the European Collection of Cell Cultures (ECACC; Porton Down, Salisbury, England).

Part A. Guidelines on the development, manufacture and control of group B streptococcal conjugate vaccines

A.1 Definitions

A.1.1 International name and proper name

The international name of the vaccine should be “group B streptococcal conjugate vaccine” with additions to indicate the serotype(s) in the vaccine. The proper name should be the equivalent of the international name in the language of the country in which the vaccine is licensed.

The use of the international name should be limited to vaccines that meet the specifications given below.

A.1.2 Descriptive definition

Multivalent GBS conjugate vaccine is a preparation of capsular polysaccharide from specific serotypes of GBS that are covalently linked to carrier protein.

A.2 General manufacturing requirements

The general manufacturing recommendations contained in WHO Good manufacturing practices for pharmaceuticals (50) and WHO Good manufacturing practices for biological products (51) should be applied to establishments manufacturing GBS conjugate vaccines with the addition of the following:

Details of standard operating procedures for the preparation and testing of GBS conjugate vaccines adopted by the manufacturer, together with evidence of appropriate validation of each production step, should be submitted for the approval of the national regulatory authority (NRA). All assay procedures used for quality control of the conjugate

vaccines and vaccine intermediates must be validated. As may be required, proposals for the modification of manufacturing and control methods should also be submitted for approval to the NRA before they are implemented.

Group B streptococci are Biosafety Level 2 (BSL-2) pathogens. The organism should be handled under appropriate conditions for this class of pathogen (52). Standard operating procedures need to be developed for dealing with emergencies arising from the accidental spillage, leakage or other dissemination of bacteria. Personnel employed in the production and control facilities should be adequately trained. Appropriate protective measures, including vaccination, should be implemented if available. Adherence to current good manufacturing practices is important to the integrity of the product, to protect workers and to protect the environment.

A.3 Control of source materials

A.3.1 Control of polysaccharide

A.3.1.1 Strains of group B streptococci

The streptococcal strains used for preparing the polysaccharide and the tests used for their characterization should be agreed with the NRA. Each strain should be from a single well-characterized stock that can be identified by a record of its history, including the source from which it was obtained, number of passages and the tests used to determine its characteristics. Each strain should have been shown to be capable of stably producing polysaccharide of the appropriate serotype. Proton nuclear magnetic resonance (^{1}H or ^{13}C NMR) spectroscopy, immunochemical tests or any other suitable method may be used for confirming the identity of the polysaccharide.

A.3.1.2 Seed lot system

The production of GBS polysaccharide should be based on a working seed lot system. Cultures derived from the working seed lots should have the same characteristics as the cultures of the strain from which the master seed lot was derived (A.3.1.1). To ensure their stability, seed lots should be stored either freeze-dried or frozen in a dedicated storage facility, as recommended in the WHO good manufacturing practices for biological products (51). Wherever possible, manufacturers are encouraged to avoid the use of materials of animal origin. If, however, materials of animal origin are used in the medium for seed production, for preservation of strain viability for freeze-drying or for frozen storage, they should comply with *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (53) and should be approved by the NRA.

A.3.1.3 Culture media used to produce group B streptococcal polysaccharide

The culture medium used to prepare bacterial seed lots and commercial vaccine lots should also be free from substances likely to cause toxic or allergic reactions in humans. Additionally, the liquid culture medium used for vaccine production should be free from ingredients that will

form a precipitate upon purification of the capsular polysaccharide. If materials of animal origin are used, they should comply with *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (53) and should be approved by the NRA.

A.4 Control of vaccine production

A.4.1 Control of polysaccharide antigen production

A.4.1.1 Single harvests

Consistency of streptococcal growth should be demonstrated by monitoring parameters such as growth rate, pH, pO_2 and the final yield of polysaccharide ; however, monitoring should not be limited to these parameters.

A.4.1.2 Control of bacterial purity

Samples of the culture should be taken before inactivation and be examined for microbial contamination. The purity of the culture should be verified by suitable methods, which should include inoculation on to appropriate culture media, including plate media that do not support growth of GBS. If any contamination is found, the culture or any product derived from it should be discarded. The inactivation process should also be adequately validated.

A.4.1.3 Purified polysaccharide

Each lot of GBS polysaccharide should be tested for identity, purity and molecular size using a combination of validated methods to provide all necessary data and any test limits or ranges not defined by a pharmacopoeia should be agreed with the NRA.

A.4.1.3.1 Polysaccharide identity

A test should be performed on the purified polysaccharide to verify its identity, such as NMR spectroscopy or suitable immunoassay. In cases where other polysaccharides are produced on the same manufacturing site, the method should be validated to show that it distinguishes the desired polysaccharide from all other polysaccharides produced on that manufacturing site.

A.4.1.3.2 Polysaccharide content

The polysaccharide content should be measured using a suitable, validated method agreed with the NRA, such as ^{1}H or ^{13}C NMR (54,55) or HPAEC-PAD (56), a suitable chemical method, or an immunochemical assay. Suitable polysacchairde reference preparations should be used when appropriate

A.4.1.3.3 Molecular size or mass distribution

The molecular size or mass distribution of each lot of purified polysaccharide should be estimated to assess the consistency of each batch. The distribution constant (K_D) should be determined by measuring the molecular size distribution of the polysaccharide at the main peak

of the elution curve obtained by a suitable chromatographic method. The K_D value or the mass distribution limits, or both, should be established and shown to be consistent from lot to lot for a given product. To ensure consistency and a defined proportion of high molecular size polysaccharide for gel filtration using HPSEC, typically at least 50% of each polysaccharide should elute at a K_D value less than a predefined value, depending on the chromatographic method used. However, if molecular weight (MW) is determined by static light scattering then there is no need for a K_D value since it is a coefficient that is dependent on the column used. As an alternative, polysaccharide MW distribution can be determined by gel permeation chromatography using a MW standard calibration curve (that is, dextran, pullulans or polyethylene oxide standards) – the number average molecular weight (Mn), the weight average molecular weight (Mw) and the size average molecular weight (Mz) should be determined to describe the distribution.

An acceptable level of consistency should be agreed with the NRA. Alternatively, calculation of the peak width at the 50% level can be used to analyse the distribution of MW. Suitable detectors for this purpose include a refractive index detector (57), alone or in combination with a static light scattering detector (for example, multi-angle laser light scattering detector). The methodology used should be validated to demonstrate sufficient resolution in the appropriate MW range. Manufacturers are encouraged to produce polysaccharides that have a consistent distribution of molecular size.

A.4.1.3.4 Moisture content

If the purified polysaccharide is to be stored as a lyophilized powder, the moisture content should be determined by suitable methods approved by the NRA and shown to be within agreed limits.

A.4.1.3.5 Protein impurity

The protein content should be determined using a suitable validated method, such as that of the method of Lowry et al., using bovine serum albumin as a reference (58), or another suitable validated method. Sufficient polysaccharide should be assayed to accurately detect protein contamination. Each lot of purified polysaccharide should typically contain no more than 1% (weight/weight) of protein. However, this may vary depending upon the serotype, and an acceptable level of protein contamination should be agreed with the NRA.

