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## **Meeting Report**

# **WHO/Health Canada Consultation on Serological Criteria for Evaluation and Licensing of New Pneumococcal Vaccines**

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## **Abstract**

New pneumococcal vaccine formulations containing more conjugated serotypes are at advanced stages of clinical development and are likely to be subject to regulatory assessment soon. There are also currently a number of initiatives to facilitate the introduction of pneumococcal conjugate vaccines in developing countries. These initiatives are dependent upon the vaccines being satisfactorily licensed and subsequently prequalified by the WHO for use by UN agencies. WHO recommendations for pneumococcal conjugate vaccine production and control were established in 2003 and published in the WHO Technical Report Series (TRS) 927, annex 2. These recommendations have served as a basis for setting up national requirements for the evaluation and licensing of pneumococcal conjugate vaccines. The Consultation aimed to provide regulators and manufacturers with further guidance regarding the serological criteria for licensing of new pneumococcal conjugate vaccines based on a review of the scientific basis for the recommendations made in TRS 927 and any recent relevant data. A major conclusion was that current recommendations continue to provide a solid basis for evaluation of pneumococcal conjugate vaccines and may be referred to when assessing new vaccines for licensure and pre-qualification. Many of the uncertainties that remain regarding serological criteria for the assessment of the potential efficacy against invasive pneumococcal disease of new pneumococcal conjugate vaccines are already considered in this document and proposals are made for handling data that are not entirely straightforward. However, it was also noted that all relevant immunological parameters should be taken into account when comparing new pneumococcal conjugate vaccines with licensed 7vPnC vaccine. In addition to the proportions of subjects that reach pre-defined antibody concentrations (by ELISA) or OPA titres, the geometric mean antibody concentrations (derived from ELISA data) or titres (derived from OPA data) and reverse cumulative distribution curves should all be presented for review. New information on assay performance and the effectiveness of currently licensed 7vPnC vaccine as assessed in routine mass immunization programs should be considered in any update of TRS 927, annex 2.

### **1. Background information and objectives of the meeting**

Pneumococcal disease is a major cause of morbidity and mortality throughout the world. A highly effective pneumococcal conjugate vaccine (PCV), offering protection against disease caused by seven virulent serotypes, was first licensed in the USA in 2000 and has since been incorporated into the immunization programs of an increasing number of developed countries. New vaccine formulations containing more conjugated serotypes are at advanced stages of clinical development and are likely to be subject to regulatory assessment in the coming year. There are also currently a number of initiatives to facilitate the introduction of pneumococcal conjugate vaccines in developing countries. These initiatives are dependent upon the vaccines being satisfactorily prequalified by the WHO for use by UN agencies.

WHO recommendations for pneumococcal conjugate vaccine production and control were established in 2003 and published in the WHO Technical Report Series (TRS) 927, annex 2 (1). These recommendations have served as a basis for setting up national requirements for the evaluation and licensing of pneumococcal conjugate vaccines.

TRS 927 proposed that new pneumococcal conjugate vaccines be compared with the licensed 7vPnC vaccine that had been evaluated for efficacy against invasive pneumococcal disease (IPD) in infants and toddlers. It was stated that the primary comparison of immune responses could be based on anti-pneumococcal antibody concentrations measured by IgG ELISA. A reference ELISA assay was developed and detailed laboratory procedures were described in a WHO Training Manual. Two WHO Reference Laboratories were established to assist with the evaluation of serological responses to new pneumococcal conjugated vaccines using this standardized methodology and to provide training in the conduct of the assay to other laboratories.

TRS 927 made suggestions for the interpretation of ELISA data that were based on analyses of immune responses to the currently licensed 7vPnC vaccine and its efficacy against IPD in infants and toddlers as demonstrated in pre-licensure clinical studies. A threshold antibody concentration (0.35 mg/l; based on the WHO ELISA method without 22F adsorption) was recommended to be used as the primary measure to compare the serotype specific immune responses to new pneumococcal conjugate vaccines or new serotypes added to the currently licensed product, but

it was stressed that this threshold antibody concentration does not necessarily predict protection in an individual subject, nor was it to be taken alone as the sole consideration for licensure. TRS 927 also recommended that opsonophagocytic activity (OPA) and immunological memory should be demonstrated at least for a subset of vaccinees and be included as an element in the assessment of new pneumococcal conjugate vaccines. It was stated that antibody concentrations measured by the standardized (WHO) ELISA that were in the range 0.20-0.35 mg/l correlated best with OPA titre of 1:8, which in turn correlated best with protective efficacy.

### **Recent developments**

During the clinical development programmes for extended valency (e.g. 10 and 13-valent) pneumococcal conjugate vaccines, several issues arose regarding a) the standardization and validation of antibody assays, b) the relative importance of ELISA and OPA data, and c) the criteria that might be used to compare these vaccines with the currently licensed 7vPnC vaccine to bridge back to the demonstration of protective efficacy against IPD. Several regulatory agencies requested that WHO re-evaluate the recommendations made in TRS 927, taking into account data available since 2003, to determine whether any significant changes were needed.

### **Objectives of the Consultation in July 2008**

The Consultation aimed to provide regulators and manufacturers with further guidance regarding the serological criteria for licensing of new pneumococcal conjugate vaccines based on a review of the scientific basis for the recommendations made in TRS 927 and taking into account any recent relevant data. In particular, the Consultation aimed to review new information on assay performance and the effectiveness of the licensed 7vPnC vaccine as assessed in routine mass immunisation programs, including the use of only two doses in infancy followed by a booster dose. The consultation focused on: a) the need for guidance on the future licensing of pneumococcal conjugate vaccines; b) clarification of the primary end-point; c) the re-evaluation of critical parameters in the ELISA; d) principles for bridging between both different ELISA methodologies and different clinical studies.

The Consultation was hosted and co-sponsored by the Health Canada and was attended by regulators, academic vaccine researchers, representatives of public health bodies and manufacturers of pneumococcal conjugate vaccines. Dr D. Wood, Coordinator of the Quality, Safety and Standards Team of the WHO Immunization, Vaccines and Biologicals Department opened the meeting by emphasizing the need for clearly defined licensing criteria for new pneumococcal vaccines at this point in time. Dr E. Griffiths (Health Canada) chaired the consultation and Dr I. Feavers (NIBSC) served as a rapporteur.

