

**RECOMMENDATIONS TO ASSURE THE QUALITY, SAFETY AND
EFFICACY OF GROUP A MENINGOCOCCAL CONJUGATE
VACCINES**

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

INTRODUCTION

Recommendations (formerly known as Requirements) for meningococcal polysaccharide vaccines were adopted by the WHO Expert Committee on Biological Standardization in 1976 (1) and amended in 1978 and 1981 (2,3). In clinical studies these vaccines have been shown to have efficacy around 90% and have proved to be highly effective in public health interventions (4). Nevertheless, their modest ability to elicit protective responses in young infants (aged < 2 years) and their inability to induce immunological memory has prevented their implementation in routine childhood immunization programmes.

Since the successful introduction of the *Haemophilus influenzae* type b (Hib) conjugate vaccines into pediatric vaccination schedules, considerable progress has been made in the development of similar conjugate vaccines based on other bacterial capsular polysaccharides including the capsular polysaccharides of *Streptococcus pneumoniae* and *Neisseria meningitidis* (5,6). Clinical trials have demonstrated that group C meningococcal (MenC) conjugate vaccines are highly immunogenic in all age groups and induce immunological memory and avidity maturation of anti-capsular antibodies (7-19). Following the introduction of the MenC conjugate vaccines in several countries in Europe, there have been documented declines in the incidence of the disease, confirming that the immune responses elicited confer protection against the infection (20). Effectiveness data from the UK and Spain (21), where the MenC conjugate vaccines were first introduced to infants with a catch-up programme in older children and young adults, have clearly shown the importance of herd immunity and have suggested that there is a need to maintain a minimum level of circulating antibody for protection despite the ability of these vaccines to elicit immune memory. Recommendations for the MenC conjugate vaccines were adopted by the WHO Expert Committee on Biological Standardization in 2004 (22).

The success of the MenC conjugate vaccines prompted the development of conjugate vaccines for other meningococcal capsular polysaccharides. These include a tetravalent A, C, Y, W135 conjugate vaccine recently licensed in some countries and a monovalent group A meningococcal (MenA) conjugate vaccine currently in clinical trial. As a result, there is a need for recommendations to assure the quality, safety and efficacy of MenA conjugates, which may be used as monovalent vaccines or may be included as components of combination vaccines.

GENERAL CONSIDERATIONS

Bacteria of the species *N. meningitidis* are gram-negative diplococci with polysaccharide capsules that have led to the identification of at least 13 different groups based on immunochemical studies. However, bacteria of groups A, B, C, Y and W135 account for almost all cases of disease (23), manifest mainly as meningitis and septicaemia. Meningococci are further classified into serotypes and subtypes on the basis of the immunologic reactivity of their outer membrane proteins (PorA and PorB), or using related genetic techniques (24). They may also be genotyped by multi-locus sequence typing (25).

Epidemics caused by *N meningitidis* of group A are usually characterized by a predominance of a single meningococcal genotype and a shift of cases towards older age groups. Although group A isolates were at one time a common cause of meningococcal disease worldwide, they are now principally responsible for recurrent epidemics in the so-called "meningitis belt" countries in sub-Saharan Africa (26,27). These epidemics occur in irregular cycles every 5–12 years, and last for two to three dry seasons, dying out during the intervening rainy seasons. During epidemics, attack rates may be as high as 400–800 per 100 000. In addition, outbreaks of group A meningococcal disease are associated with the annual Hajj pilgrimage in Saudi Arabia although W135 has also emerged as a problem in recent years.

An ideal meningococcal vaccine would offer comprehensive protection against all five of the pathogenic groups but the development of such a combined product has faced major obstacles related to the immunobiology of group B capsular antigen. Bivalent (A and C) and tetravalent (A, C, Y and W135) unconjugated polysaccharide vaccines have been widely available since the early 1970s (4). Studies carried out during the 1960s confirmed the critical role of antibody-dependent complement-mediated lysis of the meningococcus as the principal immunological mechanism of protection (28,29). The polysaccharide vaccines elicit good bactericidal antibody responses in immunologically mature individuals and have been used effectively to manage epidemics and localized outbreaks as well as to offer protection to groups, such as students and military recruits, who are regarded as being at particular risk of the disease (30–32). However, polysaccharides are T-cell-independent antigens so that they are poorly or not at all immunogenic in persons aged < 2 years, they do not induce immunological memory and their use may predispose to blunting of the immune response to subsequent doses(33–35). In contrast, experience with Hib, pneumococcal and MenC conjugate vaccines has demonstrated that chemical conjugation of the capsular polysaccharides to a protein carrier can elicit T-cell-dependent immune responses (34,36,37). As a result, these conjugated vaccines can elicit protective immune responses in infants and children aged < 2 years, with induction of immune memory and without blunting of the immune response to further doses.

SPECIAL CONSIDERATIONS

The production and control methods for conjugate vaccines are more complex than those for their unconjugated capsular polysaccharide counterparts. Polysaccharide vaccines consist of defined chemical substances that, if prepared to the same specifications, can reasonably be expected to have comparable potencies, regardless of the manufacturer. However, effective meningococcal conjugate vaccines have been developed that differ in the composition and size of the saccharide, the carrier protein employed and the chemistry used for conjugation, and the methods are largely based on the successful strategies employed for the production of Hib conjugate vaccines (38). The polysaccharide starting material may be of high molecular

weight or partly size-reduced prior to random activation and conjugation. The saccharide component of oligosaccharide-based vaccines is generated by degradation of the polysaccharide to form active functional groups, either at both terminals or at only one terminal, which may be used directly or modified prior to conjugation. A more recent approach, avoiding bacterial polysaccharide production, involves the synthesis of a short saccharide chain from readily available chemical precursors which is conjugated directly to the protein (39). A small range of carrier proteins have been used thus far in conjugate vaccine production. For example, MenA conjugate vaccines have been manufactured with diphtheria toxoid, CRM197 or tetanus toxoid as carrier proteins (40).

Since the MenA conjugate vaccines are manufactured from purified components by a defined chemical process, the strategy for the control of the vaccine relies heavily on molecular characterization and purity to ensure each vaccine lot is consistent with the specifications of the vaccine lots used in the definitive clinical trials that confirmed their safety and immunogenicity (17, 40-45). Whilst immunogenicity testing in animals forms a necessary part of vaccine development, experience gained following the licensure of the MenC conjugate vaccines suggests that a routine animal potency test is not necessary when vaccine consistency has been assured by physicochemical criteria.

It is likely that MenA conjugates may be combined with other antigens (e.g. antigens given routinely to infants and children, including other conjugated antigens) into multivalent vaccines for convenience of vaccine delivery (46). Because of the problems associated with performing physicochemical analyses on complex vaccine formulations, the manufacturer should consider which tests to perform at a production stage before formulation and on final bulks or final lots of such vaccines. The testing regimen should be in agreement with the NRA.

The role of bactericidal antibody in protection against meningococcal disease is supported by the association between the level of serum bactericidal antibodies and protection against disease in the general population and the increased risk of meningococcal disease for individuals with complement deficiencies (28,48,49). In the case of MenA disease, the protection afforded by plain polysaccharide vaccine was demonstrated in controlled clinical trials in Egypt, Finland and Sudan during the early 1970s. However, the low burden of meningococcal disease in most countries has made it difficult to conduct controlled clinical studies before licensure to evaluate the efficacy of meningococcal conjugate vaccines. To date, then, the licensure of meningococcal conjugate vaccines has been based on a) elicitation of a bactericidal antibody response; b) evidence that the immune response is T cell-dependent; and c) evidence that the conjugate vaccine is not more reactogenic than other licensed vaccines in the same target population (50-52). Guidance on the clinical evaluation of MenA conjugate vaccines is described in Part C of these recommendations.

PART A. MANUFACTURING RECOMMENDATIONS

A.1. Definitions

A.1.1 International name and proper name

The international name should be "Group A meningococcal conjugate vaccine". The proper name should be equivalent to the international name in the language of the country of origin.

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

A.1.2 Descriptive definition

A group A meningococcal conjugate vaccine consists of group A saccharide covalently linked to a carrier protein.

A.1.3 International reference materials

No international reference materials were available for quality testing of group A meningococcal conjugate vaccines at the time of preparing this document. No formally established international reference materials that would allow the standardization of assays for immune responses to group A meningococcal conjugate vaccines are currently available.

