

Annex 2

Recommendations for the production and control of meningococcal group C conjugate vaccines

Recommendations published by WHO are intended to be scientific and advisory. Each of the following sections constitutes guidance for national regulatory authorities and for the manufacturers of biological products. If a national regulatory authority so desires, these Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the national regulatory authority. 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 national regulatory authorities which may benefit from those details.



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Introduction

The 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 an efficacy in the region of 90% and have proved to be highly effective in public health interventions (4). Nevertheless, their inability to elicit protective responses in young infants or to induce good immunological memory has prevented their use in national infant immunization schedules.

Following the successful introduction of the *Haemophilus influenzae* type b conjugate (Hib) vaccines, considerable progress has been made in the development of similar conjugate vaccines based on meningococcal group C capsular polysaccharide. Controlled clinical trials have demonstrated that these vaccines are highly immunogenic in all age groups and, as T-cell dependent antigens, induce immunological memory and affinity maturation of anti-capsular antibodies (5–8, 8–17). Vaccines based on meningococcal group C conjugates have been shown to offer protective immunity following their introduction in the UK (18). Glycoconjugate vaccines are both physically and immunobiologically distinct from their unconjugated counterparts emphasizing the need for new recommendations for these products.


General considerations

Neisseria meningitidis is a significant cause of bacterial meningitis and septicaemia. Meningococci are divided into serogroups on the basis of their chemically and serologically distinct capsular polysaccharides, but only organisms belonging to one of the five groups, A, B, C, Y and W135, cause disease (19). Group A organisms cause widespread epidemic disease in the so-called “meningitis belt” countries, whereas the other four groups are responsible for endemic disease and localized outbreaks worldwide (20, 21). Disease caused by group C organisms occurs primarily in infants although outbreaks caused by group C organisms in students and military recruits have contributed to an elevated incidence of meningococcal disease in teenagers and young adults.

An ideal vaccine would offer comprehensive protection against all five of the pathogenic serogroups, but its development has faced major obstacles related to the immunobiology of the capsular antigens. Bivalent (A and C) and tetravalent (A, C, Y and W135) polysaccharide vaccines have been widely available since the early 1970s (4). Pivotal 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 (22, 23). 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 (24–26). However, vaccines based upon plain polysaccharides have serious drawbacks; their immunogenicity is age-related and they fail to elicit immunological memory, and hence a booster response on subsequent exposure to the polysaccharide (27–29). Experience with the Hib vaccine had shown that the immunogenicity of polysaccharides could be improved by chemical conjugation to a protein carrier thereby eliciting a T-cell-dependent antisaccharide response (28, 30, 31). Several different meningococcal group C conjugates have been developed that demonstrably stimulate T-cell-dependent antibody production (5, 7, 14, 32, 33).

Special considerations

The production and control of conjugate vaccines is more complex than that for their unconjugated capsular polysaccharide counterparts. Polysaccharide vaccines consist of defined chemical substances that, if prepared to the same specifications, can reasonably be ex-




pected to have comparable potencies, regardless of the manufacturer. Effective meningococcal group C conjugate vaccines have been developed that differ both in the nature of the saccharide and of the carrier protein employed. Meningococcal group C capsular polysaccharide is usually *O*-acetylated. However, 5–10% of group C case isolates produce a capsule that is not *O*-acetylated. Conjugate vaccines based on either acetylated or de-*O*-acetylated polysaccharide elicit potent serum bactericidal antibody (SBA) responses against organisms expressing either form of the group C capsule, even though the antibody responses may not be directed against the same epitopes (6, 7). Effective meningococcal group C conjugate vaccines have been manufactured with either the diphtheria toxoid CRM197 or tetanus toxoid as carrier. The manufacturer has a choice of possible carrier proteins providing that the resulting conjugate vaccine is safe and stimulates production of T-cell-dependent protective antibody in infants and young children and boostable immune responses.

As the low burden of group C meningococcal disease made phase III clinical studies unfeasible, the first meningococcal group C conjugate vaccines were licensed in the UK on the basis of their proven immunogenicity rather than their clinical efficacy. The rationale behind licensure was based on:

- the studies by Gotschlich et al. (24) that demonstrated that serum bactericidal activity could be taken as an indicator of clinical protection against group C meningococcal disease;
- the evidence from clinical trials that showed that the conjugate vaccine was highly immunogenic and induced immunological memory in all age groups; and
- experience with the Hib vaccines that had already established the safety and advantages of conjugate vaccine technology.