A.4.1.3.6 Nucleic acid impurity

Each lot of polysaccharide should contain no more than 2% by weight of nucleic acid as determined by ultraviolet spectroscopy – on the assumption that the absorbance of a 1 g/l nucleic acid solution contained in a cell of 1 cm path length at 260 nm is 20 (59) or by another validated method. Sufficient polysaccharide should be assayed to accurately determine nucleic acid contamination.

A.4.1.3.7 Pyrogen content

The pyrogen content of the purified polysaccharide should be determined and shown to be within acceptable limits agreed by the NRA. The test used should be based on an assessment of the risk that the material is potentially contaminated with endotoxin or non-endotoxin pyrogens. Where there is a risk of non-endotoxin pyrogens being present, the use of the monocyte activation test (MAT) is recommended (60,61). In cases where non-endotoxin pyrogens are unlikely to be present, endotoxin testing using the recombinant Factor C (rFC) assay or the recombinant Cascade Response (rCR) assay is recommended (62).

A.4.1.3.8 Residual process-related contaminants

The levels of residual process-related contaminants in the purified polysaccharide (for example, CTAB, formaldehyde or other bacterial inactivating agent, and antifoaming agents) should be determined and shown to be below the limits agreed with the NRA. The routine testing of each lot before release for residual process-related contaminants may be omitted once consistency of production has been demonstrated on a number of lots agreed with the NRA.

A.4.1.4 Modified polysaccharide

Purified polysaccharide is usually activated to enable conjugation; it may also be partially depolymerized or fragmented, either before or during the activation process.

A.4.1.4.1 Chemical modification

Several methods are satisfactory for the chemical activation modification of polysaccharides prior to conjugation. The method that is chosen should be approved by the NRA.

A.4.1.4.2 Extent of modification of the polysaccharide

The manufacturer should demonstrate consistency of the degree of modification of the polysaccharide, either by an assay of each batch of the polysaccharide or by validation of the manufacturing process. Depending on the conjugation chemistry used, consistency in the degree of polysaccharide activation may be determined as part of process validation and reflect characteristics of vaccine lots shown to have adequate safety and immunogenicity in clinical trials.

A.4.1.4.3 Molecular size distribution

If any size-reduction or activation steps are performed, the average size or mass distribution (that is, the degree of polymerization) of the processed polysaccharide should be measured using a suitable method and shown to be consistent. The molecular size distribution should be specified for each serotype, with appropriate limits for consistency, as the size may affect the reproducibility of the conjugation process.

A.4.2 Control of the carrier protein

A protein that is safe and, when covalently linked with polysaccharide, elicits a T-cell-dependent immune response against polysaccharide could potentially be used as a carrier protein. Suitable carrier proteins include, but are not limited to, TT, DT and CRM197.

A.4.2.1 Consistency of production of the carrier protein

The manufacturing process for a carrier protein should be shown to consistently yield batches that are suitable for the conjugation process. Adequate in-process control should be implemented to monitor critical process parameters, such as the growth rate of the microorganism, pH of production culture and the final yield of the carrier protein.

A.4.2.2 Characterization and purity of the carrier protein

Carrier proteins should be assayed for purity and concentration and tested to ensure they are nontoxic and appropriately immunogenic. All tests used to control the carrier protein should be approved by the NRA.

Preparations of TT and DT should satisfy the relevant WHO recommendations (63,64). CRM_{197} can be obtained from cultures of *Corynebacterium diphtheriae* C7/β197 (65) or by expression in other genetically modified microorganisms (66). CRM197 with a purity of not less than 90% as determined by high-performance liquid chromatography (HPLC) should be prepared by column chromatographic methods. Residual host cell DNA content should be measured and results should be within the limits approved by the NRA for the particular product. Testing for residual host cell DNA content may be omitted if adequate validation data are available. When CRM197 is produced in the same facility as DT, tests should be carried out to distinguish the CRM protein from the active toxin.

A test should be performed on the purified carrier protein to verify its identity. Mass spectrometry or a suitable immunoassay or physicochemical assay could be performed as appropriate and convenient.

Additionally, the carrier protein should be further characterized using appropriate physicochemical methods, such as: (a) sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE); (b) isoelectric focusing; (c) HPLC; (d) amino acid analysis; (e) amino acid sequencing; (f) circular dichroism; (g) fluorescence spectroscopy; (h) peptide mapping; or (i) mass spectrometry. Outcomes should be within the specifications of the carrier protein that was used to prepare the lots evaluated in the definitive clinical studies used for licensing.

A.4.2.3 Specific toxicity of carrier protein

The purified carrier protein should be tested to confirm the absence of toxicity specific to the carrier protein where appropriate (for example, when DT or TT is used as the carrier protein) using a validated non-animal method where available (e.g. the Vero cell assay for DT).

A.4.2.4 Degree of activation of the carrier protein

Adipic acid dihydrazide (ADH) or other appropriate linkers, such as N-succinimidyl-3-(2-pyridyldithio)-propionate or Hydrazine-Polyethylene Glycol-Hydrazine linker (Hz-PEG-Hz linker), can be used to modify the carrier protein (67). The level of protein modification should be monitored, quantified and be consistent. The use of an in-process control may be required. The reproducibility of the method used for modification should be validated.

The level of modification of the carrier protein by ADH can be assessed by determining the amount of hydrazide; this can be achieved by using colorimetric reactions with 2,4,6-trinitrobenzenesulfonic acid using ADH as a standard (68). Other suitable methods include fluorescent tagging followed by HPLC or quadrupole time-of-flight mass spectrometry.

A.4.3 Conjugation and purification of the conjugate

Conjugation methods involve multistep processes. Prior to demonstrating the immunogenicity of the vaccine in clinical trials, both the method of conjugation and the control procedures should be established to ensure the reproducibility, stability and safety of the conjugate.

The derivatization and conjugation processes should be monitored and analyzed for unique reaction products. Residual unreacted functional groups or their derivatives are potentially capable of reacting in vivo and may be present following the conjugation process. The manufacturing process should be validated and the limits for unreacted activated functional groups (those that are known to be clinically relevant) at the conclusion of the conjugation process should be agreed with the NRA.

After the conjugate has been purified, the tests described below should be performed to assess the consistency of the production process. These tests are critical for ensuring lot to lot consistency.

A.4.4 Control of monovalent bulk conjugates

A.4.4.1 Identity

A test should be performed on the monovalent bulk to verify its identity of both the serotype-specific polysaccharide and carrier protein. The method should be validated to show that it distinguishes the desired monovalent material from all other polysaccharides and conjugates produced on that manufacturing site.

A.4.4.2 Residual reagents

The purification procedures for the conjugate should remove any residual reagents that were used for conjugation and capping. The removal of reagents, their derivatives and reaction by-products such as ADH, phenol and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (known as EDC, EDAC or EDCI) should be confirmed using suitable tests or by validation of the purification process. The routine testing of each lot may be omitted once consistency of production has been demonstrated on a number of lots; the number should be agreed with the NRA. The specifications of the residual reagents and the quantifiable methods to be used should be agreed with the NRA.