The following reagents are available through the courtesy of manufacturers and national control or reference laboratories: Human anti-meningococcal capsular antibody reference serum CDC1992, for the standardization of serum bactericidal assays (SBA) and enzyme-linked immunosorbent assays (ELISAs); group A meningococcal polysaccharide and methylated human serum albumin for use in ELISAs; and group A specific monoclonal antibody used to confirm the identity of group A polysaccharide are available from the National Institute for Biological Standards and Control (NIBSC), Potters Bar, United Kingdom.

Any meningococcal strain expressing a group A capsule may be used in the SBA providing that it is not killed by the complement source in the absence of group A specific antibodies. Ideally, the strain used for the SBA should be representative of the prevalent clonal complex causing disease where the vaccine is to be used.

A.1.4 Terminology

The following definitions are given for the purpose of these recommendations only.

Master seed lot: A quantity of live bacterial suspension of *N. meningitidis* derived from the original strain, has been processed as a single lot and is of uniform composition. It is used for the preparation of the working seed lots. Master seed lots shall be maintained in the freeze-dried form at or below 4°C or be frozen at or below -45°C.

Working seed lot: A quantity of live bacterial suspension of *N. meningitidis* derived from the master seed lot by growing the organisms and maintaining them in aliquots in the freeze-dried form at or below 4°C or in the frozen state at or below -45°C. The working seed lot is used, when applicable, after a fixed number of passages approved by the NRA, for the inoculation of production medium.

Single harvest: The material obtained from one batch of cultures that have been inoculated with the working seed lot (or with the inoculum derived from it), harvested and processed together.

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

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

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

Bulk conjugate: A conjugate prepared from a single lot or a pool of lots of polysaccharide and a single lot or a pool of lots of carrier protein and conjugated at the same time. This is the parent material from which the final bulk is prepared.

Final Bulk: The homogenous preparation 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 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.

A.2. General manufacturing recommendations

The general manufacturing recommendations contained in Good Manufacturing Practices for Pharmaceuticals (53) and Biological Products (54) should be applied to establishments manufacturing meningococcal conjugate vaccines with the addition of the following:

Details of standard operating procedures for the preparation and testing of meningococcal conjugate vaccines adopted by the manufacturer together with evidence of appropriate validation of each production step should be submitted for the approval of the 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 to the NRA for approval before they are implemented.

N. meningitidis is a Biosafety Class 2 pathogen and represents a particular hazard to human health through infection by the respiratory route. The organism should be handled under appropriate conditions for this class of pathogen (55). Standard operating procedures need to be developed for dealing with emergencies arising from the accidental spillage, leakage or other dissemination of meningococcal organisms. Personnel employed in the production and control facilities should be adequately trained. Appropriate protective measures including vaccination should be implemented. Adherence to current Good Manufacturing Practices (53,54) is important to the integrity of the product, to protect workers and the environment.

A.3. Production control

A.3.1 Control of polysaccharide

A.3.1.1 Strains of *N. meningitidis*

The strain of *N. meningitidis* used for preparing the group A polysaccharide should be identified by a record of its history, including the source from which it was obtained and the tests made to determine the characteristics of the strain. The strain should have been shown to be capable of producing group A polysaccharide. They should also have been shown to the satisfaction of the NRA.

Serological assays and/or ¹H nuclear magnetic resonance (NMR) spectroscopy are suitable methods for the confirmation of identity of purified polysaccharide.

A.3.1.2 Seed lot system

The production of group A meningococcal polysaccharide shall be based on a seed lot system. Master seed lots should be fully characterized. Cultures derived from the working seed lots shall have the same characteristics as the cultures of the strain from which the master seed lot was derived (A3.1.1).

When a new working seed lot is prepared, the capsular polysaccharide should be characterized by appropriate methods. It is also deemed necessary to perform comparability study of a new working seed lot with the previous lot. In addition, control tests of verification of culture purity and strain identity should also be performed.

Methods to be used for confirming polysaccharide include NMR and/or serological assays or the other methods agreed by NRA.

A.3.1.3 Culture media for the production of meningococcal polysaccharide

The liquid culture medium used for vaccine production should be free from ingredients that will form a precipitate upon addition of hexadecyl trimethylammonium bromide to a concentration of 1 g l⁻¹.

The acceptability of the source(s) of any components of bovine, ovine or caprine origin used in culture media should be approved by the NRA. If any materials of animal origin are used in seed preparation or preservation, or in production, they should comply with the WHO guidelines on medicinal and other products in relation to human and animal transmissible spongiform encephalopathies (56) and should be approved by the NRA.

Manufacturers are encouraged to avoid wherever possible the use of materials of animal origin.

Human blood or blood products should not be used in culture media for propagating bacteria, either for seed or for vaccine.

A.3.1.4 Control of single harvests

A.3.1.4.1 Monitoring consistency of production

Consistency of growth of meningococcal organisms during production of single harvests should be demonstrated. Parameters to be monitored may include, but are not limited to, growth rate, temperature, pH and the final yield of group A polysaccharide. There should be predefined conditions of time and growth for termination of the fermentation for harvest of polysaccharide.

A.3.1.4.2 Control of bacterial purity

Samples of the culture from single harvests should be taken before killing 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. If any contamination is found, the culture or any product derived from it should be discarded. The killing process should be validated.

A.3.1.5 Control of purified polysaccharide

Each lot of purified group A meningococcal polysaccharide should be tested for identity and purity. A number of approaches to determine polysaccharide identity and purity give complementary but incomplete information, so the combination of methods employed to provide all necessary data should be agreed by the NRA. The purity limits given below are expressed with reference to the polysaccharide in its salt form (sodium or calcium), corrected for moisture. If the purified polysaccharide is stored as a frozen liquid, the tests must be conducted on an aliquot that has been dried. Variations in these specifications that may be appropriate if unusual salt forms are present should be agreed by the NRA.

Generally, the culture is harvested after killing the organism by heating to 56°C for 30 minutes, or by the use of a suitable inactivating agent or any other suitable method after which the polysaccharide is partially purified by precipitation with hexadecyl trimethylammonium bromide. Methods used for further purification of this intermediate should be approved by the NRA. Purified meningococcal polysaccharide and, when necessary, partially purified intermediates, are usually stored at or below -20°C to ensure stability.

A.3.1.5.1 Identity test

A test should be performed on the purified polysaccharide to verify its identity. 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 and approved by the NRA.

Serological assays and/or ¹H nuclear magnetic resonance spectroscopy provide convenient methods for this purpose (41,57) .

A.3.1.5.2 Moisture content

If the purified polysaccharide is to be stored as a lyophilised or otherwise dried powder, the moisture content should be determined by suitable methods approved by the NRA and shown to be within agreed limits. The value is used to calculate the results of the other chemical tests with reference to the dried substance. If the purified polysaccharide is stored as a frozen liquid, this applies to the aliquot that has been dried.

A.3.1.5.3 Polysaccharide content

The quantity of the purified polysaccharide can be estimated by the determination of the phosphorus content. The phosphorus content should be not less than 8.0% of the dry weight of the isolated product, as determined by the Chen assay (58,59), or other suitable methods approved by the NRA.

Other methods, such as high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) or conductivity detection (HPAEC-CD), may be used to define the quantitative composition of the purified polysaccharide but the methods should be validated for the purpose (60,61). ¹H nuclear magnetic resonance spectroscopy also provides a convenient approach to define the composition of the purified polysaccharide if an internal reference compound is included (62,63). If other methods are used, alternative specifications for polysaccharide composition may apply and should be agreed with the NRA.

A.3.1.5.4 Protein impurity

Protein content in each lot of purified polysaccharide should not be more than 1% by weight of protein, as determined by the method of Lowry *et al.*, using bovine serum albumin as a reference (3,64), or other suitable validated methods approved by the NRA.

Sufficient polysaccharide shall be assayed to detect 1% protein contamination accurately.

A.3.1.5.5 Nucleic acid impurity

Nucleic acid content in each lot of purified polysaccharide should not be more than 1% by weight of nucleic acid as determined by ultraviolet spectroscopy, on the assumption that the absorbance of a 10g^l⁻¹ nucleic acid solution contained in a cell of 1 cm path length at 260 nm is 200 (3) or by other validated methods approved by the NRA.

Sufficient polysaccharide shall be assayed to detect 1% nucleic acid contamination accurately.

