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Meeting Report

WHO Information Consultation on Acellular Pertussis, DTwP, Hepatitis B and Combination Vaccines - meeting report on acellular pertussis vaccine sessions

9-13 November 2009

Footnote: This report records the deliberations of an international group of experts, and does not necessarily represent the decisions or the stated policy of the World Health Organization and other institutions or regulatory agencies from which the experts were invited.

Summary

This report reflects the discussion and conclusions of a WHO group of experts from National Regulatory Authorities, National Control Laboratories, vaccine industries and other relevant institutions, meeting on 9-13 November 2009, in Geneva, Switzerland for the revision of WHO Guidelines for the production and control of the acellular pertussis component of monovalent or combined vaccines. Based on the recent developments and standardization in quality control methods and manufacturing process the revision of these WHO Guidelines has been initiated and the drafting group has revised the document. In the meeting the current situation of quality control methods in terms of potency and safety, and nonclinical and clinical evaluation of acellular pertussis vaccines were presented and discussed. The revised Guidelines were reviewed and recommendations on further revision to reflect the current development and ensure the quality, safety and efficacy of the vaccine were made by the participants to guide the next steps of the revision by drafting group. The revised Guidelines will be finalized and submitted to the Experts Committee on Biological Standardization of WHO for final adoption.

Introduction

The WHO informal consultation on acellular pertussis, DTwP, hepatitis B and combined vaccines was convened on 9-13 November 2009 in Geneva, Switzerland, with participants from national regulatory authorities (NRA), national control laboratories (NCL), vaccine manufacturers and academia. In 1996, WHO developed the first Guidelines for the production and control of the acellular pertussis component of monovalent or combined vaccines and they were published in the WHO Technical Report Series (TRS 878, Annex 2). This document has been used by the vaccine manufacturers and NCLs to evaluate the quality and safety of acellular pertussis vaccine. Since then, WHO has coordinated several collaborative studies to evaluate additional quality control tests and to establish international standards. In addition, WHO has organized several consultations to review the progress in this area and the outcomes of the collaborative studies. Based on the discussions at these meetings, the experts have recommended the revision to the current Guidelines. A drafting group was established to initiate the revision, the members of this group included individuals from NCLs, NRAs and academia. The drafting group revised the current Guidelines and generated a draft for reviewing by the participants of the consultation.

The meeting was opened by Dr I. Knezevic, Quality, Safety and Standards team (QSS) of the WHO Immunization, Vaccines and Biologicals (IVB) Department. Dr T. Jivapaisarnpong was appointed Chairperson and Drs B. Meade and R Gaines-Das as Rapporteurs.

Acellular pertussis vaccines in the context of WHO biological standardization
Dr. Ivana Knezevic (WHO)

Dr Knezevic opened the meeting by providing a general outline of WHO biological standardization activities relevant to the development, manufacturing and quality control of

acellular pertussis vaccines. She reminded the participants that the foundation for all decisions concerning the selection of methods and setting of specifications is scientific evidence.

Dr Knezevic provided some historical background related to acellular pertussis (aP) vaccines. The original WHO document on these vaccines was approved in 1996 as Guidelines due to the limited experience with aP vaccines at that time. The purpose of this meeting was to consider the experience and scientific evidence acquired since that time. The goal of the current revision of the Guidelines was to provide a balanced view of the scientific evidence reached through consensus of experts in the field. Due to the development of new aP vaccines there is a need for the WHO to provide guidance on the development, manufacturing, quality control testing and evaluation of these vaccines.

Dr Knezevic summarized the approaches that are available for WHO to provide guidance and standardization. These include:

- Written standards defining the testing methods and criteria for production, quality control, nonclinical, and clinical evaluation of biological products.
- Physical standards that can be used to measure activity in relative terms;
- Manuals that provide practical guidance for performing and interpreting specific quality control tests;
- Training programs and curricula that provide additional support.

Initially WHO guidance documents for specific vaccines addressed manufacturing and quality control issues. More recent documents aim to provide a broader perspective, and include scientific and regulatory considerations for development, licensing, lot release, manufacturing and post-marketing surveillance (PMS).

