

Annex 2

Recommendations for the production and control of pneumococcal conjugate vaccines

Recommendations published by WHO are intended to be scientific and advisory in nature. The parts of each section printed in type of normal size have been written in a form, such that, should a national regulatory authority so desire, they may be adopted as they stand as definitive national requirements or used as the basis of such requirements. Those parts of each section printed in small type are comments and recommendations for guidance for those manufacturers and national regulatory authorities which may benefit from additional information.

It is recommended that modifications be made only on condition that the modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the recommendations set out below.

The terms “national regulatory authority” and “national control laboratory” as used in these recommendations, always refer to the country in which the vaccine is manufactured.

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Introduction

Recommendations (formerly known as Requirements) for pneumococcal polysaccharide vaccines were drafted in 1980 but were never adopted by the WHO Expert Committee on Biological Standardization (1). Vaccines based on the capsular polysaccharides of the 23 serotypes of *Streptococcus pneumoniae* most commonly associated with human disease have been licensed in many countries (2). These vaccines have been shown to be efficacious against invasive pneumococcal disease and have proved to be effective for the protection of individuals who are at particular risk of infection. Nevertheless, their inability to elicit protective responses in young infants or to induce good immunological memory has prevented their inclusion in national infant immunization schedules.

The development of bacterial capsular polysaccharide–protein conjugates represents a major advance in prophylaxis against bacterial infections (3). Following the successful introduction of the *Haemophilus influenzae* type b conjugate (Hib) and meningococcal C conjugate (MenC) vaccines into paediatric vaccination schedules, considerable progress has been made in the development of similar conjugate vaccines based on pneumococcal capsular polysaccharides. Glycoconjugate vaccines are both physically and immunobiologically distinct from their unconjugated counterparts, emphasizing the need for recommendations specifically for these products.

General considerations

Infections caused by *S. pneumoniae*, the pneumococcus, are responsible for substantial morbidity and mortality, particularly in the very young and in the elderly (2, 4, 5). Pneumococci are grouped into serotypes on the basis of their chemically and serologically distinct capsular polysaccharides. Of the 90 pneumococcal serotypes (6), the capsular polysaccharides of the 23 most commonly associated with disease are included in the polysaccharide vaccines produced by various manufacturers. These vaccines are effective in individuals from about 2 years of age but, as they elicit T-cell independent immunity, they are not effective in younger children. In addition, they fail to induce boostable immunity and have little or no impact on nasopharyngeal carriage (7). In contrast, polysaccharide–protein conjugates have been shown to be highly immunogenic in infants and to induce T-cell dependent immunity. Several pneumococcal conjugate vaccines are now available or are at an advanced stage of development (8–10). The results of controlled clinical trials of these vaccines have

demonstrated that such conjugates are both safe and highly immunogenic, T-cell dependent antigens (11, 12). They have been shown to induce high levels of serum antibody and to offer protective immunity against invasive pneumococcal disease (13). They are effective in young children, induce immunological memory and reduce nasopharyngeal carriage of the pneumococcal serotypes included in the formulation (14). A 7-valent conjugate, manufactured using diphtheria protein CRM197 as the carrier protein for all seven serotypes, was first licensed in the USA in 2000 and has become increasingly available worldwide.

Protective levels of antibody elicited by the CRM197 conjugated vaccines against invasive pneumococcal disease have been estimated using the data from three clinical efficacy trials: one in Northern California; one among Navajo Indians and one in Soweto, South Africa. The aggregate efficacy for the seven serotypes these vaccines had in common was 93.0% (95% confidence interval, 81.0–98.2%). Using the data from enzyme-linked immunosorbent assays (ELISA) for anti-capsular polysaccharide antibody, an estimate of 0.35 µg/ml aggregated across the serotypes was associated with the point-estimate of clinical efficacy against invasive disease (see Appendix for additional details). However, this reference value is neither applicable to the determination of the protective status of the individual nor to protection against other disease end-points, e.g. pneumonia or otitis media. Practical or ethical considerations may make it impossible to perform protective efficacy trials of most new vaccine formulations. Therefore, this reference value will be important for the licensure of future products using data from immunogenicity trials.

Differences in the incidence of serotypes causing disease from one continent to another have led to the development of pneumococcal vaccine formulations consisting of increasing numbers of conjugated components (15). Recently clinical trials of 7-valent and 9-valent formulations have been completed in Finland and South Africa respectively (16–18), and further formulations with potentially greater coverage are under development (9). From a practical perspective, however, it is evident that there is a limit to the number of serotypes that can be included in such conjugate vaccine formulations and the incidence of disease-causing serotypes in the target population should be taken into consideration before vaccine development. Although geographical and temporal factors undoubtedly contribute to differences in the incidence rates between regions, the impact of differences between national epidemiological surveillance systems on case ascertainment may also prove to be a critical factor in the assessment

of pneumococcal vaccine coverage (19). The serotype composition of pneumococcal vaccines should be agreed with the national regulatory authority based on appropriate epidemiological data on the target population. The superiority of a vaccine should not be assumed on the basis of the number of serotypes included unless there is evidence that the inclusion of additional serotypes is likely to enhance its effectiveness in a particular epidemiological setting.

Special considerations

The production and control of conjugate vaccines is more complex than that of their unconjugated capsular polysaccharide counterparts. Polysaccharide vaccines consist of defined chemical substances that, if prepared to the same specifications, can reasonably be expected to have comparable potencies. Although only the 7-valent conjugate formulation has been licensed to date, experience with *H. influenzae* type b and meningococcal conjugate vaccines suggests that effective pneumococcal vaccines may be developed that differ both in the nature of the saccharide and the carrier protein employed. Vaccines are under development that utilize carrier proteins other than CRM197 and vaccine formulations could be developed in which more than one carrier is employed. The manufacturer has a choice of possible carrier proteins providing that the resulting vaccine is safe and elicits a T-cell dependent, protective immune response.

Unfortunately, the lack of a suitable animal model for all pneumococcal serotypes makes it impossible to assess the potency of these vaccines for humans on the basis of studies in animals. Consequently, it is important that new pneumococcal vaccine formulations are evaluated in humans for immunogenicity by monitoring the production of serotype-specific immunoglobulin G (IgG). Immune responses to pneumococcal vaccines have been measured using methods that determine either the total amount of antibody binding to capsular polysaccharide or the amount of functional antibody present in serum. Antibody binding is typically evaluated by the use of ELISAs or radioimmunoassays (20–22a), whereas the opsonophagocytic assay is used to measure functional antibodies (23–25). Clinical studies of conjugate vaccines have shown a good association between antibody levels measured by ELISA and protection (see Appendix). However, such studies also usually include an analysis of a subset of sera to confirm their functional (e.g. opsonophagocytic) activity. Whichever assay is used, it should be standardized so as to ensure comparability of data both between laboratories and between different clinical

studies. A set of calibration sera is available to help establish comparability between laboratories (26). As conjugate vaccines should induce a T-cell dependent immune response, this should also be evaluated during clinical trials. Indicators of T-cell dependent immunity include the production of predominantly high-avidity IgG antibody and the demonstration of a good booster response in children who have already had the primary vaccination (14).