The introduction of the vaccine was phased, first targeting the highest risk groups and providing the opportunity to obtain estimates of vaccine efficacy by comparing the incidence of disease between vaccinated and unvaccinated groups (18). The immunogenicity of the vaccine in humans should be assessed before the new vaccine is licensed.

Although it has been widely accepted since the studies by Gotschlich et al. (24) that SBA levels correlate with immune protection against group C meningococcal disease (22), there has been considerable debate during the development of meningococcal group C conjugate vaccines about the way in which the bactericidal assay should be performed (34, 35). This debate has focused mainly on whether baby rabbit serum could substitute for human serum as the source of complement in the assay, and if so how the results should be interpreted, as meningococci are more sensitive to lysis mediated by baby



rabbit complement than human complement (36–38). Whether human or baby rabbit serum is used as the source of complement, the source should be standardized following specific guidelines. Guidance on the methods available for evaluating the immune response will be published as an addendum in the light of emerging data. This guidance was subsequently established by the 53rd meeting, February 2003, of the WHO Expert Committee on Biological Standardization and published as Annex 3 of WHO Technical Report Series 926 (2004).

Because the meningococcal group C conjugate vaccines are manufactured from purified components by a clearly defined chemical process, the strategy for the control of the vaccine relies heavily on evaluation of molecular characterization and of purity to ensure that each vaccine lot is consistent with the specification of the vaccine lots used in the definitive clinical trials that confirmed their safety and immunogenicity (15, 39–41). In addition, the immunogenicity of meningococcal group C conjugate vaccines has been evaluated in mice and such data can provide an indication of the consistency and structural integrity of the vaccine (42). However, although immunogenicity testing in animals forms a necessary part of vaccine development, experience gained following the licensure of the meningococcal group C conjugates suggests that a routine animal potency test is not necessary when vaccine consistency has been assured by physicochemical criteria.

Combination vaccines containing meningococcal polysaccharide conjugate components

The introduction of meningococcal group C conjugate vaccines as an additional element of the infant immunization programme in the UK has served to highlight the need to combine paediatric vaccines for effective vaccine delivery (43). Vaccine formulations consisting of multiple components that include meningococcal group C conjugates are likely to be developed soon. If a meningococcal group C conjugate vaccine is indicated for concomitant use with other vaccines, possible effects on the clinical performance of each component in the combined vaccine, including the meningococcal group C conjugate vaccine component, should be evaluated in terms of their safety and immunogenicity.

Because of the problems associated with performing physicochemical analyses on complex vaccine formulations, the manufacturer should consider which batch release tests to perform on final bulks and final lots of such vaccines. The tests should be agreed with the national regulatory authority.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 *Proper name*

The proper name of the vaccine shall be “meningococcal group C conjugate vaccine” translated into the language of the country of use. The use of this name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 *Descriptive definition*

Meningococcal group C conjugate vaccine is a preparation of capsular polysaccharide from group C *Neisseria meningitidis* that is covalently linked to a carrier protein.

A.1.3 *International reference materials*

No formally established international reference materials that would allow the standardization of immune responses to meningococcal group C conjugate vaccines are currently available.

The following reagents are available through the courtesy of manufacturers and national control or reference laboratories: CDC1992 Reference Serum for the standardization of SBA assays and ELISAs is available from National Institute for Biological Standards and Control (NIBSC) in Europe and Centers for Disease Control in the USA; meningococcal group C polysaccharide and methylated human serum albumin for use in ELISA, and group C specific monoclonal antibody used to confirm the identity of group C polysaccharide are available from NIBSC.

A.1.4 *Terminology*

Master seed lot. A bacterial suspension of *N. meningitidis* derived from a strain that 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 should be maintained in the freeze-dried form or be frozen below -45°C .

Working seed lot. A quantity of live *N. meningitidis* organisms derived from the master seed lot by growing the organisms and maintaining them in aliquots in the freeze-dried form or the frozen state at or below -45°C . The working seed lot is used, if applicable, after a fixed number of passages, 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.

Modified polysaccharide. Purified polysaccharide that has been modified by chemical reaction or a physical process in preparation for conjugation to the carrier.