## **2. Recommendations for pneumococcal conjugate vaccines in the context of the WHO Biological Standardization Programme**

**Dr Knezevic**

As part of its standards and norms program, the WHO provides written standards that underpin the assessment of new vaccines and provide the basis of the target vaccine profile for prequalification. These written specifications provide a tool for harmonization of the specifications of biological medicines worldwide. The written standards are published as recommendations in the WHO's Technical Report Series (TRS). They are usually incorporated in national pharmacopoeias and are used as a reference by both the regulatory authorities and the vaccine manufacturers. The WHO is also responsible for the provision of the measurement standards, so-called International Standards, used to standardize biological assays globally. Both the written and measurement standards are based on scientific evidence and reflect a consensus among experts working in the field in question. They are formally ratified by the WHO's Expert Committee on Biological Standardization (ECBS). In 2003, following the licensure of the licensed 7vPnC vaccine, WHO recommendations for pneumococcal conjugate vaccine production and control were established and published in the WHO Technical Report Series (TRS) 927, annex 2 (1).

In the period since the 7vPnC vaccine was licensed, several laboratories have proposed modifications to the standardized ELISA and inevitably an increasing amount of vaccine effectiveness data have become available. Together with the subsequent development of new pneumococcal conjugate formulations, this has raised issues about the standardization and validation of antibody assays, the relative importance of ELISA and OPA data, and the criteria that might be used to compare these vaccines with the currently licensed 7vPnC vaccine.

### **3. The impact of the pneumococcal vaccines worldwide**

#### **3.1. Review of the epidemiological situation at the global level**

**Dr O'Brien**

Any prospective revision of the WHO recommendations for pneumococcal conjugate vaccine production and control has to be viewed in the context of current pneumococcal epidemiology. The global burden of pneumococcal disease and serotype distribution of disease-causing isolates were presented together with the current status of pneumococcal conjugate vaccine (PCV) introduction worldwide. The demand forecast for pneumococcal conjugate vaccines and the supply capacity for the future were reviewed.

Previous estimates of the burden of pneumococcal disease exist, published in WHO reports in 2001, 2003 and 2007. These estimates were global in nature and did not rely on a systematic review of the literature. A new effort, the Hib and Pneumococcal Global Disease Burden project, was undertaken by a collaboration between the WHO, GAVIs PneumoADIP and the Hib Initiative; the project aimed to estimate the pneumococcal disease burden in those less than five years of age, the target age group for vaccination. The aim has been to employ fully transparent methods that are comparable for *Haemophilus influenzae* type b and *Streptococcus pneumoniae*, underpinned with rigorous documentation and use the best available data. This analysis estimated that there are 14 million cases of pneumococcal disease per year with about 800 000 deaths and highlighted the major contribution of the sub-Saharan African population to the high mortality rate.

The distribution of pneumococcal serotypes causing disease differs between regions, age groups and disease syndromes. It is one of the key criteria for the development of vaccines offering an appropriate breadth of coverage. On behalf of the WHO, PneumoADIP has undertaken a global pneumococcal serotype project (GSP) to establish the serotype distribution for the PCV Target Product Profile (TPP); the PCV TPP defines the minimum characteristics for a vaccine to be considered for Advanced Market Commitment (AMC) funding. The serotypes included in the

licensed 7vPnC vaccine covers more than 50% of invasive pneumococcal disease in all regions, with the specific proportion varying by geographic region, with the lower coverage in Africa and Asia than other regions of the world. The 10-valent formulation being developed by GSK would offer a considerable improvement in serotype coverage over the 7-valent vaccine in Africa, Asia, and Latin America. It is estimated that Wyeth's 13-valent formulation, which is ultimately expected to replace the licensed 7vPnC vaccine, would cover more than 80% of invasive disease in all regions among children less than five years of age.

Pneumococcal conjugate vaccines are now starting to roll-out in developing countries. Following the meeting of its Strategic Advisory Group of Experts (SAGE) in 2007, the WHO produced a position statement on the use of pneumococcal conjugate vaccines. Currently, at least 15 countries have included 7vPnC vaccine in their infant immunization programs with high levels of private use in several others. Increased interest in PCV and the accelerated adoption of infant vaccination by countries are driven by a number of factors including the increased awareness of disease burden, the WHO/SAGE recommendation for the use of 7-valent vaccine and the availability of long-term funding for GAVI-eligible countries for the purchase of PCV. The AMC to subsidize the purchase of pneumococcal vaccines and additional GAVI funding are expected to further accelerate the demand for PCV.

The status of the PCV supply pipeline is changing and it is assumed that it will be able to fulfil forecast demand. Currently only two multinational vaccine manufacturers have PCVs that are either already licensed or will be licensed in the near future. It is estimated that there will be sufficient supply of licensed 7vPnC vaccine, which is currently undergoing the prequalification process required for supply by UN agencies, to meet projected demand until 2010. The anticipated availability of new formulations is summarized in the table. These developments in vaccine supply highlight the importance of the WHO recommendations for the timely licensure and prequalification of new products to meet projected global demand.

**Table. Overview of predicted vaccine supply over time**

Vaccine (Manufacturer)	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016-2020
<b>7-valent</b> (Wyeth) 4, 6B, 9V, 14, 18C, 19F, 23F		●								
<b>13-valent</b> (Wyeth) 4, 6B, 9V, 14, 18C, 19F, 23F plus 1, 3, 5, 6A, 7F, 19A				★	●					
<b>10-valent</b> (GSK) 4, 6B, 9V, 14, 18C, 19F, 23F plus 1, 5, 7F			★	●						
<b>Multi-valent</b> (emerging suppliers)										★
<b>New technology vaccines</b>										★

- ★ Expected Licensure (FDA, EMEA)
- Projected WHO pre-qualification

### 3.2. Further development of the pneumococcal conjugated vaccines

A number of vaccine manufacturers are involved with the further development of PCVs. Wyeth Vaccines is at an advanced stage in the clinical development of a 13-valent PCV formulation (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) that will eventually replace the company's licensed 7vPnC vaccine. Recent Phase 2 clinical trials have demonstrated immunological non-inferiority for the seven serotypes that are common to the two products. The evaluation of the new formulation has been based on the existing WHO recommendations and the performance of each serotype was assessed from percentage of responders meeting or exceeding the threshold IgG concentration of 0.35 µg/ml. The meeting was also reminded of the immense benefit of

these vaccines for public health because of their impact on the carriage of virulent (and antibiotic resistant) serotypes.

At the end of 2007 GSK submitted a licence application to the EMEA for a 10-valent PCV formulation (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F). All but two of the serotype PSs in this vaccine are conjugated to a carrier protein derived from non-typeable *H. influenzae* (NTHi). Serotypes 18C and 19F are conjugated to tetanus and diphtheria toxoids, respectively. Evidence suggests that because of the novel carrier protein the vaccine may offer some protection against otitis media caused by NTHi. The company is also interested in the development of pneumococcal proteins as part of future vaccines against pneumococcal disease.

