Annex 3

Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines

Replacement of WHO Technical Report Series, No. 927, Annex 2

Recommendations published by WHO are intended to be scientific and advisory. Each of the following sections constitutes guidance for national regulatory authorities and for manufacturers of biological products. If a national regulatory authority so desires, these Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the regulatory authority. It is recommended that any modifications to these Recommendations be made only on condition that they ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the Recommendations set out below. The parts of each section printed in small type are comments intended for the additional guidance of manufacturers and national regulatory authorities that may benefit from those details.

Introduction

These Recommendations provide guidance for the production and control of pneumococcal conjugate vaccines in Part A and for their nonclinical evaluation in Part B. Part C covers the clinical development programme applicable to pneumococcal conjugate vaccines intended primarily for the prevention of invasive pneumococcal disease (IPD) and for administration to infants and toddlers. Clinical assessment of the potential of these vaccines to prevent IPD in older children and adults (including the elderly) or to prevent other types of pneumococcal infection (e.g. pneumonia and otitis media) is not considered in any detail.

General considerations

Infections caused by *Streptococcus pneumoniae* are responsible for substantial morbidity and mortality, particularly in the very young and in the elderly (*1–3*). Pneumococci are grouped into more than 90 serotypes on the basis of their chemically and serologically distinct capsular polysaccharides. Certain serotypes are much more likely than others to be associated with clinically apparent infections, to cause severe invasive infections and to acquire resistance to one or more classes of antibacterial agents (*4*).

The capsular polysaccharides of 23 serotypes are included in licensed non-conjugated polysaccharide vaccines produced by various manufacturers. Non-conjugated pneumococcal polysaccharide vaccines elicit T-cell-independent immune responses; as a result, they do not elicit protective immune responses in children under the age of about 2 years, nor do they induce immune memory. Moreover, they have little or no impact on nasopharyngeal carriage (*5*). However, they are widely recommended for use in the elderly and in subjects from the age of approximately 2 years with underlying medical conditions that put them at high risk of developing IPD (*6*).

The development of pneumococcal conjugate vaccines, in which each of the selected bacterial capsular polysaccharides is coupled with a protein carrier molecule, has been a major advance in the prevention of IPD (*7–10*). In contrast to the 23-valent non-conjugated vaccines, conjugated vaccines induce T-cell-dependent immunity. They are consequently immunogenic in infants under 2 years of age and they elicit immune memory. Since 2006, WHO has recommended that all countries should incorporate pneumococcal conjugate vaccines in routine immunization schedules for children aged less than 2 years and has prioritized their introduction in countries with high child mortality rates and/or high rates of human immunodeficiency virus (HIV) infection (*6*).

A 7-valent pneumococcal conjugate vaccine (7vPnC) that employs CRM197 as the carrier protein for all seven serotypes was the first to be developed. It was first licensed in the USA in 2000 and has subsequently become available in some 90 countries worldwide. Pneumococcal conjugate vaccines that contain three (*11*) or six serotypes, in addition to those in the 7vPnC vaccine, have recently become available in some countries. The 10-valent vaccine includes tetanus toxoid, diphtheria toxoid or a novel protein derived from non-typable *Haemophilus influenzae* (protein D) as the carrier proteins, while the 13-valent vaccine uses only CRM197 as the carrier protein.

Vaccine efficacy against IPD has been evaluated in randomized and controlled studies in children aged less than 2 years. The studies employed the 7vPnC vaccine or an experimental 9vPnC vaccine that included all seven serotypes in the 7vPnC vaccine. At the time that these studies were initiated, no licensed pneumococcal conjugate vaccine was available; control groups therefore did not receive a pneumococcal conjugate vaccine. The studies provided data from the Gambia (*12*), South Africa (*13*) and the USA (for the general population and for native American children) (*7, 8, 14*). The 7vPnC vaccine and the 9vPnC vaccine were shown to be efficacious in preventing IPD, although serotype-specific efficacy could be estimated for only four of the serotypes.

Post-marketing effectiveness data from countries in which the 7vPnC vaccine has been introduced into the routine infant and toddler immunization programmes have shown a reduction in rates of IPD in children aged less than 2 years due to all seven vaccine serotypes and also to serotype 6A, which is not included in the vaccine (*7, 15*). In addition, routine use of the 7vPnC vaccine in infants and toddlers has been associated with reduced rates of IPD in the elderly population, indicating that there is an indirect beneficial effect (i.e. a herd immunity effect) in unvaccinated persons (*15*). Correspondingly, studies have demonstrated that the 7vPnC vaccine reduces rates of nasopharyngeal carriage of serotypes included in the vaccine and of some types that are not included. Thus far, the safety profile of 7vPnC vaccine is considered to be acceptable (*9, 10, 16, 17*).

WHO Recommendations for pneumococcal conjugate vaccine production and control were first established in 2003 and published in the WHO Technical Report Series (TRS 927, Annex 2). In that document, it was considered that practical or ethical considerations might make it impossible to perform protective efficacy trials (i.e. using an unvaccinated control group). The recommendations therefore covered the design of immunogenicity studies necessary to support the licensing of new pneumococcal conjugate vaccines (including those containing conjugated capsular polysaccharides of serotypes additional to those in the 7vPnC vaccine) intended to prevent IPD and for administration to children aged less than 2 years.

It was considered essential that the immunogenicity studies with a new pneumococcal conjugate vaccine should provide a link back to the efficacy against IPD that was demonstrated for the 7vPnC vaccine. Thus, it was recommended that immune responses to each serotype in the 7vPnC vaccine that is also included in a new pneumococcal conjugate vaccine should be directly compared in randomized clinical studies and that the primary comparison of immune responses should be based on serotype-specific IgG antibody concentrations measured by enzyme-linked immunosorbent assay (ELISA). In order to facilitate these comparisons, a WHO reference ELISA assay was established that includes pre-adsorption of sera with pneumococcal C polysaccharide (C-PS) and serotype 22F polysaccharide. Appendix 1 of this document explains these pre-adsorption steps and provides details of the validation, standardization and bridging of ELISA assays.

The immunogenicity data and estimates of vaccine efficacy against IPD across all serotypes in the 7vPnC vaccine were pooled for three of the above-mentioned randomized, controlled efficacy studies in infants and toddlers (see Table A3.1). Serum concentrations of IgG were measured using a well-characterized ELISA method (which differed from the WHO reference ELISA only in that it did not include a 22F adsorption step). For this particular ELISA protocol, it was subsequently shown that the pre-absorption of sera with C-PS and 22F had a minimal effect on estimations of IgG concentrations in a selection of sera from infants who received 7vPnC or 9vPnC vaccines (*18*). On the basis of these data, an IgG antibody concentration of 0.35 µg/ml (assessed using the WHO ELISA) was suggested as a benchmark (or threshold value) for comparing immune responses to each serotype common to the 7vPnC vaccine and a new pneumococcal conjugate vaccine (*19*). The rationale for selecting this threshold antibody concentration is described in more detail in the report of a WHO meeting (*20*). Briefly, results from three clinical trials (Table A3.1) were pooled to derive the threshold value of 0.35 µg/ml. The numbers of IPD cases in the vaccinated and unvaccinated cohorts of each trial, as well as the total number of participants, were summed and used to calculate a pooled estimate of 93% for vaccine efficacy. This vaccine efficacy was then referred to a pooled reverse cumulative distribution (RCD) curve to derive the final 0.35 µg/ml threshold (*18*). Thus, this value is not an average estimate using the trial-specific thresholds listed in Table A3.1 but is derived from the pooled RCD curve.

While this population-derived IgG antibody threshold value is considered to be a useful "benchmark", it is important that it is not interpreted to mean that achievement of ≥ 0.35 µg/ml for a specific serotype (whether included in the 7vPnC vaccine or in a new pneumococcal conjugate vaccine) predicts protection of an individual against PD due to that serotype.

Table A3.1
Estimated antibody concentration threshold using immunogenicity and vaccine efficacy (VE) data from three clinical trials

Study	Patients (per protocol)		VE observed	Estimated threshold	
	Control	PCV		µg/ml	95% CI
NCKP	10 995 (MnCC)	10 940 (7vPCV)	97.4%	0.20	(0.03, 0.67)
American Indian	2 818 (MnCC)	2 974 (7vPCV)	76.8%	1.00	(0.25, 50.00)
South Africa	18 550 (placebo)	18 557 (9vPCV)	90%	0.68	(0.03, 6.00)
Pooled (unweighted)			93%	**0.35**	(0.09, 0.89)
Pooled (weighted)			93%	**0.35**	(0.11, 0.85)

NCKP, North California Kaiser Permanente; MnCC, meningococcal group C conjugate vaccine.
Source: reference *18*.

