PNEUMOCOCCAL VACCINE POLYVALENT

1 Description definition

"Pneumococcal Vaccine Polyvalent" is a colorless liquid product containing purified capsular polysaccharides from 23 serotypes of *Streptococcus pneumoniae*. The serotype designations by the Danish naming system are 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.

2 Product control

2.1 Source materials

2.1.1 Seeds for production

S. pneumoniae seeds with serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F shall be used.

2.1.2 Culture medium

The components of *S. pneumoniae* growth should not contain any substances which have the possibility of inducing a pronounced allergic reaction in humans.

2.2 Production of the polysaccharide bulk power

2.2.1 Fermentation

Expansion of the *S. pneumoniae* seed of each serotype is performed at 37±2ºC (for serotype 9V at 36.5±1.5ºC). When the fermentation is finished, the serotypes are confirmed by the Quellung method with type-specific antisera. The culture shall not contain any other bacterial contaminants by microscopy and an appropriate culture method.

2.2.2 Inactivation

Phenol is added to *S. pneumoniae* culture medium at a concentration of 0.8w/v% or more and kept at 37±2ºC for 2 hours or more by shaking.

2.2.3 Purification for producing the bulk powder

The inactivated *S. pneumoniae* culture medium is centrifuged and filtrated for removing cell debris. By diafiltraion, polysaccharide is concentrated and nucleic acids are removed. Remaining cell debris and proteins are removed by appropriate methods. The polysaccharide is recovered by salting out in the presence of alcohol, washed and dried.

2.3 Production of the final (polyvalent) bulk

The polyvalent bulk contains each of the 23 polysaccharides in physiological saline at the concentration of 50 μg/mL. After adding phenol at the final concentration of 0.25 w/v%, the polyvalent bulk shall be passed through filter to obtain the sterile final (polyvalent) bulk.

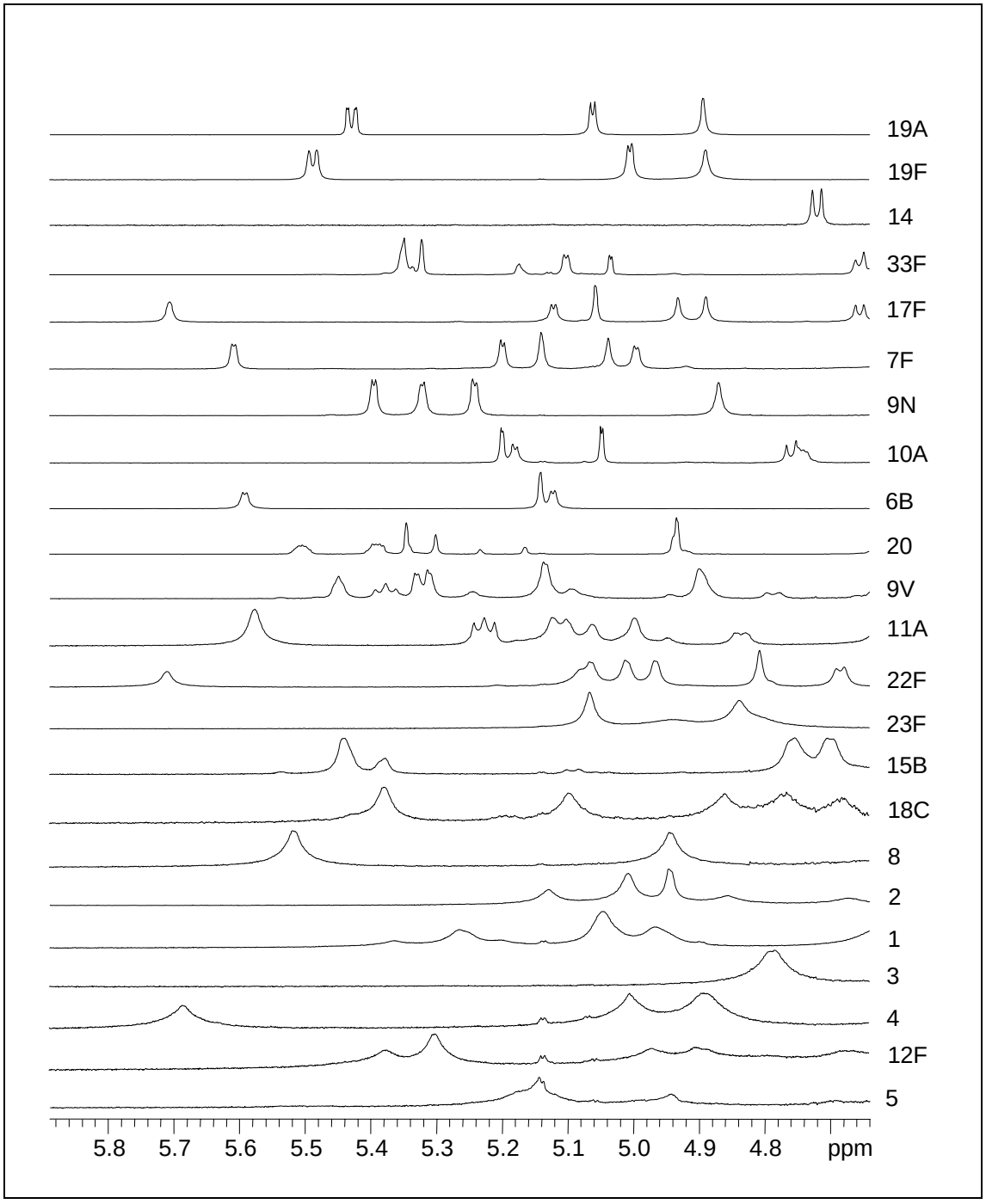
3 Control tests

3.1 Tests on the polysaccharides

Each polysaccharide shall be subjected to the tests given below.