Given the lack of a suitable animal model that will predict the potency of all pneumococcal serotypes, the strategy for the control of the vaccine is dominated by the use of tests for molecular characterization and purity. These tests focus on physicochemical criteria to ensure each vaccine lot is consistent with the specification of the vaccine lots used in the definitive clinical trials that confirmed their safety and efficacy. Animal studies form an essential part of the development of these vaccines to provide evidence that they induce T-cell dependent immunity and to characterize the immunogenicity of the vaccine during stability studies. However, an immunogenicity test in animals is not necessary for routine lot release when vaccine consistency has been assured by alternative means.

Combined vaccines containing pneumococcal polysaccharide conjugate components

The introduction of Hib and MenC conjugates as additional elements of infant immunization programmes has served to highlight the need to combine paediatric vaccines for effective vaccine delivery (27). Vaccine formulations with multiple components that include pneumococcal conjugates are likely to be developed within the next decade. If one or more conjugated pneumococcal components are indicated for co-administration with other vaccines, the possible effects on the clinical performance of each component in the vaccine, including the pneumococcal conjugate components, should be evaluated in terms of their safety and immunogenicity. Similarly, the clinical effect of concomitant administration of a pneumococcal conjugate vaccine with other vaccines at different sites should be evaluated. Because of the problems associated with performing physicochemical analyses on complex vaccine formulations, the manufacturer should consider which batch release tests are appropriate to perform on final bulks of a particular product and which tests should be performed on final lots of such vaccines. The tests should be agreed with the national regulatory authority.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 *Proper name*

The proper name of the vaccine should be “Pneumococcal conjugate vaccine” translated into the language of the country of use. The serotypes included in the vaccine should be associated with the name of the vaccine and listed in the packaging material. The use of this proper name should be limited to vaccines that satisfy the recommendations formulated below.

A.1.2 *Descriptive definition*

Multivalent pneumococcal conjugate vaccine is a preparation of capsular polysaccharide from specific serotypes of *Streptococcus pneumoniae* that are covalently linked to carrier protein.

A.1.3 *International reference materials*

No formally established international reference materials that would allow the standardization of immune responses to pneumococcal conjugate vaccines are currently available.

The following reagents are available through the courtesy of individuals, manufacturers and national regulatory or reference laboratories:

C-polysaccharide (Statens Serum Institute, Copenhagen, Denmark)

Capsular polysaccharides (American Type Culture Collection (ATCC), Manassas, Virginia, USA)

89-SF reference serum (Dr Carl Frasch, Center for Biologics Evaluation and Research, US Food and Drug Administration (CBER/FDA), Rockville, MD, USA) (22)

96DG secondary reference serum (provided by Dr David Goldblatt and distributed by National Institute for Biological Standards and Control (NIBSC), Potters Bar, Herts., England)

ELISA calibration sera (provided by Dr David Goldblatt and distributed by NIBSC, Potters Bar, Herts., England) (26)

Pneumococcal serotyping reagents (Statens Serum Institute, Copenhagen, Denmark)

HL-60 cells (ATCC, Manassas, Virginia, USA)

A.1.4 *Terminology*

Master seed lot. A bacterial suspension of *S. pneumoniae* derived from a strain that 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 the freeze-dried form or be frozen below -45°C .

Working seed lot. A quantity of live *S. pneumoniae* organisms derived from the master seed lot by growing the organisms and maintaining them in aliquots in the freeze-dried form or the frozen state at or below -45°C . The working seed lot is used, when applicable, after a fixed number of passages, for the inoculation of production medium.

Single harvest. The material obtained from one batch of cultures that have been inoculated with the working seed lot (or with the inoculum derived from it), harvested and processed together.

Purified polysaccharide. The material obtained after final purification. The lot of purified polysaccharide may be derived from a single harvest or a pool of single harvests processed together.

Modified polysaccharide. Purified polysaccharide that has been modified by chemical reaction or physical process in preparation for conjugation to the carrier.

Carrier. The protein to which the polysaccharide is covalently linked for the purpose of eliciting a T-cell dependent immune response to the pneumococcal polysaccharide.

Monovalent bulk conjugate. A conjugate prepared from a single lot or pool of lots of polysaccharide and a single lot or a pool of lots of protein. This is the parent material from which the final bulk is prepared.

Final bulk conjugate. The blend of monovalent conjugates present in a single container from which the final containers are filled, either directly or through one or more intermediate containers derived from the initial single container.

Final lot. A number of sealed, final containers that are equivalent with respect to the risk of contamination during filling and, when it is performed, freeze-drying. A final lot must therefore have been filled from a single container and freeze-dried in one continuous working session.

A.2 **General manufacturing recommendations**

The general manufacturing recommendations contained in good manufacturing practices for pharmaceuticals (28) and biological products (29) should apply to establishments manufacturing pneumococcal conjugate vaccines with the addition of the following:

Details of standard operating procedures for the preparation and testing of pneumococcal conjugate vaccines adopted by the manufacturer together with evidence of appropriate validation of each production step should be submitted for the approval of the national regulatory authority. All assay procedures used for quality control of the conjugate vaccines and vaccine intermediates must be validated. Proposals for the modification of manufacturing and control methods should also be submitted for approval to the national regulatory authority.

Streptococcus pneumoniae is a Biological Safety Level (BSL) 2 pathogen and represents a particular hazard to health through infection by the respiratory route. The organism should be handled under conditions appropriate for this class of pathogen (30). Standard operating procedures need to be developed for dealing with emergencies arising from the accidental spillage, leakage or other dissemination of pneumococcal organisms. Personnel employed in the production and control facilities should be adequately trained and appropriate protective measures including vaccination with a pneumococcal vaccine licensed for use in adults should be implemented. Adherence to current good manufacturing practices is important to the integrity of the product, to protect workers and to protect the environment.