A number of WHO documents provide general guidance and principles that apply broadly to all vaccines. The product-specific documents, such as that considered here for aP vaccines should be consistent with these general documents, but should not reiterate them. Relevant general guidelines include: a) Guidelines on stability evaluation of vaccines (2006) (1, 2), b) Guidelines on nonclinical evaluation of vaccines (2005) (3); and c) Guidelines on clinical evaluation of vaccines: regulatory expectations (2004) (4).

The WHO TRS documents are important for many reasons, but a particular application relates to the vaccines under evaluation for Prequalification (PQ). Specifically, pre-qualified vaccines are expected to meet applicable and relevant WHO Recommendations.

Although the WHO TRS documents are important, the decision-making authority for a country lies within the NRA, and is supplemented by National Pharmacopeias. However, the WHO TRS documents provide important guidance to NRA and National Pharmacopeia users, as well as to manufacturers, control laboratories, and product users. Integral to the WHO programme are the Collaborating Centers, and there has been an effort to develop a joint work plan aimed at a uniform approach to vaccine testing.

Dr Knezevic summarized specific issues that created challenges for regulation and control of aP vaccines. These include a) the diversity of preparations that had been evaluated or licensed; b) the variable composition of these preparations; c) the variable methods for detoxification of pertussis toxin (PT); d) the diversity in manufacturing methods; including co-purified and purified component vaccines; e) the use of product-specific release criteria; and f) the absence of globally-accepted criteria. Adding to the complexity and importance of an updated version of the guidance document for aP vaccines there are the possible creation of new manufacturing establishments and the introduction into clinical used of vaccines that include antigens not in the currently-licensed products.

The ground work for the current revision was initiated in previous meetings at Ferney-Voltaire (2003) (5) and St. Albans (2006) (6). WHO also supported efforts at developing a harmonized protocol for the mouse intranasal challenge assay (INCA) and further study of the modified intracerebral challenge assay (MICA) (7), an assay used for potency evaluation in some countries. Importantly, a new acellular International Standard (IS) for the MICA was approved by ECBS in 2008 (7, 8), allowing comparability among laboratories. Both MICA and INCA provided animal models that could be used for characterization of new vaccines and antigens. Regarding the mouse immunogenicity test (MIT), another assay used for product consistency monitoring (called potency testing in some countries), an outstanding issue is related to the need for clarity regarding the definition and evaluation of acceptable limits.

Dr Knezevic concluded that the goals of this consultation were to reach consensus on outstanding issues related to the aP vaccines recommendations and to plan for revision of the draft documents.

Objectives and expected outcomes of the aP vaccine track
Dr Dianliang Lei (WHO)

Dr Lei outlined specific issues related to the aP vaccine documents. He elaborated the history of aP vaccines, including the use and development of co-purified and purified component aP vaccines. Currently licensed aP vaccines have been proven to be safe and efficacious in clinical trials and routine use. He recollected that WHO had organized several collaborative studies and developed position papers to address issues and concerns raised regarding the production and evaluation of acellular pertussis vaccines. International standards for the control and evaluation of the aP vaccines have been established.

A drafting group with representation from regulators and academia was set up in 2008 to prepare the first draft of the recommendations. This draft was circulated among critical reviewers and the participants of this consultation and the resulting comments were incorporated into the current version of the Recommendations. The format for WHO Recommendations has changed in recent years, and sections providing guidance on nonclinical and clinical studies should be included in the revised Recommendations.

Dr Lei reminded participants that the objectives of the consultation were to a) review the current draft; b) review and discuss comments received from reviewers; c) propose changes; and d) agree on revisions. The aim was to provide a final version for submission to ECBS in 2010.

Acellular pertussis vaccines: current situation
Dr Dorothy Xing (NIBSC, UK)

Dr Xing provided a review of the technical aspects of the draft Recommendations. There are many products on the market throughout the world and these products are tested using a variety of methods and reference materials, some common and some product or country-specific. New manufacturers are developing new products; however full clinical efficacy studies currently do not appear to be feasible. Clear guidelines are therefore needed to provide the greatest assurance of product safety and efficacy.

