**Pneumococcal Polysaccharide Conjugate Vaccine (Adsorbed)**

Pneumococcal polysaccharide conjugate vaccine (adsorbed) is a sterile suspension of purified capsular polysaccharides obtained from *Streptococcus pneumoniae* serotypes individually conjugated to a carrier protein. The carrier protein used may vary for the various polysaccharide conjugates contained in a multivalent vaccine. The vaccine may be adsorbed on a suitable adjuvant or adsorbant.

Each serotype, produced from suitable pathogenic strains of *S. pneumoniae*, is grown in an appropriate medium.

The individual polysaccharides are purified through suitable purification methods (for example centrifugation, precipitation, ultra filtration and column chromatography). The methods applied for purification should be approved by the NRA. Each polysaccharide has a defined composition and a defined molecular size range.

The choice of polysaccharide depends on the frequency of the serotypes responsible for acute pathologies and their geographical distribution. The vaccines contain immunochemically different capsular polysaccharides.

**Production**

**General provisions**

The production method shall have been shown to yield consistently *S. pneumoniae* conjugate vaccines of adequate safety and immunogenicity in man. The production of polysaccharides and of the carrier is based on a seed lot system.

The strains of *S. pneumoniae* used for preparing the polysaccharide should be agreed with the NRA. Each strain should have been shown to be capable of producing polysaccharide of the appropriate serotype through suitable tests during seed characterization (including serotyping) and product characterization (NMR).

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.2.1).

During development studies and whenever revalidation of the manufacturing process is necessary, it shall be demonstrated by tests in animals that the vaccine consistently induces a T-cell-dependent B-cell immune response.

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

**SEED LOT**

The bacterial strains used for master seed lot 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. Cultures obtained from the working seed lot shall have the same characteristics as the strain that was used to prepare the master seed lot.
Purity of the bacterial cultures should be 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 anti-sera.

If materials of animal origin are used in the medium for seed production, preservation of strain viability for freeze-drying or for frozen storage, then they should comply with the WHO Guidelines on Transmissible Spongiform Encephalopathies and should be approved by the NRA.

**Pneumococcal polysaccharides**

Each strain of *S. pneumoniae* serotypes is individually grown in a liquid medium that does not contain high-molecular-mass polysaccharides; if any ingredient of the medium contains blood-group substances; the process is validated to demonstrate that after the purification step they are no longer detectable. The components of animal origin, if used in media, should comply with the WHO Guidelines on Transmissible Spongiform Encephalopathies and should be approved by the NRA. The consistency of growth of *S. pneumoniae* should be demonstrated by monitoring growth rate, pH and the final yield of polysaccharide.

The bacterial purity of the culture is verified by suitable methods. The culture is inactivated. Each polysaccharide is separated from the liquid culture and purified by suitable methods. If any contamination is found, the culture or any product derived from it should be discarded. The killing process should also be adequately validated. Volatile matter, including water, in the purified polysaccharide is determined by a suitable method such as thermogravimetry (2.4.31); semi-micro determination of water (2.3.43) or, where applicable, determination of solvent and/or alcohol content by spectrometry. The values are used to calculate the results of other chemical tests with reference to the dried substance, as prescribed below.

The purified polysaccharide should be stored at appropriate temperatures to ensure stability.

Only polysaccharides that comply with the following requirements may be used in the preparation of the conjugate. Percentage contents of components, determined by the methods prescribed below, are shown in the Table 1 given in Pneumococcal Polysaccharide Vaccine (Liquid/Adsorbed) Monograph.

**Identification**

Each polysaccharide is identified by an immunochemical method (2.2.14) such as countercurrent immunoelectrophoresis, rate nephelometry or any other suitable method such as ELISA or Gel diffusion or other suitable methods, for example Nuclear magnetic resonance spectrometry (2.4.34) (either H\(^1\) or C\(^{13}\)).

**Protein** (2.7.1). Depending upon the serotype used, not more than the limit approved for the product, calculated with reference to the dried substance.

**Nucleic acids** (2.7.1). Depending upon the serotype used, not more than the limit approved for the product, calculated with reference to the dried substance.
**Molecular size or molecular mass distribution.** Molecular size or molecular mass distribution is determined by size exclusion chromatography combined with an appropriate detection system. An acceptable value is established for each pneumococcal polysaccharide. Each batch should comply with this limit.

