**Group A Meningococcal Conjugate Vaccine**

Group A Meningococcal Conjugate Vaccine is a liquid or freeze-dried preparation of a polysaccharide derived from a suitable strain of *Neisseria meningitidis* type A, covalently bound to a carrier protein.

The polysaccharide, a linear copolymer, consists of partly O-acetylated repeating units of N-acetylmannosamine-6-phosphate, with a defined molecular size. The carrier protein, when conjugated to Meningitis A polysaccharide is capable of inducing a T-cell dependent B cell immune response to the polysaccharide.

**Production**

**General provisions**

*Neisseria meningitidis* group A 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. Personnel employed in the production and control facilities should be adequately trained and appropriate protective measures including vaccination should be implemented.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy. The stability of the final lots and relevant intermediates is evaluated using one or more indicator tests. Such tests may include determination of molecular size, determination of free polysaccharide in the conjugate and the immunogenicity test in suitable animal model (mice). Taking account of the results of stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

**SEED LOT**

The strain of type A *Neisseria meningitidis* used for the seed lots shall be identified by historical records that include information on their origin and by their biochemical, serological, physicochemical or molecular characteristics. The strain shall have been shown to be capable of producing the Meningococcal A polysaccharide.

The production of Meningococcal A purified polysaccharide and of the carrier protein is based on the defined seed lot systems. Master seed lot and working seed lot shall have been properly characterized and defined. Cultures derived from the working seed shall have the same characteristics as of the master seed lot. The sample culture of single harvests taken before killing shall be tested for contamination by examination of Gram stained smears and by inoculating on suitable media.

**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 liquid form, frozen at or below -45°C or can be freeze-dried and stored at or below 4°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 as liquid form frozen at or below -45°C or can be freeze-dried and stored at or below 4°C. When a new working seed lot is prepared, the capsular polysaccharide should be identified by appropriate methods such as Nuclear Magnetic resonance (NMR) spectroscopy or serological assays.

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The strain should have the following characteristics:

a) Pure culture
b) Colonies obtained from the culture are round, uniform in shape and smooth with a mucous, opalescent, greyish appearance
c) Gram staining reveals characteristic Gram-negative diplococci in ‘coffee-bean’ arrangement
d) The oxidase test is positive
e) The culture utilizes glucose and maltose
f) Suspensions of the culture agglutinate with Meningococcal A specific antisera.

PROPAGATION AND HARVEST
The working seed lot is cultured in a liquid medium that does not contain high molecular weight polysaccharide, blood group substances and free from substances precipitated by cetrimonium bromide (hexadecyltrimethylammonium bromide). If any ingredient of the medium contains blood group substances, the process shall be validated to demonstrate that after the purification step they are no longer detectable. The inoculum may undergo one or more subcultures in liquid medium before being used for inoculating the final medium. 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. The culture is inactivated, centrifuged and the polysaccharide precipitated from the supernatant by addition of cetrimonium bromide. The precipitate obtained is harvested and may be stored at -20° awaiting further purification.

PURIFIED POLYSACCHARIDE
The polysaccharides are purified, after dissociation of the complex polysaccharide and cetrimonium bromide, using suitable procedures to remove successively nucleic acids, proteins and lipopolysaccharides. The purified polysaccharide is to be stored as a lyophilised or dried powder or stored frozen at or below -20°. In case the purified polysaccharide is stored frozen, an aliquot of the purified polysaccharide should be lyophilized for testing purposes. Prepare a solution containing about 5 mg of dry polysaccharide per millilitre. The dry weight should be corrected for moisture content.

Only purified polysaccharides that comply with following requirements may be used in the preparation of conjugate bulk vaccine.

Identification and serological specificity
The identity and purity of purified polysaccharide shall be confirmed. In case where other serotypes are produced in the same manufacturing site it shall be shown that there is not more than 1% of group heterologous N. Meningitidis polysaccharide. Identity and serological specificity are determined by suitable immunochemical methods (2.2.14) and/or 1H or 13CNMR spectroscopy ( ).

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.
Polysaccharide content (Phosphorus)
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. Methods such as Chen assay (Chen, P.S. Jr Toribara T.Y. & Warner H. (1956) Micro determination of phosphorus. Analytical chemistry 28, 1756-1758) or 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.

Protein (2.7.1). Not more than 10 mg of protein per gram of purified polysaccharide for group A.

Nucleic acid (2.7.1). Not more than 10 mg of nucleic acids per gram of purified polysaccharide for group A, determined by using ultraviolet spectroscopy (2.4.7), on the assumption that the absorbance of a 10g/l nucleic acid solution contained in a cell of 1 cm path length at 260 nm is 200 or by any other suitable and validated methods.