A.4.4.3 Polysaccharide content

The polysaccharide content should be measured using an appropriate validated assay such as HPAEC-PAD (56,69), specific chemical assay or immunological method (for example, rate nephelometry, rocket electrophoresis).

A.4.4.4 Conjugated and unbound (free) polysaccharide

Only the polysaccharide that is covalently bound to the carrier protein, i.e. conjugated polysaccharide, is immunologically important for clinical protection. Each batch of conjugate should be tested for unbound or free polysaccharide to establish consistency of production and to ensure that the amount present in the purified bulk is within the limits agreed by the NRA based on lots shown to be clinically safe and efficacious.

Methods that have been used to separate unbound polysaccharide before assay, and that are potentially applicable to GBS conjugates, include hydrophobic chromatography, acid precipitation, precipitation with carrier protein-specific antibodies, gel filtration and ultrafiltration. The amount of unbound polysaccharide can be determined by specific chemical or immunological tests, or by HPAEC after hydrolysis.

A.4.4.5 Total protein and unbound (free) protein

The protein content of the purified bulk conjugate should be determined using an appropriate validated assay. Each batch should be tested for conjugated and unbound protein. The unbound protein content of the purified bulk conjugate should comply with the limit for the product that has been agreed with the NRA. Appropriate methods for determining unbound protein include HPLC and capillary electrophoresis.

A.4.4.6 Polysaccharide–protein ratio and conjugation markers

For each batch of the bulk conjugate of each serotype, the ratio of polysaccharide to carrier protein should be determined as a marker of the consistency of the conjugation chemistry. For each conjugate, the ratio should be within the range approved for each serotype-specific conjugate by the NRA and should be consistent with vaccine shown to be effective in clinical trials.

If the chemistry of conjugation results in the creation of a unique linkage marker (e.g. a unique amino acid), each batch of the bulk conjugate of that serotype should be assessed to quantify the extent of substitution of the carrier protein by covalent reaction of the polysaccharide with the carrier protein.

A.4.4.7 Absence of reactive functional groups

The validation batches should be shown to be free of reactive functional groups or their derivatives that are suspected to be clinically relevant on the polysaccharide and the carrier protein.

Where possible, the presence of reactive functional groups should be assessed for each batch. Alternatively, the product of the capping reaction may be monitored, or the capping reaction can be validated to show that reactive functional groups have been removed.

A.4.4.8 Molecular size distribution

The molecular size of the polysaccharide–protein conjugate is an important parameter in establishing consistency of production and in studying stability during storage.

The relative molecular size of the polysaccharide–protein conjugate should be determined for each bulk, using a gel matrix appropriate to the size of the conjugate. The method should be validated with an emphasis on specificity to distinguish the polysaccharide–protein conjugate from other components that may be present, e.g. unbound protein or polysaccharide. The size distribution specifications will be vaccine-specific and should be consistent with lots shown to be immunogenic in clinical trials.

Typically, the size of the polysaccharide–protein conjugate may be examined by methods such as gel filtration using HPSEC on an appropriate column. Since the ratio of polysaccharide to protein is an average value, characterization of this ratio over the molecular size or mass distribution (for example, by using dual monitoring of the column eluent) can provide further proof of the consistency of production (57).

A.4.4.9 Sterility

The bulk purified conjugate should be tested for bacterial and mycotic sterility in accordance with the methods of Part A, sections 5.1 and 5.2, of the revised WHO general requirements for the sterility of biological substances (70) or by a method approved by the NRA. If a preservative has been added to the product, appropriate measures should be taken to prevent it from interfering with the test.

A.4.4.10 Endotoxin content

To ensure an acceptable level of endotoxin in the final product, the endotoxin content of the monovalent bulk may be determined and shown to be within acceptable limits agreed by the NRA using an rFC or rCR assay.

A.4.4.11 pH

The pH of each batch should be tested, and the results should be within the established range and compatible with stability data.

A.4.4.12 Appearance

The appearance of the purified bulk conjugate solution, with respect to its form and colour, should be examined by a suitable method and should meet the established specifications. For a lyophilized preparation, the appearance should be checked after reconstitution with the appropriate diluent and should meet the established specifications.

A.4.5 Final bulk

A.4.5.1 Preparation

To formulate the final bulk, monovalent conjugate bulks may be mixed and an adjuvant, preservative and/or stabilizer added before final dilution. Alternatively, the monovalent conjugate bulks may be adsorbed to adjuvant individually before mixing them to formulate the final vaccine.

A.4.5.2 Sterility

Each final bulk should be tested for bacterial and fungal sterility as indicated in section A.4.4.9.

A.5 Filling and containers

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (50) and WHO good manufacturing practices for biological products (51) should be followed.

A.6 Control of the final product

A.6.1 Inspection of the final containers

All filled final containers should be inspected as part of the routine manufacturing process. Those containers showing abnormalities – such as vial defects, improper sealing, clumping or the presence of endogenous or exogenous particles – should be discarded.

A.6.2 Control tests on the final lot

The following tests should be performed on each final lot of vaccine (that is, in the final container) and the tests used should be validated and approved by the NRA. The permissible limits for tests listed under this section should be justified and approved by the NRA.

A.6.2.1 Appearance

The appearance of the final container and its contents should be verified using a suitable method and should meet the established criteria with respect to form and colour. For freeze-dried vaccines, their appearance should be verified before and after reconstitution, and should meet the established criteria. The test should be performed against a black, and a white, background, and according to pharmacopoeial specifications.

A.6.2.2 Identity

An identity test should be performed that demonstrates that all the intended polysaccharide serotypes and carrier protein(s) are present in the final product, unless this test has been performed on the final bulk.

A.6.2.3 Bacterial and fungal sterility

The contents of the final containers should be tested for bacterial and mycotic sterility in accordance with the methods of Part A, sections 5.1 and 5.2, of the revised WHO General requirements for the sterility of biological substances (70) or by a method approved by the NRA. If a preservative has been added to the product, appropriate measures should be taken to prevent it from interfering with the test.

A.6.2.4 Polysaccharide content

The amount of each streptococcal polysaccharide in the final containers should be determined and shown to be within the specifications agreed by the NRA. Conjugate vaccines produced by different manufacturers may differ in formulation. The specification should be justified based on the clinical lots shown to be safe and immunogenic, and approved by the NRA. The assays used are likely to be product-specific and might include chromatographic or serological methods.

A.6.2.5 Residual moisture

If the vaccine is freeze-dried, the average moisture content should be determined by methods accepted by the NRA. Values should be within the limits for the preparations shown to be adequately stable in the stability studies of the vaccine.

A.6.2.6 Endotoxin or pyrogen content

The endotoxin content of the final product should be determined using a suitable in vitro assay such as an rFC or rCR assay. The endotoxin content should be consistent with levels found to be acceptable in vaccine lots used in clinical trials and within the limits agreed with the NRA.

The need to test for non-endotoxin pyrogens should be determined during the manufacturing development process. It should also be evaluated following any changes in the production process or relevant reported production inconsistencies that could influence the quality of the product regarding its pyrogenicity. When required, the MAT should be used to measure non-endotoxin pyrogens.