3.1.1 Nuclear magnetic resonance (^{1}H) test

The D_2O reagent containing 0.01% dimethylsulfoxide (DMSO; used as an internal standard for quantitative measurements) and 0.01% sodium 3,3,4,5,5-hexadeutro-2,2-dimethly-2-silapentane-5-sulfonate (DSS-d_6; used as the internal chemical shift standard) is used. The sample is dissolved in the D_2O reagent to obtain 5 mg/mL solution for nuclear magnetic resonance (^{1}H) spectroscopy as given in JP. The region between 5.89 to 4.64 ppm of the sample spectrum is compared to that of reference spectrum of each capsular type. The correlation coefficient between spectra shall be greater than or equal to 0.95 with the homologous reference spectrum.



Reference NMR spectrum

3.1.2 *O*-acetate content test

The sample is tested by nuclear magnetic resonance (^{1}H) spectroscopy as given in Japanese Pharmacopoeia. The *O*-acetate content is calculated by the following equation. The *O*-acetate content (*O*-acetyl/polysaccharide unit molar ratio) shall be within the range of the following specification.

O-acetate content= $\frac{I_1}{3} \times \frac{n}{I_2}$

I_1: the integral of the *O*-acetate region corrected for other contributions

I_2: the integral of the polysaccharide signal (6.40-2.90 ppm) corrected for residuals

n: the number of non-exchangeable protons in the polysaccharide integration region

serotype	*O*-acetate content
1	0.3 - 1.0
7F	0.5 - 1.4
9V	0.7 - 2.2
11A	1.3 - 3.9
15B	0.5 - 1.4
17F	0.5 - 1.4
18C	0.4 - 1.3
20	1.3 - 4.0
22F	0.5 - 1.5
33F	0.4 - 1.2

3.1.3 Protein content test

By the assay given in General Tests or by equivalent assay, the protein content of each serotype shall be equal or less than 2.0%. The concentration of the polysaccharide solution in this assay is determined by high performance size exclusion chromatography with multiple-angle laser light scattering and refractive index detection.

3.1.4 Weight-average molecular mass test

By high performance size exclusion chromatography with multiple-angle laser light scattering and refractive index detection, weight-average molecular mass of each bulk powder is calculated by the following equation. The weight-average molecular mass (kDa) shall be equal or more than the following specification.

Weight-average molecular mass (M_w) = $\frac{\sum(c_i M_i)}{\sum c_i}$

ci: refractive index of slice i

Mi: molecular mass of slice i

Serotype	weight-average molecular mass (kDa)
1	370
2	770
3	610
4	270
5	250
6B	520
7F	480
8	520
9N	500
9V	690
10A	410
11A	780
12F	270
14	420
15B	570
17F	630
18C	480
19A	200
19F	420
20	320
22F	540
23F	940
33F	710

3.1.5 Serological identity test

When the sample is tested by Ouchterlony assay by using type specific antisera, precipitate shall be observed. When the sample is tested by rate nephelometory, positive reaction shall be observed with homologous antiserum and negative reaction shall be observed from heterologous antisera.

3.1.6 Endotoxin test

When the test given in General Tests is applied, endotoxin in the sample shall be equal or less than 10 EU/mg.

3.2 Tests on final lot

Following tests shall apply to each final lot.

3.2.1 Test for pH

When the test given in General Tests is applied, the pH of the sample shall be within the range between 6.0 and 7.4.

3.2.2 Test for phenol concentration

When the liquid chromatography test given in Japanese Pharmacopoeia is applied, the phenol concentration of the sample shall be within the range between 0.225-0.275w/v%.

3.2.3 Sterility test

The test given in General test shall apply.

3.2.4 General safety test

The test given in General test shall apply. The sample must meet the specification given in the approval document

3.2.5 Identity test

When the sample is tested by Ouchterlony assay, precipitate shall be observed with type specific antiserum. If necessary, the sample can be appropriately diluted with physiological saline containing 0.25 w/v% phenol. When the final lot is tested by rate nephelometory, positive reaction shall be observed with antiserum for each serotype.

3.2.6 Polysaccharide content test

Polysaccharide content in the sample is determined by rate nephelometory. Each polysaccharide concentration shall be within the range between 35-65 μg/ml.

3.2.7 Endotoxin test

When the test given in General Tests is applied, endotoxin in the sample shall be equal or less than 10 EU/mL.

4 Storage and the expiry period

The expiry date shall be two years.