A.3.1.5.6 Endotoxin content

To ensure an acceptable level of pyrogenic activity of the final product, the endotoxin content of the purified polysaccharide should be determined and shown to be within acceptable limits approved by the NRA.

An amount of less than 100 International Units of endotoxin per μg of purified polysaccharide can be achieved when measured by the *Limulus* amoebocyte lysate (LAL) test. Alternatively, a recognized pyrogenicity test can be performed in rabbits (59). Endotoxin content has also been measured by use of a chromatographic technique to determine the profile of LPS-associated lipids (65).

A.3.1.5.7 O-acetyl content

Studies to date indicate that the group A meningococcal polysaccharide used in the conjugate should be O-acetylated (66), the O-acetyl content should be monitored by colorimetric or other validated assays which approved by the NRA, to ensure consistency of production.

Bulk group A polysaccharide used for the production of licensed polysaccharide vaccine is suitable for the production of conjugate vaccine (1). O-acetyl content is conveniently determined by a colorimetric assay (67), ^1H NMR (57) or high-performance anion-exchange chromatography with conductivity detection (61). O-acetylated polysaccharide typically contains at least 2 mmol/g saccharide (>61.5% O-acetyl per repeating unit).

3.1.5.8 Molecular size distribution

The molecular size of purified polysaccharide provides an indication of the manufacturing consistency. The assay and an acceptable level of consistency should be agreed with the NRA.

The distribution constant (K_D) can 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 and/or the mass distribution limits should be established and shown to be consistent lot-to-lot for a given product chromatographed on a specific column material.

Methods such as: i) gel filtration through Sepharose CL-4B or CL-2B (or similar) in a 0.2 molar buffer using either a refractive index detector, ultraviolet, or colorimetric assay for the detection of the polysaccharide; and ii) high performance size-exclusion chromatography (HPSEC) either alone or in combination with light scattering and refractive index detectors (eg. MALLS) are suitable for this purpose (68-70). The methodology and column used should be validated to demonstrate sufficient resolution in the appropriate molecular weight range.

A.3.1.6 Control of activated saccharide

Polysaccharide preparations may be partially depolymerised either before or during the chemical modification to yield the activated saccharide.

A.3.1.6.1 Chemical activation

Several methods for the chemical activation of polysaccharides prior to conjugation may be satisfactory. The chosen methods should be approved by the NRA.

The methods used are similar to those employed in the production of conjugate vaccines against *Haemophilus influenzae* type b.

Polysaccharide may be oxidised with periodate and the periodate activated polysaccharide attached to the carrier protein directly by reductive amination, or through a secondary linker. Alternatively, the polysaccharide can be randomly activated by cyanogen bromide, or a chemically similar reagent, and a bifunctional linker added, which then allows the polysaccharide to be attached to the carrier protein directly, or through a secondary linker.

Size-reduced polysaccharides may be generated by reaction with acid and the derived oligosaccharide attached to the carrier protein directly or through a secondary linker, or by other methods.

A.3.1.6.2 Extent of activation of the saccharide

The manufacture should demonstrate control of the degree of activation of the saccharide by an assay of each batch of the saccharide.

Colorimetric and chromatographic assays and NMR spectroscopy have been used to determine the degree of activation of the saccharide (42,57,71,72).

A.3.1.6.3 Molecular size distribution

The degree of size reduction of the activated saccharide will depend upon the manufacturing process. The average size distribution (degree of polymerization) of the processed saccharide should be measured by a suitable method and should be within defined limits. The size should be specified for each type of conjugate vaccine with appropriate limits for consistency, as the size may affect the reproducibility of the conjugation process.

The molecular size may also be determined by HPSEC either alone or in combination with light scattering and refractive index detectors (eg. MALLS)(71). Other suitable methods include gel filtration, ion exchange chromatography or HPLC used together with an appropriate validated chemical assay (42,71,73).

A.3.2 Control of the carrier protein

A.3.2.1 Microorganisms and culture media for production of the carrier protein

Microorganisms to be used for the production of the carrier protein should be grown in media free from substances likely to cause toxic or allergic reactions in humans. If any materials of animal origin are used in seed preparation or preservation, or in production, they should comply with the WHO guidelines on medicinal and other products in relation to human and animal transmissible spongiform encephalopathies (56) and should be approved by the NRA.

The acceptability of the source(s) of any components of bovine, ovine or caprine origin used in culture media should be approved by the NRA.

Human blood or blood products should not be used in culture media for propagating bacteria.

Production should be based on a seed lot system with the strains identified by a record of their history and of all tests made periodically to verify strain characteristics. Consistency of growth of the microorganisms used should be demonstrated by monitoring parameters such as the growth rate, temperature, pH and final yield of appropriate protein(s).

A.3.2.2 Characterisation and purity of carrier protein

Potentially there are many proteins that could be used as carriers in meningococcal conjugate vaccines. The principal characteristics of the carrier protein should be that it is safe and elicits a T-cell-dependent immune response. Test methods used to characterize such proteins, to ensure that they are non-toxic and to determine their purity and concentration, should be approved by the NRA.

Proteins and purification methods that might be used include:

1. Tetanus or diphtheria toxoid. This must satisfy the relevant requirements published by WHO (74) and be of high purity. The purity should not be less than the value of the lots used during clinical development and agreed with the NRA (75).

2. *Diphtheria CRM 197 protein*. This is a non-toxic mutant of diphtheria toxin, isolated from cultures of *Corynebacterium diphtheriae* C7 /β197 (76). Protein of purity greater than 90% as determined by HPLC is prepared by column chromatographic methods. When produced in the same facility as diphtheria toxin, methods must be in place to distinguish the CRM 197 protein from the active toxin.

The carrier protein should also be characterized. The identity may be determined serologically. Physico-chemical methods that have been used to characterize protein include SDS-PAGE, isoelectric focusing, HPLC, amino acid analysis, amino acid sequencing, circular dichroism, fluorescence spectroscopy, peptide mapping and mass spectrometry as appropriate (70).

A.3.2.3 Processing of carrier protein

In some conjugation procedures, the carrier protein is chemically derivatized before coupling to the activated saccharide. As a measure of consistency, the extent of derivatization (or activation) of the protein needs to be determined at this stage.

Protein activation methods that have been used for conjugates include:

1. The introduction into diphtheria toxoid of a specified concentration of "spacer groups" reactive with polysaccharide.
2. The activation of carboxyl groups of tetanus toxoid by reaction with hydrazine or adipic acid dihydrazide (ADH) (77).

A.3.3 Control of purified bulk conjugate

There are a number of possible conjugation methods that might be used for vaccine manufacturing, all involve multi-step processes. Both the methods and the control procedures used to ensure the reproducibility, stability and safety of the conjugate should be established once the immunogenicity of the conjugate vaccine has been demonstrated. The derivatization and conjugation process should be monitored by analysis for unique reaction products or by other suitable means. The conditions used in the conjugation chemistry may affect the structure of the saccharide chain by causing cleavage or the loss of labile substituents.

Residual unreacted functional groups potentially capable of reacting in vivo may be present following the conjugation process. The manufacturing process should be validated to show that the reactive functional groups that remain at the conclusion of the manufacturing process, are below a limit approved by the NRA.

After the bulk conjugate has been purified, the tests described below should be performed to assess consistency of production. The tests are critical for assuring lot-to-lot consistency.

NMR spectroscopy may be used to confirm the identity and integrity of the saccharide in the conjugate (63,78,79). Alternatively, the identity could be confirmed by the use of an immunological test (e.g. ELISA).

A.3.3.1 Residual reagents

The conjugate purification procedures should remove residual reagents used for conjugation and capping. The removal of reagents and reaction by-products such as cyanide, EDAC and others depending on the conjugation chemistry, should be confirmed by suitable tests or by validation of the purification process. The acceptable limits should be approved by the NRA.

The residuals are process-specific and can be quantified by use of colorimetric and chromatographic assays. Techniques such as NMR spectroscopy (62,63) and hyphenated techniques such as LC-MS (80) may also be applied.

A.3.3.2 Conjugation markers

Where the chemistry of conjugation reaction results in the creation of a unique linkage marker (e.g. a unique amino acid), each batch should be assessed to quantify the extent of covalent reaction of the meningococcal saccharide with the carrier protein, so that the frequency of the covalent bond is given as a function of the number of saccharide repeating units or overall saccharide content.