The Serum Institute of India (SII) has plans to develop a PCV in the near future. SII is developing a 7 or 8-valent formulation based on the prevalent serotypes causing disease in India and hopes their vaccine will be in clinical development within about two and a half years. Some manufacturers have no plans to develop PCVs but have some interest in developing protein-based pneumococcal vaccines. At present, their primary interest is in the evaluation of novel PCVs in concomitant use with their own paediatric vaccine combinations.

#### **4. Serological criteria for licensing pneumococcal vaccines**

##### **4.1. Current guidelines for licensing pneumococcal vaccines**

**Dr Goldblatt**

Following the publication of the safety and efficacy data from the Northern California Kaiser Permanente (NCKP) and FinOM studies, the WHO held a series of consultations with the aim of developing serological criteria for the evaluation and licensure of new PCV formulations for infants. At one of these consultations, held at the International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD) meeting in Alaska in 2002, a preliminary analysis of the NCKP data showed that a threshold antibody concentration for protection against invasive disease could be estimated from the relationship between the point estimate of clinical efficacy and the protective antibody concentration. The relationship can be expressed as follows:

$$VE = 1 - \frac{\text{Probability of disease amongst vaccinated}}{\text{Probability of disease amongst unvaccinated}}$$

$$\therefore VE = 1 - \frac{\% \text{ vaccinated subjects with } [Ab] < Ab_{\text{protective}}}{\% \text{ unvaccinated controls with } [Ab] < Ab_{\text{protective}}}$$

It is often represented graphically in the form of a reverse cumulative distribution (RCD) curve. Given an aggregate vaccine efficacy of 97.4% for the seven serotypes included in the 7-valent PCV, the estimated threshold antibody concentration that predicted protection in the NCKP trial, after 3 doses, was 0.2µg/ml (2). This threshold was supported by other evidence. It was consistent with age-specific disease rates and for most serotypes with an OPA titre of 1:8 (notable exceptions being serotypes 19A and 7F). An issue in taking this approach was the low rates of invasive pneumococcal disease and the consequent lack of breakthrough cases for most of the serotypes. As a result, estimates of serotype-specific efficacy had wide confidence intervals or point estimates of 100%, preventing the calculation of protective concentrations. The threshold was therefore based on the assumption that the concentration of antibody required for protection is similar for all types.

At a subsequent consultation, in Geneva in 2003, the issue was revisited in light of additional data from the USA on vaccine impact and the results of clinical studies in Native Americans and in South Africa. A new threshold antibody concentration of 0.35µg/ml was derived based on the analysis of pooled immunogenicity and efficacy data from the three studies (3). This narrowed the confidence limits around the point estimate of efficacy and, as it represented data from diverse populations, was considered more appropriate for global recommendations. In this analysis there were no breakthrough cases for serotypes 6B, 18C and 23F.

There are a number of assumptions or caveats behind such analyses. They are based on the assumption that protection a) relates directly to IgG antibody concentration as measured by ELISA, b) is linked to the post primary dose, and c) is a step function. They also assume that protective levels are similar across serotypes as well as between different trials and populations.

In addition, the antibody analyses did not utilize the now recommended 22F adsorption. The correlate only relates to invasive pneumococcal disease.

The current WHO recommendations for the production and control of PCVs were drafted on the basis of the 2003 consultation. They include an appendix that details the recommended criteria for use as the primary end-point in establishing the non-inferiority of a new vaccine when compared with a licensed PCV in a head-to-head study. The geometric mean IgG concentration, as measured by ELISA without 22F adsorption, in sera collected 4 weeks after a 3 dose primary series is considered the principal licensing criterion. If an alternative ELISA is used, it should be bridged and calibrated to the recommended reference assay. A single threshold IgG concentration of 0.35µg/ml is recommended for all serotypes. For serotypes in the licensed vaccine, the percentage of responders to each serotype in the new vaccine should be compared with the percentage of responders to the same serotypes in the licensed vaccine. Non-inferiority for all serotypes is desirable but not an absolute requirement. For new serotypes, not included in the licensed vaccine, non-inferiority to the aggregate response to the serotypes in the licensed vaccine should be demonstrated. Failure of one or more serotypes should be considered on case-by-case basis. In addition to demonstrating non-inferiority with respect to the primary endpoint, the functional activity of the antibody should be demonstrated by the use of the OPA assay with a subset of sera and evidence for the induction of immunological memory should be obtained (4).

All the criteria for licensure are clearly laid out in the existing recommendations; it should not be necessary to completely re-write them. This would be a protracted process that would be likely to delay the licensure of new PCVs and potentially hinder the supply pipeline of a much needed vaccine. The existing recommendations contain all the essential components to ensure that new vaccines are safe and efficacious. The current criteria are redolent of the criteria used for licensing subsequent formulations of Hib vaccines in the USA. The Hib recommendations, which also use the proportion of vaccinees with antibody responses above a protective threshold as the primary endpoint, have proved reliable for the licensure of new vaccines and formulations. The current PCV recommendations also have sufficient flexibility for the evaluation of serotypes failing non-inferiority criteria that might enable licensing of alternative vaccines dependent upon

other criteria, such as the demonstration of OPA activity and the epidemiological relevance of a failed serotype.

The current consultation should take into account new scientific evidence and should clarify how licensing authorities should interpret clinical data against these criteria. Since 2003 data on the efficacy of 9-valent PCV has been investigated in the Gambia (5), a substantial body of effectiveness data has been accumulated following the introduction of the 7vPnC vaccine in the USA (6) and in the UK experience has been gained using a “2+1” schedule (doses at 2, 4, and 13 months) (7). It was noted in the UK that serotype 6B, which is the least immunogenic serotype after two doses in infants giving 30-50%  $\geq 0.35$   $\mu\text{g/ml}$ , was responsible for many of the breakthroughs prior to the booster dose and suggests that straying too far from this threshold results in reduced efficacy. However, other evidence suggests that vaccines inducing antibody concentrations below the 0.35  $\mu\text{g/ml}$  threshold may, for some serotypes, still exhibit some degree of protection. Opsonophagocytic activity may be a better correlate for the efficacy of some of these serotypes although this has yet to be formally tested. The key question for the current consultation was how or whether new information accrued since 2003 should be incorporated into licensing guidelines.

#### **4.2. Development of ELISA since adoption of WHO TRS 927**

**Dr Frasch**

The so-called “third generation” ELISA, cited in the WHO TRS 927 recommendations, had been adopted following the WHO consultation in 2000. It included the pre-adsorption of test serum samples with both cell wall polysaccharide (C-PS) and pneumococcal 22F capsular PS (8). However, the single antibody threshold or reference antibody concentration of 0.35  $\mu\text{g/ml}$  was defined based on ELISA data obtained without 22F adsorption. This is explained in the current recommendations, which also indicate that an equivalent threshold can be defined if 22F adsorption were used by bridging to a reference assay. The WHO consultation in 2000 had established two reference laboratories to assist those setting up and validating the reference ELISA.