Dr Xing outlined changes that have occurred since the approval of the aP vaccines Guidelines in 1996. The most important difference is that the community has much more experience with the use, regulation, and testing of aP vaccines. Human and animal studies have provided more data related to immunity, including the role of cell-mediated mechanisms. Test data in some laboratories and in an international collaborative study have shown that most of the aP vaccines used throughout the world could provide some protection in the MICA assay. A stable IS is now available for use in the MICA. A WHO collaborative study showed that although active PT influenced the protection observed, it appeared not to be essential for protection by aP vaccines in the MICA. Harmonization of the INCA allowed it to be used for vaccine and antigen characterization studies.

The WHO reagents now available include in addition to the IS for potency mentioned above, a reference antiserum for mouse antibodies (reagent 97/645), an IS of PT (JNIH-5) with defined units of bioactivity that could be used as a standard for bioactivity assays, and a pair of mouse monoclonal antibodies that recognize type 2 and type 3 fimbriae. An IS (06/140) is available for calibration of the assays for human antibodies.

Dr Xing also summarized areas where knowledge remains incomplete. For example, the nature of one or more unambiguous correlates of immunity remains elusive. Similarly, the amount of antibody that is fully protective remains uncertain. In this context, the relationship between vaccine composition, antigen contents and clinical efficacy remains unclear. Some quality criteria for a vaccine remain undefined. For example, there is no general agreement on the permissible residual content of bioactive PT and endotoxin in aP vaccines. On the other hand, although many vaccines with known efficacy pass the MICA at 4 IU/dose, it remains uncertain whether all effective vaccines would meet this specification. Finally, as more vaccines are presented in combinations with other (non-pertussis) antigens, the impact of each combination on quality of aP components is not fully understood.

This uncertainty manifest itself in the QC of aP vaccines. The heterogeneity in composition makes it impossible for a guidance document to specify the optimal composition or to provide criteria that apply to all vaccines. This has also led to the use of many product-specific reference preparations rather than general reference preparations that could be used for any product.

Dr Xing then considered the details of the test methods in use and noted by the reviewers and participants as requiring discussion.

Currently the only generally accepted test to detect residual PT activity in final product is the histamine sensitization test (HIST). Other methods, e.g. the CHO cell assay may be used in the manufacturing process. Two different HIST methodologies are used. One method uses mouse death and the other a drop in body temperature as endpoints. As currently applied, the lethal method is a limit (pass/fail) test, while the temperature method uses a quantitative approach. There is no international agreement on what constitutes a "passing" test by either method.

Two different approaches are also used for evaluation of immunological activity of aP vaccines. The MIT was designed to evaluate product consistency using antibody production in response to a defined dose of vaccine. Antibody production is evaluated using ELISA methodology, which measures binding rather than functional antibody. The reference materials, as well as acceptance criteria, are product-specific and acceptance criteria were based on the response in the test of vaccines that were used in appropriate clinical studies.

The MICA is in use as a routine release control procedure in some countries. It is a lethal challenge method that evaluates the ability of a vaccine to protect mice from an intracranial challenge. There are an international reference vaccine and a defined specification. As with the IC test used for wP vaccines, the MICA is technically challenging.

Following a decision made at a WHO ad-hoc working group meeting in NIBSC, UK (7), international collaborative studies on protection models for aP vaccines have been carried out to show if the murine Intranasal Challenge Assay (INCA) was sensitive to changes in composition of aP vaccines, and thus useful for evaluating changes done to a product. A harmonized protocol for the INCA was developed and assay shown to be transferable among laboratories. A theoretical advantage of the INCA method was that both antibody and cell mediated immunity (CMI) were shown to have a role in the model. The INCA is not used as a product release test, however, it has been shown to offer value in product evaluation.

Key comments received on the draft document
Dr Dianliang Lei (WHO)

Dr Lei reviewed key comments on the draft document received from critical reviewers, who were individuals with expertise in the evaluation of aP vaccines. These comments were discussed extensively and these discussions are included as part of this report.