Carrier. The protein to which the polysaccharide 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 pool of lots of polysaccharide and a single lot or a pool of lots of protein. This is the parent material from which the final bulk is prepared.

Final bulk. The homogeneous 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 performed, freeze-drying. A final lot must therefore have been filled from a single container and freeze-dried in one continuous working session.

A.2 General manufacturing recommendations

The general manufacturing recommendations contained in good manufacturing practices for pharmaceutical (44) and biological products (45) 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 national regulatory authority. All assay procedures used for quality control of the conjugate vaccines and vaccine intermediates must be validated. When they are required, proposals for the modification of the manufacturing and control methods should also be submitted for approval to the national regulatory authority before they are implemented.

N. meningitidis is a class 2 pathogen and represents a particular hazard to health through infection by the respiratory route. The organism should be handled under conditions appropriate for this class of pathogen (46). Standard operating procedures must 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 against *N. meningitidis* should be implemented. Adherence to the current good manufacturing practices is important to the integrity of the product, to protect workers and to protect the environment.

A.3 Production control

A.3.1 Control of the polysaccharide

A.3.1.1 Strains of *N. meningitidis*

The strain of *N. meningitidis* used for preparing the group C 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 C polysaccharide.

The strains C11 and C2241 have been shown to be suitable for group C polysaccharide.

¹H nuclear magnetic resonance spectroscopy is a suitable method for the confirmation of the identity of the polysaccharide

A.3.1.2 Seed lot system

The production of meningococcal group C 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). If materials of animal origin are used in the medium for seed production, the preservation of strain viability for freeze-drying or for frozen storage, then they should comply with the guidance given in the *Report of a WHO consultation on medicinal and other products in relation to human and animal transmissible spongiform encephalopathies (47)* and should be approved by the national control authorities.

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

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. If materials of animal origin are used they should comply with the guidance given in the *Report of a WHO consultation on medicinal and*

other products in relation to human and animal transmissible spongiform encephalopathies (47) and should be approved by the national control authorities.

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

A.3.1.4 Single harvests

Consistency of growth of meningococcal organisms should be demonstrated by monitoring growth rate, pH and the final yield of group C polysaccharide.

A.3.1.5 Control of bacterial purity

Samples of the culture should be taken before killing and examined for microbial contamination. The purity of the culture should be verified by suitable methods that should include inoculation on to appropriate culture media. If any contamination is found, the culture and any product derived from it should be discarded. The killing process should similarly be adequately validated.

A.3.1.6 Purified polysaccharide

Each lot of meningococcal group C polysaccharide should be tested for purity. The limits given below are expressed with reference to the polysaccharide in its salt form, corrected for moisture. Each manufacturer must define the limits for its own product and they must be agreed by the national control authority.

Generally, the culture is harvested after killing the organism by heating to 56°C for 10 minutes or by the use of a suitable inactivating agent; after killing the polysaccharide is partially purified by precipitation with hexadecyl trimethylammonium bromide. Methods used for further purification of this intermediate should be approved by the national regulatory authority. Purified meningococcal polysaccharide and, when necessary, partially purified intermediates, are usually stored at or below -20°C to ensure stability.

A.3.1.6.1 Identity test

A test should be performed on the purified polysaccharide to verify its identity.

A serological test and/or ¹H nuclear magnetic resonance spectroscopy provide convenient methods for this purpose (39, 48, 49).

A.3.1.6.2 Molecular size distribution

The molecular size distribution of each lot of purified polysaccharide should be estimated. 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 and/or the mass distribution

limits should be established and shown to be consistent from lot to lot for a given product. An acceptable level of consistency should be agreed with the national regulatory authority.

Suitable methods for this purpose are: gel filtration through Sepharose CL-4B or CL-2B (or similar) in a 0.2 molar buffer using a refractive index detector (3) or colorimetric assay or high-performance size-exclusion chromatography (HPSEC) either alone or in combination with light-scattering and refractive index detectors (e.g. multiple angle laser light scattering MALLS) (50).

A.3.1.6.3 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 national regulatory authority and shown to be within agreed limits.

A.3.1.6.4 Polysaccharide composition

The quality of the polysaccharide can be estimated by the determination of the sialic acid content. The sialic acid content should be not less than 80% of the dry weight of the isolated product, as determined by the resorcinol assay, using *N*-acetylneuraminic acid as a standard (51).