A.3 **Production control**

A.3.1 **Control of polysaccharide**

A.3.1.1 *Strains of Streptococcus pneumoniae*

The strains of *S. pneumoniae* used for preparing the polysaccharide should be agreed with the national regulatory authority. Each strain should have been shown to be capable of producing polysaccharide of the appropriate serotype. Each master seed lot should be identified by a record of its history, including the source from which it was obtained and the tests done to determine the characteristics of the strain.

The cultures may be examined for the following characteristics: microscopically, stained smears from a culture should appear typical of *S. pneumoniae*; the organism should grow at 37°C, but not at 25°C, and should have characteristic smooth alpha haemolytic colonies; the organism should have the ability to ferment insulin; the organism should be lysed in the bile solubility test and be sensitive to optochin; a suspension of the culture should be agglutinated or give a positive Quellung reaction with the appropriate serotyping serum.

Nuclear magnetic resonance spectrometry (either ^1H or ^{13}C) is a suitable method for the confirmation of identity of purified polysaccharide.

A.3.1.2 *Seed lot system*

The production of pneumococcal polysaccharide should be based on a working seed lot system. Cultures derived from the working seed

lots should have the same characteristics as the cultures of the strain from which the master seed lot was derived (A.3.1.1). 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 guidance given in the *Guidelines on Transmissible Spongiform Encephalopathies in Relation to Biological and Pharmaceutical Products (31)* and should be approved by the national regulatory authorities.

Manufacturers are encouraged to avoid wherever possible the use of materials of animal origin.

A.3.1.3 Culture media for the production of pneumococcal polysaccharide

The liquid culture medium used for vaccine production should be free from ingredients that will form a precipitate upon purification of the capsular polysaccharide. If materials of animal origin are used then they should comply with the guidance given in the *Guidelines on Transmissible Spongiform Encephalopathies in Relation to Biological and Pharmaceutical Products (31)* and should be approved by the national regulatory authorities.

Manufacturers are encouraged to avoid wherever possible the use of materials of animal origin.

A.3.1.4 Single harvests

Consistency of growth of *S. pneumoniae* should be demonstrated by monitoring growth rate, pH and the final yield of polysaccharide.

A.3.1.5 Control of bacterial purity

Samples of the culture 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, including plate media that do not support growth of *S. pneumoniae*. If any contamination is found, the culture or any product derived from it should be discarded. The killing process should also be adequately validated.

A.3.1.6 Purified polysaccharide

Each lot of pneumococcal polysaccharide should be tested for identity, purity and molecular size. A number of approaches to determining polysaccharide identity and purity give complementary but incomplete information, so a combination of methods should be employed to provide all necessary data and should be agreed by the national regulatory authority. The purity limits given below are expressed with reference to the polysaccharide in its salt form (sodium or calcium), corrected for moisture. Variations in these specifications

that may be appropriate if unusual salt forms are present should be agreed by the national regulatory authority.

Generally, after killing the organism, the culture is harvested and the polysaccharide isolated and purified by techniques such as fractional precipitation, chromatography, enzyme treatment and ultrafiltration. The polysaccharide is partially purified by fractional precipitation, washed, and dried to a residual moisture content shown to favour the stability of the polysaccharide. Methods used for the purification of bulk polysaccharide should be approved by the national regulatory authority. Purified pneumococcal polysaccharide and, when necessary, partially purified intermediates, are usually stored at or below -20°C to ensure stability.

A.3.1.6.1 **Polysaccharide identity**

A test should be performed on the purified polysaccharide to verify its identity. In cases where other polysaccharides are produced at the same manufacturing site, the method should be validated to show that it distinguishes the desired polysaccharide from all other polysaccharides produced at that manufacturing site.

A serological method such as countercurrent immunoelectrophoresis and/or nuclear magnetic resonance spectrometry (either ^1H or ^{13}C) is convenient for this purpose (32–34). In some cases the identity of the polysaccharide can be deduced from its composition if appropriate analytical methods are employed.

A.3.1.6.2 **Polysaccharide composition**

The composition of the polysaccharide provides information on its purity, identity and the amount of specific impurities, such as pneumococcal C-polysaccharide, that are present. Analyses should be based on the dry weight of the polysaccharide. The composition of the polysaccharide can be defined in a number of ways depending on the methodology employed and the salt form present. The specifications used should be agreed by the national regulatory authority.

Chemically, the composition of pneumococcal polysaccharides can be defined by the percentage of total nitrogen, phosphorus, uronic acid, hexosamine, methyl pentose and *O*-acetyl groups. These are usually determined by a combination of simple wet chemical tests with colorimetric read outs. Typical specifications are listed in Table 1 (35).

Other methods, such as high performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (HPAEC–PAD) applied to hydrolysates of the polysaccharide, may be used to define aspects of the quantitative composition of certain polysaccharide types, but the method should be validated for the purpose (36). ^1H nuclear magnetic resonance spectrometry also provides a convenient approach for quantitation of the composition of the purified polysaccharide if an internal reference compound is included (33, 34). The proportion of pneumococcal C polysaccharide may be determined by a combination of ^1H and ^{31}P nuclear magnetic resonance spectrometry (37, 38) or HPAEC–PAD (39).

