immunogenicity, as shown in clinical trials, is established for the particular product and each final lot must be shown to comply with this limit.

**Sterility (2.6.1).** It complies with the test for sterility.

**Bacterial endotoxins (2.6.14):** less than 25 IU per single human dose.

**ASSAY**

**Saccharide:** minimum 80 per cent of the amount of meningococcal group C polysaccharide stated on the label. The saccharide content is determined by a suitable validated assay, for example sialic acid assay (2.3.23) or anion-exchange liquid chromatography with pulsed amperometric detection (2.2.29).

**LABELLING**

The label states:
- the number of micrograms of meningococcal group C polysaccharide per human dose,
- the type and number of micrograms of carrier protein per human dose.

**MENINGOCOCCAL POLYSACCHARIDE VACCINE**

Vaccinum meningococcale polysaccharidicum

**DEFINITION**

Meningococcal polysaccharide vaccine is a freeze-dried preparation of one or more purified capsular polysaccharides obtained from one or more suitable strains of Neisseria meningitidis group A, group C, group Y and group W135 that are capable of consistently producing polysaccharides.

N. meningitidis group A polysaccharide consists of partly O-acetylated repeating units of N-acetylmannosamine, linked with 1α→6 phosphodiester bonds.

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

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

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

The polysaccharide component or components stated on the label together with calcium ions and residual moisture account for over 90 per cent of the mass of the preparation.

**PRODUCTION**

Production of the meningococcal polysaccharides is based on a seed-lot system. The production method shall have been shown to yield consistently meningococcal polysaccharide vaccines of satisfactory immunogenicity and safety in man.

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 (2.6.9).

**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.

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 utilises glucose and maltose,
- suspensions of the culture agglutinate with suitable specific antisera.

Purity of bacterial strains used for the seed lots 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 semisynthetic 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 precipitate obtained is harvested and may be stored at −20 °C 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 ethanol precipitation of the polysaccharides which are then dried and stored at −20 °C. The loss on drying is determined by thermogravimetry (2.2.34) 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 final bulk vaccine.

**Protein (2.5.16).** Not more than 10 mg of protein per gram of purified polysaccharide, calculated with reference to the dried substance.

**Nucleic acids (2.5.17).** Not more than 10 mg of nucleic acids per gram of purified polysaccharide, calculated with reference to the dried substance.

**O-Acetyl groups (2.5.19).** Not less than 2 mmol of O-acetyl groups per gram of purified polysaccharide for group A, not less than 1.5 mmol per gram of polysaccharide for group C, not less than 0.3 mmol per gram of polysaccharide for groups Y and W135, all calculated with reference to the dried substance.
**Phosphorus** (2.5.18). Not less than 80 mg of phosphorus per gram of group A purified polysaccharide, calculated with reference to the dried substance.

**Sialic acid** (2.5.23). Not less than 800 mg of sialic acid per gram of group C polysaccharide and not less than 560 mg of sialic acid per gram of purified polysaccharide for groups Y and W135, all calculated with reference to the dried substance. Use the following reference solutions.

Group C polysaccharide: a 150 mg/l solution of N-acetylneuraminic acid R.

Group Y polysaccharide: a solution containing 95 mg/l of N-acetylneuraminic acid R and 55 mg/l of glucose R.

Group W135 polysaccharide: a solution containing 95 mg/l of N-acetylneuraminic acid R and 55 mg/l of galactose R.

**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.

**Distribution of molecular size.** Examine by size-exclusion chromatography (2.2.30) using agarose for chromatography R or cross-linked agarose for chromatography R. Use a column about 0.9 m long and 16 mm in internal diameter equilibrated with a solvent having an ionic strength of 0.2 mol/kg and a pH of 7.0-7.5. Apply to the column about 2.5 mg of polysaccharide in a volume of about 1.5 ml and elute at about 20 ml/h. Collect fractions of about 2.5 ml and determine the content of polysaccharide by a suitable method. At least 65 per cent of group A polysaccharide, 75 per cent of group C polysaccharide, 80 per cent of group Y polysaccharide and 80 per cent of group W135 polysaccharide is eluted before a distribution coefficient (\(K_0\)) of 0.50 is reached. In addition, the percentages eluted before this distribution coefficient are within the limits approved for the particular product.