A unique linkage marker could be assessed on each batch (70). Saccharide to protein ratio is also a suitable conjugation marker, though not a direct measurement.

A.3.3.3 Capping markers

Each batch of bulk conjugate should be shown to be free of reactive functional groups on either the saccharide or carrier protein. Alternatively, the product of the capping reaction can be monitored or the capping reaction can be validated to show removal of reactive functional groups. Validation of the manufacturing process can eliminate the need to perform this analysis for routine control with the agreement of the NRA.

A.3.3.4 Group A meningococcal saccharide content

The group A meningococcal saccharide content should be chemically determined by means of an appropriate validated assay.

Methods that have been used include determination of phosphorus or sugar content by use of colorimetric or HPAEC-PAD assays.

A.3.3.5 Conjugated and unbound (free) saccharide

The group A meningococcal saccharide that is covalently bound to the carrier protein, i.e. conjugated saccharide, is immunologically the most important for clinical protection. Each batch of bulk conjugate should be tested for unbound or free saccharide in order 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 saccharide prior to assay, and potentially applicable to meningococcal conjugates, include hydrophobic chromatography, acid precipitation on ice, precipitation with carrier protein-specific antibodies, ultracentrifugation, gel filtration and ultrafiltration. The amount of unbound saccharide can be determined by specific chemical or immunological tests, or by HPAEC after hydrolysis.

A.3.3.6 Protein content

The protein content in the bulk conjugate should be determined by means of an appropriate validated assay (section A.3.2) and comply with limits for the particular product. Each batch of bulk conjugate should be tested for conjugated and unconjugated protein by the methods agreed with the NRA.

Appropriate methods for the determination of conjugated and unconjugated protein include HPLC, ion exchange chromatography and capillary electrophoresis or other methods.

A.3.3.7 Saccharide to protein ratio

The ratio of saccharide to carrier protein for each batch of the bulk conjugate should be determined as a marker of the consistency of the conjugation chemistry and as one measure of potency. For each conjugate, the ratio should be within the range approved for that particular conjugate by the NRA and should be consistent with vaccines shown to be effective in clinical trials.

The ratio can be determined either by independent measurement of the amounts of protein and saccharide present or by methods which give a

direct measure of the ratio. Methods include ¹H nuclear magnetic resonance spectroscopy or the use of HPSEC with dual monitoring (e.g. refractive index and UV, for total material and protein content respectively). The other methods may be used with agreement of the NRA.

A.3.3.8 Molecular size distribution

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

The relative molecular size of the saccharide-protein conjugate should be determined for each bulk conjugate, using a gel matrix appropriate to the size of the conjugate (46). The method should be validated with an emphasis on specificity to distinguish the saccharide-protein conjugate from other components that may be present, e.g. unbound protein or free saccharide. The size distribution specifications will be product-specific and should be consistent with lots shown to be immunogenic in clinical trials.

Typically the size of the saccharide-protein conjugate may be examined by methods such as gel filtration on Sepharose CL-4B, or by HPSEC on an appropriate column. Since the saccharide to protein ratio is an average value, characterisation of this ratio over the size distribution (e.g. by dual monitoring of the column eluent) can be used to provide further proof of manufacturing consistency (70).

A.3.3.9 Sterility

The purified bulk conjugate should be tested for bacterial and fungal sterility in accordance with the requirements of Part A, sections 5.1 and 5.2, of the General Requirements for the Sterility of Biological Substances as amended in 1995 (81), 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.3.3.10 Specific toxicity of carrier protein

The bulk conjugate should be tested for the absence of specific toxicity of the carrier protein where appropriate (when tetanus or diphtheria toxoids have been used). Absence of specific toxicity of the carrier protein may also be assessed through validation of the production process.

A.3.4 Control of final bulk

A.3.4.1 Preparation

The final bulk is prepared by mixing the adjuvant if used, a preservative and /or stabilizer (as appropriate) with a suitable quantity of the bulk conjugate so as to meet the specifications of vaccine lots shown to be safe and efficacious in clinical trials.

A.3.4.2 Sterility

Each final bulk should be tested for bacterial and fungal sterility as indicated in section A.3.3.9.

A.3.5 Filling and containers

The recommendations concerning filling and containers given in Good Manufacturing Practices for Biological Products (54) should be applied.

When a freeze-drying process is used for vaccine production, its validation should be submitted to the NRA for approval.

A.3.6 Control tests on final lot

Samples should be taken from each final vaccine lot to be tested and fulfill requirements of this section. All the tests and specifications, if not specifically described, should be approved by the NRA.

A.3.6.1 Identity

An identity test should be performed on each final lot.

A serological test, using antibodies specific for the purified polysaccharide may be used. Testing for the carrier protein may also be performed.

A.3.6.2 Sterility

Final containers should be tested for bacterial and fungal sterility as indicated in section A.3.3.9.

A.3.6.3 Group A meningococcal saccharide content

The amount of group A meningococcal saccharide in the final containers should be determined and shown to be within the specifications agreed by the NRA.

The conjugate vaccines produced by different manufacturers may differ in formulation. A quantitative assay for the group A meningococcal saccharide in the final containers should be carried out. The assays used are likely to be product-specific and might include phosphorus estimation by a colorimetric or HPAEC-PAD method, chromatographic or serological methods. Immunological

assays such as rate nephelometry (82) or ELISA inhibition may also be used.

A.3.6.4 Conjugated and unbound (free) saccharide

The group A meningococcal saccharide that is covalently bound to the carrier protein is immunologically the most important for clinical protection and is one measure of vaccine potency. The structural integrity of the group A saccharide component of the conjugate may be affected by the formulation and filling and therefore the final lot should be tested for unbound or free saccharide, as described in Part A, section 3.3.5. An acceptable value of free saccharide consistent with adequate immunogenicity, as shown in clinical trials, should be established for the particular product and each final lot must be shown to comply with this limit.

A.3.6.5 Molecular size distribution

The structural integrity of the group A meningococcal saccharide component of the conjugate may be affected by the formulation and filling and therefore molecular size of the saccharide-protein conjugate in the final lot should be tested, as described in Part A, section 3.3.8. This test should be included in stability studies (see Part A, section 8.1).

A.3.6.6 Residual Moisture

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

The test should be performed on 1 vial per 1000 up to a maximum of 10 vials but on no less than 5 vials taken at random from throughout the final lot. The average residual moisture content should generally be no greater than 2.5% and no vial should be found to have a residual moisture content of 3% or greater.

A.3.6.7 Pyrogen content

The vaccine in the final container should be tested for pyrogenic activity by intravenous injection into rabbits or by a *Limulus* amoebocyte lysate (LAL) test, which should be validated for this purpose. Endotoxin content or pyrogenic activity should be consistent with levels found to be acceptable in vaccine lots used in clinical trials and approved by the NRA.

A.3.6.8 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.

The adsorption of the antigen to the adjuvant should be controlled. Consistency is important and the adsorption of production lots should be demonstrated to be within the range of values found for vaccine lots shown to be clinically effective.

A.3.6.9 Preservative content

The manufacturer has a choice of possible preservatives. Considerations should be given to the stability of the chosen preservative and possible interactions between the vaccine components and the preservative. If a preservative has been added to the vaccine, the content of the preservative should be determined by a method approved by the NRA. The amount of the preservative in the vaccine dose should be shown not to have any deleterious effect on the antigen nor impair the safety of the product in humans. The preservative and its concentration should be approved by the NRA.

A.3.6.10 General safety test (Innocuity)

The requirements to test lots of meningococcal conjugate vaccine for unexpected toxicity (abnormal toxicity) should be agreed with the NRA.

Such a test may be omitted for routine lot release with the agreement of the NRA once consistency of production has been well established to the satisfaction of the NRA and when Good Manufacturing Practices are in place.

A.3.6.11 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 the clinical trials and in stability studies. For a lyophilized preparation, the pH should be measured after reconstitution with the appropriate diluent.

A.3.6.12 Inspection of final containers

Each container in each final lot should be inspected visually (manually or with automatic inspection systems), and those showing abnormalities such as improper sealing, lack of integrity and, if applicable, clumping or the presence of particles should be discarded.