Table 1

Theoretical composition of pneumococcal polysaccharides

Serotype	Total nitrogen (%) (range)	Phosphorus (%) (range)	Uronic acid ¹ (%)	Hexosamines ¹ (%)	Methyl pentose ¹ (%)	O-acetyl groups ¹ (%)
1	3.56 (3.5–6)	0 (0–1.5)	55.17 (≥45)	0	0	5.47 (≥1.8)
2	0 (0–1)	0 (0–1.0)	22.59 (≥15)	0	50.58 (≥38)	0
3	0 (0–1)	0 (0–1.0)	60.23 (≥40)	0	0	0
4	4.95 (4–6)	0 (0–1.5)	0	71.84 (≥40)	19.11 (≥10)	0
5	3.04 (2.5–6)	0 (<2)	23.59 (≥12)	44.14 (≥20)	35.22 (≥25)	0
6B	0 (0–2)	4.38 (2.5–5.0)	0	0	22.86 (≥15)	0
7F	2.28 (1.5–4.0)	0 (0–1.0)	0	33.09	26.40 (≥13)	3.5 (present)
8	0 (0–1)	0 (0–1.0)	31.70 (≥25)	0	0	0
9N	3.09 (2.2–4.0)	0 (0–1.0)	23.96 (≥20)	44.82 (≥28)	0	0
9V	1.44 (0.5–3)	0 (0–1.0)	22.33 (≥15)	20.89 (≥13)	0	8.85 (present)
10A	1.12 (0.5–3.5)	2.48 (1.5–3.5)	0	16.21 (≥12)	0	0
11A	0 (0–2.5)	3.25 (2.0–5.0)	0	0	0	13.54 (≥9)
12F	3.82 (3–5)	0 (0–1.0)	19.73 (≥15)	55.36 (≥25)	14.73 (≥10)	0
14	2.03 (1.5–4)	0 (0–1.0)	0	29.44 (≥20)	0	0
15B	1.31 (1–3)	2.89 (2.0–4.5)	0	18.94 (≥15)	0	4.01 (present)
17A	0 (0–1.5)	0 (0–3.5)	16.16 (≥10)	0	24.12 (≥20)	3.2 (present)
17F	0 (0–1.5)	2.93 (0–3.5)	0	0	30.60 (≥20)	4.06 (present)
18C	0 (0–1)	3.05 (2.4–4.9)	0	0	15.96 (≥14)	4.24 (present)
19A	2.27 (0.6–3.5)	5.04 (3.0–7.0)	0	32.98 (≥12)	26.32 (≥20)	0
19F	2.27 (1.4–3.5)	5.04 (3.0–5.5)	0	32.98 (≥12.5)	26.32 (≥20)	0
20	1.28 (0.5–2.5)	0 (1.5–4.0)	0	18.49 (≥12)	0	7.83 (present)
22F	0 (0–2)	0 (0–1.0)	21.30 (≥15)	0	31.80 (≥25)	4.22 (present)
23F	0 (0–1)	3.90 (3.0–4.5)	0	0	40.77 (≥37)	0
33F	0 (0–2)	0 (0–1.0)	0	0	0	4.24 (present)

¹ Typical specifications (35) are given in brackets.

The values are calculated using broad definitions of the classes of sugars, so, for example, "hexosamines" include 2-acetamido-2,6-dideoxyhexoses and 2-acetamido-2-deoxyuronic acids, "methylpentose" includes 2-acetamido-2,6-dideoxyhexoses and "uronic acid" includes 2-acetamido-2-deoxyuronic acids. It is not certain that such sugars would give an identical response in chemical tests used to determine the composition. The values are cited as equivalents of compounds used as references in the relevant tests. The values assume complete O-acetylation at each distinct site for O-acetylation, using published and unpublished data.

A.3.1.6.3 **Moisture content**

If the purified polysaccharide is to be stored as a lyophilized powder, the moisture content should be determined by suitable methods approved by the national regulatory authority and shown to be within agreed limits.

A.3.1.6.4 **Protein impurity**

The protein content should be determined by the method of Lowry *et al.*, using bovine serum albumin as a reference (1, 40), or another suitable validated method. Sufficient polysaccharide should be assayed to detect 1% protein contamination accurately.

Each lot of purified polysaccharide should typically contain not more than 3% by weight of protein. However, this will vary depending upon the serotype and an acceptable level of protein contamination should be agreed with the national regulatory authority.

A.3.1.6.5 **Nucleic acid impurity**

Each lot of polysaccharide should contain not more than 2% by weight of nucleic acid as determined by ultraviolet spectrophotometry, on the assumption that the absorbance of a 1 g/l nucleic acid solution contained in a cell of 1 cm path length at 260nm is 20 (1) or by another validated method.

Sufficient polysaccharide should be assayed to detect 2% nucleic acid contamination accurately.

A.3.1.6.6 **Pyrogen content**

The pyrogen content of the purified polysaccharide should be determined and shown to be within acceptable limits agreed by the national regulatory authority.

A recognized pyrogenicity test can be performed in rabbits. Alternatively, the *Limulus* amoebocyte lysate test can be performed.

A.3.1.6.7 **Molecular size distribution**

The molecular size of the purified polysaccharide in each lot provides an indication of the manufacturing consistency. An acceptable level of consistency should be agreed with the national regulatory authority and can be established either by process validation or measurement on each lot.

The distribution constant (K_D) can be determined by measuring the molecular size distribution of the polysaccharide at the main peak of the elution curve obtained by a suitable chromatographic method. The K_D value and/or the mass distribution limits should be established.

Methods such as gel filtration through Sepharose CL-4B or CL-6B (or similar) in a 0.2 molar buffer using either a refractive index detector or colorimetric assay for the detection of the polysaccharide; and high performance size-exclusion chromatography (HPSEC) with refractive index detectors either alone or in combination with light scattering (e.g. multiple angle laser light scattering (MALLS)) are suitable for this purpose (34, 41). The methodology and column used should be validated to demonstrate sufficient resolution in the appropriate molecular weight range.

A.3.1.7 **Modified polysaccharide**

Modified polysaccharide preparations may be partially depolymerized either before or during the chemical modification. The registered pneumococcal conjugate vaccines and several of the candidate vaccines use polysaccharides and oligosaccharide chains.

A.3.1.7.1 **Chemical modification**

Several methods are available for the chemical modification of polysaccharides before conjugation. The chosen method should be approved by the national regulatory authority.

The current methods used are similar to those employed in the production of conjugate vaccines against *Haemophilus influenzae* type b. For example, polysaccharide may be oxidized with periodate and the periodate-activated polysaccharide attached to free amino groups on the carrier protein by reductive amination. 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.

A.3.1.7.2 **Extent of modification of the polysaccharide**

The manufacturer should demonstrate consistency of the degree of modification of the polysaccharide, either by an assay of each batch of the polysaccharide or by validation of the manufacturing process.

A.3.1.7.3 **Molecular size distribution**

The degree of size reduction of the polysaccharide will depend upon the manufacturing process. The average size distribution (degree of polymerization) of the modified polysaccharide should be determined by a suitable method and shown to be consistent. The molecular size distribution should be specified for each serotype, with appropriate limits for consistency, as the size may affect the reproducibility of the conjugation process.

The molecular size may be determined by gel filtration on soft columns or by HPSEC using refractive index alone, or in combination with laser light scattering (e.g. MALLS) (34, 41).

A.3.2 **Control of the carrier protein**

A.3.2.1 *Microorganisms and culture media for production of carrier protein*

Microorganisms to be used for the production of the carrier protein should be grown in media free from substances likely to cause toxic or allergic reactions in humans. If any materials of animal origin are used in seed preparation or preservation or in production, they should comply with the guidance given in the *Guidelines on Transmissible Spongiform Encephalopathies in Relation to Biological and Pharmaceutical Products (31)* and should be approved by the national regulatory authority.

Production should be based on a seed lot system with the strains identified by a record of their history and of all tests made periodically to verify strain characteristics. Consistency of growth of the microorganisms used should be demonstrated by monitoring the growth rate, pH and final yield of appropriate protein(s).