**Identification and serological specificity.** The identity and serological specificity are determined by a suitable immunochemical method (2.7.1). Identity and purity of each polysaccharide shall be confirmed; it shall be shown that there is not more than 1 per cent of group heterologous N. meningitidis polysaccharide.

**Pyrogens** (2.6.8). The polysaccharide complies with the test for pyrogens. Inject into each rabbit per kilogram of body mass 1 ml of a solution containing 0.025 µg of purified polysaccharide per millilitre.

**FINAL BULK VACCINE**

One or more purified polysaccharides of 1 or more N. meningitidis groups are dissolved in a suitable solvent that may contain a stabiliser. When dissolution is complete, the solution is filtered through a bacteria-retentive filter. Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

**Sterility** (2.6.1). The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

**FINAL LOT**

The final bulk vaccine is distributed aseptically into sterile containers. The containers are then closed so as to avoid contamination.

Only a final lot that is satisfactory with respect to each of the requirements prescribed below under Identification, Tests and Assay may be released for use.

**CHARACTERS**

A white or cream-coloured powder or pellet, freely soluble in water.

**IDENTIFICATION**

Carry out an identification test for each polysaccharide present in the vaccine by a suitable immunochemical method (2.7.1).

**TESTS**

**Distribution of molecular size.** Examine by size-exclusion chromatography (2.2.30). Use a column about 0.9 m long and 16 mm in internal diameter equilibrated with a solvent having an ionic strength of 0.2 mol/kg and a pH of 7.0-7.5. Apply to the column about 2.5 mg of each polysaccharide in a volume of about 1.5 ml and elute at about 20 ml/h. Collect fractions of about 2.5 ml and determine the content of polysaccharide by a suitable method. For a divalent vaccine (group A + group C), use cross-linked agarose for chromatography R. The vaccine complies with the test if:

- 65 per cent of group A polysaccharide is eluted before \(K_0 = 0.50\),
- 75 per cent of group C polysaccharide is eluted before \(K_0 = 0.50\).

For a tetravalent vaccine (group A + group C + group Y + group W135), use cross-linked agarose for chromatography R1 and apply a suitable immunochemical method (2.7.1) to establish the elution pattern of the different polysaccharides. The vaccine complies with the test if \(K_0\) for the principal peak is:

- not greater than 0.70 for group A and group C polysaccharide,
- not greater than 0.57 for group Y polysaccharide,
- not greater than 0.68 for group W135 polysaccharide.

**Water** (2.5.12). Not more than 3.0 per cent, determined by the semi-micro determination of water.

**Sterility** (2.6.1). It complies with the test for sterility.

**Pyrogens** (2.6.8). It complies with the test for pyrogens. Inject per kilogram of the rabbit’s mass 1 ml of a solution containing:

- 0.025 µg of polysaccharide for a monovalent vaccine,
- 0.050 µg of polysaccharide for a divalent vaccine,
- 0.10 µg of polysaccharide for a tetravalent vaccine.

**ASSAY**

Carry out an assay of each polysaccharide present in the vaccine.

For a divalent vaccine (group A + group C), use measurement of phosphorus (2.5.18) to determine the content of polysaccharide A and measurement of sialic acid (2.5.23) to determine the content of polysaccharide C. To determine sialic acid, use as reference solution a 150 mg/l solution of N-acetylneuraminic acid R.

For a tetravalent vaccine (group A + group C + group Y + group W135) a suitable immunochemical method (2.7.1) is used with a reference preparation of purified polysaccharide for each group. The vaccine contains not less than 70 per cent and not more than 130 per cent of the quantity of each polysaccharide stated on the label.

**LABELLING**

The label states:

- the group or groups of polysaccharides (A, C, Y or W135) present in the vaccine,
- the number of micrograms of polysaccharide per human dose.