**Bacterial endotoxin** (2.2.3). Not more than 0.5 IU of endotoxin per microgram of polysaccharide.

**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. Wherever validation studies have demonstrated removal of residual reagents, the test on polysaccharides may be omitted.

**Composition tests.** Depending upon the chemical composition of a pneumococcal polysaccharide serotype, following tests may be applicable. The values are within the limits approved. Suitable limits for some pneumococcal polysaccharide serotype are given in pneumococcal polysaccharide vaccine. These limits are based on colorimetric estimations and are determined on dried weight basis. These composition tests may be determined using other suitable methods such as NMR. Suitable limits for such tests may be approved by NRA.

**Pyrogen** (2.2.8). Complies with the test for Pyrogen, the limits shall be fixed based on the clinical development batches. This test can be omitted if bacterial endotoxin test is validated.

**MODIFIED PNEUMOCOCCAL POLYSACCHARIDE**

Before conjugation the polysaccharide can be depolymerised by chemical or mechanical means followed by a concentration step to obtain polysaccharides of a desired molecular size range. The chemical method chosen should be approved by the NRA. Polysaccharides or depolymerized polysaccharides are modified by an activation process. Only modified polysaccharides that comply with the following requirements may be used in the preparation of the conjugate.

**Molecular size or molecular mass distribution.** In the case of a size-reduced modified pneumococcal polysaccharide, the molecular size or molecular mass distribution is determined by size exclusion chromatography combined with an appropriate detection system. An acceptable value is established for each modified pneumococcal polysaccharide. Each batch should comply with this limit.

**Degree of oxidation.** Where applicable the degree of oxidation is represented by the ratio of moles of saccharide repeat unit per mole of aldehyde and determined by a suitable method. The values are within the limits approved for each serotype.

**CARRIER PROTEIN**

The carrier protein is produced by culture of suitable (including inducible recombinant) micro-organisms with verified purity. The culture is inactivated. The strain used for the production of carrier protein is revealed for its identity, origin and short history. The strain used is approved by the National Regulatory Authority. Components of
animal origin, if used, should comply with WHO guideline on Transmissible spongiform Encephalopathies. The certificate for TSE-free should be issued.

The protein carrier should also be characterized before using for conjugation as a carrier protein (one time activity during consistency batches). 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 NRA.

The carrier protein is purified by a suitable method. Suitable tests are carried out, for validation or routinely, to demonstrate that, where applicable, the product is free from specific toxins.

Where Diphtheria toxoid is used, it is produced as described in the monograph *Diphtheria vaccine adsorbed* and complies with the requirements prescribed therein for bulk purified toxoid, except that the test for sterility is not required.

Where CRM 197 is used as the carrier protein, it is not less than 90 per cent pure, determined by a suitable method.

Where tetanus toxoid is used as the carrier protein, it is produced as described in the monograph *Tetanus vaccine adsorbed* and complies with the requirements prescribed therein for purified bulk toxoid, except that the antigenic purity is not less than 1500 Lf per milligram of protein nitrogen and that the test for sterility is not required.

Where protein D is used, a specific strain of *E.coli* is modified by a plasmid carrying the protein D coding sequence in order to express this outer surface protein of Haemophilus influenza. The modified strain is grown in a suitable liquid medium to express protein D. At the end of cultivation, protein D is purified by suitable methods. The product contains not less than 95 per cent of protein D.

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

**Identification**

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

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

**Bacterial Endotoxins** (2.2.3). Not more than 1 IU per microgram of protein.

**Carrier protein**: Not less than 90 per cent of the total protein content, determined by a suitable method.

**Residual Reagents**: Appropriate method to be used to ascertain the absence or to establish the limit of reagent/chemical used during purification. Wherever validation studies have demonstrated removal of residual reagents, the test on carrier protein may be omitted.

**MONOVALENT BULK CONJUGATE**
The conjugate is obtained by the covalent binding of activated polysaccharides to the carrier protein.

The conjugate purification procedures are designed to remove residual reagents used for conjugation. The removal of residual reagents is confirmed by suitable tests or by validation of the purification process.

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 content.** The polysaccharide content is determined by a suitable physical or chemical method or by an Immunochemical method (2.2.14). The value complies with the requirement approved for each serotype.

**Protein.** The protein content is determined by a suitable physical or chemical method. The value complies with the requirement approved for each serotype.

