# Draft New monograph for Comments and Inclusion in The Indian Pharmacopoeia

## DRAFT NEW MONOGRAPH FOR COMMENTS

This draft new monograph contain text for inclusion in the Indian Pharmacopoeia (IP). The content of this draft document is not final, and the text may be subject to further revisions prior to publication in the IP. This draft does not necessarily represent the decisions or the stated policy of the IP or Indian Pharmacopoeia Commission (IPC).

Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the monograph.

**Please send any comments you may have on this draft document to** lab.ipc@gov.in/ biologics-ipc@gov.in **before the last date for comments.**

### Document History and Schedule for the Adoption Process

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**Meningococcal Group A, C, W135, Y and X Conjugate Vaccine**

Meningococcal Group A, C, W135, Y and X conjugate vaccine is a freeze-dried preparation, or a preparation consisting of freeze-dried and liquid components, of purified capsular polysaccharides covalently linked to a carrier protein. The polysaccharides are obtained from suitable strains of Neisseria meningitidis group A, group C, group W135, group Y and group X that are capable of consistently producing polysaccharides.

*N. meningitidis* group A polysaccharide consists of partly *O*-acetylated repeating units of *N*- acetyl mannosamine, linked with α-(1→6) phosphodiester bonds.

*N. meningitidis* group C polysaccharide consists of partly *O*-acetylated repeating units of sialic acid, linked with α-(2→9) glycosidic bonds.

*N. meningitidis* group W135 polysaccharide consists of partly *O*-acetylated alternating units of sialic acid and d-galactose, linked with α-(2→6) and α-(1→4) glycosidic bonds.

*N. meningitidis* group Y polysaccharide consists of partly *O*-acetylated alternating units of sialic acid and d-glucose, linked with α-(2→6) and α- (1→4) glycosidic bonds.

*N. meningitidis* group X polysaccharide consists of N-acetylglucosamine-4-phosphate residues held together by α- (1→4) phosphodiester bonds.

The carrier protein used may vary for the various polysaccharide conjugates contained in a multivalent vaccine.

Production

General provisions

The production method shall have been shown to yield consistently meningococcal group A, C, W135, Y and X conjugate vaccines of adequate safety and immunogenicity in man. The production of the meningococcal polysaccharides and of the carrier protein(s) is based on a seed-lot system.

The production method is validated to demonstrate that the product, if tested, would comply with tests for safety and efficacy.

During development studies, it shall be demonstrated that the vaccine consistently induces a T-cell-dependent B-cell immune response to each polysaccharide. If the manufacturing process is modified, it shall be demonstrated by appropriate *in vitro* methods that the characteristic properties of the vaccine are not affected.

The stability of the conjugated bulks and/or final lot and meningococcal polysaccharides is evaluated using suitable indicator tests. Such tests may include determination of molecular size, quantification of polysaccharide content and free polysaccharide content in the conjugate.

Considering 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 LOTS

The strains of *N. meningitidis* used for the master seed lots shall be identified by historical records that include information on their origin and by their biochemical and serological characteristics.

### Master Seed

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 working seed lots. Master seed lots shall be maintained in liquid form, frozen at or below - 45° or can be freeze-dried and stored at or below 4°.

### Working Seed

The working seed lots are cultured on solid or liquid media that do not contain blood-group substances or ingredients of mammalian origin. The inoculum may undergo 1 or more subcultures in liquid medium before being used for inoculating the final medium. The liquid media used and the final medium are semi-synthetic and free from substances precipitated by cetrimonium bromide (hexadecyltrimethylammonium bromide) and do not contain blood-group substances or high-molecular-mass polysaccharides.

Cultures from each working seed lot shall have the same characteristics as the strain that was used to prepare the master seed lot. The strains have the following characteristics:

- colonies obtained from a culture are rounded, uniform in shape and smooth with a mucous, opalescent, greyish appearance;
- Gram staining reveals characteristic Gram- negative diplococci in ‘coffee-bean’ arrangement;
- the oxidase test is positive;
- the culture utilizes glucose and maltose;
- suspensions of the culture agglutinate with suitable specific antisera.

The bacterial purity of the culture is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

## PROPAGATION AND HARVEST

The working seed lots are cultured on solid media that do not contain blood-group substances or ingredients of mammalian origin. The inoculum may undergo 1 or more subcultures in liquid medium before being used for inoculating the final medium. The liquid media used and the final medium are semi-synthetic and free from substances precipitated by cetrimonium bromide (hexadecyltrimethylammonium bromide) and do not contain blood-group substances or high- molecular-mass polysaccharides.