Histamine sensitization assay based on temperature measurement in Staten Seruminstitut
Dr Peter Hubrechts (Staten Seruminstitut, Denmark)

Dr Hubrechts presented work done on the development and validation of a temperature based assay for residual PT and suggested that a test of this type be included in the Recommendations. The goal of the study was to evaluate an endpoint method for PT bioactivity that used dermal temperature as a nonlethal outcome measure. The method was shown to have a greater sensitivity than the lethal test and to detect 12.5 ng of active PT. A test vaccine inducing a post

challenge temperature decrease no larger than the temperature decrease induced by a reference material known to contain 50 ng of active PT was considered to "pass" the test.

Shu-min Zhang (Chinese NCL)

A presentation given by Dr D Lei prepared by Dr Zhang described the current status of manufacturing and control of DTaP vaccines in China. Dr Zhang noted that China began a transition to DTaP vaccines in 1996. Currently, 6 manufacturers have licensed products, and an additional 5 companies have applied for a license. These were co-purified products produced using a strain (called CS) isolated in China. The regulatory, manufacturing and testing requirements for these vaccines are defined in the Chinese Pharmacopeia, 3rd Volume, 2005 and are based on the requirements in the Japanese Minimum Requirements of Biological Products (Japanese Minimum Requirements). Requirements include the MICA for measuring potency, evaluation of toxicity by measurement of a decrease in body weight, lymphocytosis promoting activity, and histamine-sensitizing activity. Dr Zhang noted that some foreign-manufactured vaccines were imported into China. Many of these manufacturers used the MIT for assessment of potency. When imported into China, the Chinese NRA tested for potency using the MICA, and lots accepted for importation must meet the Chinese specification for potency.

Review of Part A - Manufacturing recommendations and Appendix
Dr Dorothy Xing (NIBSC, UK)

Dr Xing reviewed the content of the draft manufacturing recommendations (Part A), the appendices (methodological considerations) and the reviewers' comments on these. Vaccine manufacturers provided valuable comments regarding the manufacturing process.

The immunological activity assays, MICA and MIT were reviewed and discussed. Experiences for both assays were presented by the regulators and the advantages and limitations of both methods were discussed in detail. The participants agreed that it was important to include both methods in the document. Either MICA or MIT may be sufficient to evaluate the immunological activity of a licensed vaccine for routine release, as approved by the NRA.

Data related to the sensitivity of the histamine sensitization test (HIST) were presented. The Japanese Minimum Requirements specified an upper limit corresponding to 1.09 IU/dose and the Chinese Pharmacopoeia specified a limit of 2.00 IU/dose for this test. A collaborative study (EDQM BSP076) that included the two HIST methods was conducted and preliminary data were presented (9). The study included various DTaP vaccines and DTaP-based combination vaccines. Both the WHO and BP reference preparations of PT were used in the study. Once the study is finalized, the data may be useful for establishing limits. However, some participants in the study present at the consultation expressed concern at the perceived high variability of the activity estimates.

Background for Part B: Nonclinical evaluation of acellular pertussis vaccines
Dr Bruce Meade (Meade Biologics, USA)

Dr Meade introduced the issues to be addressed in the new sections, part B (Nonclinical) and part C (Clinical) and outlined the strategies proposed in the current draft. Key concepts were provided in the WHO Guidelines on Nonclinical Evaluation of Vaccines (3*)*. These guidelines define nonclinical evaluation of vaccines as "all *in vivo* and *in vitro* testing performed before and during clinical development of vaccines." Preclinical evaluations represent a subset of the nonclinical testing "carried out prior to the first testing of vaccines in humans." The guidance further state that nonclinical testing is "a prerequisite to the initiation of clinical trials and includes product characterization, proof of concept/immunogenicity studies and animal safety testing." The extent of such testing is guided by the experience with the product and generally will be more extensive for vaccines that have not been previously licensed and used in humans.

