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Report

WHO Working Group on standardization and control of acellular pertussis vaccines

St. Albans, United Kingdom

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World Health Organization

Abstract

This report reflects the discussion and conclusions of a WHO group of experts from national regulatory authorities, national control laboratories, vaccine industry and other relevant institutions involved in standardization and control of acellular pertussis vaccines, held on 16-17 March 2006, in St. Albans, UK. Following previous discussions (Bethesda, 2000; Ferney-Voltaire, 2003; Geneva, 2005) and collection of relevant data for quality control, on the one hand, and clinical evaluation of acellular pertussis vaccines, on the other, this meeting was intended to review the scientific basis for the revision of WHO guidelines adopted in 1996 (1). The discussion on animal protection models, immunogenicity and toxicity testing was focused on three main aspects: value of the assay for the purpose of licensing and/or lot release; validity criteria and potential optimisation of the assays. The group agreed that establishment of JNIH-3 as a potential International Standard (IS) for modified intracerebral challenge assay should be under consideration. It was suggested that the inclusion of a reference vaccine such as JNIH-3 in the intranasal challenge model could improve the standardisation of this assay. It was proposed that the development of stable reference vaccines for immunogenicity testing should be encouraged. Further collection of the data from the countries with established lot release of acellular pertussis vaccines will be undertaken to prepare a solid basis for recommendations on toxicity tests. In the context of recommendations for clinical assessment of new vaccines, the group emphasised the importance of comparability studies with antigens that have already undergone efficacy trials in the past. The outline for the section on clinical evaluation of acellular pertussis vaccines was presented and after the consultation further additions were made. Post-marketing surveillance was recognized as an important part of overall vaccine evaluation and a unique opportunity to understand vaccine performance in the population and to establish a link with quality control.

1. Introduction

Following adoption of revised recommendations for whole cell pertussis vaccines in 2005, a revision of guidelines for acellular pertussis vaccine has been initiated by WHO. The meeting of the WHO working group on pertussis vaccines was opened by Dr Ivana Knezevic, Scientist, Quality, Safety and Standards Unit of Immunization, Vaccines and Biologicals Department, WHO, Geneva, who stressed the importance of defining recommendations for production, control and evaluation of acellular pertussis vaccines at this point in time. Acellular pertussis vaccines had been introduced against a background of a variety of formulations with no globally agreed standard and with no generally accepted animal model for potency assessment. Current products in Europe, North America and Japan have undergone clinical efficacy trials and consistency criteria were established using the data generated for the clinical trial batches. The approach based on comparability with lots of proven clinical efficacy has been in place during the past 10-15 years. However, in the context of new vaccines, currently under development, it is important to consider data generated in the interim and to define recommendations for vaccine potency accordingly.

As part of the process towards the revision of the WHO recommendations for production and control of acellular pertussis vaccines, several activities have been undertaken in recent years. In particular, the WHO collaborative studies conducted from 2000 - 2002 provided a number of clarifications in terms of the value of variety of challenge models in assessing protective efficacy of vaccines. The outcomes of the study were presented to the working group in May 2003. On that occasion, all aspects of quality control were discussed and development of position papers on each assay was recommended as a first step towards revision of the guidelines. Two years later, the consultation held in March 2005 considered clinical assessment of new acellular pertussis vaccines and identified difficulties in their licensing. The latter is due to the lack of correlates of protection, on the one hand, and the complexity of comparison between new vaccines and those tested in efficacy trials conducted in the 1980s and 1990s on the other hand. Moreover, the absence of globally accepted specifications for production and control poses many challenges for both manufacturers and regulators worldwide, in particular in developing countries.

Dr Knezevic indicated that acellular pertussis vaccines recently licensed in Korea and China and those under development in India and China should serve as the examples for review of key issues to be considered for licensing and lot release in future. These new products require a clear approach for quality control (QC) as well as non-clinical and clinical evaluation. The use of reference materials for potency and toxicity testing and potential role of International Standards are essential elements in the effort towards establishment of some acceptance criteria for the vaccines in question. Moreover, potential improvements of QC tests for existing vaccines should also be considered.