Visual inspection of the appearance of the freeze-dried or liquid vaccine should be described with respect to the form and colour. In case of freeze-dried vaccines, visual inspection should be performed on the freeze-dried vaccine, on its diluent and on the reconstituted vaccine.

A.4 Records

The recommendations in Section 8 of Good Manufacturing Practices for Biological Products (54) should be applied.

A.5 Retained samples

The recommendations in Section 9.5 of Good Manufacturing Practices for Biological Products (54) should be applied.

A.6. Labelling

The recommendations in Section 7 of Good Manufacturing Practices for Biological Products(54) should be applied with the addition of the following.

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

- the amounts of group A meningococcal saccharide and carrier protein contained in each single human dose;
- the word "adsorbed", if applicable;
- the name and address of the manufacturer;
- the recommended storage temperature and the expiry date if kept at that temperature;
- if the vaccine is freeze-dried, that after its reconstitution it should be used immediately unless data have been provided to the licensing authority that it may be stored for a limited time;
- the volume and nature of the diluent to be added in order to reconstitute a freeze-dried vaccine, specifying that the diluent should be supplied by the manufacturer or approved by the NRA.

In addition, the label printed on or affixed to the container, or the label on the carton, or the leaflet accompanying the container shall contain the following:

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

A.7. Distribution and transport

The recommendations in Section 8 of Good Manufacturing practices for Biological Products (54) should be applied.

A.8. Stability, storage and expiry date

A.8.1 Stability

Adequate stability studies form an essential part of the vaccine development studies, they should be designed to help setting up the shelf-life of the vaccines.WHO is developing further guidance on the stability studies of vaccines and general principles should be followed.

The stability of intermediate products such as the purified polysaccharide and the bulk conjugate before mixing into the final bulk should be demonstrated. The stability of the vaccine in its final form, with final containers and at the recommended storage temperatures should be demonstrated to the satisfaction of the NRA for at least three lots of final product

manufactured from different independent bulk conjugates. In addition, real time stability study should be conducted on at least one final container lot produced per year.

The saccharide component of conjugate vaccines may be subject to gradual hydrolysis at a rate which may vary depending upon the type of conjugate, the type of formulation or adjuvant, the types of excipient and conditions of storage. The hydrolysis may result in reduced molecular size of the meningococcal saccharide component, a reduction in the amount of the saccharide bound to the carrier protein and or in a reduced molecular size of the conjugate.

The structural stability of the saccharide chains and of the protein carrier vary between different conjugate vaccines as found for group C meningococcal conjugate vaccines (44,46,83).

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 free saccharide content as a percentage of the total saccharide should be determined. The vaccine should meet the recommendations for final product up to the expiry date.

Molecular sizing of the final product and estimation of free saccharide may be carried out to ensure the integrity of the conjugate (46).

The level of adsorption, where applicable, should be shown to be within limits agreed by the NRA, unless data are available to show that the immunogenicity of the final product is not dependent upon adsorption of the antigen to the adjuvant.

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

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.

If the manufacturers consider to include the vaccine vial monitor (VVM) into the label, they should include appropriate studies to identify how the VVM should be used (103).

A.8.2 Storage conditions

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

Storage of both liquid and freeze-dried vaccines at a temperature of 2–8°C has been found to be satisfactory.

A.8.3 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.8.1.

PART B. NONCLINICAL EVALUATION OF NEW MENINGOCOCCAL CONJUGATE VACCINES

Nonclinical testing is a prerequisite for initiation of clinical studies in humans and includes immunogenicity studies (proof of concept) and safety testing in animals. The vaccine lots used in nonclinical studies should be adequately representative of the formulation intended for clinical investigation and, ideally, should be the same lots used in clinical studies. If this is not feasible, then the lots used clinically should be comparable to those used in the nonclinical studies with respect to potency, stability and other characteristics of quality.

Details on the design, conduct, analysis and evaluation of nonclinical studies are available in WHO guidelines for nonclinical evaluation of vaccines (84)

The capacity of a meningococcal conjugate vaccine to elicit a serum bactericidal antibody response in mice may be used to establish a preclinical proof of concept. However, this should also be shown in a second animal species. The studies should demonstrate the ability of the conjugate vaccine to boost the bactericidal antibody response. The ability of antisera raised against a candidate MenA conjugate vaccine to confer passive protection in an infant rat model of meningococcal meningitis would provide further preclinical evidence of the potential protective efficacy against invasive MenA infections (85).

PART C. CLINICAL EVALUATION OF GROUP A MENINGOCOCCAL CONJUGATE VACCINES

C.1 General considerations for clinical studies

In general, clinical trials should adhere to the principles described in the WHO guidelines on good clinical practice (86).

General principles described in the WHO guideline on regulatory expectations for clinical evaluation of vaccines apply to Men A conjugate vaccines and should be followed (87). Some of the issues that are specific to conjugate vaccines and/or particularly to the clinical development program for MenA conjugate vaccines are discussed in the following sections and should be read in conjunction with the general guidance mentioned above.

These recommendations should be viewed in the light of further data on the safety and immunogenicity of MenA conjugate vaccines and any relevant data on other types of conjugate vaccines that may become available in the near future.

C.1.1 Scope of the studies

For reasons discussed in section C.2, it is not considered necessary that the protective efficacy of MenA conjugate vaccines should be studied pre-licensure. Instead, it is expected that the pre-licensure assessment of the likely protective efficacy of conjugated MenA vaccines will be based on immune responses. The clinical development program should be conducted in populations that are representative of those in which the vaccine is expected to be deployed. The early development program should include identification of an optimal dose of

conjugated antigen and a suitable immunization schedule together with a preliminary assessment of vaccine safety.

The general approach to confirmatory clinical study design will depend on whether or not it is considered appropriate to compare immune responses elicited by candidate MenA conjugate vaccines and licensed unconjugated MenA-containing vaccines in the population(s) chosen for investigation. This is discussed in sections C.1.2, C.1.3 and C.2.2. In addition, methodological and statistical considerations described in the WHO guideline on regulatory expectations for clinical evaluation of vaccines should be taken into account (sections B2 and B3 in reference 87).

For convenience of administration it is very likely that at least some MenA conjugate constructs will be combined with other antigens (e.g. those required for the infant immunization series) into a single product. Also, monovalent or combined MenA conjugate vaccines will likely need to be given concomitantly with other vaccines that are routinely administered to various age groups. Therefore pre-licensure studies should adequately explore the potential for immune interference with respect to combination of MenA conjugates with other antigens into a single vaccine and/or concomitant administration of MenA conjugate-containing vaccines with other vaccines as appropriate to the population investigated (see C.2.5).

In the post-licensure period, there should be plans in place to further assess vaccine safety and to investigate antibody persistence, the potential need for and timing of booster doses and the effectiveness of the vaccine during routine use (see C.2.3 and C.3).

C.1.2 Studies that compare conjugated with unconjugated MenA-containing vaccines

In age groups in which high proportions of vaccinees develop presumed protective antibody responses to licensed unconjugated MenA vaccines and antibody persists for several years, the assessment of a candidate MenA conjugate vaccine should be based on comparing immune responses between groups given conjugated and unconjugated saccharides (see C.2.2). Studies should plan to compare antibody persistence between groups and responses to a further dose of conjugate (see C.2.3). Also, antibody responses to the conjugated MenA vaccine should be evaluated in persons who have and have not received unconjugated vaccine in the past during routine or emergency vaccination programs. Subjects with various elapsed times since their last dose of unconjugated vaccine should be included.

C.1.3 Studies that compare vaccinated and unvaccinated groups

It has been documented that infants may develop antibody in response to unconjugated MenA polysaccharide from about the age of 2-3 months. However, antibody titres may be low and transient in infants compared to older children. Also, following vaccination of children up to about 18-24 months of age with unconjugated MenA vaccines, the duration of antibody persistence appears to be shorter than that observed in older children, adolescents and adults. Therefore, depending on the geographic regions and the population in which the studies are conducted and the risk of disease in unvaccinated infants, it may be appropriate that at least some studies in infants compare administration of conjugated MenA vaccine with no MenA vaccine (see C.2.2). The inclusion of a control group of unvaccinated infants has the advantage of allowing for assessment of changes in maternal antibody over time and the acquisition of anti-MenA antibody as a result of natural exposure to the organism and/or to

cross-reacting antigens during the period of study. Studies should compare antibody persistence between groups and response to a further dose of MenA conjugate (see C.2.3).