Bacterial endotoxin (2.2.3). Not more than 100 Endotoxin units per µg of purified polysaccharide group A.

Pyrogens (2.2.8). Complies with the test for pyrogens. Alternatively, pyrogen test can be performed in rabbits. Inject each of the rabbit with 1ml per kg body weight of solution containing 0.025 µg of purified polysaccharide per ml, and the purified polysaccharide complies with the test for pyrogens. This test can be omitted if bacterial endotoxin test is validated for group A.

O-Acetyl groups (2.7.1). Not less than 2 mmol of O-acetyl groups per gram of purified polysaccharide group A. O-acetyl content is determined by a colorimetric assay, 1H NMR or high-performance anion-exchange chromatography with conductivity detection.

Molecular size distribution. Examine by gel filtration or high performance size exclusion chromatography (HPSEC) (2.4.16), using agarose for chromatography or cross-linked agarose for chromatography either alone or in combination with light scattering and refractive index detectors (eg. Multiple angle laser light scattering (i.e MALLS) or any other suitable method. Use a column 30 cm long and 10 mm in internal diameter equilibrated with a solvent having an ionic strength of 0.2 mol/kg and a pH of 7.0 to 7.5.

At least 65 per cent of group A purified polysaccharide is eluted before a distribution coefficient (Kd) of 0.50 is reached.

Calcium. If a calcium salt is used during purification, a determination of calcium is carried out on the purified polysaccharide; the content is within the limits approved for the product.

Residual reagents. Where applicable, tests are carried out to determine residues of reagents used during inactivation and purification. An acceptable value for each reagent is established and each batch of purified polysaccharide must be shown to comply with this limit. Where validation studies have demonstrated removal of residual reagent, the test on each batch may be omitted.
ACTIVATED SACCHARIDE

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

Chemical activation. Several methods for the chemical activation of polysaccharides prior to conjugation may be satisfactory. The chosen method should be approved by the national regulatory authority. 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.

Extent of activation of the saccharide. The manufacturer should demonstrate control of the degree of activation of the saccharide by an assay of each batch of the saccharide. Colorimetric, chromatographic assays and NMR spectroscopy have been used to determine the degree of activation of the saccharide.

Molecular size distribution. 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 be determined by HPSEC either alone or in combination with light scattering and refractive index detectors such as Multiple angle laser light scattering (MALLS). Other suitable methods include gel filtration and ion exchange chromatography.

Carrier protein

The carrier protein is chosen in a way so that when the polysaccharide is conjugated it is able to induce a T-cell-dependent immune response. The carrier proteins are produced by culture of suitable microorganisms; the bacterial purity of the culture is verified; the culture may be inactivated; the carrier protein is purified by a suitable method.

Only a carrier protein that complies with the following requirements may be used in preparation of the conjugate.

Identification

The carrier protein is identified by a suitable immunochemical method (2.2.14).

Sterility (2.2.11). Carry out the test for sterility using for each medium 10 ml or the equivalent of one hundred doses, whichever is less.

Diphtheria toxoid. Diphtheria toxoid is produced as stated under Diphtheria vaccine (Adsorbed) and complies with the requirements prescribed there for bulk purified toxoid.
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*Tetanus toxoid.* Tetanus toxoid is produced as stated under Tetanus vaccine (Adsorbed) and complies with the requirements prescribed there for bulk purified toxoid except that the antigenic purity is not less than 1500 Lf per mg of protein nitrogen.

*Diphtheria protein CRM 197.* Suitable tests are carried out, for validation or routinely, to demonstrate that the product is non-toxic. The protein obtained contains not less than 90.0 per cent of diphtheria CRM197 protein, when prepared by liquid chromatography (2.4.14) or any other suitable method. The carrier protein shall be characterized by a suitable chemical or physicochemical method like SDS-PAGE, HPLC, isoelectric focusing, amino acid sequencing, circular dichroism, fluorescence spectroscopy, peptide mapping or mass spectroscopy, as appropriate.

**OMP (Meningococcal group B outer membrane protein complex)**

OMP complex of *Neisseria meningitidis* complies with the following requirement for lipopolysaccharide and pyrogens.

*Lipopolysaccharide.* Not more than 8.0 per cent of lipopolysaccharide, determined by a suitable method.

*Pyrogens* (2.2.8). Inject into each rabbit 0.25 µg of OMP per kg body weight, for determining the pyrogenic effect.