It was recognized that a threshold based on opsonophagocytic assay (OPA) titres (which reflect functional antibody) might also be suitable for comparing immune responses between vaccines, and it was recommended that OPA data should be generated for a subset of vaccinated subjects in clinical studies. The limited data obtained during the protective efficacy studies conducted with the 7vPnC vaccine indicated that an IgG concentration ≥0.2 µg/ml (determined without 22F pre-adsorption of sera) corresponded approximately to an OPA titre ≥1:8 for some serotypes (*20*). Methods for determining OPA are also discussed in Appendix 1.

Prompted by issues raised during the development of newer pneumococcal conjugate vaccines since the publication of TRS 927 in 2003, WHO held a consultation in 2008 (*21*) to consider new scientific evidence and discuss the need to provide revised guidance for manufacturers and licensing authorities. Inter alia, the consultation reviewed effectiveness data obtained with various immunization schedules for the 7vPnC vaccine in Canada (*22*), the United Kingdom (*23*) and the USA (*7*). Technical developments in ELISA and OPA methods, variability between assays and the need for standardization were considered. The importance of bridging new assays to the WHO reference ELISA method when determining IgG concentrations was discussed, along with the option of establishing an assay-specific alternative threshold value to ≥0.35 µg/ml.

During the 2008 consultation, some data were provided that supported the use of the IgG antibody threshold as a benchmark value. For example, data from the United Kingdom had shown that only 30–50% of infants reached the threshold of ≥ 0.35 μg/ml against 6B after two doses of 7vPnC vaccine at 2 and 4 months of age and that this was associated with vaccine failures due to 6B in the interval between the second dose and the third dose at 13 months. However, previous and newer data suggested that IgG antibody concentrations below 0.35 μg/ml may be sufficient to prevent IPD due to some serotypes. In addition, some data suggested that OPA titres against certain serotypes (e.g. 19A) correlated better with estimates of effectiveness than IgG concentrations when measured using the WHO reference assay (*24*).

Overall it was considered that some of the information accrued since 2003 merited incorporation into updated WHO Recommendations for pneumococcal conjugate vaccines. Most of the revisions pertain to the clinical assessment of new pneumococcal vaccines.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 Proper name

The proper name of the vaccine shall 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 specifications 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 control or reference laboratories:

- C-polysaccharide (Statens Serum Institut, Copenhagen, Denmark);
- capsular polysaccharides (American Type Culture Collection, Manassas, VA, USA);

- 89-SF reference serum (Center for Biologics Evaluation and Research, Washington, DC, USA);
- 96DG secondary reference serum (provided by Dr David Goldblatt and distributed by National Institute for Biological Standardization and Control, Potters Bar, England);
- ELISA calibration sera (provided by Dr David Goldblatt and distributed by National Institute for Biological Standardization and Control, Potters Bar, England);
- pneumococcal serotyping reagents (Statens Serum Institut, Copenhagen, Denmark);
- HL-60 cells (American Type Culture Collection, Manassas, VA, USA or European Collection of Cell Cultures, Porton Down, Salisbury, England).

A.1.4 Terminology

The definitions given below apply to the terms used in these Recommendations. They may have different meanings in other contexts.

Master seed lot. A bacterial suspension of *Streptococcus 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 *Streptococcus pneumoniae* organisms derived from the master seed lot by growing the organisms and maintaining them in aliquots in the freeze-dried form or 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 requirements

The general manufacturing recommendations contained in Good manufacturing practices for pharmaceutical products (*25*) and Good manufacturing practices for biological products (*26*) should be applied 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 (NRA). All assay procedures used for quality control of the conjugate vaccines and vaccine intermediates must be validated. As may be required, proposals for the modification of manufacturing and control methods should also be submitted for approval to the NRA before they are implemented.

Streptococcus pneumoniae is a Biosafety Level 2 (BSL-2) pathogen and represents a particular hazard to health through infection by the respiratory route. The organism should be handled under appropriate conditions for this class of pathogen (*27*). 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 NRA. 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 made 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 inulin; 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 (NMR) spectroscopy (either ^{1}H 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, for preservation of strain viability for freeze-drying or for frozen storage, they should comply with *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*28*) and should be approved by the NRA.

Wherever possible, manufacturers are encouraged to avoid 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, they should comply with *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*28*) and should be approved by the NRA.

Wherever possible, manufacturers are encouraged to avoid 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 NRA. 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 NRA.

> Generally, after the organism is killed, 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 its stability. Methods used for the purification of bulk polysaccharide should be approved by the NRA. 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 on the same manufacturing site, the method should be validated to show that it distinguishes the desired polysaccharide from all other polysaccharides produced on that manufacturing site.

> A serological method such as countercurrent immunoelectrophoresis and/or NMR spectroscopy (either ^{1}H or ^{13}C) is convenient for this purpose (*29–31*). In some cases, if appropriate analytical methods are employed, the identity of the polysaccharide can be deduced from its composition.

A.3.1.6.2 *Polysaccharide composition*

The composition of the polysaccharide provides information on its purity, identity and the amounts 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 (Table A3.2). The specifications used should be agreed by the NRA.

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 tabulated below (*32*); they may be adapted when other methods such as ^{1}H-NMR are used.

Other methods, such as high-performance anion exchange chromatography (HPAEC) with electrochemical detection, 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 (*33*). NMR spectroscopy (^{1}H) is also a convenient means of quantitatively defining the composition of the purified polysaccharide if an internal reference compound is included (*30*, *31*). The proportion of pneumococcal C polysaccharide may be determined by a combination of ^{1}H and ^{31}P NMR spectroscopy (*34*, *35*) or HPAEC-PAD (*36*).

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 NRA 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 (*37*), 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 NRA.

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 spectroscopy – on the assumption that the absorbance of a 1 g/l nucleic acid solution contained in a cell of 1 cm path length at 260 nm is 20 (*38*) – or by another validated method.

> Sufficient polysaccharide shall 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 NRA.

> 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 each lot of purified polysaccharide provides an indication of the manufacturing consistency. An acceptable level of consistency should be agreed with the NRA and can be established either by process validation or by 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 suitable for this purpose include: gel filtration through Sepharose CL-4B or CL-6B (or similar) in a 0.2 M buffer using either a refractive index detector or colorimetric assay for 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) (*31, 39*). 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. Pneumococcal conjugate vaccines use polysaccharides and oligosaccharide chains.

A.3.1.7.1 *Chemical modification*

Several methods for the chemical modification of polysaccharides prior to conjugation may be satisfactory. The chosen method should be approved by the NRA.

The methods used currently 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; a bifunctional linker is added, which then allows the polysaccharide to be attached to the carrier protein either 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. Depending on the conjugation chemistry used, consistency in degree of polysaccharide activation may be determined as part of process validation or reflected by characteristics of vaccine lots shown to have adequate safety and immunogenicity in clinical trials.

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) (*31*, *39*). An alternative method shown to correlate to molecular size distribution (e.g. measurement of viscosity) may be used to show consistency with size reduction of the polysaccharide.

Table A3.2

Theoretical composition of pneumococcal polysaccharides[a]

Serotype	Total nitrogen (%) (range)	Phosphorus[a] (%) (range)	Uronic acid (%)	Hexosamines (%)	Methyl pentose (%)	*O*-acetyl groups (%)
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 (≥5)	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)

continues

Table A3.2 *continued*

Serotype	Total nitrogen (%) (range)	Phosphorus[a] (%) (range)	Uronic acid (%)	Hexosamines (%)	Methyl pentose (%)	*O*-acetyl groups (%)
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	40.77 (≥37)	0
33F	0 (0–2)	0 (0–1.0)	0	0	0	4.24 (present)

[a] Theoretical value with suggested range in parentheses, based on published structures. These are calculated using broad definitions of the classes of sugars; so, for example "hexosamine" includes 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 probable reference compounds used in such compositional tests. The values assume complete *O*-acetylation at each distinct site for *O*-acetylation, using published and unpublished data.

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 *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*28*) and should be approved by the NRA.

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

There are many proteins that could potentially 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 NRA.

Proteins and purification methods that might be used include:

Tetanus or diphtheria toxoid. This must satisfy the relevant Requirements published by WHO (*40*) and be of high purity (*41*).

Diphtheria CRM 197 protein. This is a non-toxic mutant of diphtheria toxin, isolated from cultures of *Corynebacterium diphtheriae* C7/β197 (*42*). 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.