The following sections provide further details regarding some of the data to be provided.

C.2 Assessment of the immune responses

C.2.1 Antibody assays

Antibody responses to MenA conjugate vaccines can be assessed by:

- Assay of serum bactericidal antibody (SBA), which measures functional antibody and should be regarded as the primary means of assessing the immune response
- Assay of MenA-specific antibody (e.g. measured with Enzyme-linked immunosorbent assay [ELISA]), which can be used as a secondary measure of immune responses.

The assays used should be fully validated.

Additional investigations of antibody quality may include measurement of antibody avidity. These assays may assist in assessment of maturation of the immune responses (e.g. in response to booster doses of the conjugate).

C.2.1.1 Assay of serum bactericidal antibody (SBA)

Although SBA titres are considered to be the most important for the assessment of the likely protective efficacy of the vaccine, there are only limited data available on anti-MenA SBA titres that might correlate with at least short-term protection against the disease.

In a prospective study in US army recruits, Goldschneider *et al.* (28) observed a strong correlation between development of group C meningococcal (MenC) disease, which was the only group circulating at that time in the population studied, and anti-MenC SBA titres <1:4 (measured using human complement; hSBA) at the time of entry into basic training. Interestingly, recruits who developed MenC disease were also much less likely to have SBA titres of at least 1:4 to MenA and MenB when starting basic training. The application of this same SBA cut-off to predict protection against MenA disease was based on the observation that hSBA titres $\geq 1:4$ seemed to correlate approximately with clinical protection against group A, B or C meningococcal disease based on studies with sera from persons aged from 0-26 years and data on disease epidemiology. Thus, hSBA titres $\geq 1:4$ against either group A or C meningococci have been proposed by the authors to correlate with at least short-term protection against invasive disease due to these organisms.

Further information on the correlation between SBA titres and protection against invasive meningococcal disease has emerged following the introduction of MenC conjugate vaccines in the United Kingdom in 1999. A re-evaluation of the correlates of protection for MenC disease was performed (88,89) using a large database on effectiveness and the results of SBA testing in which baby rabbit complement (rSBA) was employed (90,91). The source of complement in SBA assays affects the results since higher SBA titres

are obtained with the majority of sera when baby rabbit complement is used rather than human complement (92). From the estimates of effectiveness by age group in the UK and the immunogenicity data obtained from clinical trials with three MenC conjugate vaccines it was proposed that rSBA titres of 1:8 using the UK reference laboratory methodology correlated with short-term protection (92). The threshold for MenA conjugate vaccine remains to be established.

C.2.1.2 Assay of MenA-specific antibody (e.g. ELISA)

Depending on the methodology, the assay may measure total MenA-specific anti-capsular antibody or measure only IgG, IgG subclasses or IgM. However, only a proportion of the capsule-specific antibody detected may be functional and functionality would be affected by antibody isotype and avidity. The concentration of anti-capsular antibody required for protection against meningococcal disease is not known with any degree of certainty.

In one Finnish study, an anti-capsular antibody concentration of 2 μ g/ml measured by radioimmunoassay (RIA) appeared to correlate with protection following vaccination with unconjugated MenA polysaccharide (93, 94).

C.2.2 Criteria for assessment of immune responses

Despite the considerations above, there is currently no very well established immunological correlate of protection for MenA disease. Therefore, the overall assessment of the SBA titres elicited by a MenA conjugate vaccine should take into account proportions of vaccinees who achieve levels presumed to be protective (depending on the mode of assay), reverse cumulative distributions, increments in titres from pre- to post-vaccination and GMTs. If data on MenA-specific antibody concentrations are also generated, a similar approach should be taken in the assessment.

The immune response parameter chosen for the primary assessment of immunogenicity in any one study will depend on whether the candidate MenA conjugate vaccine is to be compared with a licensed unconjugated vaccine or with an unvaccinated group. This will in turn reflect the population selected for investigation as discussed in C.1.2 and C.1.3 and the anticipated pre-existing SBA titres that may reflect any prior vaccinations and natural exposure. Whichever parameter is considered to be primary (see below), between-group comparisons based on the other parameters (as above) should also be presented.

In the situation described in C.1.2 there may be a high rate of pre-existing circulating antibody. Therefore, the focus should be on demonstrating that the proportion of vaccinees in the conjugate vaccine group that achieves a pre-defined increment in SBA titre from pre- to post-vaccination (e.g. a \geq 4-fold rise) is non-inferior to that in the unconjugated vaccine group. The pre-defined margin of non-inferiority should be carefully justified and should take into account previous data regarding responses to unconjugated MenA vaccines in the population(s) chosen for study (e.g. described by age, geographical location).

In the situation described in C.1.3 there are several ways in which the potential protective efficacy conferred by a candidate MenA conjugate might be assessed. These include, but are not necessarily limited to:

- Comparison of the proportions of vaccinees reaching SBA titres considered likely to be protective at least in the short term and also of

the proportions reaching a selected higher titre (chosen according to the SBA assay used) between those vaccinated with the candidate MenA conjugate and the unvaccinated group. In this case the proportions in the vaccinated cohort that reach the pre-defined cut-offs should be superior to those in the unvaccinated group. The pre-defined margin of superiority should be carefully justified.

- Comparison of the proportions of vaccinees reaching pre-defined titres (e.g. as described in the paragraph above) and/or GMTs between infants given the MenA conjugate vaccine and older persons (e.g. children at least 3 years old) given unconjugated vaccine. Ideally, the older cohort given the unconjugated vaccine should be included as an additional arm in a study that provides a direct comparison between vaccinated and unvaccinated infants in order to provide a contemporary control group. In this way, sera from all three study groups (i.e. conjugated or unconjugated vaccine and unvaccinated) can be assayed in the same laboratory using the same methodology. In this case the responses in vaccinated infants should be non-inferior to those observed in older persons given unconjugated vaccine.

Data collected from the unvaccinated group on maternal antibody and the natural acquisition of antibody during the first few years of life should be taken into account when assessing the overall risk-benefit relationship for the candidate MenA conjugate vaccine in infants.

C.2.3 Antibody persistence and the need for booster doses

The possibility that induction of an immune memory response would be an important component of protection afforded by conjugate vaccines led to previous recommendations that their ability to elicit immunological memory should be assessed during clinical studies (7,22,38). However, despite the demonstrated ability of licensed Hib, MenC and pneumococcal conjugate vaccines to elicit immune memory, the relative importance of this phenomenon for longer-term protection against invasive infections with these organisms has been questioned in recent years. For example, one year following routine infant immunization with monovalent MenC conjugate vaccines in the UK effectiveness has been demonstrated to decline (95). This observation, together with the rapid decline of MenC-specific SBA titres after the primary series (96) and the rapid onset of meningococcal infection in vaccine failures (97) has pointed to a lesser importance of immune memory relative to circulating bactericidal antibody concentrations for maintaining protection against invasive meningococcal infections.

As a result, the documentation of antibody persistence after administration of MenA conjugate vaccines by following SBA titres over time is considered to be extremely important. Pre-licensure, these data may be limited (e.g. to around 6-12 months post-primary series). Therefore, at the time of initial licensure, plans should be in place to follow antibody levels in selected cohorts. Together with information on effectiveness (see C.3), these data should indicate whether booster doses are needed and, if so, when they should be given to maintain protection.

Despite the shift in emphasis from immune memory to persistence of adequate antibody concentrations for assessing long-term protection, it is still considered important that the characterization of the immune response to the priming dose(s) should include demonstration of an anamnestic response to a booster dose of a MenA conjugate vaccine when administered

at least 6 months after completion of the primary series. It is also recommended that changes in the avidity of MenA-specific IgG from pre- to post-primary series and before and after a booster dose of conjugate vaccine should be evaluated at least in a subset of vaccinees.

The investigation of the induction of immune memory during the primary series has often been assessed by administration of a challenge dose of unconjugated saccharide at least 6 months later. The challenge dose has usually consisted of a small amount of an appropriate licensed unconjugated saccharide vaccine. However, there is no licensed monovalent unconjugated MenA saccharide vaccine that could be used to provide such a challenge dose*. This means that, if performed, the challenge would have to be made with reduced doses of a licensed unconjugated MenA/C or A/C/W135/Y vaccine.