A.3.2.2 *Characterization and purity of the carrier protein*

Potentially there are many proteins that could be used as carriers in pneumococcal conjugate vaccines. The principal characteristics of the carrier protein should be that it is safe and, in the conjugate, elicits a T-cell dependent immune response against the polysaccharide. 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 the national regulatory authority.

Proteins and purification methods that might be used include:

1. *Tetanus or diphtheria toxoid*. This must satisfy the relevant requirements published by WHO (42) and be of high purity (43).
2. *Diphtheria CRM 197 protein*. This is a non-toxic mutant of diphtheria toxin, isolated from cultures of *Corynebacterium diphtheriae* C7/β197 (44). Protein purity should be greater than 90% as determined by an appropriate method. When produced in the same facility as diphtheria toxin, methods must be in place to distinguish the CRM 197 protein from the active toxin.

The protein carrier should also be characterized. The identity may be determined serologically. Physicochemical methods that may be used to characterize protein include sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE), isoelectric focusing, high-performance liquid chromatography (HPLC), amino acid analysis, amino acid sequencing, circular dichroism, fluorescence spectrophotometry (fluorimetry), peptide mapping and mass spectrometry as appropriate (34).

A.3.3 **Control of monovalent bulk conjugates**

There are a number of possible conjugation methods that might be used for vaccine manufacture; all involve multi-step processes. Both

the method and the control procedures used to ensure the reproducibility, stability and safety of the conjugate should be established for licensing. The derivatization and conjugation process should be monitored by analysis for unique reaction products or by other suitable means. The conditions used in the conjugation process may affect the structure of the polysaccharide chain by causing the loss of labile substituents. Unless the combination of tests used to characterize the bulk monovalent conjugates confirm that the structure is maintained, a test to demonstrate identity of the intact polysaccharide should be performed.

Residual activated functional groups potentially capable of reacting *in vivo* may be present following the conjugation process. The manufacturing process should be validated to show that the activated functional groups do not remain at the conclusion of the manufacturing process and any residual groups are below a limit approved by the national regulatory authority.

After the conjugate has been purified, the tests described below should be performed in order to assess consistency of manufacture. The tests are critical for assuring lot-to-lot consistency.

A.3.3.1 *Identity*

A test should be performed on the monovalent bulk to verify its identity. The method should be validated to show that it distinguishes the desired monovalent material from all other polysaccharides and conjugates produced at that manufacturing site.

A.3.3.2 *Residual reagents*

The conjugate purification procedures should remove residual reagents used for conjugation and capping. The removal of reagents and reaction by-products such as cyanide, 1-ethyl-3,3-(3-dimethylaminopropyl)-carbodiimide (EDAC) and others, depending on the conjugation chemistry, should be confirmed by suitable tests or by validation of the purification process.

A.3.3.3 *Polysaccharide–protein ratio and conjugation markers*

For each batch of the bulk conjugate of each serotype, the ratio of polysaccharide to carrier protein should be determined as a marker of the consistency of the conjugation chemistry. For each conjugate, the ratio should be within the range approved for that particular conjugate by the national regulatory laboratory and should be consistent with vaccine shown to be effective in clinical trials.

For pneumococcal conjugate vaccines the ratio is typically in the range of 0.3–3.0 but varies with the serotype. The ratio can be determined either by independent measurement of the amounts of protein and polysaccharide

present (corrected for unbound protein and unbound polysaccharide), or by methods that 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 ultraviolet, for total material and protein content, respectively).

If the chemistry of conjugation results in the creation of a unique linkage marker (e.g. a unique amino acid), each batch of the bulk conjugate of that serotype should be assessed to quantify the degree of substitution of the carrier protein by covalent reaction with the modified pneumococcal polysaccharide.

The structural complexity and structural differences between the pneumococcal serotypes are such that in most cases a simple conjugation marker will not be able to be identified.

A.3.3.4 *Capping markers*

Each batch should be shown to be free of activated functional groups on either the chemically modified polysaccharide or carrier protein. Alternatively, the product of the capping reaction can be monitored, or the capping reaction can be validated to show removal of unreacted functional groups. Validation of the manufacturing process during vaccine development can eliminate the need to perform this analysis for routine control.

A.3.3.5 *Conjugated and unbound (free) polysaccharide*

Only the pneumococcal polysaccharide that is covalently bound to the carrier protein, i.e. conjugated polysaccharide, is immunologically important for clinical protection. Each batch of conjugate should be tested for unbound or free polysaccharide in order to ensure that the amount present in the purified bulk is within the limits agreed by the national regulatory authority based on lots shown to be clinically safe and efficacious.

Methods that have been used to separate unbound polysaccharide prior to assay, that are potentially applicable to pneumococcal conjugates, include hydrophobic chromatography, acid precipitation, precipitation with carrier-protein-specific antibodies, gel filtration and ultrafiltration. The amount of unbound polysaccharide can be determined by specific chemical or immunological tests, or by HPAEC after hydrolysis.

A.3.3.6 *Protein content*

The protein content of the conjugate should be determined by means of an appropriate validated assay and should comply with limits for the particular product. Each batch should be tested for conjugated and unbound protein.

If possible, the unconjugated protein should also be measured. Appropriate methods for the determination of conjugated and unconjugated protein include HPLC or capillary electrophoresis.

A.3.3.7 *Molecular size distribution of polysaccharide–protein conjugate*

The molecular size of the polysaccharide–protein conjugate is an important parameter in establishing consistency of production and in studying stability during storage.

The relative molecular size of the polysaccharide–protein conjugate should be determined for each bulk using a gel matrix appropriate to the size of the conjugate. The method should be validated with an emphasis on having sufficient specificity to distinguish the polysaccharide–protein conjugate from other components that may be present, e.g. unbound protein or polysaccharide. The size-distribution specifications will be vaccine-specific and should be consistent with lots shown to be immunogenic in clinical trials.

Typically the size may be determined by gel filtration on Sepharose CL-2B, or by HPSEC on an appropriate column. Because the polysaccharide–protein ratio is an average value, characterization of this ratio over the conjugates with their size distribution (e.g. by dual monitoring of the column eluent) can be used to provide further proof of manufacturing consistency (46).

A.3.3.8 *Sterility*

The bulk purified conjugate should be tested for bacterial and mycotic sterility in accordance with the recommendations of Part A, sections 5.1 and 5.2, of the revised Requirements for the Sterility of Biological Substances (47) or by a method approved by the national regulatory authority. If a preservative has been added to the product, appropriate measures should be taken to prevent it from interfering with the test.

