Executive Summary

Background information

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.

TRS 927 proposed that new pneumococcal conjugate vaccines be compared with the licensed vaccine that had been evaluated for efficacy against invasive pneumococcal disease (IPD) in infants and toddlers (i.e. Prevenar). 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 the 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 Prevenar and the efficacy of this vaccine against IPD in infants and toddlers as demonstrated in pre-licensure clinical studies. A single threshold antibody concentration (0.35 mg/l; based on the WHO ELISA method) was recommended to be used in the primary comparison of immune responses to different pneumococcal conjugate vaccines but it was stressed that this threshold does not necessarily predict protection in an individual subject. TRS 927 also recommended that opsonophagocytic activity (OPA) should be measured, that immunological memory should be demonstrated at least for a subset of vaccinees and that the
findings should be taken into account along with ELISA data 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 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 Prevenar in order to bridge back to the demonstration of protective efficacy against IPD. Several regulatory agencies requested that WHO re-consider the recommendations made in TRS 927, taking into account data available since 2003, to determine whether any significant changes were needed.

**Aim 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 any recent relevant data. In particular, the Consultation aimed to review new information on assay performance and the effectiveness of Prevenar as assessed in routine mass immunisation programs, including the use of only two doses in infancy followed by a booster dose.

The Consultation was hosted and co-sponsored by the Health Canada and was attended by regulators, vaccine researchers, academics, representatives of public health bodies and manufacturers of pneumococcal conjugate vaccines.

**Main outcomes of the Consultation**

During the Consultation a number of conclusions were drawn and several proposals for amendment of TRS 927 were made in order 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 Prevenar 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 Prevenar 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 Prevenar. The existing recommendation to compare immune responses to additional serotypes with the aggregate immune response to the seven serotypes in Prevenar 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. All relevant immunological parameters should be taken into account when comparing new pneumococcal conjugate vaccines with Prevenar. 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, due to concerns regarding 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 mg/l (measured using the WHO reference 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 mg/l is to be used as a primary endpoint for the purposes of sample size determination, the proportion of those receiving Prevenar that will exceed this threshold will need to be estimated. It was considered that this will be difficult in new populations and is further complicated by the fact that the proportions will differ for each serotype. It was considered 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 purified polysaccharide antigens for coating of wells and 22F pre-adsorption of sera, 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 Prevenar.

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 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 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 Prevenar 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.

Next steps

1. A Meeting Report will be prepared within the next two months and submitted for publication in the scientific literature;
2. 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;
3. 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;
4. 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;
5. Further 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.