Depletion of immunologic memory and antibody hyporesponsiveness have been observed after a dose of unconjugated MenC vaccine, particularly in young children (98-100). Although the clinical consequences of these observations are not clear, the use of a conjugated MenA booster to assess immunologic memory circumvents any concern there might be regarding challenge with an unconjugated vaccine that contains MenC saccharide in addition to MenA saccharide.

* The term of "challenge dose" refers to the administration of unconjugated polysaccharide to mimic encounter with the organism.

C.2.4 Immune responses to carrier protein

To date, proteins such as a non-toxic diphtheria toxin molecule (CRM197), diphtheria toxoid and tetanus toxoid have been used in the production of various meningococcal conjugate vaccines. Administration of these conjugated saccharides alone has been found to result in measurable amounts of antibody to the carrier proteins but not to a sufficient extent that routine immunization schedules for diphtheria or tetanus could be amended. Co-administration of these conjugates with routine vaccines containing diphtheria and tetanus toxoids has generally enhanced the total levels against these antigens (depending on the carrier). These issues should be investigated for any new conjugate vaccine and should take into account the functionality of the antibody to the carrier. If notable increases in anti-diphtheria or anti-tetanus toxin antibody titers are observed under these circumstances then consideration should be given to the potential for adverse events to occur (e.g. as a result of hyper-immunisation).

For any novel proteins that may be used to manufacture conjugate vaccines (i.e. those are not already components of existing licensed vaccines), the immune response to the carrier should be explored. Any foreseeable potential clinical significance of the findings should be discussed and further studies conducted as necessary.

C.2.5 Combined vaccines and concomitant administration with other vaccines

C.2.5.1 Combined vaccines

It is already well documented that immune responses to certain types of conjugated antigens are lower when they are combined with some other antigens in pre-formulated products

compared to separate but concomitant and/or separate and non-concomitant administration (e.g. lower responses to Hib conjugates when they are combined with acellular pertussis components). In some instances the immune response to a conjugated antigen has been shown to be lower when it is mixed with other antigens only immediately before injection. More recently it has become apparent that there may be particular problems of immune interference when more than one conjugated antigen is included in the same combined vaccine. Therefore, if a candidate MenA conjugate vaccine is to be included in a combination product, with or without other conjugated saccharide(s), there should be an adequate exploration of the potential for immune interference to occur.

Ideally, studies should aim to demonstrate non-inferiority of SBA responses to the candidate MenA conjugate when included in the combined vaccine compared to administration alone. The pre-defined margins for non-inferiority require careful justification. However, such studies might become very complex in design if the combined formulation containing the MenA conjugate will include one or more other antigens not previously combined together. Thus, due to problems of feasibility, alternative study designs may have to be considered. For example, if there is already a MenA conjugate vaccine approved for use in the age group of interest for the test product then a comparison of anti-MenA SBA titres between the test and licensed vaccines may suffice for reassurance that there is no important adverse effect on immune responses to the candidate MenA conjugate when it is included in the combined product. Whatever the study design, the immune responses to all the antigens in the final combined formulation should be shown to be satisfactory. If there is any immune interference observed with respect to any of the combined antigens, the possible clinical implications should be carefully considered before proceeding with clinical development.

C.2.5.2 Concomitant administration

In recent years, it has also become apparent that concomitant administration of some types of conjugates with other vaccines in routine use, including other conjugated vaccines, may give rise to detectable immune interference although the clinical significance of the observed phenomena is not always clear(101). Examples include depression of anti-MenC SBA GMTs on co-administration with acellular pertussis vaccines and higher anti-Hib responses when Hib-tetanus toxoid (PRP-T) conjugates are co-administered with MenC-T conjugates compared to co-administration with MenC-CRM197 conjugates.

Therefore it is important that immune responses to candidate MenA conjugate vaccines (whether monovalent or in a combined vaccine) should be evaluated on co-administration with other vaccines that are representative of types that, for convenience and compliance reasons, are very likely to be given at the same clinic visits. Responses to other co-administered antigens should also be evaluated. The approach to these studies is based primarily on demonstrating non-inferiority of responses to antigens when vaccines are co-administered compared to each vaccine given alone, with careful justification of pre-defined non-inferiority margins.

C.3 Post-marketing studies and surveillance

The information in the application dossier is likely to be restricted to studies in certain geographical areas and to populations with particular demographic features. Also, the total population evaluated for safety in pre-licensure clinical studies may be limited such that only those adverse events that occur at a frequency of at least 1/1000 persons vaccinated can be

described with any degree of confidence (section B.7.4. in reference 87). Therefore, it is considered critically important that well-developed plans are in place prior to licensure for the assessment of vaccine safety and effectiveness during routine use in the post-marketing period.

Issues to be explored include the assessment of longer-term antibody levels in selected cohorts (as mentioned in C.2.3) and the impact of vaccination on meningococcal carriage. Depending on the choice of, and the immune response to, the carrier protein special attention may need to be paid to the possibility that the safety profile of the vaccine could be affected.

In reality, sound and comprehensive safety and effectiveness data cannot be collected by the manufacturers alone. Therefore, there should be planned collaborations between Market Authorization Holders (MAHs) and national and international public health bodies.

All data collected should be submitted to the responsible regulatory authorities at regular intervals so that any implications for the marketing authorization can be assessed.

PART D. RECOMMENDATIONS FOR NATIONAL REGULATORY AUTHORITIES

D.1 General

NRAs are responsible for ensuring that products released for public distribution are evaluated properly and meet international standards of quality and safety, and NRAs are expected to exercise control functions published by WHO in a competent and independent manner, backed up with enforcement power.

The general recommendations for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (102) should be applied.

In addition, as part of overall vaccine evaluation, the NRAs are responsible for the nonclinical and clinical assessment of Men A conjugate vaccines (as mentioned in parts B and C of this document) and it is essential that adequate resources and procedures are in place to ensure a comprehensive evaluation of the data. The role of NRAs as well as general principles for nonclinical and clinical evaluation of vaccines are described in WHO guidance documents (84, 87) and should be considered.

D.2 Official lot release and certification

A vaccine lot should be released only if it fulfils national requirements and/or Part A of these Recommendations.

A statement signed by the appropriate official of the NRA should be provided at the request of the manufacturing establishments and should certify whether or not the lot of vaccine in question satisfies all national requirements as well as Part A of these Recommendations. The certificate should state the number under which the lot was released, and the number appearing on the labels of the containers. Importers of MenA conjugate vaccines should be provided a copy of the official national release document. The purpose of the certificates is to facilitate the exchange of vaccines between countries.

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www.who.int/vaccines-documents/)

APPENDIX 1

MODEL SUMMARY PROTOCOL FOR MANUFACTURING AND CONTROL OF GROUP A MENINGOCOCCAL CONJUGATE VACCINE

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

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

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

Summary information on final lots

International name of product _____
Commercial name _____
Product license (Marketing Authorization) No. _____
Country _____
Name and address of manufacturer _____

Final packing lot number _____
Type of containers _____
Number of containers in this packing lot _____
Final container lot number _____
Number of filled containers in this final lot _____
Date of manufacturing (Filling or lyophilizing, if applicable) _____
Nature of final product (adsorbed) _____
Preservative and nominal concentration _____
Volume of each recommended single human dose _____
Number of doses per final container _____

Summary of the composition:

(Include a summary of the qualitative and quantitative composition of the vaccine per human dose including the conjugate, any adjuvant used and other excipients.)

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

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

Detailed information on manufacture and control

SUMMARY OF STARTING MATERIALS

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

A 3.1 CONTROL OF GROUP A MENINGOCOCCAL POLYSACCHARIDE

Strain

Identity of meningococcal strain used in vaccine _____
Origin and short history _____
Authority that approved the strain _____
Date approved _____

Master seed lot

Lot No. _____
Date of master seed lot was established _____

Working seed lot

Lot No. _____
Date of working seed lot was established _____
Control tests on working seed lot _____
Date of reconstitution of seed lot _____

Culture media for the production of meningococcal polysaccharide

Any components of animal origin

Certificate for TSE-free

Control of single harvests

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity and identity, the method and date of killing, the method of purification, and the yield of purified polysaccharide.

Control of purified polysaccharide

Lot No.