An important consideration in evaluating subsequent developments in the ELISA is that the ELISA is only of value as a primary assay as long as it has a strong predictive value for a positive OPA, the surrogate for protective immunity. Since the adoption of the recommendations in WHO TRS927, the need to routinely use a combination of C-PS and 22F PS for pre-absorption has been validated by a number of laboratories. A study by Henckaerts I *et al.* demonstrated that seropositivity measured by a standardized OPA was predictive for the serotype-specific IPD efficacy of pneumococcal conjugate vaccines at a threshold titre of  $\geq 1:8$  (9). Moreover, 22F adsorption improves the correlation between IgG binding and opsonophagocytic titres.

The mechanism by which the 22F adsorption increases serotype specificity has recently been elucidated. Observations that 22F reactive antibodies develop rapidly with age and that their concentration varies by geographic region implied that they might be induced by a carbohydrate antigen. Skovsted, *et al.* identified the active component in the 22F PS as C-PS containing two, rather than one, phosphocholine per repeat unit. As a result there is an additional unique epitope on C-PS, which means that both forms of cell wall polysaccharide (CWPS and CWPS2) are needed to assure type specificity (10). The adsorption potential of CWPS2 was superior to 22F CPS and adsorption with CWPS2 alone could not replace adsorption with CWPS. These observations remain to be evaluated by other laboratories.

Another important development since the WHO recommendations were adopted has been the multiplex antibody binding assay, which enables the antibody response to multiple pneumococcal PSs to be analyzed in a single reaction (11). There are clear advantages to the use of this approach for the evaluation of multivalent vaccine formulations, including a substantial reduction in the volume of serum required and the number of assays that can be processed at once. The 9-plex microsphere assay developed by Lal *et al.* proved to be linear over a 24-fold serum dilution range and comparison of single and 9-plex assays revealed no evidence of interference between microspheres. Inhibition with pneumococcal serotype PSs showed that the assay was specific and when samples were assayed there was a good correlation of the 9-plex assay with the ELISA.

### **4.3. Discussion**

#### **Diversity of ELISAs and ELISA reagents**

In the ensuing discussion concern was expressed about the diversity of ELISAs and ELISA reagents currently used to evaluate antibody responses to PCVs. Some suggested that the current recommendations should be redrafted to compel laboratories to use the reference assay. However, in line with the recommendation of the WHO Consultation held in 2000, others argued in favour of retaining a performance-based approach in which a laboratory should use the QC serum panel to demonstrate that their assay performed similarly to the reference assay in a bridging study. Despite these different views, there was broad consensus that it was important to retain the link with the original ELISA and efficacy data. From a regulatory perspective, the critical factor was that non-inferiority comparisons were made using the same assay in the same laboratory. In addition, concern that the higher protective antibody threshold observed in clinical trials held in developing countries might be attributable to 22F-adsorbable antibodies was not supported by the available evidence.

#### **Efficacy evaluation of pneumococcal conjugate vaccines and the interpretation of the efficacy data**

Because of paucity of IPD cases in the vaccinated individuals, some simplifying assumptions are required when establishing the link between antibody concentration and IPD. When determining the threshold concentration of 0.35 for pneumococcal conjugate vaccines, these limitations were clearly laid out (2). However, the implications of these assumptions to pooling efficacy trial data from different epidemiological settings have not been thoroughly investigated.

The calculation of the threshold concentration was based on a step function, a highly conservative model for evaluating future vaccines that may underestimate the actual efficacy of future vaccines, since it is plausible that some proportion of the population below the threshold may also benefit from the vaccine to some extent.

## **5. Outcomes of the studies conducted by the vaccine manufacturers: experience with ELISA**

### **5.1. GSK**

**Dr Poolman**

There is general agreement that protection against invasive pneumococcal disease is threshold-driven. The current 0.35 µg/mL non-inferiority threshold is a population-based correlate of protection and was derived using an ELISA that only included CPS adsorption. Antibody concentrations determined using alternative methods have to be bridged to derive an equivalent threshold. The 2003 WHO recommendations recommended that the assay used should be calibrated against a reference assay. In line with WHO 2003 recommendations, GSK compared its modified ELISA (see below) including 22F adsorption with the WHO reference assay. GSK established that a threshold of 0.20 µg/ml in their assay was equivalent to the 0.35 µg/ml reference threshold and that the percentage responders with titres  $\geq 0.20$  µg/ml in the GSK assay was equivalent to the percentage responders with titres  $\geq 0.35$  µg/ml determined by the reference ELISA. The application of 22F adsorption in ELISA with different panels of sera was reviewed. In the WHO reference laboratory at the Institute of Child Health (London), post-immunisation serum samples from children immunised with an 11-valent PCV formulation showed a reduction in GMC when adsorbed with 22F PS (12). Similarly, 22F adsorption had a significant impact on the serotype specific GMCs determined for sera from South African infants immunised with a 9-valent PCV (13). Analysis of the NCKP infant sera showed that 22F adsorption had a limited impact, although there was a clear impact on the control sera from children receiving MenC conjugate vaccine. Possible reasons for the variable magnitude of 22F effect included higher levels of non-specific antibodies in regions either with shorter immunization schedules, perhaps because of higher levels of maternal antibodies, or higher early colonization rates (naturally acquired non-PS abs). It was suggested that the 22F adsorption ELISA may decrease currently perceived regional differences and measure the response to vaccination more accurately. It is also necessary to get a better understanding of regional differences with respect to OPA responses.

It is accepted that contamination of capsular PS with other immunogens varies among different vaccine lots and that the effectiveness of adsorbents like 22F PS in neutralizing antibodies to the contaminants will therefore also vary (14). GSK compared the performance of its own capsular PS preparations in ELISA with those obtained from ATCC. Data obtained using paediatric pre-immunisation sera showed the use of GSK PSs with 22F adsorption was for some serotypes more effective at reducing non-specific antibody binding than ATCC PSs with 22F adsorption. They conclude that the combination of using GSK-PS as the coating antigen with 22F adsorption provides highly specific ELISA. This has been validated according to ICH guidelines and calibrated using the qualification sera panel. The GSK ELISA with 22F adsorption was bridged to the reference assay using serum samples from the POET study (12). There was good concordance between the assays and the results demonstrated that a single threshold of 0.20 µg/ml in the GSK assay was equivalent to the protective threshold of 0.35 µg/ml in the (non-22F adsorbed) reference assay.