A.3.3.9 *Specific toxicity of carrier protein*

The bulk conjugate should be tested for the absence of specific toxicity of the carrier protein where appropriate (e.g. when tetanus or diphtheria toxoids have been used). Absence of specific toxicity of the carrier protein may also be assessed through validation of the production process.

A.3.3.10 *Endotoxin content*

To ensure an acceptable level of endotoxin in the final product, the endotoxin content of the monovalent bulk may be determined and shown to be within acceptable limits agreed by the national regulatory authority.

A.3.4 **Final bulk**

A.3.4.1 *Preparation*

To formulate the final bulk, monovalent conjugate bulks may be mixed together and an adjuvant, a preservative and/or stabilizer is

added before final dilution. Alternatively, the monovalent conjugate bulks may be adsorbed to adjuvant individually before mixing them to formulate the final vaccine.

A.3.4.2 *Sterility*

Each final bulk should be tested for bacterial and mycotic sterility as indicated in section. A.3.3.7.

A.3.5 *Filling and containers*

The recommendations concerning filling and containers given in Annex 1, Section 4 of Good Manufacturing Practices for Biological Products (39) should be applied (29).

A.3.6 *Control tests on final product*

A.3.6.1 *Identity*

An identity test should be performed that demonstrates that all of the intended pneumococcal polysaccharide serotypes are present in the final product, unless this test has been performed on the final bulk.

A serological test, using antibodies specific for the purified polysaccharide may be used.

A.3.6.2 *Sterility*

The contents of final containers should be tested for bacterial and mycotic sterility as indicated in section A.3.3.8.

A.3.6.3 *Pneumococcal polysaccharide content*

The amount of each pneumococcal polysaccharide in the final containers should be determined, and shown to be within the specifications agreed by the national regulatory authority.

The conjugate vaccines produced by different manufacturers differ in formulation. A quantitative assay for each of the pneumococcal polysaccharides in the final container should be carried out. The assays used are likely to be product-specific and might include chromatographic or serological methods. Immunological assays such as rate nephelometry (48) or ELISA inhibition may be used.

A.3.6.4 *Residual moisture*

If the vaccine is freeze-dried, the average moisture content should be determined by methods accepted by the national regulatory authority. Values should be within limits for the preparations shown to be adequately stable in the stability studies of the vaccine.

The test should be performed on 1 vial per 1000 up to a maximum of 10 vials but on no less than 5 vials taken at random from throughout the final lot. The average residual moisture content should generally be no greater than 2.5% and no vial should be found to have a residual moisture content of 3% or greater.

A.3.6.5 *Endotoxin content*

The vaccine in the final container should be tested for endotoxin content by a *Limulus* amoebocyte lysate (LAL) test. 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 national regulatory authority.

A.3.6.6 *Adjuvant content*

If an adjuvant has been added to the vaccine, its content should be determined by a method approved by the national regulatory authority. The amount and nature of the adjuvant should be agreed with the national regulatory authority. If aluminium compounds are used as adjuvants, the amount of aluminium should not exceed 1.25 mg per single human dose.

A.3.6.7 *Preservative content*

The manufacturer has a choice of possible preservatives. Consideration should be given to the stability of the chosen preservative and possible interactions between the vaccine components and the preservative. If a preservative has been added to the vaccine, the content of preservative should be determined by a method approved by the national regulatory authority. The amount of preservative in the vaccine dose should be shown not to have any deleterious effect on the antigen or to impair the safety of the product in humans. The preservative and its concentration should be approved by the national regulatory authority.

A.3.6.8 *General safety test (innocuity)*

The requirement to test lots of pneumococcal conjugate vaccine for unexpected toxicity (abnormal toxicity) should be agreed with the national regulatory authority.

Such a test may be omitted for routine lot release once consistency of production has been well established to the satisfaction of the national regulatory authority and when good manufacturing practice is in place.

A.3.6.9 *pH*

If the vaccine is a liquid preparation, the pH of each final lot should be tested and shown to 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.

A.3.6.10 *Inspection of final containers*

Each container in each final lot should be inspected visually (manually or with automatic inspection systems), and those showing abnor-

malities, lack of integrity and, if applicable, clumping or the presence of particles should be discarded.

A.4 **Records**

The recommendations in section 8 of Good manufacturing practices for biological products (39, Annex 1) should be applied (29).

A.5 **Retained samples**

The recommendations in section 9.5 of Good manufacturing practices for biological products (39, Annex 1) should be applied (29).

A.6 **Labelling**

The recommendations in section 7 of Good manufacturing practices for biological products (39, Annex 1) should be applied with the addition of the following (29).

The label on the carton or the leaflet accompanying the container should indicate:

- the pneumococcal serotype and carrier protein present in each single human dose;
- the amount of each conjugate present in a single human dose;
- the temperature recommended during storage and transport;
- if the vaccine is freeze-dried, that after its reconstitution it should be used immediately unless data have been provided to the licensing authority that it may be stored for a limited time; and
- the volume and nature of the diluent to be added in order to reconstitute a freeze-dried vaccine, specifying that the diluent should be supplied by the manufacturer and approved by the national regulatory authority.

A.7 **Distribution and transport**

The recommendations in section 8 of Good manufacturing practices for biological products (39, Annex 1) should be applied (29).

A.8 **Stability, storage and expiry date**

A.8.1 **Stability testing**

Adequate stability studies form an essential part of the vaccine development process. The stability of the vaccine in its final form and at the recommended storage temperatures should be demonstrated to the satisfaction of the national regulatory authority with final containers from at least three lots of final product made from different independent bulk conjugates.

Given the complexity of these multivalent vaccines, other approaches may be used, with the approval of the national regulatory authority.

The polysaccharide component of conjugate vaccines may be subject to gradual hydrolysis at a rate that may vary depending upon the type of conjugate, the type of formulation or adjuvant, the types of excipient and conditions of storage. The hydrolysis may result in reduced molecular size of the pneumococcal polysaccharide component, a reduction in the amount of the polysaccharide bound to the protein carrier and in a reduced molecular size of the conjugate.

The structural stability of the oligosaccharide chains and of the protein carrier vary between different conjugate vaccines.

Tests should be conducted before licensing to determine the extent to which the stability of the product has been maintained throughout the proposed validity period. The vaccine should meet the specifications for final product up to the expiry date.

Molecular sizing of the final product may be carried out to ensure the integrity of the conjugate. The antigen content of each serotype conjugate may be determined by a quantitative serological assay.