Protein D derived from non-typable *Haemophilus influenzae*. The routine release should include tests to confirm identity and purity of the protein as approved by the NRA, supplemented by additional data to characterize the protein.

> The protein carrier should also be characterized. The identity may be determined serologically. Physicochemical methods that may be used to characterize protein include sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE), isoelectric focusing, high-performance liquid chromatography (HPLC), amino acid analysis, amino acid sequencing, circular dichroism, fluorescence spectroscopy, peptide mapping and mass spectrometry as appropriate (*31*).

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 chemistry may affect the structure of the polysaccharide chain by causing the loss of labile substituents. Unless the results of the tests used to characterize the bulk monovalent conjugate can provide information on structural changes, an explicit identity test on the polysaccharide present 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 no activated functional groups remain at the conclusion of the manufacturing process or that the level of any remaining groups is below a limit approved by the NRA.

After the conjugate has been purified, the tests described below are usually performed on non-adsorbed conjugate bulks. Alternatively, they may be performed on adsorbed monovalent conjugate bulks, e.g. in case individual conjugate bulks are adsorbed to adjuvant before final formulation of the vaccine. 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 on 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.

> The residuals are process-specific and can be quantified by use of colorimetric and chromatographic assays. Techniques such as NMR spectroscopy and hyphenated techniques such as liquid chromatography–mass spectrometry may also be applied.

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 NRA and should be consistent with vaccine shown to be effective in clinical trials.

> For pneumococcal conjugate vaccines the ratio is typically in the range 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, or by methods that give a direct measure of the ratio. Methods include 1H NMR spectroscopy or the use of HPSEC with dual monitoring (e.g. refractive index and UV, 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 extent of substitution of the carrier protein by covalent reaction of the pneumococcal polysaccharide with the carrier protein.

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

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 the 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 establish consistency of production and to ensure that the amount present in the purified bulk is within the limits agreed by the NRA based on lots shown to be clinically safe and efficacious.

> Methods that have been used to separate unbound polysaccharide before assay, and 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 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 and capillary electrophoresis.

A.3.3.7 Molecular size distribution

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 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 examined by gel filtration on Sepharose CL-2B or by HPSEC on an appropriate column. Since the polysaccharide–protein ratio is an average value, characterization of this ratio over the size distribution (e.g. by dual monitoring of the column eluent) can be used to provide further proof of manufacturing consistency (*43*).

A.3.3.8 Sterility

The bulk purified conjugate should be tested for bacterial and mycotic sterility in accordance with the requirements of Part A, sections 5.1 and 5.2, of the revised General requirements for the sterility of biological substances (*44*) or by a method approved by the NRA. 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 NRA.

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, preservative and/or stabilizer 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.3.3.8.

A.3.5 Filling and containers

The recommendations concerning filling and containers given in Good manufacturing practices for biological products should be applied (*26*).

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 and carrier protein(s) 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 NRA.

The conjugate vaccines produced by different manufacturers differ in formulation. A quantitative assay should be carried out for each of the pneumococcal polysaccharides in the final container. The assays used are likely to be product-specific and might include chromatographic or serological methods. Immunological assays such as rate nephelometry (*45*) or ELISA inhibition may be used.

Assessment of the content of each serotype in the final vaccine may be difficult and may require complex methodologies not available to national control laboratories (NCLs). In the event that testing is performed in the framework of lot release by NCLs, measurement of the total polysaccharide content could therefore be authorized.

A.3.6.4 Residual moisture

If the vaccine is freeze-dried, the average moisture content should be determined by methods accepted by the NRA. Values should be within the 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 test (LAL). 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 NRA.

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 NRA. The amount and nature of the adjuvant should be agreed with the NRA. 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 NRA. 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 NRA.

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 NRA.

> Such a test may be omitted for routine lot release once consistency of production has been well established to the satisfaction of the NRA 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 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 abnormalities such as improper sealing, 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 (*26*) should be applied.

A.5 Retained samples

The recommendations in section 9.5 of Good manufacturing practices for biological products (*26*) should be applied.

A.6 Labelling

The recommendations in section 7 of Good manufacturing practices for biological products (*26*) should be applied with the addition of the following:

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 showing that it may be stored for a limited time;
- 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 NRA.

A.7 Distribution and transport

The recommendations in section 8 of Good manufacturing practices for biological products (*26*) should be applied.

A.8 Stability, storage and expiry date

A.8.1 Stability testing

Adequate stability studies form an essential part of the vaccine development studies. These studies should follow the general principles outlined in Guidelines on stability evaluation of vaccines (*46*). The stability of the vaccine in its final form and at the recommended storage temperatures should be demonstrated to the satisfaction of the NRA 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 NRA.

The polysaccharide component of conjugate vaccines may be subject to gradual hydrolysis at a rate that may vary with the type of conjugate, the type of formulation or adjuvant, the type of excipients and conditions of storage. The hydrolysis may result in reduced molecular size of the pneumococcal polysaccharide component, in 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 not be feasible. However, to ensure that the integrity of the conjugate is preserved, molecular sizing may be carried out at an intermediate level, before formulation of the multivalent vaccine. 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 NRA, 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 NRA.

A.8.2 Storage conditions

Storage conditions should be based on stability studies and approved by the NRA.

> 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 NRA 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. Nonclinical evaluation of new pneumococcal conjugate vaccines

Details on the design, conduct, analysis and evaluation of nonclinical studies are available in WHO guidelines on nonclinical evaluation of vaccines (*47*).

Nonclinical testing is a prerequisite for the initiation of clinical studies in humans and includes immunogenicity studies (proof of concept) and safety testing in animals. The vaccine lots used in nonclinical studies should be adequately representative of the formulation intended for clinical investigation and, ideally, should be the same lots used in clinical studies. If this is not feasible, the lots used clinically should be comparable to those used in the nonclinical studies in terms of potency, stability and other characteristics of quality.

With specific regard to pneumococcal conjugate vaccines, studies in animals would be expected to provide data on immune responses to the vaccine as part of the routine assessment of toxicokinetics. No single species can be recommended for these studies but manufacturers may find it useful to look at the data that have been generated for licensed pneumococcal conjugate vaccines that are in the public domain. It is important to appreciate that these data do not reliably predict a dose or range of doses of antigens that might be appropriate for study in humans, but such studies should demonstrate that a new pneumococcal vaccine elicits boostable immune responses in animals.

Part C. Clinical evaluation of pneumococcal conjugate vaccines

C.1 Considerations for clinical studies

This section addresses some issues that are specific to, or particularly relevant for, the clinical development of pneumococcal conjugate vaccines. The recommendations made should be considered in conjunction with the general principles described in Guidelines on clinical evaluation of vaccines: regulatory expectations (*48*) and should be viewed in the light of data on the safety, immunogenicity and effectiveness of pneumococcal conjugate vaccines that may become available in the future.

The section does not make any recommendations for the selection of serotypes to be included in a new pneumococcal conjugate vaccine. The selection process should take into consideration the relative frequencies of serotypes that cause IPD in the target population in different geographical regions.

Section C.2 considers the content of the clinical development programme applicable to pneumococcal conjugate vaccines intended primarily for the prevention of IPD and for administration to infants and toddlers. For reasons explained in General considerations, the potential efficacy of new pneumococcal conjugate vaccines for preventing IPD in this age group will be assessed based on studies of immune responses. Specific consideration is given to the immune response parameters of interest, the selection of licensed comparator vaccines, comparisons of immune responses to serotypes included in a new vaccine and in licensed comparator(s), and evaluation of immune responses to serotypes that are included only in a new vaccine.

Section C.3 briefly considers the clinical assessment of the potential for pneumococcal conjugate vaccines to prevent IPD in older children and adults (including the elderly) and to prevent non-invasive pneumococcal infections (e.g. pneumonia or otitis media).

Section C.4 considers the data on safety and effectiveness that should be collected following first approval of a new pneumococcal conjugate vaccine.

C.2 Assessment of immune responses

C.2.1 Assays to assess serotype-specific antibody responses

Immune responses to pneumococcal conjugate vaccines can be assessed by:

- Determination of serotype-specific IgG antibody geometric mean concentrations (GMCs) based on measurement of binding to polysaccharides (e.g. using an ELISA method). Appendix 1 provides a detailed consideration of the development and standardization of ELISA methods, including:
  - alternative methods to ELISA for measurement of serotype-specific IgG concentrations;
  - the need to use a reference standard and quality control (QC) sera for IgG assays;
  - the need to bridge new assays (whether ELISA or not) to the WHO reference assay and the option of deriving alternative threshold values when using new assays that correspond to 0.35 μg/ml based on a well-justified rationale.
- Determination of serotype-specific functional antibody titres using an OPA (*49*). The conduct of OPAs is addressed in Appendix 1.