A.6.2.7 Adjuvant content

If an adjuvant has been added to the vaccine, its content should be determined by a method approved by the NRA. The amount and nature of the adjuvant should be agreed with the NRA. If aluminium compounds are used as adjuvants, the amount of aluminium should not exceed 1.25 mg per single human dose.

A.6.2.8 Preservative content

If a preservative has been added to the vaccine, the content of preservative should be determined by a method approved by the NRA. The amount of preservative in the vaccine dose should be shown not to have any deleterious effect on the antigen or to impair the safety of the product in humans. The preservative and its concentration should be approved by the NRA.

A.6.2.9 pH

If the vaccine is a liquid preparation, the pH of each final lot should be tested and shown to be within the range of values found for vaccine lots shown to be safe and effective in clinical trials and in stability studies. For a lyophilized preparation, the pH should be measured after reconstitution with the appropriate diluent.

A.6.2.10 Osmolality

The osmolality of the final lots should be determined and shown to be within the range considered to be safe for intramuscular administration to humans and agreed with the NRA. The test for osmolality may be omitted once consistency of production is demonstrated or justification is provided, with the agreement of the NRA.

A.6.3 Control of diluents

The general guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (50) should be followed during the manufacture and quality control of the diluents used to reconstitute the vaccine. An expiry date should be established for the diluents based upon stability data. For lot release of the diluent, tests should be done to assess its appearance, identity, volume and sterility, and the concentrations of its key components.

A.7 Records

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (50) and WHO good manufacturing practices for biological products (51) should be followed as appropriate for the level of development of the vaccine.

A.8 Retained samples

The recommendations in section 9.5 of *WHO Good manufacturing practices for biological products* (51) should be applied.

A.9 Labelling

The recommendations in section 7 of WHO Good manufacturing practices for biological products (51) should be applied with the addition of the following:

The label on the carton or the leaflet accompanying the container should indicate:

- the streptococcal serotypes and carrier protein present in each single human dose;
- the amount of each conjugate present in a single human dose;
- the temperature recommended during storage and transport;
- if the vaccine is freeze-dried, that after its reconstitution it should be used immediately unless data have been provided to the licensing authority showing that it may be stored for a limited time;

- the volume and nature of the diluent to be added to reconstitute a freeze-dried vaccine, specifying that the diluent should be supplied by the manufacturer and approved by the NRA.

A.10 Distribution and transport

The guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (50) and WHO good manufacturing practices for biological products (51) should be followed.

Shipments should be maintained within specified temperature ranges, and packages should contain cold-chain monitors. Further guidance on these and related issues is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (71).

A.11 Stability, storage and expiry date

The relevant guidance provided in WHO good manufacturing practices for biological products (51) should be followed. Any statements concerning storage temperature and expiry date that appear on primary or secondary packaging should be based on experimental evidence and should be approved by the NRA.

A.11.1 Stability testing

Adequate stability studies are an essential part of the vaccine development studies. These studies should follow the general principles outlined in *WHO Guidelines on stability evaluation of vaccines* (72) and *WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions* (73). The shelf-life of the final product and the hold time of each process intermediate (such as the purified polysaccharide, the carrier protein and the purified bulk conjugate) should be established based on the results of real-time, real-condition stability studies, and approved by the NRA.

The stability of the vaccine in its final container and at the recommended storage temperature should be demonstrated to the satisfaction of the NRA on at least three lots of the final product manufactured from different bulk conjugates. In addition, a real-time real-condition stability study should be conducted on at least one final container lot produced each year.

A protocol should be established and followed for each stability study which specifies the stability-indicating parameters to be monitored, as well as the applicable specifications. Some stability-indicating parameters may change over the shelf-life as discussed below. The specifications should take into consideration the expected quality of the vaccine at the end of shelf-life and should be linked to lots demonstrated to be safe and effective/immunogenic in clinical trials.

The polysaccharide component of conjugate vaccines may be subject to gradual hydrolysis at a rate that may vary with the type of conjugate, the type of formulation or adjuvant, the type of excipients and conditions of storage. The hydrolysis may result in reduced

molecular size of the polysaccharide component, in a reduction in the amount of the polysaccharide bound to the protein carrier and in a reduced molecular size of the conjugate.

Tests should be conducted before licensing to determine the extent to which the stability of the product has been maintained throughout the proposed validity period. The vaccine should meet the specifications for final product up to the expiry date.

If applicable, the residual moisture should be monitored as part of stability testing and release testing.

Where applicable, the level of adsorption of the conjugate to the adjuvant should be shown to be within the limits agreed with the NRA, unless data show that the immunogenicity of the final product does not depend on the adsorption of the antigen to the adjuvant.

Accelerated stability studies may provide additional supporting evidence of the stability of the product or other product characteristics, or both, but are not recommended for establishing the shelf-life of the vaccine under a defined storage condition.

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

The statements concerning storage temperature and expiry date appearing on the label should be based on experimental evidence, which should be submitted for approval to the NRA.

A.11.2 Storage conditions

Storage conditions should be based on stability studies and approved by the NRA.

A.11.3 Expiry date

The expiry date should be approved by the NRA and based on the stability of the final product as well as the results of the stability tests referred to in section A.9.1.

Part B. Nonclinical evaluation of group B streptococcal conjugate vaccines

This section addresses the pharmacological and toxicological testing of a new candidate GBS vaccine. In line with the Scope of this document, guidance is provided on nonclinical evaluation of vaccine candidates that are built on the capsular polysaccharides (CPS) as antigens with intended use in pregnant persons in order to protect their newborns. The guidance provided in this section should be read in conjunction with the principles outlined in the WHO *guidelines on nonclinical evaluation of vaccines* (12) and WHO *guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines* (13).

B.1 Primary pharmacodynamics

The ability of a new GBS vaccine to induce immune responses should be carefully evaluated. For the sake of operational reason, the manufacturer may consider conducting the initial studies in non-pregnant adult female animals, using relevant species that have been reported to respond to GBS vaccines, such as CD-1 mice, rats, or rabbits (56,74-77). In general, the testing should include the clinically intended route of administration and dose regimes, unless otherwise

justified. The immune parameters to be evaluated should focus on the binding and functional antibodies (e.g. opsonophagocytic) specific for GBS of homologous serotypes/CPS present in the vaccine formulation, as major outcomes, using sera of the vaccinated animals. The immune responses to each antigen component included in the final vaccine formulation should be demonstrated. The studies may include exploration of dose-response by testing of various dose levels of the vaccine, to help the selection of doses in early human trials. For a vaccine candidate that contains adjuvant, the benefit of including it in the vaccine formulation should be demonstrated. Similarly, if a clinically intended dosing regimen includes 2 vaccine doses to be given at a specific interval, the favorable effect of the second dose administration on vaccine immunogenicity should be investigated. It is expected that a promising vaccine formulation will be able to raise stronger and long-lasting antibody responses after the completion of vaccination course.