Other methods, such as high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), may be used to define the quantitative composition of the purified polysaccharide, but the methods should be validated for the purpose (42). If other methods are used, alternative specifications for sialic acid may apply and should be agreed with the national regulatory authority.

A.3.1.6.5 Protein impurity

Each lot of purified polysaccharide should contain not more than 1% by weight of protein, as determined by the method of Lowry et al., using bovine serum albumin as a reference (3, 52), or by another suitable validated method.

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

A.3.1.6.6 Nucleic acid impurity

Each lot of purified polysaccharide should contain not more than 1% by weight of nucleic acid as determined by ultraviolet spectroscopy, on the assumption that the absorbance of a 10 g l⁻¹ nucleic acid solution contained in a cell of 1 cm path length at 260 nm is 200 (3), or by another validated method.

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

A.3.1.6.7 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 limits agreed as being acceptable by the national regulatory authority.

Less than 100 International Units of endotoxin per μg of polysaccharide when measured by the *Limulus* amoebocyte lysate test can be achieved in the production process. Alternatively, a recognized pyrogenicity test can be performed in rabbits.

A.3.1.6.8 O-acetyl content

The meningococcal group C polysaccharide used in the conjugate may be either O-acetylated or de-O-acetylated (53). For the O-acetylated form, the O-acetyl content should be monitored (by colorimetric or other validated assay) to ensure consistency of production. Similarly for the de-O-acetylated form, the absence of O-acetylation should be demonstrated to ensure consistency of production.

Bulk group C polysaccharide used for the production of licensed polysaccharide vaccine is suitable for the production of conjugate vaccine (7). O-acetyl content is conveniently determined by a colorimetric assay or ^1H NMR. O-acetylated polysaccharide typically contains at least 1.5mmol/g saccharide.

A.3.1.7 Modified polysaccharide

Modified polysaccharide preparations are usually partially depolymerized either before or during the chemical modification.

A.3.1.7.1 Chemical modification

Several methods for the chemical modification of polysaccharides prior to conjugation have been found to be satisfactory. The chosen method should be approved by the national regulatory authority.

Suitable methods include:

- Production of size-reduced polysaccharides by controlled acid hydrolysis and size fractionation. The resulting oligosaccharide fraction is reductively aminated and activated by coupling through the amine group to a functional linker, bis-N-hydroxysuccinamide ester of adipic acid. The reaction between the activated oligosaccharide and the protein generates the conjugate vaccine.
- Size-reduced polysaccharides are produced by periodate oxidation generating aldehyde groups. Upon mixing with the carrier protein Schiff's bases form between the aldehyde groups of the oligosaccharide and the amino groups of the protein, which can be reduced to form stable covalent bonds by treatment with sodium cyanoborohydride.

As part of the in-process controls, the processed polysaccharide to be used in the conjugation reaction may be assessed for the number of functional groups introduced.

A.3.1.7.2 Molecular size distribution

The degree of size reduction of the polysaccharide will depend upon the manufacturing process. The average size distribution (degree of polymerization) of the processed polysaccharide should be measured by a suitable method. 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 using MALLS detection (54). Other suitable methods include gel filtration, ion exchange chromatography or high-pressure liquid chromatography (HPLC) used together with an appropriate validated chemical assay.

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 guidance given in the *Report of a WHO consultation on medicinal and other products in relation to human and animal transmissible spongiform encephalopathies* (47) and should be approved by the national regulatory authority.

Production should be based on a seed lot system and the strains should be 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 the growth rate, pH and final yield of appropriate protein(s).

A.3.2.2 *Characterization and purity of the carrier protein*

Proteins that have been used as carriers in meningococcal conjugate vaccines licensed to date include tetanus toxoid and the non-toxic mutant of diphtheria toxin (CRM197). The 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 national control authority.

Proteins and purification methods that might be used include:

- *Tetanus or diphtheria toxoid*. This must satisfy the relevant requirements published by WHO (55) and be of high purity. The purity should be at least 1500 Lf/mg (Lf = limit of flocculation) protein (nondialysable) nitrogen (56).
- *Diphtheria CRM197 protein*. This is a non-toxic mutant of diphtheria toxin, isolated from cultures of *Corynebacterium diphtheriae* C7/β197 (57). 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 CRM197 protein from the active toxin.