The meeting was set up to discuss the above issues and to identify action points towards revision of the WHO guidelines for acellular pertussis vaccines. The

outcomes of the consultation would be presented to the Expert Committee on Biological Standardization at its meeting in October 2006.

The consultation appointed Dr Roland Dobbelaer a Chairman and both Drs Michael Corbel and Dorothy Xing as Rapporteurs.

Dr R. Dobbelaer (SIPH, Belgium) pointed out that there is great interest in development of acellular pertussis / or acellular pertussis-based combination vaccines and highlighted the scientific importance of this area. He stressed a need to answer the following questions:

- How QC of existing vaccines could be improved?
- How should new products and new combinations be evaluated?
- How to carry out clinical and non-clinical evaluation of new vaccines?
- The scientific basis for QC tests?

2. Current status of quality control methods

Dr M. Corbel (NIBSC, UK) summarised key issues in current quality control of acellular pertussis vaccines and highlighted the technical aspects of these assays as well as difficulties in the interpretation of the results. Current routine control tests for acellular pertussis vaccines hinge on characterisation of antigens, assay of immunogenicity, assay of pertussis toxin by histamine sensitisation and identity testing of antigenic components. General problem areas for acellular pertussis vaccines include the diversity of preparations, variation in antigen composition between manufacturers and the use of different detoxification methods and purification processes. This diversity in manufacturing and formulation adds further complexity to the quality control aspects. There are no globally accepted reference preparations and release criteria; thus current reference materials and release criteria are product specific. The basis of current regulatory approaches is to demonstrate that newly manufactured lots are comparable either with lots shown to have acceptable clinical trial performance or lots equivalent to these clinical trial lots. As the composition of acellular pertussis vaccines varies widely between manufacturers/products, this causes difficulty in standardisation of the laboratory tests for control of these vaccines. There is evidence suggesting that both cellular and humoral immune responses contribute to protection in humans, and thus the development of laboratory methods and animal models for evaluation of new acellular pertussis vaccines/combinations which detect both types of immune response is necessary. Currently, a modified intra-cerebral challenge assay (MICA, modified Kendrick test) is used in Japan, Korea and China as the potency assay for release with a specification ≥4 unit/dose. In other countries, no official potency test is used at present and use of animal protection models is limited to monitoring protection against circulating *B.pertussis* strains. They are also used for characterization of new vaccines and new vaccine combinations containing acellular pertussis antigens as well as for investigation of post-marketing incidents. An immunogenicity test is used for monitoring consistency of production in routine control procedures with comparison with a clinical trial lot (or equivalent). However, the definition of equivalency is

difficult, therefore there is no defined common specification. The relevance to protection of such immunogenicity models is unknown and they are to be considered for consistency monitoring only. Following the decision made at the WHO Ad Hoc Working Group meeting (1998, NIBSC, UK), international collaborative studies on protection models for acellular pertussis vaccines were initiated in 1999 and the outcome of these studies was discussed in subsequent WHO working group meetings (2-4). The harmonised procedure for the intra-nasal challenge assay (INCA), established in 2003, has been shown to be effective for monitoring the activity of different vaccines and is transferable between laboratories. Future work on optimising experimental conditions to allow calculation of a relative potency is needed and a reference vaccine needs to be agreed.

Currently, the safety tests for acellular pertussis vaccines include: assay of residual active pertussis toxin (PT) and reversibility of detoxification using the histamine sensitisation assay, endotoxin assay and general toxicity test. The absence of other toxins at significant levels (heat labile toxin, adenyl cyclase toxin, tracheal cytotoxin) is built into process validation. Dr. Corbel highlighted the factors which could influence safety tests. He emphasised the outstanding issues for the histamine sensitisation test, such as large variation in test performance and no agreed defined sensitivity for the assay and suggested that these could be improved by:

- Including a reference group in each assay and narrowing the range of permissible sensitivity;
- expressing the results in international unit (IU) of PT activity to allow traceability to the IS;
- establishing limits for a “safe” amount of PT activity, based on a panel of products with a known safety history.