Opsonopagocytic antibody (OPA) assays measure functional antibodies and have an important role in the evaluation of pneumococcal vaccines. A single non-inferiority ELISA threshold has a number of limitations. It fails to take account of serotype differences in the protective threshold or differences in immune responses in different regions. The GSK OPA assay is based on CDC protocol, modified to improve robustness and throughput. It has been validated in accordance with ICH guidelines. The data presented indicated that the percentage of responders achieving an OPA antibody titre of  $\geq 8$  reflected protection against invasive pneumococcal disease. In contrast, the 22F inhibition ELISA tended to overestimate (19F) or underestimate (6B, 23F, 6A) IPD effectiveness for particular serotypes (9). Serotype 3 presented a particular problem. It is difficult to detect OPA activity with serotype 3 isolates, which are typically highly encapsulated. GSK used an atypical isolate (SSI 3/1) that expressed less capsule. They were concerned that the serotype 3 assay is the only OPA assay that uses an atypical strain, for all other serotypes typical clinical isolates were used. The OPA assay with the atypical SSI 3/1 strain was not suitable to measure the absence of protective serotype 3 immunity against AOM.

The ELISA assay can be made more specific for anti-capsular antibody by adding adsorption with type 22F PS to previous C-PS adsorption (15). Two published studies have examined the impact of double adsorption with C-PS and 22F PS on the immunological threshold of protection established in 2003 for the 7vPnC vaccine (123). In Wyeth's study the objectives were to assess whether such an assay modification would a) affect the estimated antibody concentration or b) change the protective correlate. In meeting these two objectives four key principles for bridging were applied: 1) The sera used had to come from infants immunized with the vaccine which has demonstrated clinical efficacy (i.e. the currently licensed 7vPnC vaccine); 2) Sufficient numbers of sera from immunized and control infants should be used, ideally from the same study population in which clinical efficacy was demonstrated; 3) The WHO reference assay and the modified assay must be run in the same laboratory, changing only a single variable (i.e. 22F adsorption); and 4) The statistical methodology and calculations should follow those used for the original estimate (including data from controls and single-adsorbed reference serum).

Sera from 368 infants from the NCKP efficacy trial were re-assayed with both single and double adsorbent ELISA, permitting the use of the same statistical methodology and calculations as had been applied for the determination for the original estimate. The sera were post dose 3 (i.e. 7 months of age). There were 188 and 180 sera in the vaccine and control groups respectively. The single (16) and double (15) adsorbant assays were run in parallel. The results showed that double adsorption resulted in small reductions in pneumococcal antibody in infants immunized with 7vPnC vaccine but significant reductions in the unimmunized controls. Recalculation of the protective concentration following double adsorption resulted in a small decline from 0.35  $\mu$ /ml to 0.32  $\mu$ /ml (3). Similar results were obtained using sera from the Native American (7vPnC vaccine) and South African (9vPnC vaccine) studies. Double adsorption also had a minimal impact on the measurement of the antibody concentrations to the six additional serotypes in the 13-valent PCV (1, 3, 5, 6A, 7F and 19A).

The pneumococcal reference serum 89-SF, which has assigned serotype-specific IgG concentrations, is used to ensure the consistent quantitation of serotype-specific IgG levels in both adult and pediatric sera. The 89-SF reference standard was derived from a pool of adult donors immunized with 23-valent pneumococcal PS vaccine. The serotype-specific IgG assignments were developed using a single C-PS adsorption to remove non-specific antibody and double adsorption is therefore not recommended. Adult sera have been shown to contain non-specific antibodies that are removed by adsorption with 22F PS. Thus 22F adsorption of the reference standard 89-SF would be expected to reduce the serotype-specific IgG levels detected for each of the serotypes. Consequently, if the reference standard 89-SF is double adsorbed with 22F and C-PS, and the original single adsorbent derived serotype-specific IgG assignments retained, all serotype-specific IgG assignments for test sera will be inappropriately inflated. This was examined by treating the reference standard 89-SF as an unknown sample in the standard ELISA and the titres were determined for the serotypes in the 13-valent PCV. The results showed that double adsorption of the reference standard inflated the GMC values for some serotypes by more than 25%. This would make it impossible to distinguish between vaccines in non-inferiority studies because the percent of responders whose sera meet or exceed the protective threshold are already high. Lowering the protective threshold or inflating assay results may allow less immunogenic and, therefore, potentially less protective vaccines to meet non-inferiority requirements. The current protective threshold applies only to invasive disease and the impact on mucosal infections, which appear to require higher antibody for protection, is therefore more likely to be reduced by the use of a less immunogenic vaccine.

Although the OPA has the advantage of measuring functional antibody levels, it is a complex assay, dependent upon a number of components that at best can only be partially defined. Evidence was presented that OPA data for serotypes 7F and 19A correlate poorly with protection in PCV7 immunized infants on the basis of percent achieving a titre of 1:8 suggesting that this threshold was not applicable for some serotypes. This lack of correlation at 1:8 reflects the ten-fold differences in OPA GMTs between serotypes and the fact that it is a minimal threshold for activity implying that anyone who responds is protected. A standardized or well-characterized

assay is needed that can be bridged to other assays and the intrinsic variability of the assay needs to be overcome. The OPA also needs to be bridged to efficacy trials.

### **5.3. Merck**

**Dr Sikkema**

Biomarker development and vaccine development are invariably interlinked. Immunological surrogates of protection such as toxin neutralization, viral neutralization, hemagglutination inhibition and serum bactericidal activity represent typical vaccine biomarkers. They usually correspond to an efficacy study or post-introduction analyses of vaccine effectiveness. The rationale for the use of such surrogates was that they offered earlier, as well as easier or more frequent, measurement of endpoints. They should also offer more precise measurement and hence reduce the sample sizes needed for studies. The use of reliable immunological surrogates as vaccine biomarkers should lead to faster and quicker decision making, benefiting those most in need of new products. This raises the question of whether the OPA meets these criteria.

Immune surrogates of protection depend not only on the quantity of antibody but also the quality of the antibody response. Antibody functionality will depend on many factors including the isotype, subclass and avidity. Antibody persistence has also been shown to be important for the prevention of disease (e.g. Hib and MenC). Immunological memory can also be a critical element of a protective immune response but memory alone may not be sufficient to prevent a rapidly developing infection.

Biomarker evaluation and reproducibility are affected by analytical issues in the laboratories performing assays. The pneumococcal ELISA with double adsorption was validated at Merck in 1998. The company uses C-PS with serotype 25 and 72 PSs (types not in 23-valent vaccine), which give comparable inhibition to 22F PS. The 89-SF reference serum was calibrated comparing with and without adsorbants and the reference was treated in the same way as the test sera in the subsequent assays.