The carrier protein should also be characterized. The identity may be determined serologically. Physicochemical methods that may be 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 (58).

A.3.3 **Control of bulk purified conjugate**

A number of methods of conjugation are currently in use; all involve multi-step processes. Both the method and the control procedures used to ensure the reproducibility, stability and safety of the conjugate should be established once the immunogenicity of a particular meningococcal conjugate vaccine has been demonstrated. The derivatization and conjugation process should be monitored by analysis for unique reaction products or by other suitable means.

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 no activated functional groups remain at the conclusion of the manufacturing process.

After the conjugate has been purified, the tests described below should be performed in order to assess consistency of manufacture. 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 (59, 60)

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, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDAC) and phenol should be confirmed by suitable tests or by validation of the purification process.

A.3.3.2 *Conjugation markers*

Where the chemistry of the 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 polysaccharide with the carrier protein, so that the frequency of the covalent bond is given as a function of the number of polysaccharide repeating units or overall polysaccharide content.

A unique linkage marker could be assessed for each batch or, alternatively, the manufacturing process should be validated to demonstrate that it yields conjugate with a level of substitution that is consistent from batch to batch.

The ratio of saccharide to protein is also a suitable conjugation marker, though not a direct measurement.

A.3.3.3 Capping markers

Each batch should be shown to be free of unreacted functional groups on either the chemically modified polysaccharide or the carrier protein.

Where possible, unreacted functional groups should be assessed for each batch. Alternatively, the product of the capping reaction can be monitored or the capping reaction can be validated to show removal of unreacted functional groups.

A.3.3.4 Polysaccharide content

The content of meningococcal polysaccharide should be chemically determined by means of an appropriate validated assay.

Methods that have been used for the determination of the meningococcal polysaccharide content include the resorcinol assay or HPAEC-PAD detection.

A.3.3.5 Conjugated and unbound (free) polysaccharide

Only the meningococcal polysaccharide that is covalently bound to the carrier protein (i.e. conjugated polysaccharide) is immunologically important for clinical protection and excessive levels of unbound polysaccharide could potentially result in immunological hyporesponsiveness to group C polysaccharide.

Each batch of conjugate should therefore be tested for unbound or free polysaccharide to ensure that the amount present in the purified bulk is within the limits agreed by the national control authority based on that present in lots shown to be clinically safe and efficacious.

Methods that have been used to assay unbound polysaccharide include gel filtration; ultrafiltration and hydrophobic chromatography; ultracentrifugation with HPAEC-PAD, or colorimetric detection (42).

A.3.3.6 Protein content

The protein content of the conjugate should be determined with an appropriate validated assay (section A.2). Each batch should be tested for conjugated and unbound protein.

The unconjugated protein content is normally <5%. Appropriate methods for the determination of conjugated and unbound protein include HPLC or capillary electrophoresis.

A.3.3.7 Polysaccharide to protein ratio

The polysaccharide to protein ratio of the conjugate should be calculated. For each conjugate, the ratio should be within the range approved for that particular conjugate by the national regulatory

authority and should be consistent with the ratio in vaccine that have been shown to be effective in clinical trials.

A.3.3.8 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 (42). The method should be validated with an emphasis on its 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 that of lots shown to be immunogenic in clinical trials.

Typically the size of the polysaccharide–protein conjugate may be examined by gel filtration on Sepharose CL-4B. Suitable alternative methods are acceptable.

Since the saccharide: protein ratio is an average value, determination of this ratio over the size distribution can be used to provide further proof of manufacturing consistency (58).

A.3.3.9 Sterility

The bulk purified conjugate should be tested for bacterial and mycotic sterility in accordance with the requirements of Part A, sections 5.1 and 5.2, of the revised Requirements for Biological Substances (61), or by a method approved by the national regulatory authority. 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 (e.g. 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 Final bulk

A.3.4.1 Preparation

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



A.3.4.2 Sterility

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

A.3.5 Filling and containers

The recommendations concerning filling and containers given in Annex 1, Section 4 of *Good manufacturing practices for biological products* should be applied (45).

A.3.6 Control tests on final product

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.

A.3.6.2 Sterility

The contents of final containers should be tested for bacterial and mycotic sterility as described in section A.3.3.9.