However, it is important to note that, the interpretation of results of the initial studies in non-pregnant animals should be with caution, if the serological assays used for the *in vitro* quantification of GBS/CPS-specific antibodies cannot distinguish the different types of antibodies, including IgG and IgM. In fact, both IgG and IgM types of the antibodies could be raised by the vaccination with a GBS vaccine, whereas it is the IgG antibody that is known to be efficiently passed on to their pups from mothers via the placenta. Therefore, the nonclinical evaluation of vaccine immunogenicity by using the cord blood or blood of the pups born to the vaccinated dams will provide most direct information. When such studies are pursued, consideration should be given to the timing of vaccination in female animals. In such an animal model, too early vaccination with a relatively weak immunogenic formulation may end up with low IgG concentrations achieved in the newborn at birth. Accordingly, whether execution of animal vaccination prior to mating, or to include an additional dose administered during early pregnancy to mount the GBS/CPS-specific IgG antibody response, should be considered on a case-by-case basis, depending on the anticipated performance of the vaccine candidate from the initial studies.

It is a prerequisite that adequate information on vaccine immunogenicity is made available before entering into human clinical trials with a new GBS vaccine.

So far, there is no well-established immune correlate of protection against invasive GBS in neonates and infants. A neonatal challenge model (74,75) was recently used to assess the ability of vaccinated female mice to pass on maternal antibodies *in utero* which protect their neonatal pups against a lethal challenge with virulent GBS. This model could be useful for specific information on achieving newborn protection by a new GBS vaccine after maternal immunization in animals, although acknowledging that such data may not reliably predict the product performance in humans.

B.2 Pharmacokinetics

Studies to determine serum or tissue concentrations of vaccine components are normally not needed. However, the understanding of distribution, quantity, and clearance of the administered vaccine components following administration can be helpful in case of using novel adjuvants, and new formulations (12,13).

B.3 Toxicology studies

Details on the design, conduct, analysis and evaluation of nonclinical toxicology studies are available in *WHO guidelines for nonclinical evaluation of vaccines* (1) and *WHO guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines* (13). In addition, regional documents on considerations for developmental toxicity studies for preventative and therapeutic vaccines for infectious disease indications (78) may also be informative.

Safety testing in animals is a prerequisite for the initiation of clinical studies in humans. The vaccine lots used in pivotal toxicity studies should be adequately representative of the formulation intended for clinical investigation and, ideally, should be the same lots used in clinical studies. If this is not feasible, the lots used clinically should be comparable to those used in the nonclinical studies in terms of potency, stability and other characteristics of quality.

Part C. Clinical evaluation of group B streptococcal conjugate vaccines

C.1 Introduction

Clinical trials with GBS polysaccharide-protein conjugate vaccines should be conducted in accordance with the principles described in the *WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products* (79) and the *WHO Guidelines on clinical evaluation of vaccines: regulatory expectations* (14).

This section addresses the clinical evaluation of GBS polysaccharide-protein conjugate vaccines intended for maternal immunization with the primary aim of preventing invasive GBS disease in neonates and infants. The clinical programme that is outlined assumes that initial licensure rests on safety and immunogenicity data and that vaccine efficacy is inferred from the proportions of cord blood samples that have anti-capsular IgG concentrations at or above threshold values specific to serotypes and to EOD and LOD that have been derived from sero-epidemiological studies. Therefore, this section is applicable only to GBS vaccines that are expected to provide protection against invasive GBS disease by elicitation of anti-capsular antibody. While the clinical programme envisaged does not include conduct of a pre-licensure vaccine efficacy trial, it anticipates that there are plans in place at time of licensure for conduct of vaccine effectiveness studies.

C.2 Immunogenicity trials

C.2.1 Assays

General guidance on the use and validation of assays to determine immune responses to vaccines is provided in the WHO Guideline on clinical evaluation of vaccines; regulatory expectations (2). Whenever possible, results should be reported in International Unitage along with information about the performance of any relevant International Standard(s).

C.2.1.1 Anti-capsular IgG

The assay that is applied to sera obtained in clinical trials should be the same as or bridged to the assay(s) used in the sero-epidemiological studies from which the threshold values to be applied to cord blood anti-capsular IgG concentrations were derived. Adequate controls should be used to define a valid test and justify pooling of data across assay runs.

C.2.1.2 Opsonophagocytic antibody (OPA)

The method used to calculate OPA endpoint titres should be provided. Generally, it is recommended that the endpoint should be derived from the linear portion of the titration curve.

C.2.2 Trial population and design

C.2.2.1 Nonpregnant women of childbearing age

The first clinical trials are expected to provide data on safety and immunogenicity in nonpregnant female participants of childbearing age who are randomised to receive candidate vaccine formulations or placebo. It is highly desirable and generally expected that a single dose of a candidate polysaccharide-protein conjugate vaccine will suffice such that a second dose does not elicit a potentially clinically important increment in antibody levels. However, the safety and immunogenicity of second doses given within intervals relevant to the intended use (i.e. after no more than 4-8 weeks has elapsed since the first dose) should be investigated in one or more of the early trials.

The immunogenicity data obtained in non-pregnant women of childbearing age should be used to select the vaccine formulation(s) to be tested in pregnant women. Before progressing to trials in pregnant female participants, the safety data obtained from non-pregnant female participants should be considered sufficient to rule out any major concerns for use during pregnancy, such as risk for high fever in the first days after vaccination.

C.2.2.2 Pregnant women

The minimum and maximum periods of gestation for enrolment into trials in pregnant women should cover the intended range for use. It is generally recommended that the duration of pregnancy at time of enrolment is determined from an ultrasound examination that was conducted during or after week 18 of gestation. Reflecting the peak period of placental transfer of IgG, and assuming that only one dose of vaccine is required, it is recommended that participants should have completed at least 24 weeks and no more than 36 weeks of gestation when vaccinated. Consideration should be given to enrolling adequate cohorts at weeks 24-28, 28-32 and 32-36 weeks of gestation when vaccinated to provide insight into any effect there may of gestational stage to maternal immune response and to cord blood antibody levels.

Furthermore, at least in initial trials in pregnant women, sponsors may wish to limit enrolment to singleton pregnancies with no known fetal abnormalities and no evidence of placental insufficiency. If women who test positive for HIV are to be excluded from the first clinical trial(s), consideration should be given to generating sufficient immunogenicity data in HIV-positive women so that they may be included in Phase 3 trials with or without stratification at randomisation by HIV status.

C.2.2.2.1 Dose-finding trials

Dose-finding safety and immunogenicity trials in pregnant women should include randomisation to candidate vaccine(s) or to placebo until such time that it may be considered inappropriate to withhold a licensed GBS vaccine from the control group. If withholding a licensed GBS vaccine is no longer possible, consideration should be given to administering placebo followed by the licensed control vaccine after a short delay (e.g. 2-4 weeks) in the control group so that placebo-controlled safety data can be generated. In this case, the test group(s) would receive vaccine followed by placebo to maintain the double-blind design.

The final vaccine formulation for Phase 3 trials in pregnant women should be selected from the infant cord blood data obtained in the dose-finding trial(s). The final candidate vaccine should maximize the proportions of neonates born to vaccinated mothers who have cord blood anti-capsular IgG concentrations above the selected serotype-specific threshold values for EOD and/or LOD whilst maintaining an acceptable safety profile. Additionally, assuming that it is still possible to include randomisation to placebo, the final candidate vaccine should maximize the difference in cord blood anti-capsular IgG concentrations between infants born to vaccinated and unvaccinated mothers.