Date of manufacturing

Volume

Identity

Date of Test

Method

Specification

Result

Moisture content

Date of Test

Method

Specification

Result

Polysaccharide content

Date of Test

Method

Specification

Result

Protein impurity

Date of Test _____
Method _____
Specification _____
Result _____

Nucleic acid impurity

Date of Test _____
Method _____
Specification _____
Result _____

Endotoxin content

Date of Test _____
Method _____
Specification _____
Result _____

O-acetyl content

Date of Test _____
Method _____
Specification _____
Result _____

Molecular size distribution

Date of Test _____
Method _____
Specification _____
Result _____

Control of activated saccharide

Lot No. _____
Method for activation _____

Extent of activation

Date of Test _____
Method _____
Specification _____
Result _____

Molecular size distribution

Date of Test _____
Method _____
Specification _____
Result _____

A.3.2 CONTROL OF THE CARRIER PROTEIN

Microorganisms used

Identity of strain used in carrier protein production _____
Origin and short history _____
Authority that approved the strain _____
Date approved _____

Master seed lot

Lot No. _____
Date of master seed lot was established _____

Working seed lot

Lot No. _____
Date of working seed lot was established _____
Control tests on working seed lot _____
Date of reconstitution of seed lot _____

Culture media for production of carrier protein

Any components of animal origin _____
Certificate for TSE-free _____

Tests on carrier protein

Identity

Date of Test _____
Method _____
Specification _____
Result _____

Purity

Date of Test _____
Method _____
Specification _____
Result _____

Toxicity (if applicable)

Date of Test _____
Method _____
Specification _____
Result _____

Extent of derivatization (if applicable)

Date of Test _____
Method _____
Specification _____
Result _____

A.3.3 CONTROL OF PURIFIED BULK CONJUGATE

Production details of bulk conjugate

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

Tests on purified bulk conjugate

Residual reagents

Date of Test _____
Method _____
Specification _____
Result _____

Conjugation markers

Date of Test _____
Method _____
Specification _____
Result _____

Capping markers

Date of Test _____
Method _____
Specification _____
Result _____

Group A meningococcal saccharide content

Date of Test _____
Method _____
Specification _____
Result _____

Conjugated and unbound (free) saccharide

Date of Test _____
Method _____
Specification _____
Result _____

Protein content

Date of Test _____

Method _____
Specification _____
Result _____

Saccharide to protein ratio

Date of Test _____
Method _____
Specification _____
Result _____

Molecular size distribution

Date of Test _____
Method _____
Specification _____
Result _____

Sterility

Method _____
Media _____
Volume tested _____
Date of inoculation _____
Date of end of test _____
Specification _____
Result _____

Specific toxicity of carrier protein (if applicable)

Method _____
Strain and type of animals _____
Number of animals _____
Route of injection _____
Volume of injection _____
Quantity of protein injected _____
Date of start of test _____
Date of end of test _____

Specification _____
Result _____

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

A.3.4 CONTROL OF FINAL BULK

Lot No. _____

Name and nature of adjuvant, if used _____

Lot No. _____

Final concentration in the final bulk _____

Name and nature of preservative, if used _____

Lot No. _____

Final concentration in the final bulk _____

Name and nature of stabilizer, if used _____

Lot No. _____

Final concentration in the final bulk _____

Test on final bulk

Sterility

Method _____

Media _____

Volume tested _____

Date of inoculation _____

Date of end of test _____

Specification _____

Result _____

A.3.5 FILLING AND CONTAINERS

Lot No. _____

Date of sterile filtration _____
Date of filling _____
Volume of final bulk filled _____
Filling 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 period of storage approved _____
Storage temperature and period _____

A.3.6 CONTROL TESTS ON FINAL LOT

Tests on final lot

Appearance before reconstitution (if applicable)¹

Date of Test _____
Method _____
Specification _____
Result _____

Appearance (after reconstitution)

Date of Test _____
Method _____
Specification _____
Result _____
Lot number of diluent used _____

Identity

Date of Test _____
Method _____
Specification _____
Result _____

Sterility

Method _____
Media _____
No. of containers tested _____

Date of inoculation _____
Date of end of test _____
Specification _____
Result _____

Group A meningococcal saccharide content

Date of Test _____
Method _____
Specification _____
Result _____

Conjugated and unbound (free) saccharide

Date of Test _____
Method _____
Specification _____
Result _____

Molecular size distribution

Date of Test _____
Method _____
Specification _____
Result _____

Residual moisture¹

Date of Test _____
Method _____
Specification _____
Result _____

Pyrogen content

Date of Test _____
Method _____
Specification _____
Result _____

Adjuvant content (if applicable)²

Date of Test _____
Nature and concentration of adjuvant per human dose _____
Method _____
Specification _____
Result _____

Preservative content (if applicable)

Date of Test _____
Method _____
Specification _____
Result _____

General safety test

Date of Test _____
Method _____
Specification _____
Result _____

pH

Date of Test _____
Method _____
Specification _____
Result _____

Immunogenicity (if applicable)³

Method _____
Strain and type of animals _____
Number of animals (per group) _____
Route of injection _____
No. and volume of injections _____
Quantity of MenA saccharide injected _____
Date of start of test _____
No. of pre/post immunization sera tested _____
Date of end of test _____
Specification _____
Result _____

Stability⁴

Indicate separately all relevant details including changes in the proportion of free saccharide, molecular size distribution, pH, residual moisture and immunogenicity in accelerated degradation tests, and after storage for the maximum period claimed for the product at the recommended temperature.

SEPARATE ADJUVANT⁵

Summary of production details of adjuvant

When an adjuvant suspension is provided for the reconstitution of lyophilized vaccine a summary protocol of production and control details should be provided. The information provided and tests performed will depend on the adjuvant used.

Summary information on adjuvant

Name and address of manufacturer	_____
Nature of the adjuvant	_____
Lot No.	_____
Date of manufacturing	_____
Expiry date	_____

Tests on adjuvant

Adjuvant content

Date of Test	_____
Method	_____
Specification	_____
Result	_____

Appearance

Date of Test	_____
Method	_____
Specification	_____
Result	_____

pH

Date of Test	_____
Method	_____
Specification	_____
Result	_____

Pyrogenicity⁶

Date of Test _____
Method _____
Specification _____
Result _____

Sterility

Method _____
Media _____
No. of containers tested _____
Date of inoculation _____
Date of end of test _____
Specification _____
Result _____

¹ Only applies to lyophilized vaccines

² Only applies when adjuvant is present in the final container

³ Needed only for a sufficient number of batches to validate the production method. Not required for routine lot release

⁴ Needed only for sufficient batches to validate production method and proposed shelf-life.

⁵ This section is only required when adjuvant is provided separately for the resuspension of a lyophilized vaccine.

⁶ A pyrogen test of the adjuvant is not needed if a pyrogen test was performed on the adjuvant reconstituted vaccine.

CERTIFICATION BY THE MANUFACTURER

Name of head of control of the manufacturer _____

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

I certify that lot No. _____ of Group A Meningococcal Conjugate Vaccine, whose number appears on the label of the final containers, meets national requirements and satisfies Part A of the WHO Recommendations to assure the quality, safety and efficacy of group A meningococcal conjugate vaccines (WHO TRS).

Signature _____

Name (typed) _____

Date _____

APPENDIX 2

Model certificate for the release of group A meningococcal conjugate vaccines

This certificate is to be provided by the National Regulatory Authority of the country where the vaccines have been manufactured, upon request by the manufacturer

Certificate N° _____

LOT RELEASE CERTIFICATE

The following lot(s) of group A meningococcal conjugate vaccine produced by _____¹ in _____², whose numbers appear on the labels of the final containers, meet all national requirements³ and Part A⁴ of the WHO Recommendations to assure the quality, safety and efficacy of group A meningococcal conjugate vaccines (WHO TRS _____), revised _____⁵, and comply with Good Manufacturing Practices for Pharmaceutical Products⁶ and Good Manufacturing Practices for Biological Products⁷.

As a minimum, this certificate is based on examination of the summary protocol of manufacturing and control.

Final Lot No. _____

No. of released human doses in this final lot _____

Expiry date _____

The Director of the National Regulatory Authority (or Authority as appropriate):

Name (Typed) -----

Signature -----

Date -----

¹ Name of manufacturer

² Country of origin

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

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

5 WHO Technical Report Series, No. ____, YYYY, Annex __.

6 WHO Technical Report Series, No. 823, 1992, Annex 1.

7 WHO Technical Report Series, No. 822, 1992, Annex 1.

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