Dr. Corbel also pointed out that, subject to correlation with clinical performance in respect of reactogenicity, there would be a preference for *in-vitro* assays with a clear end point such as the fluorometric HPLC to measure PT S1 activity and the binding assay to measure integrity of the B oligomer receptor binding sit, currently under evaluation.

He concluded that quality control tests on acellular pertussis vaccines are still dominated by the concept of product specificity and await the development of more generally applicable and standardised methods and release criteria. There is a need to agree a definition for an effective potency assay and to identify a suitable reference preparation. A specific assay for residual active PT needs to be identified and the use of a reference preparation should be encouraged. There is also a need to define an acceptable upper limit for active residual PT and endotoxin content.

3. Respiratory challenge models

3.1. Value of INCA

Details of intranasal and other respiratory challenge models were discussed in 2003 and published elsewhere (2-4). Transferability of the INCA was demonstrated in the

second phase of the collaborative study using a harmonised test protocol. It is clear that the model can distinguish immunized mice from non-immunized but protective efficacy in the animal cannot be quantified. Dr N. Guiso (Institut Pasteur, France) emphasized that the INCA is able to detect differences between lots within one product as well as between different products, in terms of cfu reduction. However, current experimental design is not appropriate as a routine assay for determining vaccine potency. Dr. Guiso also pointed out that it is important to monitor the consistency of acellular pertussis vaccine products via clinical surveillance. The INCA assay is currently used by two manufacturers in Europe for characterization of their vaccines. The group agreed that the test is useful:

- To assess potential impact of changing formulation and/or manufacturing process;
- During development of new formulations;
- To assess potential interactions in new combinations;
- As a parameter for stability studies;
- To establish lot consistency;
- For comparing immunity induced by one particular vaccine against infection due to different clinical isolates
- To study events detected during post-marketing surveillance.

It was agreed that the INCA should not be proposed as a potency assay for routine lot release testing. However, inclusion of JNIH-3 as a reference material in the assay and potential expression of quantitative results against the reference was considered as a potential improvement of the assay design. Data should be collected from manufacturers and other experts who are able to provide such information. Although providing product specific information, the INCA may serve as a useful tool for characterisation of the biological activity of purified antigens and formulated vaccines. However, in the absence of unequivocal data for correlation with protection in humans, the model may not be appropriate for comparison of different products and the interpretation of superiority/inferiority between them.

3.2. Validity criteria for INCA

The standardisation of the assay is an important issue, otherwise it would be difficult to use this assay for characterisation of new products or for performing pre-clinical evaluation. Furthermore, inclusion of a reference to standardise the assay and/or calculate relative potency for characterisation of vaccines would provide useful parameters for setting up validity criteria. The establishment of JNIH-3 as a potential reference for the INCA should be considered. It was agreed that validity criteria for the assay should be described in the revision of guidelines for acellular pertussis vaccines. Data should be collected from manufacturers/ control laboratories and the data from collaborative studies should be re-visited.

3.3. Optimisation of the INCA

Dr N.Guiso mentioned that the methods used for preparing the challenge inoculum made of *B. pertussis* strain 18323 suspension for INCA challenge can vary between laboratories and this could affect the outcome of the test. Other factors such as mouse

strain, changes in feed source or quality, housing, etc, could alter the immune response. The group agreed that further recommendations on the quality control of challenge strain 18323 should be considered. Dr Y Horiuchi (NIID, Japan) presented data on overall calculation based on only one sampling time (day 5) in comparison to multiple sampling times (day 0, day 5 and day 8), which did not show a significant difference based on the information from the previous two collaborative studies. This may indicate that the assay could be further optimised to reduce the experimental time and animal usage. Inclusion of a reference vaccine (e.g. JNIH-3) to allow calculation of relative potency could add further weight for validation purposes and reliable characterisation and re-characterisation of products from time to time by laboratory evaluation.