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

**Free saccharide.** Unbound polysaccharide is determined after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods.

A value consistent with adequate immunogenicity as shown in clinical trials is established for each serotype and each lot must be shown to comply with this limit.

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

**Molecular size or molecular mass distribution.** Molecular size or molecular mass distribution is determined by size exclusion chromatography combined with an appropriate detection system. An acceptable value is established for the bulk conjugate of each polysaccharide. Each batch should comply with this limit.

**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 conjugate polysaccharide must be shown to comply with this limit. Where validation studies have demonstrated removal of residual reagent, the test on conjugate polysaccharide may be omitted.

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

**Specific toxicity of the carrier protein.** It complies with the test for specific toxicity carried out using a validated method where appropriate (e.g. when tetanus or diphtheria toxoids have been used). Absence of specific toxicity of the carrier protein may also be accessed through validation of the production process.

**Bacterial endotoxins** (2.2.3). Not more than 0.75 IU of endotoxin per microgram of polysaccharide.
**ADSORBED MONOVALENT BULK CONJUGATE**

An aluminium containing adjuvant may be added to each of the monovalent bulk conjugates prior to formulation of the final bulk. Once the conjugates are adsorbed on a sterile adjuvant, sterility is assured by aseptic processing.

Only an adsorbed monovalent bulk conjugate that complies with the following requirements may be used in the preparation of the final bulk vaccine.

**Identification**

Each adsorbed polysaccharide conjugate is identified by an immunochemical method (2.2.14) or other suitable methods.

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

**Saccharide.** The polysaccharide content is determined by a suitable physical or chemical method or by an Immunochemical method (2.2.14). The value complies with the requirement approved for each serotype.

**Free saccharide.** Centrifuge the adsorbed monovalent bulk conjugate. In the supernatant the unbound polysaccharide is determined after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic liquid chromatography, ultrafiltration or other validated methods. An acceptable value consistent with adequate immunogenicity as shown in clinical trials is established for each serotype and each lot must be shown to comply with this limit.

**Degree of Adsorption.** If any adjuvant is used the degree of Adsorption of each polysaccharide conjugate is assessed.

**FINAL BULK VACCINE**

A final Bulk Vaccine may be formulated from the individually adsorbed monovalent bulk conjugate, or from the mixture of the monovalent bulk conjugates that is adsorbed on an aluminium-containing adjuvant.

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

Only a final bulk vaccine that complies with the following requirements and is within the limits approved for the particular product may be used in the preparation of the final lot.

**Sterility** (2.2.11). It complies with the test for sterility, carried out using 10 ml for each medium 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

Carry out an identification test to determine each polysaccharide content by a suitable immunochemical method (2.4.14) or by any other suitable validated method.

Carry out an identification test to determine carrier protein(s) present in the vaccine by a suitable validated method.

Tests

pH (2.4.24). If the vaccine is a liquid preparation, the pH of each final lot should 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.

Total protein content. The protein content of the final vaccine should be determined by means of an appropriate validated assay and comply with limits for the particular product.

Total saccharide content. The total saccharide protein content of the final vaccine should be determined by means of an appropriate validated assay and comply with limits for the particular product.

Extractable volume. Not less than the nominal volume of the intended dose.

Aluminium (2.3.9). Not more than 1.25 mg per single human dose.

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

Bacterial endotoxins (2.2.3): Not more than 12.5 IU per single human dose, unless otherwise justified and authorized.

Preservative content. If the preservative is added in the vaccine, its amount should be determined using a validated method approved by NRA.

General safety test (Innocuity). The final vaccine should comply with the test for abnormal toxicity for immunosera and vaccines for human use.

ASSAY

Saccharide content. The polysaccharide content for each serotype is determined by a suitable immunochemical method (for example, nephelometry assay or enzyme linked immunosorbent assay (ELISA)). The vaccine contains not less than 70 per cent and not more than 130 per cent of the quantity stated on the label for each polysaccharide. The confidence limits \( P = 0.95 \) are not less than 80 per cent and not more than 120 per cent of the estimated content.
**Labeling.** The label states (1) the pneumococcal serotype and carrier protein present in each single human dose; (2) the number of micrograms of each polysaccharide per single human dose; (3) the type and nominal amount of carrier protein per single human dose; (4) if applicable, the name and amount of adsorbent; (5) if applicable, that the vaccine must be shaken before use; (6) if applicable, that the vaccine must not be frozen; (7) temperature recommended during storage and transport; (8) route of administration.