When comparing immune responses to pneumococcal conjugate vaccines following completion of the infant immunization series, it is recommended that the primary analysis should be based on IgG concentrations (see C.2.2.1). Secondary analyses should include a comparison of OPA titres (see C.2.2.2). The assessment of immune responses to booster doses is discussed in section C.2.3.

C.2.2 Evaluation of immune responses following the primary series

C.2.2.1 Selection of licensed comparator(s)

As long as the 7vPnC vaccine that has been evaluated in clinical studies of protective efficacy remains on the market it is recommended that the immune responses to this vaccine and to a new pneumococcal conjugate vaccine should be directly compared in prospective randomized studies in infants. Such studies provide the basis for bridging the protective efficacy conferred by the 7vPnC vaccine against IPD that was demonstrated in randomized controlled studies and in post-licensure studies of effectiveness to the new vaccine on the basis of comparable serotype-specific immune responses.

It is expected that the 7vPnC vaccine will become unavailable at some time in the future. Comparisons of immune responses should therefore be made between a new vaccine and at least one licensed vaccine for which immune responses were directly compared with the 7vPnC vaccine during the clinical development programme. Thus, licensure of a new pneumococcal conjugate vaccine would be based on a “bridge to a bridge” back to the data on efficacy and effectiveness for the seven serotypes in the 7vPnC vaccine.

Selection of the licensed pneumococcal conjugate vaccine(s) to be used as the comparator(s) will require very careful justification and must be discussed with NRAs. It is recommended that preference be given to selecting licensed comparators for which some effectiveness data are already available that lend support to the immunogenicity data on which their approval was based, together with a substantial safety database. Consideration should also be given to choosing licensed comparator(s) that have the highest number of serotypes in common with the new vaccine.

NRAs may be reluctant to approve a new pneumococcal conjugate vaccine on the basis of comparison with vaccines that are not actually licensed in their countries. However, once several pneumococcal conjugate vaccines have been approved in various countries, it may not be feasible for a new vaccine to be compared with every licensed vaccine. It is recommended that NRAs consider the acceptability of the licensed comparators used in clinical studies based on all the data available in the public domain regarding their safety, immunogenicity and effectiveness.

Whatever the licensed comparator(s) selected for clinical studies, comparisons of immune responses should follow the guidance provided in the following sections. Assessments of immune responses to serotypes that are and are not common to the new vaccine and the licensed comparator(s) require different approaches as described in C.2.2.3.

C.2.2.2 Schedules and populations

Immune responses to pneumococcal conjugate vaccines vary according to the schedule used, the population studied, and the antigen composition and nature of the vaccines that are administered concomitantly. It is not feasible to study new vaccines with every possible schedule in current use or in a very large range of geographical regions, nor is it possible to evaluate the effects of concomitant administration with a large range of vaccines in routine use (see section C.2.5). Manufacturers should justify the relevance of the clinical data provided to each country in which approval is sought and should discuss the basis for extrapolation of the findings.

For example, immune responses following a 2, 3 and 4 months schedule within a specific population are usually lower than response following a more relaxed 3-dose schedule (e.g. 2, 4 and 6 months). Documentation of satisfactory immune responses with the former schedule therefore supports the expectation that satisfactory immune responses would also be observed with the latter schedule. However, the local and systemic reactogenicity associated with a vaccine may also differ between schedules within a specific population, so that there is still a need to collect some safety data with other schedules proposed for approval (e.g. 2, 4 and 6 months).

Manufacturers may also choose to investigate immune responses after two doses in infancy (such as dosing at 2 and 4 months or 3 and 5 months). An exploration of immune responses after two or three doses in infants is to be encouraged since it is possible that, for certain vaccines administered according to specific schedules, there is no advantage in a third dose. The importance of assessing immune responses to additional doses after completion of any infant immunization series is addressed in section C.3.

C.2.2.3 Primary analysis

In the following sections the references to percentages reaching IgG concentrations ≥0.35 μg/ml are based on the WHO reference ELISA, as explained in General considerations and in Appendix 1. It is recognized in section C.2.1 and in Appendix 1 that it may be acceptable for manufacturers to employ an alternative and well-justified threshold value when using a specific in-house assay. Any alternative threshold value that is proposed should be shown to correspond to 0.35 μg/ml in a well-conducted bridging assay against the WHO reference ELISA. If the justification for using an alternative threshold value is considered to be acceptable, it would be used wherever the text that follows mentions 0.35 μg/ml.

The primary analysis should be based on IgG concentrations measured approximately 4 weeks after completion of the primary infant immunization series; IgG responses to serotypes shared between a new vaccine and the licensed comparator and to serotypes found only in a new vaccine should be regarded as co-primary but the analyses require different approaches as described below. The predefined margins of non-inferiority for each end point should be justified (*50*, *51*) and the effects of multiplicity should be taken into consideration in the statistical analysis plan. It is essential that the sample size is large enough to provide adequate power for the planned analyses; however, manufacturers may be able to provide justification for basing the calculation of sample size on a specific parameter if the total study size would otherwise become unmanageable. Such proposals need to be reviewed on a case-by-case basis.

There should at least be a measurable immune response to each serotype included in the new vaccine. Protocols should propose a definition for a measurable response that takes into account the performance characteristics of the assay.

For the serotypes common to the new vaccine and the licensed comparator

The end-points used in the primary analysis should be:

- the percentage of subjects with IgG ≥0.35 μg/ml, *and*
- the serotype-specific IgG GMC ratios.

It may be that the IgG responses to one or more serotypes meet the predefined non-inferiority criteria applied to percentages reaching the threshold value but do not meet the predefined non-inferiority criteria applied to the comparison of GMCs – or vice versa. In this situation, meeting one of the two sets of criteria should be considered adequate for approval. If IgG responses for one or more serotypes fail to meet both sets of criteria, the NRA should take into consideration the disease burden associated with the serotype(s) when considering whether or not to approve the vaccine. In addition, if effectiveness data are already available for use of the new vaccine in other countries or regions, these may be used to assist the decision-making process. It may also be helpful to take into account the secondary immunogenicity analyses.

For serotypes found only in the new vaccine

Based on the serotype-specific demonstration of efficacy and effectiveness of the 7vPnC vaccine, there is a reasonable rationale for comparing proportions that achieve ≥0.35 μg/ml against each serotype contained only in the new vaccine with any serotype in the licensed comparator that achieves the lowest percentage ≥0.35 μg/ml.

In the event of failure to elicit an IgG response to one or more serotypes that is at least comparable with the lowest response to any of the serotypes common to both vaccines, the issues mentioned above with regard to disease burden and any existing effectiveness data would again need to be taken into account.

If the NRA considers that, in the situations described above, it would still be appropriate to approve the new vaccine it is recommended that:

- The prescribing information makes clear the possible limitations of vaccine efficacy.
- Attention should be paid to the feasibility of estimating vaccine effectiveness in the post-approval period for the specific serotype(s) for which the predefined criteria were not met. The post-approval data may be used to indicate that the immune responses to the serotype(s) are sufficient to confer some protection against IPD. The feasibility and speed with which data could be generated will depend on the frequency of IPD associated with the serotype(s) in question. The generation of effectiveness data is considered in section C.2.4 below.

C.2.2.4 Secondary analyses

IgG concentrations

Since there is no definitive serotype-specific immunological correlate of protection established for pneumococcal conjugate vaccines, it is most important that the primary analysis of immune responses following completion of the infant immunization series is accompanied by other comparisons, including RCD plots. For any serotype that is common to the vaccines that have been compared, the RCDs should be carefully scrutinized for any divergence of the curves. If divergence is observed, it is recommended that attention be given to the feasibility of generating serotype-specific vaccine effectiveness data, as mentioned above and in section C.2.4. RCD plots should also be generated for serotypes found only in the new vaccine but the review of these data should be seen as exploratory.

OPA data

The functional antibody responses (based on OPA assay data) to individual serotypes should be determined in a randomized subset of vaccinated subjects within some or all of the clinical studies. The OPA assay used by an individual manufacturer should be well validated. Issues surrounding the conduct of OPA assays are considered in Appendix 1.