4. Modified Intracerebral Challenge Assay (MICA)

4.1. Value of MICA

Currently, the MICA is used in Japan, China and Korea and possibly other Asian countries. The test employs a whole cell pertussis vaccine as standard; however different countries use different whole cell pertussis vaccine preparations in the calculation of relative potency of acellular pertussis vaccine. This model has been shown to work reliably and is able to provide a quantitative measure of the protective efficacy in animals. Dr Y. Horiuchi (NIID, Japan) reviewed the Japanese data on quality control of acellular pertussis vaccines. Japanese acellular pertussis vaccines comprise mainly PT and FHA. He pointed out that there are no clinical data available on the role of each antigen in regard to their immunogenicity and their relation to protection. In Japan, the MICA, which measures overall protection, is used for quality control of all products. Products that have passed this test have been considered efficacious as is evidenced by the effectiveness of the immunisation program in Japan since the introduction of acellular pertussis vaccines in the 1980s.

Dr S. Zhang (NICPBP, China) highlighted that there are six manufacturers in China who currently produce acellular pertussis vaccines (mainly PT and FHA). As in Japan, the MICA is the Chinese Pharmacopoeia official potency test for lot release and the specification for products to pass the test is $\geq$4 units/dose with a lower limit at 95% confidence interval of $\geq$2 units. Recently, acellular pertussis vaccine products have also been imported from European/North American manufacturers. According to Chinese regulations, these products have to be tested in the MICA. He pointed out that although to date, there have been no major problems for these products to meet the specification in the MICA and introducing a common standard based on acellular pertussis vaccine would be more reasonable than the current individual whole cell pertussis vaccine standards used in each country.

Dr Lee (FDA, Korea) added that MICA is part of licensing and lot release of acellular pertussis vaccines in South Korea. For this purpose, whole cell pertussis vaccine is used as a reference material.

4.2. Validity criteria for MICA

The group agreed that there is a need for establishment of an international vaccine standard for MICA. JNIH-3 is a potential candidate for this purpose since it has been included in previous collaborative studies for both the MICA and respiratory challenge models and the data are traceable. However, there are still issues that need to be addressed in this respect. The relationship of JNIH-3 to co-purified products needs to be determined. Re-calculation of vaccine potencies in terms of JNIH-3, in comparison with the calculation against whole cell vaccine reference needs to be carried out by re-visiting data from collaborative studies. Establishment of assay parameters/validity criteria in terms of regression, parallelism need to be considered. Careful consideration should also be given in some countries (eg, Japan, China), to the switching from current whole cell vaccine standards to an acellular pertussis vaccine based standard in terms of pharmacopoeial minimum requirement of ≥4 unit/dose for the vaccine lot to pass the test.

4.3. Optimisation of the MICA

Inconsistency of results in different mouse strains using whole cell standards were observed. Standardization of MICA by introducing acellular standard e.g. JNIH-3 needs to be explored. It is hoped that the use of an acellular vaccine as standard for MICA could improve consistency between laboratories. Further validation of the assay by comparing the results obtained using an acellular vaccine standard with those obtained using whole cell vaccine is to be undertaken.

5. Immunogenicity assays

The immunogenicity test has been in use for monitoring consistency of production as a routine release control procedure in both Europe and North America and the group felt that the assay was valuable for this purpose. Because of the diversity of acellular pertussis vaccines, the test is product-specific. Currently, for regulatory purposes, manufacturers are requested to provide adequate validity criteria for the in house-reference preparation. Issues that have been raised include:

- For the existing products, maintaining the link between routine production and the original lots of proven clinical efficacy through the use of consecutive in-house reference preparations remains difficult, nor is it clear how to link the data to the efficacy trials, especially in the case of a single dilution assay.
- The relevance of mouse immunogenicity data for the evaluation of the protective activity of new acellular pertussis products remains unclear.

The group discussed the possibility/need of setting up a stable common reference vaccine e.g. a five component vaccine for the immunogenicity test. A functional assay is available to measure PT-neutralising antibodies, however, there are no functional assays for antibodies to the other pertussis antigens. The ELISAs used in the immunogenicity test measure binding rather than functional activity, and the relationship of antibody binding activity to protection is unknown. It is also unknown if the antibody responses generated by vaccines produced differently in respect to their antigen composition, concentration, detoxification methods and formulations would be comparable in an immunogenicity assay. In the absence of this information,

establishment of a common reference vaccine for all products for immunogenicity assay would be difficult.