The desorption of antigen from aluminium-based adjuvants, if used, may take place over time. The level of adsorption should be shown to be within limits agreed by the national regulatory authority, unless data are available to show that the immunogenicity of the final product is not dependent upon adsorption of the antigen to the adjuvant.

Accelerated stability studies may provide additional supporting evidence of the stability of the product but cannot replace real-time studies.

When any changes are made in the production procedure that may affect the stability of the product, the vaccine produced by the new method should be shown to be stable.

The statements concerning storage temperature and expiry date appearing on the label should be based on experimental evidence, which should be submitted for approval to the national regulatory authority.

A.8.2 Storage conditions

Storage conditions should be based on stability studies and approved by the national regulatory authority.

Storage of both liquid and freeze-dried vaccines at a temperature of 2–8°C has been found to be satisfactory. The stability of pneumococcal conjugate components varies with serotype of the capsular polysaccharide.

A.8.3 **Expiry date**

The expiry date should be approved by the national regulatory authority and based on the stability of the final product as well as the results of the stability tests referred to in section A.8.1.

Part B. Requirements for national regulatory authorities

B.1 **General**

The general recommendations for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (29) should be applied.

B.2 **Official release and certification**

A vaccine lot should be released only if it fulfils national requirements and/or Part A of these Recommendations.

A statement signed by the appropriate official of the national regulatory authority should be provided at the request of the manufacturing establishments and should certify that the lot of vaccine in question satisfies all national requirements as well as Part A of these Recommendations. The certificate should state the number under which the lot was released by the national controller, and the number appearing on the labels of the containers. Importers of pneumococcal conjugate vaccines should be given a copy of the official national release document. The purpose of the certificates is to facilitate the exchange of vaccines between countries.

B.3 **Reactogenicity and immunogenicity of vaccine in humans**

The national regulatory authority should satisfy itself that adequate control of the pneumococcal conjugate vaccine has been achieved. Clinical data supporting consistency of vaccine production should be obtained prior to registration of the product. Several different lots of the product should be used during the clinical studies and shown to give similar immune responses. Such studies may need to be repeated if changes in production are made, or when the pneumococcal conjugate is intended to be part of a new combination vaccine formulation. The national regulatory authority should ensure that the studies include an adequate number of subjects to provide statistically valid data on reactivity and immunogenicity. The pneumococcal conjugate vaccines are manufactured from purified components by a clearly defined chemical process. Any changes in production or formulation of the vaccine should be reported to the national regulatory authority,

which will decide whether additional clinical data are required on a case-by-case basis. Such a review should take into account the likelihood of such changes affecting the quality, the consistency, the structural integrity and the immunogenicity of the product, and consider the possible cumulative effect of multiple modifications that individually may be regarded as minor.

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Appendix

Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants

The lack of a definitive serological correlate of protection and the multiplicity of antigens involved, especially because the clinical efficacy of several of the individual serotypes represented in the only licensed vaccine has not been established, have been an obstacle for licensure of new formulations or combinations of pneumococcal conjugate vaccines.

WHO undertook a series of consultations to develop serological criteria for the evaluation and licensure of new formulations, combinations or different vaccination schedules for pneumococcal conjugate vaccines. At a consultation held in Alaska in May 2002, a preliminary analysis of data from the efficacy trial in northern California was presented. The results of the analysis showed that a threshold antibody concentration for protection against invasive disease could be estimated using a few simplifying assumptions, and the following relationship between the point estimate of clinical efficacy (VE) and a protective antibody concentration:

$$VE = 1 - \frac{\text{Probability of disease in Vax group}}{\text{Probability of disease in control group}}$$
$$\therefore VE = 1 - \frac{\% \text{ of Vax subjects with } [Ab] < Ab_{\text{protective}}}{\% \text{ of control subjects with } [Ab] < Ab_{\text{protective}}}$$

where Vax group is the vaccinated group and [Ab] is concentration of antibody.

The threshold antibody concentration of 0.20 µg/ml thus derived was supported by a number of other observations. These included:

- the threshold corresponded with the threshold opsonophagocytic antibody titre of 1:8;
- it predicted age-specific disease rates;
- it was consistent with available data from passive immunization using bacterial polysaccharide immune globulin (BPIG) to prevent pneumococcal otitis media and invasive pneumococcal disease;
- it appeared to discriminate clearly between vaccinees who had received conjugate and controls in immunogenicity studies;

- infants with antibody above the threshold showed evidence of priming and a booster response to a subsequent dose of vaccine. The rationale for selecting the threshold antibody concentration is described in more detail in the proceedings of a WHO meeting (1).

On the recommendations arising from this consultation, this analysis was repeated using the pooled immunogenicity and efficacy data from all the completed trials of pneumococcal conjugate vaccines to narrow the confidence limits around the point-estimate of efficacy and to allow additional populations to be represented. The threshold antibody concentration derived from the pooled analysis using the methods described previously was 0.35 µg/ml. Opsonophagocytic antibody titres were available from two of the three studies and analysis of the data showed that antibody concentrations in the range of 0.20–0.35 µg/ml correlated best with an opsonophagocytic antibody titre of 1:8, which in turn correlates best with protective efficacy. The results of the pooled analysis were presented at a second consultation held in June 2003, which was attended by experts in pneumococcal epidemiology and vaccine evaluation, as well as representatives of regulatory agencies. On the basis of the data presented at this consultation, the criteria listed in the following section were recommended for use as a relevant value to establish non-inferiority of a new vaccine when compared to a vaccine against invasive pneumococcal disease that is already licensed. These criteria should not be used to evaluate vaccines against other clinical end-points, e.g. pneumonia and otitis media. It should be noted that immunological responses to pneumococcal conjugate vaccines may vary significantly by population, and a new candidate vaccine shown to be inferior to the licensed vaccine in one population may nevertheless be non-inferior in a second population and may therefore be acceptable in the second population.

The development of standardized assays to evaluate serological responses to new pneumococcal conjugate vaccines has been long pursued by WHO through many consultations. Agreement was reached at a WHO Workshop held in Geneva in 2000 to select one well-characterized pneumococcal ELISA protocol as a reference or benchmark assay for laboratories evaluating serological responses to pneumococcal vaccines and to make the link with the pivotal clinical protection studies carried out during the licensure of the first seven-component conjugate vaccine. Two WHO reference laboratories have been established to help other laboratories set up and standardize their own pneumococcal ELISA and to ensure the comparability and acceptability of the serological data. These reference laboratories

are located at the Institute of Child Health, London, England , and at the Bacterial Respiratory Pathogen Reference Laboratory, The University of Birmingham, Alabama, USA. The detailed protocol for the pneumococcal ELISA, developed with technical assistance from Wyeth Vaccines, Rochester, New York, USA, is available through the Internet site at: www.vaccine.uab.edu.