At present, the interpretation of OPA data is made difficult by the fact that, while reaching a titre ≥1:8 indicates the presence of functional antibody, a titre that might correlate with protection against IPD due to any one serotype is unknown. For this reason it is recommended that comparisons of OPA titres that are common to the new vaccine and the licensed comparator focus on serotype-specific geometric mean titre (GMT) ratios. In addition, the serotype-specific RCD plots should be compared. OPA GMTs and RCD plots should also be generated for serotypes found only in the new vaccine but the review of these data should be seen as exploratory.

C.2.2.5 Other possible analyses

Manufacturers may choose to evaluate other parameters that are of interest but would not currently be seen as essential for study and inclusion in the application dossier. These include:

- antibody avidity; and
- effects on nasopharyngeal carriage, which may be assessed before and/or after initial approval.

C.2.3 Post-primary series (booster) doses

C.2.3.1 Immune memory

The clinical development programme should generate data to demonstrate that a new pneumococcal conjugate vaccine induces an immune memory response during the infant immunization series. These data can be obtained as part of the assessment of immune responses to booster doses of the new vaccine (see below). Administration of a non-conjugated pneumococcal vaccine (e.g. 23-valent polysaccharide vaccine) to children aged less than 2 years, who received conjugated vaccine in infancy, for the purpose of assessing prior induction of immune memory is not recommended. There are concerns that this practice may result in immune hyporesponsiveness on further encounters with pneumococcal polysaccharides (i.e. on natural exposure or on receipt of further doses of a pneumococcal conjugate vaccine).

C.2.3.2 Rationale for assessing responses to post-primary series (booster) doses

The effectiveness data currently available from the routine use of the 7vPnC vaccine in developed countries are based on administration of 2 or 3 doses during infancy and a booster dose in the second year of life (from 11 months onwards). Experience gained with other polysaccharide conjugate vaccines has indicated the importance of immunological memory, adequate circulating antibody, and indirect (herd) protection to provide protection against invasive disease. Although clinical trials in developing countries have demonstrated the efficacy of the experimental 9vPnC vaccine over approximately 3–6 years following administration to infants on the EPI schedule without a subsequent dose in the second year of life (*52*) it remains to be seen whether this immunization strategy will provide long-term protection against IPD comparable with that achieved by regimens that employ a post-primary series booster dose. In addition, children at particular risk of IPD and/or with immunodeficiency probably need a post-primary series booster dose (*53*).

Clinical development programmes for new pneumococcal conjugate vaccines should therefore include studies in which immune responses to booster doses are measured and compared with responses to a licensed comparator(s) in a predefined secondary analysis. However, the optimal timing of the booster dose is unknown and probably varies according to the schedule and the concomitant vaccines in the infant immunization series. In most cases, booster doses are given at least 6 months after the last dose of the primary series and between the ages of 12 and 24 months but in some settings there may be reasons to boost earlier (e.g. at around 9 months). Ideally, clinical studies should investigate administration of booster doses at various times, although it must be recognized that it is not feasible to examine all possible permutations. Some of these data may be generated after initial approval of a new vaccine.

It is recommended that subsets of subjects be identified for longer-term follow-up of persistence of immunity after administration of booster doses. These data may be provided after first approval. Waning of antibody concentrations over time is inevitable and should not be interpreted per se to indicate the need for a booster dose. It is important that longer-term antibody concentrations be viewed in conjunction with effectiveness data to assess the potential need for additional doses later in life to maintain protection.

C.2.3.3 Comparisons of immune responses to booster doses

The evaluation of immune responses to booster doses should be based primarily on comparisons of immune responses at approximately 4 weeks post-booster dose between groups of children who received the same pneumococcal conjugate vaccine (i.e. either the new vaccine or the licensed comparator) for the primary series and for boosting. Induction of immune memory during infancy should be associated with higher post-boost antibody concentrations in subjects who received a primary series in infancy than in age-matched unvaccinated children. If there is already routine use of licensed pneumococcal conjugate vaccine(s) in infants at study sites, it will not be impossible to compare responses to a single dose in the second year of life between previously vaccinated and unvaccinated groups for serotypes that are common to both vaccines. However, an assessment of booster responses to any additional serotypes in the new vaccine could be made by administering it to a subset of children who received the licensed comparator in infancy.

Measurement of pre- as well as post-boost antibody concentrations necessitates collection of an extra blood sample and is not considered to be necessary in all studies. However, it is preferred that at least some information on pre-boost antibody concentrations and/or titres should be generated during the clinical development programme. One possible way to do this is to randomly assign subjects to provide either a pre-boost or a post-boost blood sample at the time of initial randomization to vaccine group. These data allow changes in antibody levels from post-primary series to pre-booster to be assessed. In most studies, post-boost blood samples are obtained 4 weeks after the dose. The increase in antibody levels would be expected to start very early in those who are already primed. Some exploration of immune responses at less than 4 weeks post-booster dose in randomized subsets could be informative.

Immune responses to booster doses of pneumococcal conjugate vaccines would be expected to be very high for each of the serotypes included in the vaccine given in the infant immunization series. For this reason, comparisons between vaccine groups based on percentages reaching serotype-specific IgG concentrations ≥0.35 μg/ml or OPA titres ≥1:8 (or other relatively low cut-off values) would not be helpful because they would not adequately detect any differences between vaccine groups. It is therefore recommended that the comparisons of responses to booster doses focus on the ratio of the post-booster value to the post-primary value for the IgG GMCs or the OPA GMTs. It is also important that RCD plots are provided and inspected for any divergence in the curves.

C.2.4 Immune responses to carrier proteins

To date, the carrier proteins used in licensed pneumococcal conjugate vaccines have included a non-toxic diphtheria toxin molecule (CRM197), diphtheria toxoid, tetanus toxoid and Protein D from *Haemophilus influenzae*.

Administration of pneumococcal conjugate vaccines that employ diphtheria or tetanus toxoid or CRM197 as carrier(s) has been found to enhance the relevant anti-toxin antibody levels, but not to a sufficient extent to replace routine immunization with diphtheria or tetanus toxoid-containing vaccines. Co-administration of a new pneumococcal conjugate vaccine with routine infant and toddler vaccines (i.e. containing diphtheria and tetanus toxoids) could result in high anti-toxin levels. Careful attention should be paid to the reactogenicity observed in these circumstances of use since increased rates of some reactions could be associated with high anti-toxin levels. As discussed in section C.2.5, data should be generated on anti-toxin levels on co-administration of a new pneumococcal conjugate vaccine with representative licensed vaccines in routine use.

It is possible that a carrier protein itself might elicit an immune response that confers some protection against an infectious disease. If a manufacturer wished to pursue such a claim, an appropriate clinical development programme would need to be discussed with NRAs.

C.2.5 Concomitant administration with other vaccines

Accumulation of data on the safety and immunogenicity of new pneumococcal conjugate vaccines when co-administered with other infant and toddler vaccines is essential. Concomitant administration of polysaccharide conjugates with other vaccines in routine use, which can include other conjugated vaccines, may give rise to lower immune responses to one or more of the co-administered antigens (i.e. immune interference), although the clinical significance of the observed phenomena is not always clear. The data on the effects of co-administration that are available at the time of initial licensure may be expanded in post-approval studies. It is sufficient that only some of the clinical studies include a formal assessment of the effects of co-administration on immune responses.

Studies of the effects of co-administration should include vaccines that are representative of types that, for reasons of convenience and compliance, are very likely to be given at the same clinic visits during routine use of a new pneumococcal conjugate vaccine. Because of the very large range of licensed vaccines that may need to be co-administered with pneumococcal conjugate vaccines in infants and toddlers, using a variety of schedules, it is not feasible for manufacturers to study every possible permutation. Immune responses to the conjugated pneumococcal serotypes and to the co-administered antigens should be evaluated. Limitations of sera volumes commonly make it necessary to perform an additional randomization step to select sera to be used in the different antibody assays.

The range and design of studies should take into account the following general statements regarding schedules and co-administered vaccines:

- If there is no potentially clinically significant effect on immune responses observed on concomitant administration using an early infant schedule (e.g. 6, 10 and 14 weeks, or 2, 3 and 4 months), it is unlikely there any such effect would be observed on co-administration using more relaxed schedules (such as 2, 4 and 6 months) in a similar population, since the magnitude of immune responses is generally higher in the latter case. In contrast, an extrapolation of no effect observed with co-administration on a relaxed schedule to use on an early infant schedule in a similar population is not possible.

- If no potentially clinically significant effects on immune responses are observed on concomitant administration of a new pneumococcal vaccine with a complex vaccine (e.g. a hexavalent vaccine containing DTaP, IPV, HBV and Hib), with or without a meningococcal conjugate vaccine, it is reasonable to extrapolate the findings to co-administration with less complex vaccines (i.e. containing a lower total number of antigens, such as DTaP–IPV–Hib) – but not vice versa.