**Bulk conjugate**

Meningococcal A purified polysaccharide is chemically modified to enable conjugation; it is usually partly depolymerised either before or during this procedure. Reactive functional groups or spacers may be introduced into the carrier protein or polysaccharide prior to conjugation. The conjugate is obtained by the covalent binding of polysaccharide and carrier protein. Where applicable, unreacted but potentially reactogenic functional groups are made unreactive by means of capping agents; the conjugate is purified to remove reagents. Where validation studies have demonstrated removal of a residual reagent, the test on bulk conjugate may be omitted.

*Only a bulk conjugate that complies with the following requirements may be used in preparation of the final bulk vaccine.*

**Residual reagents.** Removal of residual reagents such as cyanide, EDAC (ethyldimethylaminopropylcarbodiimide) and others depending on the conjugation chemistry is confirmed by suitable tests or by validation of the purification process. The residuals are process-specific and can be quantified by use of colorimetric and chromatographic assays. Techniques such as NMR spectroscopy may also be applied.

**Group A meningococcal saccharide content.** The group A meningococcal saccharide content is determined by assay of phosphorus (2.7.1) or sugar content by use of colorimetric or HPAEC-PAD assays.

**Conjugated and unbound (free) saccharide.** 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 and 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

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amount of unbound saccharide can be determined by specific chemical or immunological tests or by HPAEC after hydrolysis.

**Protein content** (2.7.1). The protein content in the bulk conjugate should be determined by means of an appropriate validated assay and comply with limits for the particular product. Each batch of bulk conjugate should be tested for conjugated and unconjugated protein by a suitable method. Appropriate methods for the determination of conjugated and unconjugated protein include HPLC, ion exchange chromatography and capillary electrophoresis or other methods.

**Saccharide to protein ratio.** Determine ratio by calculation.

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 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.

**Molecular size distribution.** Molecular size distribution is determined by gel filtration or size exclusion chromatography (2.4.16), using a gel matrix, appropriate to the expected size of the conjugate.

**Sterility** (2.2.11). Carry out the tests for sterility using for each medium 10 ml or equivalent of one hundred doses, whichever is less.

**FINAL BULK VACCINE**

An adjuvant, an antimicrobial preservative and a stabilizer may be added to the bulk conjugate before dilution to the final concentration with a suitable diluent.

Only a final bulk vaccine that complies with the following requirement may be used in preparation of the final lot.

**Antimicrobial preservative.** Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

**Sterility** (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

**FINAL LOT**

The final lots are filled in suitable containers, under stringent aseptic conditions.

Only a final lot that is satisfactory with respect to each of the requirements given under Identification, Tests and Assay may be released for use. Provided the test for antimicrobial preservative has been carried out on the final bulk vaccine, it may be omitted on the final lot.

**Identification**

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The vaccine is identified by a suitable immunochemical method (2.2.14) for purified polysaccharide group A and the carrier protein.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Group A meningococcal saccharide. Not less than 80.0 per cent and not greater than 120.0 per cent of the amount of polysaccharide stated on the label as determined by a phosphorus assay (2.7.1) or immunochemical method (2.2.14) or by any other suitable method like colorimetry or by HPAEC-PAD. Immunological assays such as rate nephelometry or ELISA inhibition may also be used.

Unbound (Free) polysaccharide. Unbound polysaccharide is determined after removal of the conjugate for example by 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 immunochemical method (2.2.14) or by HPAEC after hydrolysis.

Molecular size distribution. Molecular size distribution is determined by gel filtration or size exclusion chromatography (2.4.16) using a gel matrix, appropriate to the expected size of the conjugate.

Water (2.3.43). Not more than 3.0 per cent.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit’s mass a quantity of the vaccine equivalent to 0.025 µg of polysaccharide.

Or by a Limulus amoeocyte 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.

Aluminium (2.3.9). Not more than 1.25 mg per single human dose. When aluminium compounds are used as an adjuvant.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the amount shown to be efficacious and is not greater than 115.0 per cent of that stated on the label.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

pH (2.4.24). The pH of the vaccine, reconstituted if necessary, is within the range approved for the product.

Labelling. The label states (1) the number of micrograms of group A meningococcal saccharide per human dose, (2) the type and nominal amount of carrier protein per single human dose, (3) the name and address of the manufacturer, (4) the recommended storage temperature and the expiry date if kept at that temperature, (5) in case of freeze-dried preparation, the direction for reconstitution and the time within which the vaccine must be used after reconstitution, (6)
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the adjuvant used for the preparation of the vaccine; (7) name and amount of diluents for reconstitution, in case of freeze-dried preparation.