Dose-finding trials in pregnant women should include determination of maternal anti-capsular IgG at approximately 4 weeks post-vaccination with additional samples collected at least from a subset to describe antibody kinetics. Documenting the antibody decay curve in vaccinated women during and following delivery (e.g. for 3-6 months) may give an early indication of the need to re-vaccinate women during each pregnancy. Consideration should be given to investigating the safety and immunogenicity of revaccination during a subsequent pregnancy whenever the opportunity arises in the post-approval period.

C.2.2.2.2 Phase 3 trials

It is desirable that Phase 3 trials are conducted in several countries and geographical regions to provide a broad assessment of any variations in immune responses. Consideration may also be given to stratification of enrolment by country and/or region.

The primary immunological readout in Phase 3 trials should be the cord blood anti-capsular IgG concentrations with calculation of proportions of neonates with levels for each of EOD and LOD at or above the threshold values for each serotype included in the vaccine.

The primary analyses should estimate the predicted vaccine efficacy against each of EOD and LOD due to any of the vaccine serotypes derived from the proportions of neonates born to each of vaccinated or unvaccinated mothers who have cord blood IgG concentrations

above the selected threshold values. Additional primary analyses should estimate the predicted serotype-specific vaccine efficacy for EOD and for LOD.

The sample size calculation should consider the precision of the estimates of predicted vaccine efficacy as well as ensuring that the total mother-infant pairs exposed to the final candidate vaccine provide an adequate safety database.

It is recommended that sponsors and NRAs should pre-agree the desirable lower bounds of the 95% confidence intervals around the estimates of predicted vaccine efficacy for each of EOD and LOD. The predicted vaccine efficacy is likely to vary by serotype and by EOD and LOD. Therefore, the final licensing decision must be based on the totality of the data and the anticipated overall protection against invasive GBS disease that might be anticipated in the individual NRA's jurisdiction.

Depending on the carrier protein in the GBS polysaccharide-protein conjugate vaccine, the infant immune responses to relevant routine infant vaccines should be determined at least for a subset. For example, if the carrier protein is CRM197, infant immune responses to diphtheria toxoid and to any conjugate vaccine that has CRM197 as the carrier protein should be determined.

If randomisation to placebo is no longer considered appropriate, it may be appropriate to design the Phase 3 trial to bridge the candidate vaccine to the efficacy of a licensed GBC polysaccharide-protein conjugate vaccine. In this case, the control group would receive a licensed GBS vaccine for which estimates of vaccine efficacy have been reported from one or more vaccine effectiveness studies and the trial would provide a formal comparison of proportions achieving at least the threshold levels of anti-capsular IgG in test and control groups.

Analysis of cord blood anti-capsular IgG levels by gestation elapsed at time of maternal vaccination and by time elapsed between maternal vaccination and delivery may assist in determining the recommendations made in the prescribing information. Documenting the post-natal antibody decay curve in infants until titres are below the selected threshold levels may give an early indication of the maximum duration of protection that might possibly be expected.

The secondary immunological readouts should include the OPA titres in cord blood and the maternal anti-capsular IgG levels at 4 weeks after vaccination. Depending on prior data accrued, it may be sufficient to determine obtain these data from a randomly selected subset of samples. Follow-up samples should be obtained, as described in dose-finding trials.

As applicable to each immunological readout, secondary descriptive analyses should include the seroconversion rates, geometric means and geometric mean fold increases.

C.3 Efficacy trials

For reasons discussed under *General considerations*, it is considered that initial licensure of GBS polysaccharide-protein conjugate vaccines would not require conduct of a pre-licensure vaccine efficacy trial. It will be for the individual NRA to determine the acceptability of this approach after considering the reliability and relevance of published threshold values of cord blood anti-capsular Ig antibody that may be used to infer vaccine efficacy.

If initial licensure based on the application of threshold anti-capsular antibody levels is not considered acceptable for any reason, NRAs and sponsors are referred to the WHO Guideline on clinical evaluation of vaccines; regulatory expectations (14), which provides guidance on the design of efficacy trials.

C.4 Vaccine effectiveness studies

It is essential that there are plans in place at time of initial licensure to estimate vaccine effectiveness. The aim of these studies should include estimating vaccine effectiveness by serotype and by EOD and LOD. Furthermore, whenever possible, these studies should include collection of cord blood samples and determination of anti-capsular IgG to support or revise the threshold values that may predict protection against invasive GBS. Potentially, at least subsets of these samples could be used to determine OPA titres to attempt to further evaluate correlations with protection against invasive GBS.

It is anticipated that certain countries will include a GBS polysaccharide-protein conjugate vaccine in routine antenatal care shortly after initial licensure along with national programmes designed to collect all the data needed to estimate vaccine effectiveness by serotype and for EOD and LOD. While the license holders will likely not be involved in such programmes, they should be aware of the likely timelines for reporting the results and should include such studies in the list of post-licensing activities. Moreover, there may be some jurisdictions in which the responsible NRAs request that the license holder conducts a vaccine effectiveness study in co-operation with the public health authorities or, at least, undertakes the determination of cord blood anti-capsular IgG concentrations. Each NRA should receive a comprehensive list of all such studies, each stating the role (if any) of the license holder, which should be updated at regular intervals.

C.4.1 Study design

There are several possible designs that may be considered, as discussed in the WHO Guideline on clinical evaluation of vaccines: regulatory expectations (14).

The population to be vaccinated will reflect the prescribing information and the decisions made by the relevant public health authority on any exclusions to be made from the target population.

To support the estimates of vaccine effectiveness, there should be high confidence in the completeness of disease surveillance along with guidance on the samples to be obtained from all suspected cases of invasive GBS disease. The method of case ascertainment should be tailored to the healthcare system but should be as comprehensive as possible, including instructions to pregnant women, infant care-givers and relevant healthcare professionals, especially midwives, obstetricians and paediatricians, on trigger signs and symptoms for possible invasive GBS disease. The primary case definitions for GBS EOD and LOD should require both clinical and laboratory criteria to be met. Laboratories should be competent to process samples and arrange shipping to designated centres for determination of serotypes.

C.4.2 Study analysis

It is recommended that vaccine effectiveness studies should be designed primarily to estimate overall vaccine efficacy against invasive GBS disease and, assuming enough cases are accrued, to also estimate efficacy by serotype for EOD and for LOD separately. The data should be explored for efficacy according to timing of vaccination during gestation and time elapsed between vaccination and delivery. If possible, in accordance with the antenatal population targeted by the routine vaccination programme, the data should be explored to identify factors that might reduce efficacy, such as placental insufficiency and HIV infection.

Vaccine effectiveness studies also present the possibility to explore any potential benefit of GBS polysaccharide-protein conjugate vaccines on maternal complications in later pregnancy or in the peri-partum period as well as rates of pre-term birth or stillbirth. If these endpoints are incorporated into vaccine effectiveness studies, there must be adequate case definitions supported by clinical and laboratory criteria.