The most straightforward way to assess the effect of co-administration on immune responses is by means of random selection of sera obtained from subjects who have received a new or a licensed pneumococcal vaccine along with exactly the same routine vaccine(s) on the same schedule within the same study. This approach assumes that the licensed comparators used in these studies have already been approved for co-administration with the types of infant or toddler vaccines that have been selected for study. Thus, it also assumes that any immune interference that may have been observed in studies with the licensed comparator(s) was not considered to be potentially clinically significant.

The primary objective of these studies would be the same as in all other studies that directly compare immune responses between a new pneumococcal conjugate vaccine and licensed comparator(s); the focus would therefore be on the responses to the pneumococcal serotypes as already described.

Comparisons of immune responses to all other co-administered antigens should be listed among the preplanned secondary analyses. If the results indicate that immune responses are lower to one or more of the antigens on co-administration with a new pneumococcal conjugate vaccine than with the licensed vaccine(s), NRAs will need to consider the potential clinical consequences on a case-by-case basis. Consideration should be given to prior data on co-administration of each antigen with the 7vPnC or other licensed comparator. There may be a greater concern if the selected licensed comparator had itself depressed the immune response to an antigen compared with the 7vPnC vaccine.

Any increase in adverse reactions that is observed on co-administration will need to be weighed against the convenience of administering multiple vaccines during a single health-care contact.

C.2.6 Studies in special populations

Certain underlying conditions (e.g. immunodeficiency and asplenia) predispose to pneumococcal infections. Some, but not all, populations with these conditions may also mount lower than usual immune responses to pneumococcal conjugate vaccines. In populations with a high prevalence of conditions predisposing to IPD, clinical studies may be conducted specifically to assess the safety and immunogenicity of new pneumococcal vaccines. These studies may be performed before or after initial licensure and should include an assessment of OPA titres in a subset of sera.

C.3 Other possible indications for use

On the basis of safety and immunogenicity studies alone, there is currently no rationale for approving new pneumococcal conjugate vaccines for prevention of IPD following administration to subjects older than 2 years or for prevention of pneumonia or otitis media. This is because no immunological correlate of protection has been identified and the available data do not support a recommendation for any threshold value that might be used as a benchmark. The clinical development programmes to support these indications require different approaches but no definitive guidance can be given regarding the clinical studies that should be performed.

Pneumococcal conjugate vaccines have been approved for the prevention of otitis media and pneumonia caused by *S. pneumoniae*. Thus far, approval of pneumococcal conjugate vaccines for the prevention of these indications has been based on efficacy and effectiveness data.

No pneumococcal conjugate vaccine is yet approved for prevention of pneumococcal disease in the elderly but an efficacy study was in process at the time of preparation of this annex. It is possible that efficacy and immunogenicity data obtained during this study may eventually allow for approvals based on a comparison of safety and immunogenicity data only.

C.4 Post-marketing studies of safety and effectiveness

The manufacturer has a responsibility to assess safety and effectiveness following initial approval of a new pneumococcal vaccine. NRAs should ensure that adequate plans are in place regarding these activities at the time of first licensure of a new pneumococcal conjugate vaccine. Basic principles for conducting post-licensure studies and surveillance are outlined in the Guidelines on clinical evaluation of vaccines: regulatory expectations (*48*). Specific commitments should be made by manufacturers to provide data to NRAs on a regular basis and in accordance with national regulations. The data that are collected and submitted to the responsible NRAs should be assessed rapidly so that action can be taken if there are implications for the marketing authorization.

The collection of reliable and comprehensive data on effectiveness involves close cooperation between manufacturers and public health authorities. Pre- and post-approval discussions between vaccine manufacturers responsible for placing the product on the market and national and international public health bodies are therefore essential for ensuring that reliable effectiveness data are collected in the post-marketing period in selected countries/regions. Robust estimates of effectiveness can be obtained only in geographical locations in which a new pneumococcal conjugate vaccine has been introduced into routine immunization programmes and where suitable infrastructure is in place to identify cases of IPD. Publications produced by the WHO Expanded Programme on Immunization are important sources of information to assist in the monitoring of vaccine effectiveness once new vaccines are introduced into immunization programmes. A manual outlining approaches to monitoring the impact of *S. pneumoniae* conjugate vaccination on pneumococcal disease burden was being developed.

At present it is not known whether subjects who have completed an infant immunization series and received a subsequent booster dose will require further booster doses to maintain long-term protection against all serotypes in the vaccine. The need for further doses may depend on several factors such as waning antibody levels, the lack of natural boosting associated with low rates of circulation of some or all serotypes in the vaccine, the numbers of cases identified from disease surveillance, and estimates of herd immunity that result from routine use. It is also important to assess the effects of widespread vaccination on the incidence of IPD caused by non-vaccine serotypes to evaluate any beneficial effects and/or evidence of serotype replacement following vaccine introduction. The duration of monitoring of effectiveness will need to be reviewed continually since it should be driven by the findings.

Part D. Recommendations for national regulatory authorities

D.1 General

The general recommendations for control laboratories contained in Guidelines for national authorities on quality assurance for biological products (*54*) should be applied.

D.2 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 NRA 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 release certificate should state the number under which the lot was released by the NRA 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. (See appendices 2–4 for the summary protocol for manufacturing and control of pneumococcal conjugate vaccine; certification by the manufacturer and a model certificate for the release of pneumococcal conjugate vaccines.)

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 physicochemical characterization and purity. These tests focus on 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 immunogenicity.

D.3 Consistency of manufacture

The NRA should satisfy itself that adequate control of the manufacturing, shipping and storage of the pneumococcal conjugate vaccine has been achieved. NRAs may consider that a formal clinical lot-to-lot consistency study is not necessary if adequate and satisfactory data are provided to support consistency of manufacture. However, several different lots of the product should be used in randomized studies and should elicit comparable immune responses in similar populations.

Pneumococcal conjugate vaccines are manufactured from purified components by a clearly defined validated chemical process. Any changes in production or formulation of the vaccine should be reported to the NRA and a decision regarding the potential need for additional clinical data should be made on a case-by-case basis. The decision should take into account the likelihood of such changes affecting the quality, consistency, structural integrity and immunogenicity of the vaccine and should consider the possible cumulative effect of multiple modifications that individually may be regarded as minor.

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Appendix 1

Methodological considerations: quantification of IgG antibodies for type-specific pneumococcal capsular polysaccharide in human sera

Introduction

This appendix provides guidance on the standardization and validation of methods for measurement of pneumococcal serotype-specific IgG antibody concentrations and functional antibody titres.

Measurement of serotype-specific IgG antibody

Serotype-specific IgG antibody should be the primary parameter used to compare the immune responses to new and licensed pneumococcal conjugate vaccines.

Assay development

The ELISA that was used to evaluate serotype-specific IgG concentrations in sera obtained from subjects enrolled into the three protective efficacy trials with the 7vPnC or experimental 9vPnC vaccines included a pre-adsorption step with pneumococcal C-polysaccharide (C-PS) to reduce the content of non-serotype-specific antibody. On the basis of data from the three studies using the original ELISA method, a threshold IgG antibody concentration of ≥0.35 µg/ml was recommended for use as a benchmark when comparing immune responses between vaccines (*1*).

It was subsequently shown that the concentration of non-serotype-specific antibody in adult sera can be reduced further by pre-adsorption with both C-PS and 22F polysaccharide. This double pre-adsorption approach is recommended because it reduces the potential for over-estimation of serotype-specific IgG antibody concentrations and improves the correlation between results of the ELISA and OPA titres (*2*, *3*). This ELISA was established as the WHO reference assay; the detailed protocol for the method is available elsewhere (*4*).

The original ELISA method that was used to establish the threshold value of ≥0.35 µg/ml differed thus from the WHO reference ELISA in that it did not include a 22F pre-adsorption step. It was later shown that, using the original ELISA protocol, pre-adsorption of sera with C-PS and 22F had a minimal effect on estimations of IgG concentrations in a selection of sera from vaccinated infants compared with the original assay that used only C-PS pre-adsorption (*5*).