The current draft of the Recommendations uses the terms new or novel with respect to both antigens and vaccine formulations. When using the terms, there are two general possibilities: 1) the new antigen is one of the antigens in the currently licensed products (i.e., PTxd, FHA, PRN, FIM2/3); however the antigen is produced by a new process, from a new production strain, and/or by a new manufacturer, or 2) the new antigen of interest is not one of the antigens in currently licensed products. One approach is to use the term "new" to describe antigens in the first category and "novel" to refer to antigens in the second category; in any case clarity and consistency of terminology is needed to avoid confusion. Additional nonclinical and clinical testing is likely to be required for antigens for which there is limited clinical experience. Although many scenarios are possible for new vaccine formulations, some of the more likely scenarios include: 1) vaccine has formulation similar to vaccine with proven efficacy, but is missing one of antigens; 2) vaccine has a formulation similar to vaccine with proven efficacy, but includes a novel antigen; or 3) vaccine is produced from same antigens as used in a currently licensed product, but has different amounts of one or more antigens (e.g., Tdap).

The current draft states that there are no laboratory tests, animal models, and/or human immune responses that can provide complete assurance that a newly-developed acellular pertussis vaccine will be adequately safe and effective. Thus, some reviewers have expressed the need for caution when considering a transition from whole-cell (wP) to acellular pertussis vaccines. Specifically, wP vaccines are safe, effective, and cheaper to produce than aP vaccines. Thus, although these WHO documents should offer a path for approval of new aP vaccines, this path should not be unduly easy or artificially streamlined. Therefore the goal for manufacturers of new aP vaccines is to accumulate a substantial body of nonclinical and clinical evidence supporting the conclusion that the vaccine is likely to be safe and effective for the proposed use.

The draft document suggests a sequential approach to collection of this supporting evidence, beginning with comprehensive nonclinical (preclinical) testing. Meaningful characterization and testing can be conducted at different production stages, and could include genetic analysis of production strains, as well as characterization studies of the purified antigens before and after chemical treatment, the individual antigens before and after aluminum adsorption, and the antigens combined with other vaccine components. Some manufacturers co-purify the aP antigens rather than individually purifying them. Although this introduces challenges to characterization studies for co-purified antigens, meaningful evaluations can be conducted at similar stages of production. For co-purified aP antigens, the composition (i.e., the relative proportion of each antigen) should be defined.

Characterization studies should be extensive and include evaluations of purity, residual toxin activity, bioactivity of purified antigens (when possible), reactivity with specific antibodies (polyclonal and monoclonal), induction of binding and functional antibodies, and induction of protective activity in animal models. An important limitation for co-purified antigens or antigen mixtures that include PTxd is that PTxd appears to be highly protective in many animal models. Thus, when present, PTxd severely limits the ability of the models to detect a contribution to protection by other antigens. Various mouse protection models can be employed in nonclinical studies, including MICA, intranasal, and lethal and non-lethal aerosol challenge models. Currently, assays that measure functional antibody are available only for some of antigens, specifically for PTxd (PT-neutralization assay in CHO cells) and FIM2/3 (whole-cell agglutination assay).

Background for Part C - Clinical evaluation of acellular pertussis vaccines
Dr Bruce Meade (Meade Biologics, USA)

With respect to clinical development (Part C), the draft document suggests a sequential approach to collection of the evidence supporting safety and efficacy, beginning with the comprehensive nonclinical (preclinical) testing outlined above, followed by a progression of clinical evaluations. Although efficacy trials appear very difficult, if not impossible, safety and immunogenicity trials of adequate design and size are possible and should be conducted. These would include small scale human safety and immunogenicity studies, larger scale human safety and immunogenicity studies, and pivotal human safety and immunogenicity studies. Finally, because the tools for clinical evaluation are limited, post-marketing surveillance will be essential. The document assumes that only those vaccines with extensive nonclinical testing (as per part B) would be considered for clinical evaluation, with the local NRA responsible for evaluating adequacy of nonclinical information.

The Recommendations should provide guidance on issues related to the design and evaluation of the clinical studies. Most studies are expected to be comparative studies, thus the choice of a comparator vaccine is a particularly important issue, because the potential comparator vaccines differ substantially in formulation and composition. However, guidance is also needed related to the post-immunization data that should be collected for safety and immunogenicity assessment, including the specific time points for sample collection, assays to be used, and the endpoints for evaluation.