The group found that comparison with a calibrated reference preparation that can be traced back to lots of proven safety and efficacy is still relevant. However, the reliability of such an approach after more than a decade is questionable. It was agreed that manufacturers with existing products should be encouraged to develop individual stable vaccine reference preparations for the immunogenicity test to ensure reliable comparison linked to a clinical trial lot e.g. by a lyophilised reference preparation. Manufacturers who develop new products should be encouraged to prepare stable clinical lots for this purpose. It is also recommended that properly designed validation studies should be carried out before a single dilution assay was applied and acceptable pass criteria should be defined for each product.

6. Toxicity tests

6.1. Histamine sensitization tests (HIST)

Dr Y. Horiuchi (NIID, Japan) summarised the Japanese experience on the toxicity test using HIST based on measurement of rectal temperature and its relation to clinical surveillance data on acellular pertussis vaccines. The surveillance data suggesting that the residual pertussis toxin in the vaccine is relevant to clinical safety. However, the sensitivity of different mouse strains used in HIST is variable. Therefore the assay needs to be carefully evaluated to be sure that it is fit for its purpose. He also pointed out that a clinical trial of new products is essential for proving safety. However, there are limits to the number of lots and subjects that can be evaluated in clinical studies, and therefore it would be difficult to study adequately possible lot-to-lot variation in clinical safety. Additionally, the enrolment of subjects who have extremely high sensitivity, i.e. those most likely to show a severe adverse reaction, cannot be assured. Therefore, a trial in which no cases of rare, severe adverse events are observed cannot be taken as proof that these reactions could not occur following immunisations. He emphasised the essential importance of both the toxicity test in the laboratory control testing and ongoing clinical surveillance to evaluate the safety level of residual pertussis toxin in the vaccine.

Dr. D. Xing (NIBSC, UK) pointed out that there are still unresolved issues in HIST. They include the following: the detailed mechanism of HIST is not clear, the information on clinically safe levels of residual PT in vaccines is not available, and the sensitivity/or detection limits of HIST performed by individual laboratories are different. Additionally, it is unknown if the interaction between PT and components/or formulation factors of combination vaccine could affect the outcome of HIST. There were difficulties in setting up pass criteria (except Japan, 1.09IU). She showed the data from the JNIH-5 collaborative study which indicated that different PT reference preparations have different specific bioactivities, ranging from 0.12 to 1.05 IU per ng of protein. Thus, the study concluded that without use of a common reference preparation, inter-laboratory comparison is not possible (Geometric Coefficient of Variation is 300% for LD50 without reference, 30% for relative potency).

Dr R. Dobbelaer (SIPH, Belgium) described a forthcoming EDQM collaborative study on HIST. This is the test used for routine batch release for both DTaP and DTaP based combination vaccines. HIST specifications were initially set up for DTaP products and some difficulties had been experienced in getting certain DTaP based combination vaccines to pass these criteria recently. Furthermore the potential interactions between PT and other components/or formulation factors in the final combination vaccine are unknown. Since DTaP based combination vaccines have been widely used in Europe for some time, it is now possible to examine a panel of such vaccines with a known record of clinical safety for their levels of reactivity in HIST. Standardization of HIST is important to provide assurance on quality control and also to allow possible comparison of data from different labs. The aim of this study is to:

- Calibrate the current EP PT standard in terms of the 1st WHO IS and to express its activity in IU and through this to establish the assay sensitivity in each laboratory.
- Assess levels of reactivity of combination vaccines with a known history of clinical safety in EU market.
- Optimise the current design of the HIST.

The group expressed interest in learning the outcome of this study which should provide useful information for the revision of the WHO guidelines for acellular pertussis vaccines. The group also suggested an investigation of the possible interactions between PT and other components (eg, DT, IPV, Hib, HBsAg) in combined vaccines in the HIST. For this purpose, spiking studies could provide valuable information.