Despite the establishment and widespread recognition of the WHO reference ELISA, several laboratories have developed their own in-house ELISA methods that include modifications of the original assay protocol. Experience gained with various in-house methods has demonstrated that relatively small changes in assay methodology, such as the source of polysaccharides used to coat the wells, can affect assay performance. In addition to these modified ELISA methods, new assays that measure serotype-specific IgG concentrations have been developed. For example, multiplex antibody binding assays can be used to determine multiple serotype-specific IgG concentrations simultaneously and so reduce the volumes of sera required from individual subjects. All in-house assays used in immunogenicity studies designed to evaluate protection against IPD need to be bridged to the WHO reference assay in order to maintain the link between immune responses to vaccination and the demonstration of protective efficacy against IPD conferred by the seven conjugated polysaccharides in the 7vPnC vaccine.

Reference laboratories

Two WHO reference laboratories have been established to facilitate the standardization of ELISA methods. These are located at the Institute of Child Health, London, England, and at the Bacterial Respiratory Pathogen Reference Laboratory, University of Birmingham, Birmingham, AL, USA.

Reagents

A reference serum (89-SF; Center for Biologics Evaluation and Research) and a quality control panel of sera (National Institute for Biological Standards and Control) have been established using blood samples collected from adults vaccinated with 23-valent polysaccharide vaccine. While the applicability of an adult reference serum when determining IgG concentrations in sera obtained from vaccinated infants has been debated, it was not considered feasible to produce an infant reference serum pool.

The 89-SF serum has assigned serotype-specific IgG concentrations that were developed using a single C-PS adsorption step (*6*). Addition of a 22F pre-adsorption step would be expected to reduce serotype-specific IgG concentrations and is therefore not recommended. Indeed, it has been shown that pre-adsorption with both C-PS and 22F resulted in inflation of the GMC values for some serotypes by more than 25%. Because of the depletion of supplies of 89-SF, a replacement reference serum is being developed and will be bridged to 89-SF. The new reference (ref 007sp) will be calibrated against the 89-SF after adsorption with both C-PS and 22F polysaccharides.

The assigned IgG concentrations in the QC panel of sera were based on pre-adsorption with C-PS and 22F; the sera should therefore be subjected to double pre-adsorption before use. It is expected that a new QC panel of sera will be established to assist in the standardization of new assays and to monitor assay performance.

Assay validation

In the clinical development programme for each new pneumococcal conjugate vaccine, it is essential that validated assays be conducted in centralized laboratories. Assay validation involves demonstrating that the performance characteristics of the method meet the requirements for the intended use of the method. The protocols for assay validation studies should identify, and justify the choice of, parameters to be studied, and include the predefined acceptance criteria. There should be a detailed description of processing and storage of samples, reference standards and reagents, and generation of the calibration curve.

Extensive general guidance is available regarding assay validation and is also applicable to ELISA methods for estimating pneumococcal serotype-specific IgG concentrations; detailed guidance is therefore not given here. However, validation studies should adequately describe the following attributes of the assay:

- specificity,
- accuracy,
- precision (including repeatability, intermediate precision and reproducibility),
- detection limit,
- quantitation limit,
- linearity,
- range,

and robustness should be documented during assay development.

Assay standardization and bridging to the original ELISA

Inter-laboratory assay variation can be attributed to the laboratory protocol (i.e. the reagents, the reference standards, and the conditions and times for protocol steps) and the data reduction method used (i.e. non-parallelism between standard and serum dilution curves, the functions used to model standard curves, and the calculation protocols).

In-house methods for measurement of serotype-specific IgG concentrations should be evaluated using a performance-based approach that allows laboratories to optimize certain assay parameters and reduce rates of systematic errors. One approach to evaluating assay performance is to determine IgG concentrations for the reference and QC panel sera using the in-house method and compare these values with the assigned concentrations (*7*). In this way, the data can be used to estimate the level of agreement between the in-house assay and the WHO reference ELISA. That is, if the results are within predefined and justified acceptance criteria, it can be expected that the in-house assay will generate results from unknown sera that are comparable with those obtained using the WHO reference assay.

A statistical approach has been proposed for comparisons of performance between in-house and WHO reference assays (*7*). Alternative statistical methods that may be used to determine the agreement between laboratories or between one assay and the QC panel sera include Lin's concordance correlation coefficient and other regression procedures (e.g. a Deming regression). It is recommended that laboratories should obtain expert statistical advice when undertaking these comparisons.

As explained above, in-house assays used to evaluate immune responses to pneumococcal conjugate vaccines intended for administration to infants and toddlers for the prevention of IPD need to be adequately and carefully bridged to the original ELISA protocol in order to maintain the link to the protective efficacy that has been demonstrated for the 7VPnC vaccine. Each in-house method that is to be used to evaluate protection against IPD by assessing serotype-specific IgG concentrations in sera obtained from vaccinated infants should be adequately bridged to the WHO ELISA.

The bridging process requires a study that is specifically designed to demonstrate comparable performance between the in-house assay and the WHO reference ELISA. Bridging studies should employ sera obtained from infants who have received the 7vPnC vaccine or, if this vaccine is no longer available, a suitable alternative licensed vaccine that contains at least the seven serotypes in the 7vPnC vaccine. The statistical approach to analysis of bridging study results is similar to that used for routine assay standardization.

Based on the performance of an-in-house assay and the results of a bridging study, a laboratory may consider it appropriate to apply an alternative assay-specific threshold value when analysing the data as recommended in section C.2. The use of an alternative assay-specific threshold value would require a very detailed and robust justification of threshold equivalency, and it is recommended that this be discussed with NRAs before the clinical development programme reaches the stage of analysis of IgG concentration data.

Determination of functional antibody using OPA

An OPA provides a measurement of functional antibody, and it is recommended that functional antibodies be assessed in subsets of sera obtained in clinical studies with all new pneumococcal conjugate vaccines. However, there is no very well established threshold value that could be used to assist in interpretation of the data; the reasons for this include the lack of standardization of these complex assays, which are intrinsically variable because of the need to use reagents of a biological nature (e.g. cells, bacteria and complement) (*8*). A standardized and well-characterized assay, against which all other OPA methods can be bridged, is therefore needed.

A multi-laboratory study to assess comparability of OPA assays began in 2007. The five participating laboratories used the same assay protocol and method of data analysis that were in routine use at each site. Each laboratory received 24 samples and ran its OPA method for either 7 or 13 serotypes. All five laboratories used HL 60 effector cells that were differentiated using a similar protocol. Two laboratories used a multiplex platform and four used baby rabbit serum as the source of complement. There was no consistency among laboratories with regard to the pneumococcal isolates used in the assay.

Notwithstanding the diversity of the assays in use, the results obtained by the five laboratories showed a good level of agreement. There was particularly good agreement on the samples with negative titres, although the agreement on actual titres was poor.

It is clear that further standardization efforts are needed to permit comparison of OPA results from different laboratories and across clinical studies. Improvements in assay performance, such as the establishment of a reference serum, will provide more reliable results. Ultimately these efforts should also facilitate assessment of the correlation between OPA titres and protection. However, even using a single assay in one laboratory, the available data with the 7vPnC vaccine showed up to 10-fold differences in OPA GMTs between serotypes, suggesting that serotype-specific correlates will probably need to be derived.

Correlation between IgG concentrations and functional antibody (OPA titres)

Although it is recommended that the primary analysis of immune responses should be based on IgG concentrations, the serotype-specific functional antibody is regarded as the surrogate of protection. Thus, IgG concentrations determined by in-house assays should be assessed for correlation with OPA titres using sera from subjects who receive the new and reference pneumococcal conjugate vaccines in at least one clinical study.

References

1. Recommendations for the production and control of pneumococcal conjugate vaccines. In: *WHO Expert Committee on Biological Standardization. Fifty-fourth report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 927), Annex 2.
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4. Wernette CM et al. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. *Clinical and Diagnostic Laboratory Immunology*, 2003, 10:514–519.

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7. Quataert SA et al. Assignment of weight-based antibody units to a human antipneumococcal standard reference serum, lot 89-S. *Clinical and Diagnostic Laboratory Immunology*, 1995, 2:590–597.

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Appendix 2

Summary protocol for manufacturing and control of pneumococcal conjugate vaccine

The following protocol, which is intended for guidance, indicates the information that should be provided as a minimum by the manufacturer to the national regulatory authority.

Information and tests may be added or deleted as required by the national regulatory authority. It is thus possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO Recommendations for a particular product should be given in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a lot release certificate from the national regulatory authority of the country in which the vaccine was produced, stating that the product meets national requirements as well as the recommendations in Part A of this document.