The bacterial purity of the culture is verified by methods of suitable sensitivity. These may include

inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

The cultures are centrifuged and the polysaccharides precipitated from the supernatant by addition of cetrimonium bromide. The obtained precipitate is harvested and may be stored at − 15° or below awaiting further purification.

**PURIFIED POLYSACCHARIDES**

The polysaccharides are purified, after dissociation of the complex of polysaccharide and cetrimonium bromide, using suitable procedures to remove successively nucleic acids, proteins and lipopolysaccharides.

The final purification step consists of suitable procedure precipitation of the polysaccharides, which are then stored frozen at−15° or below or dried in vaccum and stored at −15°C or below. The loss on drying is determined by thermogravimetry (2.4.31) or another suitable method and the value is used to calculate the results of the other chemical tests with reference to the dried substance.

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

**Protein** (2.7.1 or other suitable methods): maximum 10 mg per gram of purified polysaccharide, calculated with reference to the dried substance.

**Nucleic acids** (2.7.1 or other suitable methods): maximum 10 mg per gram of purified polysaccharide, calculated with reference to the dried substance.

***O*-Acetyl groups** (2.7.1): minimum 2 mmol per gram of purified polysaccharide for group A, calculated with reference to the dried substance. The Meningococcal group C, W and Y used in the conjugate may be either O-acetylated or de-O-acetylated. 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.

**Phosphorus** (2.7.1): minimum 80 mg per gram of purified polysaccharide for group A and group X, calculated with reference to the dried substance.

**Sialic acid** (2.7.1). minimum 800 mg per gram of purified polysaccharide for group C and minimum 560 mg per gram of purified polysaccharide for groups W135 and Y, all calculated with reference to the dried substance, using *N-acetylneuraminic acid* to prepare the reference solution.

**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 particular product. Where validation studies have demonstrated removal of calcium, the test on purified polysaccharides may be omitted.

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

may be omitted.

**Molecular-size or molecular-mass distribution**. The molecular-size or molecular-mass distribution is determined by size-exclusion chromatography (2.4.16) combined with an appropriate detection system. An acceptable value is established for each purified polysaccharide. Each batch must be shown to comply with this limit.

**Identification and serological specificity**. The identity and serological specificity are determined by a suitable immunochemical method (2.2.14) or another suitable method, for example $^{1}H$ nuclear magnetic resonance spectrometry (2.4.34). Identity and purity of each polysaccharide shall be confirmed; it shall be shown that there is not more than 1 per cent m/m of group-heterologous *N. meningitidis* polysaccharide. Test for serological specificity of polysaccharide with respect to heterologous N. Meningitidis polysaccharide can be omitted after establishment of Change over procedure and process validation.

**Bacterial endotoxins** (2.2.3). The content is within the limits approved by the competent authority for the particular product.

**Water** (2.3.43). Where applicable, the values are within the limits approved for each group, determined by a suitable method. If loss on drying (by TGA or other suitable methods) is performed on the purified polysaccharide, residual water determination is not necessary.

## MODIFIED MENINGOCCOCAL POLYSACCHARIDES

Before conjugation, the polysaccharides can be depolymerized by chemical or mechanical means followed by a concentration step to obtain polysaccharides of a desired molecular size range. Polysaccharides or depolymerized polysaccharides are modified by an activation process.

**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 if applicable. Colorimetric, chromatographic assay 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 limit. 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 PROTEINS

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 microorganism; the bacterial purity of the culture is verified. The culture may be inactivated. The carrier protein is purified by a suitable method.

**Identification**

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

**Diphtheria CRM197 protein.** 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% of diphtheria CRM197 protein, when tested by liquid chromatography (2.4.14) or any suitable method. The carrier protein shall be characterized by a suitable chemical or physicochemical method like SDS-PAGE, HPLC, isoelectric focusing, aminoacid sequencing, circular dichroism, fluorescence spectroscopy, peptide mapping or mass spectroscopy, as appropriate.

**Recombinant CRM197**. The protein obtained contains not less than 90% of recombinant CRM197 protein, when tested by liquid chromatography (2.4.14) or any 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.

**Diphtheria toxoid**. Diphtheria toxoid is produced as stated under Diphtheria vaccine (Adsorbed) and complies with the requirements prescribed there for bulk purified toxoid.