Primary end-point

The following criteria are recommended for use as the primary end-point for demonstration of non-inferiority against a registered vaccine:

- IgG antibody concentration, as measured by ELISA, in sera collected 4 weeks after a three-dose primary series is considered to be the optimal primary end-point and main licensing parameter.
- A single threshold or reference antibody concentration is recommended for use for all pneumococcal serotypes. A reference antibody concentration of 0.35 µg/ml, that has been determined through a pooled analysis of data from the efficacy trials with invasive disease end-points that have been completed to date, is recommended (1, 2). This threshold does not necessarily predict protection in an individual subject.
- The reference value is defined on the basis of data obtained using ELISA without pre-adsorption with serotype 22F. Antibody concentrations determined using an alternative method will need to be bridged to this method to derive an equivalent threshold concentration. It is recommended that the assay used be calibrated against a reference assay (3).
- Direct clinical comparison of the registered (established) vaccine with the new one is the preferred method for evaluating new vaccine formulations.
- The percentage of responders (those in whom post-immunization antibody concentration is above the threshold) should be used as the criterion to determine non-inferiority.
- For the serotypes present in a registered vaccine, the percentage of responders to each serotype in the new formulation or combination should be compared with the percentage of responders to the same serotype in the registered vaccine in the same population. Non-inferiority to antibody response for each of the serotypes in the registered vaccine is desirable, but not an absolute requirement. Registration of products in which one or more serotypes do not meet non-inferiority criteria would have to be decided on an individual basis.

- Serotypes not contained in a registered formulation may be evaluated for non-inferiority to the aggregate response to the serotypes in the registered vaccine. Failure of one or more new serotypes to meet this criterion may be considered on an individual basis (see example given above).

Additional criteria that must be met to support registration

In addition to showing non-inferiority with respect to the primary end-point, additional data to demonstrate the functional capacity of the antibody and induction of immunological memory in a subset of the sera are required for registration.

Functional antibodies

- Opsonophagocytic activity as measured by opsonophagocytic assay after a three-dose priming series is required to demonstrate the functionality of antibodies.
- The method used to demonstrate opsonophagocytic activity should be comparable to the reference assay (4).

Immunological memory

- Evidence of memory should be demonstrated. One possible method is to administer a booster dose of pneumococcal polysaccharide vaccine and to compare concentrations between age-matched unprimed and primed individuals; data from non-concurrent controls may be sufficient for the purposes of comparison.
- A full dose of polysaccharide vaccine should be used at this stage because the use of a reduced dose of the polysaccharide vaccine as a booster has not been sufficiently tested.
- Avidity of antibodies is also a useful marker for immunological memory.

The following reference reagents and quality control and reference materials are available for the serological assays (also available at: <http://www.vaccine.uab.edu/information.htm>) (see also Tables A1, A2a and A2b).

Pneumococcal ELISA calibration sera: To obtain an aliquot of each of the 12 sera please contact (email preferred):

Dr David Goldblatt

Email: d.goldblatt@ich.ucl.ac.uk

WHO Pneumococcal Reference Laboratory
Institute of Child Health

Table A.1

Materials for pneumococcal assays

Name	Provider	Address	Fax Number	E-mail/web site
C-polysaccharide	Statens Serum Institute	5 Artillerivej DK-2300 Copenhagen S Denmark	45-3268-3167	Serum@ssi.dk
Capsular polysaccharide	American Type Culture Collection (ATCC)	10801 University Boulevard Manassas, VA, 20110-2209 USA	1-703-365-2750	http://www.atcc.org/
89-SF (standard)	Dr Carl Frasch	Center for Biologics Evaluation and Research, US Food and Drug Administration Bethesda, MD USA	1-301-402-2776	Frasch@cber.fda.gov
ELISA Calculation program	Mr Brian Plikkaytis	Centers for Disease Control and Prevention Atlanta, GA USA	1-404-639-2780	http://www.cdc.gov/ncidod/dbmd/bimb/elisa.htm

30 Guilford Street
London WC1N 1EH, England

Sera are stored at, and will be distributed by, the National Institute of Biological Standards and Control, Potters Bar, Herts., England.

Table A.2a

Values assigned to 89-SF (reference serum)

Type ^a	Total antibody (mg/l)	IgG (mg/l) ^b	IgM (mg/l)	IgA (mg/l)
1	10.7	6.3	1.7	1.4
3	7.9	2.4	0.6	4.3
4	7.0	4.1	1.4	1.2
5	10.0	5.8	4.2	1.2
6B	24.3	16.9	3.0	1.5
7F	7.3	5.2	1.9	1.1
9V	10.2	6.9	1.6	1.7
14	37	27.8	1.2	1.9
18C	6.7	4.5	1.3	0.8
19F	18.8	13.0	3.2	2.02
23F	11.9	8.1	0.7	1.3

^a Serotypes 1 and 5 are included in a typical 9-valent vaccine and serotypes 3 and 7F are included in a typical 11-valent vaccine.

^b Source: reference 5, confirmed by CBER, USFDA. The value assigned for 19F is subject to further confirmation.

Table A.2b

Values assigned to 89-SF for additional serotypes

Type ^a	Total Ig (mg/l)	IgG (mg/l)	IgM (mg/l)	IgA (mg/l)
2	21.4	12.2	5.1	3.9
8	11.5	5.1	2.0	2.0
9N	12.7	7.8	2.4	2.1

References

1. Jodar L et al. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine*, 2003, 21:3265–3272.
2. Chang I et al. Serological predictors against invasive pneumococcal disease: a summary of three pneumococcal conjugate vaccine efficacy trials. Paper presented to Consultation Meeting on WHO Guidelines for Production and Quality Control of Pneumococcal Conjugate Vaccines. Geneva, 4–5 June 2003.
3. *Training manual for enzyme linked immunosorbent assay for the quantitation of Streptococcus pneumoniae serotype specific IgG (Pn PS ELISA)*. Available at: <http://www.vaccine.uab.edu/>

4. **Romero-Steiner S et al.** Multilaboratory evaluation of a viability assay for the measurement of opsonophagocytic antibodies specific to the capsular polysaccharide of *Streptococcus pneumoniae* (Pnc). *Clinical and Diagnostic Laboratory Immunology* 2003, **10**:1019–1024.
5. **Quataert et al.** *Clinical and Diagnostic Laboratory Immunology*. 1995, **2**:590–597.