Summary information on final lots

International nonproprietary name of product ______________________

Commercial name ______________________

Product licence (marketing authorization) number ______________________

Country ______________________

Name and address of manufacturer ______________________

Final packing lot number ______________________

Type of containers ______________________

Number of containers in this packing lot ______________________

Final container lot number ______________________

Number of filled containers in this final lot ______________________

Date of manufacture (filling) ______________________

Nature of final product (adsorbed) ______________________

Preservative and nominal concentration ______________________

Volume of each recommended single human dose ______________________

Number of doses per final container ______________________

Summary of the composition (include a summary of the qualitative and quantitative composition of the vaccine per human dose including the conjugate, any adjuvant used and other excipients):

Shelf-life approved (months) ______________________

Expiry date ______________________

Storage conditions ______________________

The following sections are intended for the reporting of the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production; thus, if any test has to be repeated, this must be indicated. Any abnormal results should be recorded on a separate sheet.

Detailed information on manufacture and control

Summary of starting materials

It is possible that a number of bulk lots are used to produce a single final lot. A summary of the bulk polysaccharide, activated polysaccharide, bulk carrier protein and bulk conjugate lots that contribute to the final lot should be provided.

Control of pneumococcal polysaccharides

Strain

Identity of *Streptococcus pneumoniae* strain used in vaccine ______________________

Origin and short history ______________________

Authority that approved the strain ______________________

Date approved ______________________

Master seed lot

Lot number ______________________

Date working seed lot was established ______________________

Working seed lot

Lot number ______________________

Date working seed lot was established ______________________

Control tests on working seed lot ______________________

Culture media for the production of pneumococcal polysaccharides

Any components of animal origin ______________________

Certificate for TSE-free ______________________

Control of single harvests

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity and identity, the method and date of bacterial killing, the method of purification, and the yield of purified polysaccharide.

Control of purified polysaccharide

Lot number ______________________________
Date of manufacture ______________________________
Volume ______________________________

Identity

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Moisture (for lyophilized intermediates)

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Polysaccharide content

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Protein impurity

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Nucleic acid impurity

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Endotoxin content

Date of test ______________________________

Method ______________________________

Specification ______________________________

Result ______________________________

O-*acetyl content (for relevant polysaccharides)*

Date of test ______________________________

Method ______________________________

Specification ______________________________

Result ______________________________

Molecular size distribution

Date of test ______________________________

Method ______________________________

Specification ______________________________

Result ______________________________

Control of modified polysaccharide (if applicable)

Lot number ______________________________

Method for activation ______________________________

Extent of modification

Date of test ______________________________

Method ______________________________

Specification ______________________________

Result ______________________________

Molecular size distribution

Date of test ______________________________

Method ______________________________

Specification ______________________________

Result ______________________________

Control of carrier protein

Microorganisms used

Identity of strain used in carrier protein production ______________________________

Origin and short history ______________________________

Authority that approved the strain ______________________________

Date approved ______________________________

Working seed lot

Lot number ______

Date working seed lot was established ______

Control tests on working seed lot ______

Date of reconstitution of seed lot ______

Culture media for production of carrier protein

Any components of animal origin ______

Certificate for TSE-free ______

Tests on carrier protein

Identity

Date of test ______

Method ______

Specification ______

Result ______

Purity

Date of test ______

Method ______

Specification ______

Result ______

Extent of derivatization (if applicable)

Date of test ______

Method ______

Specification ______

Result ______

Antigenic activity (for protein D derived from non-typable Haemophilus influenzae*)*

Date of test ______

Method ______

Specification ______

Result ______

Control of monovalent bulk conjugate

Production details of bulk conjugates

List the lot numbers of the individual polysaccharides and the carrier protein(s) used in the manufacture of the conjugate vaccine, the production procedure, date of manufacture and yield.

Tests on purified bulk conjugates

The tests listed below are usually performed on non-adsorbed conjugate bulks. Alternatively, they may be performed on adsorbed monovalent conjugate bulks, e.g. in case conjugate bulks are adsorbed to adjuvant individually before final formulation of the vaccine.

Residual reagents (if applicable)

Date of test ____
Method ____
Specification ____
Result ____

Pneumococcal polysaccharide content

Date of test ____
Method ____
Specification ____
Result ____

Identity

Date of test ____
Method ____
Specification ____
Result ____

Free polysaccharide content

Date of test ____
Method ____
Specification ____
Result ____

Protein content

Date of test ____
Method ____
Specification ____
Result ____

Free protein content (if applicable)

Date of test ____
Method ____
Specification ____
Result ____

Ratio of polysaccharide to protein

Date of test ______________________________

Method ______________________________

Specification ______________________________

Result ______________________________

Molecular size distribution

Date of test ______________________________

Method ______________________________

Specification ______________________________

Result ______________________________

Sterility

Method ______________________________

Media ______________________________

Volume tested ______________________________

Date of inoculation ______________________________

Date of start of test ______________________________

Date of end of test ______________________________

Specification ______________________________

Result ______________________________

Specific toxicity of carrier protein (if applicable)

Method ______________________________

Strain and type of animals ______________________________

Number of animals ______________________________

Route of injection ______________________________

Volume of injection ______________________________

Quantity of protein injected ______________________________

Date of start of test ______________________________

Date of end of test ______________________________

Specification ______________________________

Result ______________________________

Depending upon the conjugation chemistry used to produce the vaccine, suitable tests should also be included demonstrating that residual reagents and reaction by-products are below a specified level.

Control of final bulk

Lot number ______________________________

Name and nature of adjuvant, if used __________
Lot number __________
Final concentration in the final bulk __________

Name and nature of preservative, if used
Lot number __________
Final concentration in the final bulk __________

Name and nature of stabilizer, if used
Lot number __________
Final concentration in the final bulk __________

Test on final bulk
Sterility
Method __________
Media __________
Volume tested __________
Date of inoculation __________
Date of end of test __________
Specification __________
Result __________

Filling and containers

Lot number __________
Date of sterile filtration __________
Date of filling __________
Volume of final bulk filled __________
Filling volume per container __________
Number of containers filled (gross) __________
Date of lyophilization (if applicable) __________
Number of containers rejected during inspection __________
Number of containers sampled __________
Total number of containers (net) __________
Maximum period of storage approved __________
Storage temperature and period __________

Control tests on final product

Tests on final lot
Appearance
Date of test __________
Method __________

Specification ______________________________
Result ______________________________

Identity

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Sterility

Method ______________________________
Media ______________________________
No. of containers tested ______________________________
Date of inoculation ______________________________
Date of end of test ______________________________
Specification ______________________________
Result ______________________________

Serotype-specific pneumococcal polysaccharide content

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Endotoxin content

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Total polysaccharide content

Date of test ______________________________
Method ______________________________
Specification ______________________________
Result ______________________________

Adjuvant content

Date of test ______________________________
Nature and concentration of adjuvant per
human dose ______________________________

Method ____
Specification ____
Result ____

Preservative content (if applicable)

Date of test ____
Method ____
Specification ____
Result ____

General safety test (if applicable)

Date of test ____
Method ____
Specification ____
Result ____

pH

Date of test ____
Method ____
Specification ____
Result ____

Appendix 3

Certification by the manufacturer

Name of the manufacturer ______________________________

Certification by person from the control laboratory of the manufacturing company taking overall responsibility for the production and control of the vaccine.

I certify that Lot No. ______________ of Pneumococcal Conjugate Vaccine, whose number appears on the label of the final containers, meets national requirements and satisfies Part A of the WHO Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines (WHO TRS 977).

Signature ______________________________
Name (typed) ______________________________
Date ______________________________

Appendix 4

Model certificate for the release of pneumococcal conjugate vaccines

This certificate is to be provided by the national regulatory authority of the country where the vaccines have been manufactured, upon request by the manufacturer.

Certificate No. ______________________

LOT RELEASE CERTIFICATE

The following lot(s) of pneumococcal conjugate vaccine produced by ______________________[1] in ______________________,[2] whose numbers appear on the labels of the final containers, meet all national requirements[3] and Part A[4] of the WHO Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines (WHO TRS 977),[5] and comply with Good manufacturing practices for pharmaceutical products[6] and Good manufacturing practices for biological products.[7]

As a minimum, this certificate is based on examination of the summary protocol of manufacturing and control.

Final lot No.	No. of released human doses in this final lot	Expiry date

Director of the national regulatory authority (or other authority as appropriate):

Name (typed) ____
Signature ____
Date ____

[1] Name of manufacturer.
[2] Country of origin.
[3] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national regulatory authority.
[4] With the exception of provisions on distribution and shipping, which the national regulatory authority may not be in a position to assess.
[5] WHO Technical Report Series, No. 977, 2013, Annex 3.
[6] WHO Technical Report Series, No. 823, 1992, Annex 1.
[7] WHO Technical Report Series, No. 822, 1992, Annex 1.