To improve the efficiency of processing samples from clinical studies, Merck has developed an electrochemiluminescence (ECL) platform for the evaluation of sera. In this assay the pneumococcal PSs are spotted onto the carbon surface of a 96 well plate with formats of up to 10 spots per well. The ECL assay has been validated with eight serotypes (3, 4, 6B, 9V, 14, 18C, 19F, and 23F) to assess its reproducibility, parallelism, precision and specificity. Assay validity criteria as well as limits of quantitation (LOQ) and detection (LOD) were established. The pneumococcal ECL assay exhibits a wide dynamic range (>1000 fold) and the ability to read titres down to cut-off of 0.1 µg/mL, or lower. It correlates well with the internationally accepted pneumococcal ELISA and proved to be reproducible in a WHO Reference laboratory setting using the QC serum panel. The ECL assay has the potential to be extremely useful for the quantification of IgG responses to multiple pneumococcal serotypes simultaneously, while conserving serum volume.

## **6. Current understanding of the surrogates of protection**

### **6.1. OPA - review of the data from collaborative study      Dr Carlone /Dr Plikaytis**

A WHO meeting to discuss the standardization of the pneumococcal OPA was held in Geneva at the end of January 2007. This workshop addressed a number of concerns. The principal issue was whether there was an OPA that was sufficiently standardized for the evaluation of PCVs and posed the question of whether, following a previous interlaboratory study, the assay described by Steiner et al. was sufficiently standardized (4). Other issues included the comparability of single and multiplex assays and the diversity of assay formats used in different laboratories. The workshop decided to conduct a small multilaboratory study. There would be no attempt to make the laboratories use a standard assay. Each would use their own assay protocol and method of data analysis. In addition, all the titres would be sent to Dr Nahm for recalculation using the same (interpolated) method and all data would be sent to Dr Plikaytis for statistical analysis. In March 2007, 24 blinded samples sent to five laboratories, which ran the OPA for either 7 or 13 serotypes. There was a preliminary discussion of the results of this study at the recent ISPPD

conference in Iceland and there were plans for the group to reconvene in Geneva for a full discussion at a date yet to be determined.

No two laboratories performed the assay in exactly the same way, although all used HL-60 effector cells differentiated using a similar protocol. Two laboratories used a multiplex platform; three used a singleplex assay. Most (4/5) used baby rabbit serum as the source of complement but there was no consistency between laboratories with regard to the pneumococcal isolates used in the assay. The results show that the OPA has a higher degree of variability than ELISA, which is perhaps not surprising as the ELISA was more rigidly controlled across laboratories. There was no attempt to standardize the OPA in this study. Given this and the diversity of the assay formats, there was a good level of agreement in the results among the five laboratories. It was suggested that further standardization would reduce variability but was unlikely to change point estimates of protection. Evidence suggested that there would have been higher agreement between laboratories had there been acceptance criteria for the assay data. For example, if results were excluded where there was a fourfold difference between replicate samples (titre > 4), then in general the result remains the same but the length of 95% confidence intervals reduced and number of outliers are reduced.

## **6.2. Discussion**

The ensuing discussion reinforced the potential importance of functional antibody assays, such as the OPA, for the licensure of future vaccines (e.g. for type 19A it seems OPA better reflects efficacy as compared to ELISA). Further IPD-based efficacy studies comparing a PCV to a non-PCV arm will be impractical. The US CFR allows data from the biologically closest functional assay to be used for licensure backed up with phase 4 effectiveness studies. A similar approach has been adopted in Europe. For example, the licensure of MenC conjugates in the UK was based on serum bactericidal antibody data backed up with post-licensure effectiveness data.

The collaborative study had provided some reassurance that different laboratories could get similar results and there was good agreement between laboratories on the samples with negative titres even though the agreement on actual titres was poor. This level of consistency was attributed to the long association of both OPA and ELISA development in the same laboratories.

It was suggested that it is important to standardize the HL-60 effector cells and the way in which they were induced to differentiate. There was some discussion about the possibility of further refining the assay whilst avoiding a major standardization effort. The critical issue, however, was the need to define a protective threshold value.

The titre of 1:8 indicates the presence of functional antibodies in serum but it was important to be able to relate this to protection and to the ELISA threshold. The recommendations should also define how to bridge new OPAs to the collaborative study. Moon Nahm indicated that his laboratory now had data on sera from 2000 children and would try using them to obtain a threshold value. Others suggested that it would be helpful if the companies were also to make their data available for analysis. The ELISA is currently more precise than the OPA. Support was expressed for the existing recommendations, in which the ELISA provides the primary endpoint with the OPA data providing reassurance that the antibody response is functional.

The use of a reference serum to bridge between OPAs was discussed. Supplies of the 89-SF reference serum, currently used in ELISA, are becoming depleted. The decision was taken by CBER, NIAID and PATH in 2006 to produce a new human serum pool to replace 89-SF with a reference serum that was also suitable for use with the OPA. The protocol was executed in 2007 and serum was collected from 275 volunteers, 4 and 8 weeks after immunization with the 23-valent PS vaccine Pneumovax. A number of individual sera will be retained to supplement the current QC serum panel as it runs out. David Wood suggested that assigning International Units for OPA to the new reference serum would facilitate the standardization of the assay by eliminating the use of end-point titres.

## **7. Conduct and evaluation of ELISA assays - directions for future**

### **7.1. Comparability of alternative assays with WHO methodology B. Plikaytis**

Inter-laboratory assay variation can be attributed to two sources: 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). Given the number of

manufacturers and research centres engaged in vaccine research and development, it is hard to expect everyone to use exactly the same assay protocol. However, the pneumococcal vaccine research community managed to agree on a consensus ELISA, the currently recommended WHO reference assay. It was accepted that individual laboratories would modify or optimize their assays and the WHO workshop in 2000 agreed that a “performance based” approach should be adopted for pneumococcal ELISA. A panel of QC sera is used to calibrate assay and data reduction methods. Results are then compared to assigned values and, if the results are within an acceptable degree of tolerance, the laboratory could be expected to generate results comparable with other labs running the WHO reference assay (17). Since the reference assay was developed in the mid 1990s, laboratories have deviated from the original protocol. The most critical deviation has been the use of 22F adsorption of 89-SF, which is used as the assay standard, but we are also soon expecting the introduction of a replacement for the 89-SF reference serum.

There is a clear distinction between bridging between different assays and bridging between different correlates of protection. In a bioassay bridging study, a parameter of interest within the protocol is directly compared with a changed version of that parameter in a revised protocol to evaluate the effect of that change on the performance of the assay. The expectation is to obtain the same result as the reference assay. In a correlate of protection bridging study there is no expectation that the result should be the same for both assays (e.g. assays with and without 22F adsorption, or the introduction of a new reference serum). Correlate of immunity bridging studies should be conducted within the same laboratory to eliminate inter-laboratory variability. It would be best if such studies used a series of serum samples from the original phase 3 trials to retain the link with clinical efficacy.