In countries that already had comprehensive surveillance for invasive GBS disease in place prior to introducing vaccination, assuming that the annual incidence of disease was documented to be generally stable, there is the potential to compare rates of invasive GBS disease before and after implementing routine vaccination as well as examining the proportions of cases due to individual serotypes and any differential effects there may have been on EOD vs. LOD. If the policy on use of IAP is modified at any time after introduction of routine vaccination in a specific country or region, a vaccine effectiveness study may also be used to examine any change in incidence of EOD that may occur.

Ideally, cord blood samples should be obtained from all neonates during the study for determination of serotype-specific anti-capsular IgG and at least randomly selected subsets of samples should be investigated for OPA titres. Analyses of these data may provide support for maintaining or revising the anti-capsular IgG threshold values applied at the time of initial licensure and/or may support additional interpretive criteria.

C.5 Safety aspects

The critical safety data for GBS polysaccharide-protein conjugate vaccines pertain to the safety profile in pregnant women. The threshold for determining tolerability of a vaccine during pregnancy is usually lower than that applicable to non-pregnant adults. The risk of local and systemic reactions to vaccination, including fever, should be assessed in non-pregnant women before proceeding to vaccinate pregnant women. The data should not suggest any undue concerns for use during the second and third trimesters. For example, the rates of fever, especially high fever, should be low to negligible.

The rates of premature delivery, complications of pregnancy or labour and the condition of infants at birth should be compared between the vaccinated and unvaccinated groups along with post-natal development up to the age of approximately 6 months.

In larger Phase 3 trials, provided that no signals arose in earlier trials, post-natal developmental assessments may be conducted in a randomised subset of infants.

It is recommended that serious adverse events should be documented for 6 months after maternal vaccination and for 6 months post-natal in their infants.

Part D. Guidelines for NRAs

D.1 General

The guidance for NRAs and national control laboratories (NCLs) given in the *WHO Guidelines for national authorities on quality assurance for biological products* (80) and *WHO Guidelines for independent lot release of vaccines by regulatory authorities* (81) should be followed. These guidelines specify that no new biological product should be released until consistency of lot manufacturing and quality has been established and demonstrated by the manufacturer.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety or efficacy of the conjugate vaccine, should be discussed with and approved by the NRA.

For control purposes, the relevant international reference preparations currently in force should be obtained for the purpose of calibrating national, regional and working standards as appropriate. The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release.

Consistency of production has been recognized as an essential component in the quality assurance of vaccines. The NRA should carefully monitor production records and quality control test results for clinical lots, as well as for a series of consecutive lots of the final bulk and final product.

D.2 Official release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations (81).

A summary protocol for the manufacturing and control of GBS conjugate vaccines, based on the model summary protocol provided below in Appendix 1 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA/NCL in support of a request for the release of a vaccine for use. This protocol may also be referred to as the Product Specification File.

A lot release certificate signed by the appropriate NRA/NCL official should then be provided if requested by the manufacturing establishment, and should certify that the lot of vaccine meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot, including the basis of the release decision (by summary protocol review or independent laboratory testing). The purpose of this official national lot release certificate is to facilitate the exchange of vaccines between countries and should be provided to importers of the vaccines.

A model NRA/NCL Lot Release Certificate for GBS conjugate vaccines is provided below in Appendix 2.

Authors and acknowledgements

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Appendix 1

Model summary protocol for the manufacturing and control of group B streptococcal conjugate vaccines

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by the manufacturer to the NRA or NCL.

Information and tests may be added or omitted as necessary with the approval of the NRA or NCL. In cases where the testing method is different from the one listed in this model protocol, it should be approved by the NRA.

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

The section concerning the final product should be accompanied by a sample of the label and a copy of the leaflet (package insert) that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it should also be accompanied by a lot release certificate (see Appendix 2) from the NRA or NCL of the country in which the vaccine was produced and/or released, stating that the product meets all national requirements as well as Part A of these WHO Guidelines.

1. Summary information on final lot

International name of product: _____

Commercial name: _____

Product licence (marketing authorization) number: _____

Country: _____

Name and address of manufacturer: _____

Nature of final product: _____

Final packaging lot number: _____

Type of container: _____

Final container lot number: _____

Number of containers in this final lot: _____

Number of doses per final container: _____

Volume of each recommended single human dose: _____

Preservative used and nominal concentration: _____

Summary of composition (*include a summary of the qualitative and quantitative composition of the vaccine per single human dose; including the conjugate, any adjuvant used and other excipients*):

_____

_____

Shelf-life approved (months): _____

Date of manufacture: _____

Expiry date: _____

Storage conditions: _____

2. Detailed information on manufacture and control

The following sections are intended for reporting 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 had to be repeated, this information must be indicated. Any abnormal results must be recorded on a separate sheet.

Summary of source materials

It is possible that a number of bulk lots may be used to produce a single final lot. A summary of the bulk polysaccharide, activated saccharide, bulk carrier protein and bulk conjugate lots that contributed to the final lot should be provided.

Control of serotype-specific GBS polysaccharides

Bacterial strain

Identity of bacterial strains used (i.e. for each serotype): _____

Origin and short history: _____

Authority that approved the strain: _____

Date approved: _____

Bacterial culture media for seed-lot preparation and polysaccharide production

Free from ingredients that form precipitate when CTAB is added: _____

Free from toxic or allergenic substances: _____

Any components of animal origin (list): _____

Certified as TSE-free: _____

Master seed lot

Lot number: _____

Date master seed lot established: _____

Working seed lot

Lot number: _____

Date working seed lot established:_____

Type of control tests used on working seed lot:_____

Date seed lot reconstituted:_____

Control of single harvests

For each single harvest, indicate the medium used; the dates of inoculation; the temperature of incubation; the dates of harvests and harvest volumes; the results of tests for bacterial growth rate, pH, purity and identity; the method and date of inactivation if used; the method of purification; and the yield of purified polysaccharide.

Control of purified GBS polysaccharides

Lot number:_____

Date of manufacture:_____

Volume:_____

Identity

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Polysaccharide content

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Molecular size or mass distribution

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Moisture content

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Protein impurity

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Nucleic acid impurity

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Pyrogen content

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Residues of process-related contaminants

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Control of modified polysaccharide

Lot number:_____

Method of chemical modification:_____

Extent of activation for conjugation

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Molecular size or mass distribution

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Control of carrier protein

Microorganisms used

Identity of strain used to produce carrier protein:_____

Origin and short history:_____

Authority that approved the strain:_____

Date approved:_____

Bacterial culture media for seed-lot preparation and carrier-protein production

Free from ingredients that form precipitate when CTAB is added:_____

Free from toxic or allergenic substances:_____

Any components of animal origin (list):_____

Certified as TSE free:_____

Master-seed lot

Lot number:_____

Date master-seed lot established:_____

Working-seed lot

Lot number:_____

Date established:_____

Type of control tests used on working-seed lot:_____

Date seed lot reconstituted:_____

Control of carrier-protein production

List the lot numbers of harvests: indicate the medium used; the dates of inoculation; the temperature of incubation; the dates of harvests and harvest volumes; the results of tests for

bacterial growth rate, pH, purity and identity; the method and date of inactivation; the method of purification; and the yield of purified carrier protein. Provide evidence that the carrier protein is nontoxic.