There is no definitive understanding of the immunological mechanisms responsible for vaccine-induced protection, and no immunological test(s) that adequately predict protection for all products. Household contact studies associated with two efficacy trials showed lowest attack rates in subjects who were antibody positive in multiple assays (as measured by ELISA) at time of exposure. However, challenges in applying this information remain for two reasons: 1) ELISA assays measure binding activity and may not measure the protective antibody, and 2) clinical studies typically measure antibody at peak (4 to 6 weeks post-immunization) rather than pre-exposure. Recently, an international reference serum became available, and human immunogenicity data can now be reported in IU.

Summary of the general discussion

The current version of the recommendations was thoroughly reviewed and issues were discussed by the participants. The following section is a summary of the issues discussed and consensus reached.

Manufacturing considerations

The Recommendations must differentiate genetically inactivated PT from chemically-inactivated PT, because some of the QC tests used for chemically-inactivated PT may not apply to genetically inactivated PT. In addition, the stability of the gene sequence that codifies for the mutant PT should be verified on working seed unless there are appropriate/adequate validation studies indicating that strain characterization at an earlier stage is adequate.

Clarification is needed to highlight that this document covers only antigens produced from *B. pertussis* and that although other approaches are possible (e.g., antigens produced in *B. bronchiseptica* or *E. coli*) they are not being considered.

The Recommendations should provide the opportunity to license new aP vaccines. However, DTwP are vaccines with proven safety and efficacy and transition to DTaP offers significant challenges and therefore needs to be carefully planned to provide assurance of safety and efficacy of the DTaP vaccine.

Product specifications are defined by process validation studies and performance of the clinical lots. The interpretation of "clinically effective" should be clarified as well as the approach taken to determine specifications for newly approved vaccines. One suggested approach was to include a statement indicating that specifications must be established based on the approaches defined in parts B and C. These specifications should be based on data for similar products already in clinical use and by the performance of the new vaccines in the selected relevant tests.

There was extensive discussion of the quality control tests outlined in Part A, including the recommended levels of endotoxin. There was agreement that data are not available to set an absolute limit for endotoxin that applies to all aP products at present. Guidance based on available information, such as that included in Pharmacopeias and other compendia should be provided. Specifications should be established during licensing, based on consultation with NRA. Clarification should be provided on the stage or stages at which endotoxin should be measured.

The terminology used for antigen bulks and final formulated bulk requires clarification; specifically, the different stages and options should be clarified. For example, there could be confusion between individually-purified pertussis antigens (e.g., pertussis toxoid, filamentous hemagglutinin, or pertactin), the acellular pertussis bulk concentrate that includes all of the

pertussis antigens, and the final formulated bulk that includes all other antigens and components (e.g., diphtheria toxoid, tetanus toxoid, inactivated polio vaccine (IPV), aluminum adjuvant).

Assays

It was agreed that the recommendations should indicate that there are different quality control assays currently used to assess vaccine potency and residual toxicity. Limitations and advantages should be included for some of the assays described.

Different NRAs have taken different approaches for the evaluation of aP vaccines, and some have considered each vaccine as a unique product to be evaluated individually with product-specific tests and specifications. Global specifications offer advantage, however, the participants recognized that there are considerable difficulties with this approach and that data to develop such specifications are not available at the present time.

Immunological activity assays

There was considerable discussion contrasting the two different immunological activity assays, the MICA and MIT. It was agreed that their current applications should be noted and that methodological considerations be provided as an appendix.

The document should address alternative approaches to evaluate immunological activity indicating that only one test method may be needed for a given product. The choice of assay and the specifications should be based on the product characteristics and performance, with assay and specifications approved by the NRA.

Available reference materials cited in the document should be listed in the general considerations section, because many of the preparations are used in the assays discussed in the nonclinical and clinical evaluation sections. In addition, a statement should be included indicating that in-house reference preparations should be calibrated against WHO reference standards, if applicable.