As it is most likely that new vaccines without efficacy data will be compared with a licensed vaccine using a protective threshold, the threshold value is critical providing the only link with efficacy. The use of a threshold also gives us the ability to evaluate serotypes in the new vaccine that are not in licensed vaccine. There are a number of issues to be considered surrounding the use of a threshold value:

- 1) The current recommendations advise against the use of the threshold to predict individual protection; there are not enough breakthrough cases and the immunogenicity samples are not representative of the population;
- 2) The variance of the threshold cannot be incorporated in the definition of an endpoint in non-inferiority studies because the threshold and confidence interval are not calculated statistics. They are inferred from the measured vaccine efficacy and confidence interval and the RCD curve;
- 3) Can the threshold be refined as new studies are published? Threshold is valuable but it will be a challenge to specify how it is used in future immunogenicity trials.

## **7.2. Discussion**

The use of an adult reference serum for clinical trials among infants was briefly debated but it was agreed that it would be impossible to produce an infant reference serum pool.

There was concern that although the 0.35 µg/ml threshold in ELISA provided a benchmark, it did not help the licensing authorities deal with serotypes that failed to meet this threshold. Several speakers expressed the view that the functional assay was critical for the evaluation of new pneumococcal vaccines. However, it was also accepted that we did not yet have a reliable threshold titre or value for the OPA. It was argued that although a titre of 1:8 has limitations, there is at least some evidence that it predicts protection for some serotypes. The preference for a functional assay indicated that perhaps the WHO recommendations should be revised to recommend the use of OPA as the primary assay; however, this raised the question of whether there was sufficient scientific evidence to support such a change. The consensus was that, in the absence of a robust threshold, it was not yet possible to make such a change and that there was very little time in which to define a reliable OPA threshold. It was concluded that both antibody binding and functional assays should be performed, and if they were contradictory the functional OPA data may provide a more reliable indicator of protection. This approach would be

consistent with the preference for functional antibody data but in this case the licensing authorities needed guidance on how to interpret OPA data. It was emphasized that such guidance should be clear and based on scientific evidence, and care should be taken not to ruin the current guidance.

It was suggested that the use of a reference level might solve the problems associated with a threshold. When the new reference serum is bridged to the 89-SF reference, the assignment of International Units for use in the OPA should be considered.

The meeting unanimously endorsed the work of the two WHO reference laboratories and was strongly in favour of their continued support.

## **8. Regulatory considerations for pneumococcal conjugate vaccines - round table discussion**

The discussion of regulatory issues concentrated on difficulties surrounding the assessment of the immunological responses to candidate pneumococcal conjugate vaccines with extended valency. In particular, the focus was on the use of immunological data to predict protective efficacy against invasive pneumococcal disease (IPD) in infants and toddlers due to serotypes included in candidate vaccines. The importance of using immunological data to maintain a link between efficacy against IPD as demonstrated for currently licensed 7vPnC vaccine in infants and toddlers was reaffirmed and stressed. Regulatory requirements for approval of extended valency vaccines in the prevention of IPD in older subjects, pneumonia or otitis media were not discussed during this meeting.

Since the development of the WHO TRS 927 (Annex 2) and widespread licensing of the 7vPnC vaccine for prevention of IPD in infants and toddlers, it has been generally agreed that the clinical development of extended valency vaccines will be based on studies that directly compare immune responses to the candidate vaccine and currently licensed 7vPnC vaccine for all serotypes that are included in both vaccines. Immune response data are also expected for any

serotypes in the candidate vaccine that are not included in currently licensed 7vPnC vaccine and should be comparable to the 7vPnC serotypes.

**The main difficulties facing regulatory agencies include:**

- Selection of the primary immunological parameter(s) to be compared for the serotypes found in the candidate vaccine and in currently licensed 7vPnC vaccine;
- The immunological response criteria that should be met when comparing responses to serotypes shared between the candidate vaccine and currently licensed 7vPnC vaccine;
- Criteria for assessing immune responses to serotypes found only in the candidate vaccine;
- Assurance that immunological responses are assayed appropriately.

TRS 927 proposed that the primary comparison of immune responses could be based on serotype-specific anti-pneumococcal antibody concentrations measured by IgG ELISA. Although regulatory agencies have sometimes agreed to proposals from companies that comparative studies should focus on percentages reaching serotype-specific IgG concentrations  $\geq 0.35 \mu\text{g/ml}$ , it became clear that regulators do not regard this criterion as being definitive. Indeed, the many limitations of the  $\geq 0.35 \mu\text{g/ml}$  cut-off value (e.g. uncertain applicability across all serotypes, in different populations) are widely recognized by agencies but it has been accepted as a benchmark since the available data do not support firm recommendations for alternative criteria.

Some regulatory agencies consider that comparisons of functional antibody responses are of primary importance (i.e. in this case opsonophagocytic antibody [OPA]) and it was discussed that companies should adequately investigate functional antibody responses and explore the correlation between ELISA and OPA. As the interpretation of OPA data with regard to predicting protective efficacy is unclear, TRS 927 states that IgG in the range 0.20-0.35 mg/l correlated with OPA titres of 1:8, which correlated best with protective efficacy for some serotypes. Therefore, at least an assessment of subjects reaching this cut-off should be performed.

Given the uncertainties regarding interpretation of ELISA and OPA data, a full presentation of all possible immune response parameters is very important (e.g. including percentages reaching various antibody concentrations/titres, reverse cumulative distribution curves, comparisons of seroconversion rates [however defined], GMCs/GMTs). Antibody persistence and evidence for priming (i.e. responses to conjugate vaccine booster doses) may help to support the evaluation of post-primary immune responses. The limitations of the predictive capacity of pre-licensure immunogenicity data strongly support the need for comprehensive post-marketing studies of vaccine performance. Data on longer-term protection against IPD caused by all serotypes are needed due to the potential for waning immunity, lack of natural boosting due to low rates of circulation and strain replacement.

Particular problems arise regarding the selection of appropriate non-inferiority criteria for comparing responses (whether by ELISA or OPA) to each serotype that is common to the candidate vaccine and the licensed 7vPnC vaccine and in dealing with situations in which pre-defined non-inferiority criteria are met for some but not all serotypes. The interpretation of immune responses to serotypes found only in the candidate vaccine is also difficult. While TRS 927 suggests comparisons with the aggregate immune response to the seven serotypes in the licensed 7vPnC vaccine, which is not unreasonable, comparisons with the lowest immune response to any of the serotypes in the licensed 7vPnC vaccine may also be acceptable.

For both ELISA and OPA assays the regulatory agencies expressed several concerns regarding the reliability of the assays. In particular, the need to use ELISA assays that are adequately bridged to the WHO reference assay and the current lack of standardization of OPA methodologies. It was recognized that ongoing efforts may not resolve all the issues in time to assist in the assessment of the first few applications for extended valency vaccines.