A.3.6.3 Meningococcal polysaccharide content

The amount of each meningococcal group C conjugate in the final containers should be determined, shown to be within the limits specified by the national regulatory authority, and be plus or minus 20% of the stated content.

The conjugate vaccines produced by different manufacturers differ in formulation. A quantitative assay for the meningococcal polysaccharide in the final container is likely to be product-specific. Colorimetric methods, chromatographic methods (including HPLC), or serological methods may be used.

A.3.6.4 Residual moisture

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

The test should be performed on one vial per 1000 up to a maximum of 10 vials but on no less than five 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.3.6.5 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 test. 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 national regulatory authority.

A.3.6.6 Adjuvant content

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

The consistency of adsorption of the antigen to the adjuvant is important and the adsorption of production lots should be demonstrated to be within the range of values measured in vaccine lots shown to be clinically effective.

A.3.6.7 Preservative content

If a preservative has been added to the vaccine, its content should be determined by a method approved by the national regulatory authority. 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 national regulatory authority.

A.3.6.8 General safety test (innocuity)

The requirement to test lots of meningococcal conjugate vaccine for unexpected toxicity (abnormal toxicity) should be agreed with the national regulatory authority. Such a test may not be required if another animal test (e.g. a test for immunogenicity) is to be performed and the test for unexpected toxicity can be omitted for routine lot release once consistency of production has been well established to the satisfaction of the national regulatory authority and when good manufacturing practice is in place.

A.3.6.9 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 shown to be safe and effective for vaccine lots in the clinical trials and in stability studies. For a lyophilized preparation, the pH should be measured after reconstitution with the appropriate diluent.

A.3.6.10 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.

A.4 **Records**

The recommendations in section 8 of good manufacturing practices for biological products, (Annex 1) should be applied (45).

A.5 **Retained samples**

The recommendations in section 9.5 of good manufacturing practices for biological products (Annex 1) should be applied (45).

A.6 **Labelling**

The recommendations in section 7 of good manufacturing practices for biological products (Annex 1) should be applied with the addition of the following (45).

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

- the amounts of meningococcal polysaccharide and carrier protein contained in each single human dose;
- the temperature recommended during storage and transport;
- that if the vaccine is freeze-dried it should be used immediately after its reconstitution unless data have been provided to the licensing authority to indicate that it may be stored for a limited time; and
- 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 or approved by the national control authority.

A.7 **Distribution and transport**


The recommendations in section 8 of good manufacturing practices for biological products (Annex 1) should be applied (45).

A.8 **Stability testing, storage and expiry date**

A.8.1 **Stability testing**

Adequate stability studies form an essential part of the vaccine development studies. The stability of the polysaccharide before conjugation should be demonstrated. The stability of the vaccine in its final form and at the recommended storage temperatures should be demonstrated to the satisfaction of the national regulatory authority with final containers from at least three lots of final product from different bulk conjugates.

The polysaccharide component of conjugate vaccines may be subject to gradual hydrolysis at a rate that may vary depending upon the type



of conjugate, the type of formulation or adjuvant, the type of excipients and the conditions of storage. The hydrolysis may result in reduced molecular size of the meningococcal polysaccharide component, a reduction in the amount of the polysaccharide bound to the protein carrier and in a reduced molecular size of the conjugate.

The structural stability of the oligosaccharide chains and of the protein carrier vary between different conjugate vaccines (42).

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 unbound polysaccharide or bound polysaccharide content as a percentage of the total polysaccharide should be determined. The vaccine should meet the recommendations for the final product (see Part A, sections A.3.3.5 and A.3.3.8) up to the expiry date.

Molecular sizing of the final product may be carried out to ensure the integrity of the conjugate.

The desorption of antigen from aluminium-based adjuvants, if used, may take place over time and should be investigated and shown to be within the limits agreed by the national regulatory authority.

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 procedure 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 that appear on the label should be based on experimental evidence, which should be submitted for approval to the national regulatory authority.

A.8.2 **Storage conditions**

Storage conditions should be based on stability studies and approved by the national regulatory authority.

Storage of both liquid and freeze-dried vaccines at a temperature of 2–8 °C has been found to be satisfactory. Group C conjugate vaccines have generally proved to be stable over a wide range of storage temperatures, although some formulations have been shown to be affected by repeated freeze-thawing.

A.8.3 **Expiry date**

The expiry date should be approved by the national control authority 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. Recommendations for national regulatory authorities

B.1 General

The general recommendations for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (45) should be applied.