Residual active pertussis toxin assays

The HIST assays evaluating residual active pertussis toxin activity of vaccines for safety were discussed in depth. The value of monitoring residual PT activity using a quantitative assay rather than an endpoint (pass/fail) assay was discussed. Specifically, a quantitative result may allow better tracking and trending of data to maintain product consistency and may provide a result that could be investigated to determine whether it has a relationship with clinical safety. However, some participants had over 15 years of successful experience with the endpoint test and did not see an incentive to move to a quantitative assay as long as the sensitivity of the end-point lethal assay is routinely verified by use of an appropriate control.

The participants felt that there are several aspects of the HIST assays that merit discussion and clarification in the Recommendations, including:

- The use of the international standard PT for calibrating the sensitivity of the assay system. Specifically, it should be recommended that assay sensitivity be reported in IU of PT bioactivity.
- The WHO Recommendations should be clear with respect to the manufacturing stage at which the HIST assay is required (e.g. antigen bulk, final formulated bulk, final product, etc.).

- Participants agreed that the WHO Recommendations will not provide an upper limit for amount of bioactive PT in aP vaccines as measured by HIST, as there are insufficient data to make a firm recommendation. Ideally, limits are based on data from lots with acceptable safety in adequately designed clinical trials and should be approved by the NRA.

- Some laboratories have attempted to develop an alternative to the HIST assay by using a control/reference vaccine in the assay system in addition to the test vaccine and the PT control material, and some participants suggested that this approach should be included in the Recommendations. There was no consensus that a reference vaccine was required; however, this remains an option for laboratories.

- Approaches to address each aspect of the "3R" approach for animal testing (reduction, refinement, and replacement) were discussed.

  Refinement: A response to histamine can be evaluated through lethal vs. non-lethal (drop in body temperature) endpoints. In addition, drop in temperature appears to provide a more sensitive endpoint. However, even when using the drop in temperature method, some deaths do occur. In the Japanese method, these animals are included in the test by measuring the body temperature of the dead mice at 30 minutes.

  Reduction: Both the lethal and the temperature measurement methods can be adapted to a quantitative or limit test format which requires fewer animals per test. The Danish Staten Serum Institut group, for example, reported a pass/fail endpoint method using drop in body temperature as the readout.

  Replacement: It was noted that some laboratories are working on alternative non-animal based assays to replace the mouse HIST. One option under evaluation is to combine the results from two *in vitro* assays, namely, an enzymatic activity assay (for A subunit activity) and a binding assay (for B-oligomer glycoprotein binding activity). However, the available data are not sufficient to allow replacement of the *in vivo* mouse assay at the moment. Further work to evaluate this approach was encouraged.

- Document should be clear with respect to the manufacturing stage at which the HIST assay is required (e.g. antigen bulk, final formulated bulk, final product, etc.).

Nonclinical

The nonclinical section is a new addition to the published Guidelines. It was agreed that this section should contain a description of the characterization studies necessary for the approval of new vaccines, among them a thorough characterization of the acellular pertussis component that includes the determination of residual toxic activity and impurities, and proof of concept protection studies using animal challenge models. It is expected that many manufacturing parameters will be established through appropriate process validation studies. For new products, a justification/rationale for formulation should be provided.

The nonclinical program of a new/novel vaccine should adhere to the WHO recommendations outlined in the nonclinical evaluation guideline (4).

For vaccines based on genetically inactivated PT, the evaluations should include characterization of gene stability, gene sequence, residual toxin bioactivity etc.

A recommendation was made to include more details regarding the need for a comparator vaccine or reference preparation when conducting nonclinical studies, along with suggestions regarding the evaluation criteria to be used.

There was no consensus on whether manufacturers need to purify the antigens in co-purified mixtures for nonclinical studies; however it was agreed that a set of tests for co-purified antigens will be listed in the recommendations to provide guidance on the approach to characterize these complex formulations. It should be noted that this information could be requested by the NRA. In addition, the document should clearly state that vaccines should have no detectable heat-labile toxin (HLT)/dermonecrotic toxin (DNT) activity.

Clinical

In general, the design goal for clinical study of new vaccines is comparability to an existing vaccine for which efficacy has been established.