**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.

**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 8.8 per cent of lipopolysaccharide, determined by a suitable method.

**Pyrogens** (2.2.8) or **Bacterial endotoxins (BET)** (2.2.3). Inject into each rabbit 0.25 µg of OMP per Kg body weight, for determining the pyrogenic effect. It complies with the test for pyrogens or, preferably and where justified and authorised, with a validated in vitro test such as the bacterial endotoxin test.

**MONOVALENT BULK CONJUGATE**

The conjugate is obtained by the covalent binding of activated polysaccharides to the appropriate carrier protein.

Only a bulk conjugate that complies with the following requirements may be used in the preparation of the final bulk vaccine. For each test, limits of acceptance are established and each batch of conjugate must be shown to comply with these limits.

**Saccharide**. The saccharide content is determined by a suitable physical or chemical method or by an

immunochemical method. The value complies with the requirement approved for each conjugate.

**Protein**. The protein content is determined by a suitable physical or chemical method (for example, 2.7.1 or other suitable methods). The value complies with the requirement approved for each conjugate.

**Saccharide-to-protein ratio**. Determine the ratio by calculation. The value complies with the requirement approved for each conjugate.

**Free saccharide**. The free polysaccharide content is determined after separation from the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration, or other validated methods. The value complies with the requirement approved for each conjugate.

**Free carrier protein**. The free carrier protein content is determined by a suitable method, either directly or by calculation from the results of other tests. The value complies with the requirement approved for each conjugate.

**Residual reagents**. Where applicable, suitable tests are carried out to determine residues of reagents used during conjugation. An acceptable value for each reagent is established for the particular product and each batch of monovalent bulk conjugate must be shown to comply with this limit. Where validation studies have demonstrated removal of residual reagents, the test on monovalent bulk conjugate may be omitted.

**Molecular size or molecular-mass distribution**. The molecular-size or molecular-mass distribution is determined by size-exclusion chromatography (2.4.16) combined with an appropriate detection system. An acceptable value is established for each conjugate. Each batch must be shown to comply with this limit. Where justified and authorized, the test may be carried out on the monovalent bulk conjugate only.

**Identification**. Each polysaccharide conjugate is identified by an immunochemical method (2.2.14) or any another suitable validated method.

**FINAL BULK VACCINE**

Where a final bulk vaccine is formulated as a release intermediate, it complies with the following requirement and is within the limits approved for the particular product.

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

**Sterility** (2.2.11). It complies with the test for sterility, carried out using 10ml or the equivalent of 100 doses for each medium, whichever is less.

**FINAL LOT**

Only a final lot that is within the limits approved for the particular product and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

**Identification**

Conjugate from each group present in the vaccine is identified by a suitable immunochemical method (2.2.14) or any another suitable validated method.

**Tests**

**Free saccharide**. The free polysaccharide content for each group is determined by a suitable physico-chemical (for example liquid chromatography (2.4.14)) or immunochemical (for example enzyme-linked immunosorbent assay (ELISA) (2.2.14) method. The free polysaccharide content is within the limits approved by the competent authority for the particular product. Where justified and authorized, the test may be carried out on the monovalent bulk conjugate only.

**Molecular size or molecular-mass distribution**. The molecular-size or molecular-mass distribution is determined by size-exclusion chromatography (2.4.16) combined with an appropriate detection system. An acceptable value is established for each conjugate. Each batch must be shown to comply with this limit. Where molecular-size or molecular-mass distribution is validated and determined on the monovalent conjugate bulks, the test on the final lot may be omitted.

**Sterility** (2.2.11). It complies with the test for sterility.

**Water** (2.3.43). Not more than 3.0 per cent of moisture content by thermogravimetry, Karl Fischer or any other suitable method.

**Bacterial endotoxins** (2.2.3). The content is within the limits approved by the competent authority for the particular product.

**Antimicrobial preservative**. Where applicable, determine the amount of antimicrobial preservative by chemical method or any other suitable method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

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

**Assay**

**Saccharide**. The total polysaccharide content for each group is determined by a suitable physico-

chemical (for example liquid chromatography (2.4.14)) or immunochemical (for example enzyme-linked immunosorbent assay (ELISA) (2.2.14) method.

The content of each group is within the limits approved by the competent authority for the particular product.

**LABELLING**

The label states:

– the nominal amount of polysaccharide for each group (A, C, W135, Y and X) per single human dose;

– the type and amount of carrier protein per single human dose