B.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 national regulatory authority should be provided at the request of the manufacturing establishments, and should certify that 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 by the national controller, and the number appearing on the labels of the containers. Importers of meningococcal group C conjugate vaccines should be given a copy of the official national release document. The purpose of the certificates is to facilitate the exchange of vaccines between countries.

B.3 Reactivity and immunogenicity of vaccine in humans

The national regulatory authority should satisfy itself that adequate control of the meningococcal group C conjugate vaccine has been achieved. Manufacturing consistency for vaccine lots used in the clinical trials should be demonstrated and well documented. These lots should be adequately representative of the formulation intended for marketing. Clinical data may be required to support the demonstration of manufacturing consistency. Such studies may need to be repeated if changes in production are made, or when the meningococcal conjugate is part of a new combination vaccine formulation. The national regulatory authority should ensure that the studies are performed in an adequate number of subjects to obtain statistically valid data on reactivity and immunogenicity. The meningococcal group C conjugate vaccines are manufactured from purified components by a clearly defined chemical process. Any changes in production or formulation of the vaccine should be reported to the national control authority, which will decide on a case-by-case basis whether additional clinical data are required. Such a review should take into account the likelihood of changes in production or formulation affecting the quality, the consistency, the structural integrity or the immunoge-

nicity of the product, and should also consider the possible cumulative effect of multiple modifications that individually may be regarded as minor.

Two types of assay are useful for measuring antibody responses to vaccination. The studies by Gotschlich et al. demonstrated that a serum bactericidal titre of $\geq 1:4$ measured with human complement is an indicator of clinical protection against group C meningococcal disease (22). The SBA thus provides a good surrogate measure of protective immunity. However, subsequent standardization of this assay uses rabbit complement, which has been shown to increase the titres. Nevertheless, there is a general consensus that when baby rabbit serum is used as the source of complement, SBA titres of $< 1:8$ are predictive of susceptibility to invasive meningococcal disease and titres of $1:128$ are highly predictive of protection. Currently, there is uncertainty as to whether titres between $1:8$ and $1:64$ can be a measure of protective immune response and further serological data should be obtained. In the UK, a combination of additional indicators was used to assess immune response to license a meningococcal group C conjugate vaccine. These included:

- evidence of a fourfold rise in antibody titre between pre- and postimmunization sera;
- demonstration of immunological memory; and
- evidence of increased avidity of serogroup C-specific antibody (36).

The ELISA is an antigen-binding assay and has less variability than the SBA which is a bioassay. The ELISA measures total or isotype-specific serum antibody responses. However, the results of the “standardized” ELISA (62) frequently have not correlated with measurements of SBA. Modifications of the standardized ELISA including the use of more purified polysaccharide, derivatized antigens, and incorporating chaotropic agents in the serum-diluting buffer can improve the correlation (63).

In light of emerging data, guidance on study design for the evaluation of new stand-alone or combined meningococcal group C conjugate vaccines and serological methods will be the subject of a WHO consultation to be published as an addendum to these recommendations¹.

Immunization with meningococcal group C conjugate vaccines also primes for the ability to generate memory antibody responses upon

¹ This Addendum was subsequently approved by the WHO Expert Committee on Biological Standardization at its fifty-third meeting (WHO Technical Report Series, No. 926, 2004).



subsequent exposure to plain meningococcal polysaccharide (5). Although unproven, the ability of an immunized person to generate a memory antibody response upon exposure to the pathogen may be an important second mechanism of protection, particularly when serum antibody concentrations are below the protective threshold.

Some Hib polysaccharide–protein conjugate vaccines show lower immunogenicity, compared with administration of Hib conjugate vaccine alone, when given in a manufactured combination vaccine; or when mixed with another vaccine immediately before injection; or when administered at the same time as, but as a separate injection to, certain other vaccines. National regulatory authorities should ensure that the data made available to them are relevant to individual national immunization programmes, so that appropriate recommendations may be made regarding co-administration of vaccines.

For combinations of meningococcal group C conjugate vaccines and other antigens, that are either pre-combined or intended to be given by mixing immediately before injection, the national regulatory authority should ensure that there are adequate studies to demonstrate that there is no clinically significant interference with the induction of immunological memory by the meningococcal group C conjugate component.

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