The importance of selection of an appropriate comparator vaccine was noted, although it may be challenging to identify a formulation that is similar with respect to antigen composition, source and concentration, is a licensed vaccine with proven efficacy or effectiveness, and can be used in the study population. The potential drift in protective activity, from the model vaccines to the new vaccines, which may occur when comparator vaccines have not been assessed in clinical efficacy trials, was also discussed.

Clinical studies should be designed as non-inferiority trials using predefined primary and secondary endpoints. The Recommendations should provide clear guidance on the parameters that need to be taken into consideration for evaluating immunogenicity (e.g. geometric mean concentration, or GMC, percent with defined-fold increase, and/or percent above specified threshold). Additional information on the induction of cellular immunity may be informative but interpretation of data from these assays is not straight forward. It was noted that the document should also comment on issues related to interference, immune enhancement, and interactions.

Whenever possible, assessment of functional antibodies should complement the measurement of antibodies that bind the antigens. Clinical evaluation of vaccine formulations that contain novel antigens (e.g. not previously tested in humans) will require a more in depth clinical program.

The importance of case definitions when assessing the overall protective efficacy of aP vaccines was highlighted. A recent systematic review in Sweden of clinical studies of aP vaccines revealed that these vaccines are efficacious and safe. Although new efficacy trials are generally not feasible, there may be a window of opportunity to do a study in neonates during the interval prior to the initiation of the infant doses (2-3 months) where disease is still observed.

A suggestion was made that the Recommendations should discuss the evaluation of infant doses and booster doses in separate sections, since they require evaluation of different parameters.

General issues

Concerns were raised regarding the use and interpretation of the term, "consistency" (e.g. consistency of immunogenicity, lot-to-lot consistency, and consistency between studies) and "equivalence" and "non-inferiority". These terms need to be defined in order to be evaluable. To address the issue, a suggestion was made to add following wording to the introductory section of the document: "In this document, where the terms equivalence or consistency are used without further definition or qualification, it is the responsibility of the NRA to set the requirements for these. In setting such requirements, it is not sufficient to use only a statistical test that gives the result that the two materials (vaccines) compared are not significantly different from one another, since such a test places no limits on the magnitude of the difference."

Proposed timeline for revision of recommendations for acellular pertussis vaccines

- Nov 2009 - Feb 2010: Drafting Group to prepare and update the current draft document based on the comments made by participants and the discussions at the consultation. If needed, additional discussions of the Drafting Group could be organized by teleconference and e-mails.

- March - May 2010: Review of the updated draft by participants of the consultation, and experts from NRAs, industry and academia.

- June - July: The document will be posted in the WHO website for public consultation prior to submission to ECBS.

- August: Incorporation of comments received from the public consultation and preparation of the document for submission to ECBS fall meeting in 2010.

Abbreviations:

aP: acellular pertussis (vaccine)
BP: British Pharmacopeia
CHO: Chinese hamster ovary (cell)

CMI: cell mediated immunity
DNT: dermonecrotic toxin
DTaP: diphtheria, tetanus and acellular pertussis vaccine
DTwP: diphtheria, tetanus and whole cell pertussis vaccine
ECBS: Expert Committee on Biological Standardization
EDQM: The European Directorate for the Quality of Medicines & HealthCare
ELISA: enzyme linked immunosorbent assay
FHA: filamentous hemagglutinin
FIM: fimbriae
GMT: geometric mean titer
HIST: histamine sensitization test
HLT: heat-labile toxin
IC: intracerebral challenge
INCA: intranasal challenge assay
IPV: inactivated polio vaccine
IS: international standard
IU: international unit
IVB: Department of Immunization, Vaccines and Biologicsals
MICA: modified intracerebral challenge assay
MIT: mouse immunogenicity test
NRA: national regulatory authorities
NCL: national control laboratory
OPV: oral polio vaccine
PMS: post-marketing surveillance
PQ: prequalification
PRN: pertactin
PT: pertussis toxin
PTxd: pertussis toxoid
QC: quality control
QSS: Quality, Safety and Standard team
Tdap: diphtheria, tetanus and acellular pertussis vaccine for adult use
TRS: Technical Report Series
WHO: World Health Organization
wP: whole cell pertussis (vaccine)

References

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