Purified carrier protein

Lot number:_____

Date produced:_____

Identity

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Protein impurity

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Nucleic acid impurity

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Modified carrier protein

Lot number:_____

Date produced:_____

Method of modification:_____

Specific toxicity (if appropriate)

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Extent of activation

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Control of purified bulk conjugate

Production details of bulk conjugate

List the lot numbers of the saccharide and carrier protein used to manufacture the conjugate vaccines, the production procedure used, the date of manufacture and the yield.

Tests on purified bulk conjugate

Identity

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Residual reagents

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Polysaccharide content

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Conjugated and unbound (free) polysaccharide

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Total protein and unbound (free) content

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Ratio of polysaccharide to protein

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Conjugation markers

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Absence of reactive functional groups (capping markers)

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Molecular size or mass distribution

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Bacterial and fungal sterility

Method used:_____

Media:_____

Volume tested:_____

Date of inoculation:_____

Date of end of test:_____

Specification:_____

Result:_____

Endotoxin content

Date tested:_____

Method used:_____

Specification:_____

Result:_____

pH

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Appearance

Date tested:_____

Method used:_____

Specification:_____

Result:_____

Depending on the conjugation chemistry used to produce the vaccine, tests should also be included to demonstrate that amounts of residual reagents and reaction by-products are below a specified level.

Control of final bulk

Lot number:_____

Date prepared:_____

Preservative (if used)

Name and nature:_____

Lot number:_____

Final concentration in the final bulk:_____

Stabilizer (if used)

Name and nature:_____

Lot number:_____

Final concentration in the final bulk:_____

Adjuvant (if used)

Name and nature:_____

Lot number:_____

Final concentration in the final bulk:_____

Tests on final bulk

Bacterial and fungal sterility

Method used:_____

Media:_____

Volume tested:_____

Date of inoculation:_____

Date of end of test:_____

Specification:_____

Result:_____

Filling and containers

Lot number:_____

Date of sterile filtration:_____

Date of filling:_____

Volume of final bulk:_____

Volume per container:_____

Number of containers filled (gross):_____

Date of lyophilization (if applicable):_____

Number of containers rejected during inspection:_____

Number of containers sampled:_____

Total number of containers (net):_____

Maximum duration approved for storage:_____

Storage temperature and duration:_____

Control tests on final lot

Inspection of final containers

Date tested: _____

Method used: _____

Specification: _____

Results: _____

Appearance before reconstitution:[1] _____

Appearance after reconstitution: _____

Diluent used: _____

Lot number of diluent used: _____

Tests on final lot

Identity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Sterility

Method used: _____

Media: _____

Number of containers tested: _____

Date of inoculation: _____

Date of end of test: _____

Specification: _____

Result: _____

Polysaccharide content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

[1] This applies to lyophilized vaccines.

Moisture content[2]

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Endotoxin or pyrogen content

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Adjuvant content and degree of adsorption (if applicable)

Date tested: _____

Nature and concentration of adjuvant per single human dose: _____

Method used: _____

Specification: _____

Result: _____

Preservative content (if applicable)

Date tested: _____

Method used: _____

Specification: _____

Result: _____

pH

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Osmolality

Date tested: _____

[2] This applies only to lyophilized vaccines.

Method used:_____

Specification:_____

Result:_____

Control of diluent (if applicable)

Name and composition of diluent:_____

Lot number:_____

Date of filling:_____

Type of diluent container:_____

Appearance:_____

Filling volume per container:_____

Maximum duration approved for storage:_____

Storage temperature and duration:_____

Other specifications:_____

Control of adjuvant[3]

Summary of production details for the adjuvant

When an adjuvant suspension is provided to reconstitute a lyophilized vaccine, a summary of the production and control processes should be provided. The information provided and the tests performed depend on the adjuvant used.

Summary information for the adjuvant

Name and address of manufacturer:_____

Nature of the adjuvant:_____

Lot number:_____

Date of manufacture:_____

Expiry date:_____

Tests on the adjuvant

Adjuvant content

Date tested:_____

Method used:_____

Specification:_____

Result:_____

[3] This section is required only when an adjuvant is provided separately to reconstitute a lyophilized vaccine.

Appearance

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Purity or impurity

Date tested: _____

Method used: _____

Specification: _____

Result: _____

pH

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Pyrogenicity[4]

Date tested: _____

Method used: _____

Specification: _____

Result: _____

Sterility

Method used: _____

Media: _____

Number of containers used: _____

Date of inoculation: _____

Date of end of test: _____

Specification: _____

Result: _____

[4] A pyrogen test of the adjuvant is not needed if a pyrogen test was performed on the adjuvanted reconstituted vaccine.

3. Certification by the manufacturer

Name of head of production and/or quality control (typed)_____

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

I certify that lot no. _____ of multivalent GBS conjugate vaccine, whose number appears on the label of the final containers, meets all national requirements and satisfies Part A[5] of the WHO Guidelines to assure the quality, safety and efficacy of GBS conjugate vaccines.[6]

Signature_____

Name (typed)_____

Date_____

4. Certification by the NRA/NCL

If the vaccine is to be exported, attach the model NRA/NCL Lot Release Certificate for GBS conjugate vaccines (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.

[5] With the exception of provisions on distribution and transport, which the NRA may not be in a position to assess.
[6] WHO Technical Report Series, No. XXXX, Annex 2.

Appendix 2

Model NRA/NCL Lot Release Certificate for group B streptococcal conjugate vaccines

This certificate is to be provided by the NRA or NCL of the country in which the vaccine has been manufactured, on request by the manufacturer.

Certificate no._____

The following lot(s) of GBS conjugate vaccine produced by

_____[7]

in _____,[8] whose numbers appear on the labels of the final containers, meet all national requirements[9] and Part A[10] of the WHO Guidelines to assure the quality, safety and efficacy of group B streptococcal conjugate vaccines,[11] and comply with WHO good manufacturing practices for pharmaceutical products: main principles;[12] WHO good manufacturing practices for biological products;[13] and the WHO Guidelines for independent lot release of vaccines by regulatory authorities.[14]

The release decision is based on_____[15]

Final lot number_____

Number of human doses released in this final lot_____

Expiry date_____

[7] Name of manufacturer.
[8] Country of origin.
[9] If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA or NCL.
[10] With the exception of provisions on distribution and transport, which the NRA or NCL may not be in a position to assess.
[11] WHO Technical Report Series, No. XXXX, Annex X.
[12] WHO Technical Report Series, No. 986, Annex 2.
[13] WHO Technical Report Series, No. 999, Annex 2.
[14] WHO Technical Report Series, No. 978, Annex 2.
[15] Evaluation of the product-specific summary protocol, independent laboratory testing and/or specific procedures laid down in a defined document, and so on as appropriate.

The certificate may also include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and/or common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date);
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate.

The Director of the NRA/NCL (or other appropriate authority)

Signature_____

Name (typed)_____

Date_____