Overall, regulators are faced with many uncertainties regarding the basis for approval of extended valency vaccines intended to prevent IPD in infants and toddlers but these uncertainties should not impede the ability to consider the licensure of new products which are essential for meeting the vaccine needs globally. While TRS 927 remains a useful document it was proposed that a revision might usefully expand on several issues, even if there is no definitive advice that can be given. Meanwhile it would be useful for regulators to exchange views on minimum

licensing criteria that should apply regarding indications for prevention of IPD in infants and toddlers.

## **9. Conclusions and way forward**

### **9.1. Consensus statements**

During the Consultation a number of conclusions were drawn and several proposals for amendment of TRS 927 were made to reaffirm, clarify and provide further guidance on specific issues as follows:

#### ***Serological data and interpretative criteria***

1. Current recommendations published in WHO TRS 927 continue to provide a solid basis for evaluation of pneumococcal conjugate vaccines and may be referred to when assessing new vaccines for licensure and pre-qualification. Many of the uncertainties that remain regarding serological criteria for the assessment of the potential efficacy against IPD of new pneumococcal conjugate vaccines are already considered in this document and proposals are made for handling data that are not entirely straightforward.
2. The importance of maintaining a link between efficacy against IPD as demonstrated for the currently licensed 7vPnC vaccine in infants and toddlers was reaffirmed and stressed. Since the two extended valency pneumococcal conjugate vaccines that are most advanced in development each contain the seven serotypes in the licensed 7vPnC vaccine the immune responses (ELISA and OPA) to these shared serotypes should be compared in clinical studies that include head to head comparisons between new vaccines and the licensed 7vPnC vaccine. The existing recommendation to compare immune responses to additional serotypes with the aggregate immune response to the seven serotypes in the

licensed 7vPnC vaccine remains a reasonable approach but there are alternative approaches that could be considered.

3. It was proposed to place more emphasis on the assessment of OPA data in parallel with ELISA data due to the importance of assessing the functional antibody responses elicited by vaccination. It is important to explore the relationship between ELISA and OPA. Information on the degree of correlation between these assays should be included in the evidence to support licensure.
4. Other relevant immunological parameters should be taken into account when comparing new pneumococcal conjugate vaccines with licensed 7vPnC vaccine. In addition to the proportions of subjects that reach pre-defined antibody concentrations (by ELISA) or OPA titres, the geometric mean antibody concentrations (derived from ELISA data) or titres (derived from OPA data) and reverse cumulative distribution curves should all be presented for review.
5. Further guidance should be provided on the assessment of induction of immunological memory. In particular, it should address concerns regarding the induction of hyporesponsiveness. It was proposed that the use of conjugated rather than plain polysaccharide pneumococcal vaccine should be recommended in future for the assessment of priming of the immune system by previous doses.
6. While the percentages of subjects reaching the reference antibody concentration of at least 0.35 µg/ml (measured using the WHO reference non-22F ELISA) remains a useful benchmark and should continue to be taken into account, there is a need for TRS 927 to further clarify the reasons why this cut-off should not be regarded as a definitive immunological correlate of protection.

7. Comparisons of immunogenicity would usually be designed to demonstrate non-inferiority but it was reaffirmed that, as already stated in TRS 927, it may not be necessary to reach pre-defined non-inferiority criteria for all 7 serotypes that can be directly compared.
8. If the percentages of subjects that reach antibody concentrations of at least 0.35 µg/ml is to be used as a primary endpoint for the purposes of sample size determination, the proportion of those receiving currently licensed 7vPnC vaccine that will exceed this threshold will need to be estimated in the same population for each serotype. It was suggested that a more detailed consideration of possible approaches to the determination of sample size would be a useful addition to TRS 927.

### *Assays*

9. The Consultation reaffirmed the ongoing need for WHO Reference Laboratories and the critical importance of their continued support for the standardization of ELISA and OPA between laboratories.
10. For estimation of IgG antibody concentrations the use of the WHO reference ELISA or assays that have been adequately bridged to the WHO reference ELISA method was emphasized. It was agreed that there is a need to clarify the minimum requirements for an adequate bridging study. The need to compare assays on a performance basis was reaffirmed. In addition, it was recognised that there is room for improvement with regard to the statistical analysis of the data generated by different ELISA methodologies.
11. Multiple modifications by laboratories to the WHO ELISA have led to a number of difficulties in comparing results obtained by different laboratories as well as the overall interpretation of the data. It is clear that all modifications of the assay need to be carefully

monitored and considered in the interpretation of the results. Some modifications, such as the use of more highly purified polysaccharide antigens for coating of wells and 22F pre-adsorption of reference sera, appear to have a more significant impact on the results than certain other changes. Importantly, it was proposed that all modifications of the standardized WHO ELISA should be appropriately bridged to the original assay using sera obtained from infants at approximately 4 weeks after completion of a 3-dose primary series of licensed 7vPnC vaccine.

12. Replacement of the reference serum 89-SF is ongoing. The values assigned to this standard were based on the standardized WHO ELISA. Assays used for the calibration of the replacement for the 89-SF standard will need to be appropriately bridged to the WHO assay to provide continuity of the unitage. It is expected that the new reference serum pool will also be utilized as a reference standard for the OPA, and thus the calibration exercise should also examine the fitness of the candidate material for this purpose.
13. Methodological considerations for the determination of OPA titres were also discussed. Preliminary analysis of the outcomes of a small collaborative study showed good inter-laboratory agreement in spite of the differences in testing methodologies. It appeared that the assay is relatively robust but is currently (i.e. in the absence of an internal assay standard) considered to be less precise than ELISA.

### *Post-marketing data*

14. The limitations of the predictive capacity of pre-licensure immunogenicity data strongly support the need for post-marketing studies. In particular, the impact of routine vaccination on pneumococcal serotypes additional to those in the currently licensed 7vPnC vaccine needs to be assessed in comprehensive studies of vaccine performance. Data on longer-term protection against IPD caused by all serotypes are needed due to the

potential for waning immunity, lack of natural boosting due to low rates of circulation and strain replacement.

## **9.2. Way forward**

1. Outcomes of the meeting as well as a plan of action to address clarifications in TRS 927 will be reported to the Expert Committee on Biological Standardization at its meeting in October 2008;
2. Drafting group(s) will be set up to initiate revision of the TRS 927, appendix of the annex 2, and to provide further guidance on licensing criteria;
3. Further discussions on the calibration of the replacement for 89-SF as a reference serum, standardization of the OPA and availability of HL60 cells will be conducted in coming months;
4. Consultation on the overall evaluation of pneumococcal conjugate vaccines is planned in 2009, the date and venue will depend of the outcomes of fundraising activities that are going on.

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