6.2. Endotoxin

Current WHO Guidelines states that the manufacturing process should be designed to reduce the level of lipooligo-saccharide (LOS) endotoxin from *B. pertussis* associated with the antigens constituting the vaccine. The LOS content of the final bulk pertussis vaccine is usually measured for lot release by means of the Limulus Amoebocyte Lysate (LAL) test. LOS content should not exceed the amount present in lots shown to be safe in clinical trials. After the WHO pertussis working group meeting in 2000, a survey on the endotoxin test performed by laboratories who produce/ or perform control tests on pertussis vaccines was carried out. Data from fourteen laboratories indicated that the current specification for endotoxin content in acellular pertussis vaccines are most commonly defined in the individual product licences and varied between regulatory bodies. For instance, 2 EU/dose was specified in the Japanese Pharmacopoeia, while the EP limit was stated to be less than or equivalent to 100 EU/dose. Dr. Y. Horiuchi pointed out that there is no detectable endotoxin level in Japanese acellular pertussis vaccines and Dr. D. Xing presented the data on endotoxin levels for 51 lots from four different types of products which all showed endotoxin levels below 10 IU/ml. There was a general agreement that as different assay methods e.g. LAL assay and the rabbit pyrogen assay, were currently used for determination of endotoxin levels, there is insufficient information to allow the setting of an upper limit for endotoxin at this stage. Additional data generated from other countries e.g. China,

India, would be required. It did not appear necessary to standardise the test procedure for endotoxin determination at the current stage. However, the group agreed that for GMP purposes, the endotoxin test would be still a key test at both antigen purification and final bulk stages.

6.3. Other tests

Dr. Y. Horiuchi pointed out that the CHO cell clustering assay method might be inadequate for evaluating aldehyde detoxification of PT. Even after a mild treatment with formaldehyde, PT markedly lost its CHO cell clustering activity while about 10 % of original HS activity is still retained. Therefore, CHO cell clustering assay cannot predict the *in vivo* activity of the aldehyde treated PT.

Dr D. Xing described the work at NIBSC on "Development of an enzymatic-HPLC assay in combination with a carbohydrate binding assay as alternative to the HIST for pertussis vaccines". The HPLC method is used for determination of the ADP-ribosylation activity of the A subunit of PT and the binding assay is used for determination of B subunit activity. She showed evidence that both of these activities are important for the *in vivo* activity seen in HIST. She pointed out that the HPLC and binding activities from different types of vaccines can be variable. Therefore, it is unlikely that a common specification for all products could be set up. For the purpose of monitoring product consistency, a product-dependent specification should be set up and further validation on a large number of batches of each product would be required.

7. Clinical evaluation of new acellular pertussis vaccines

Following discussion by the WHO working group on clinical evaluation of pertussis vaccines in March 2005, a draft with key issues for licensing new acellular pertussis vaccines was prepared. Dr M. Powell (MHRA, UK) presented the draft "Prerequisites for licensing new acellular pertussis vaccines" as an outline for the clinical section in the recommendations. The group agreed upon the principles summarized in the draft and identified issues that require further consideration. Some of the difficulties relating to the clinical assessment of new acellular pertussis vaccines include the variety of antigens that may be chosen for vaccine development and the fact that protective efficacy cannot be predicted from serological responses because there is no consensus regarding the immunological correlates of protection. It was agreed that a section on the pre-licensure regulatory expectations for new acellular pertussis vaccines should be incorporated into the recommendations. Furthermore ongoing post-marketing surveillance is important to monitor the effectiveness of existing acellular pertussis vaccines as well as to confirm the effectiveness of new vaccines. Thus, a section on appropriate post-marketing surveillance should be also included in the revised recommendations.

7.1. Anticipated Scenarios

At least three different scenarios in the clinical development of new acellular pertussis vaccines, prior to licensure, were identified.

- An aP containing vaccine for which an established aP component (i.e. same pertussis purified antigen(s) manufactured by the same company using the same processes, and formulated the same way) and that has been found suitably efficacious in a clinical efficacy trial has been used for formulation of another vaccine.

- An aP-containing vaccine for which the aP pertussis antigen composition is the same as studied in a previous protective efficacy trial but for which some or all antigens are not made by the same manufacturer and/or by the same process as the vaccine tested in a previous protective efficacy study.

- An aP containing vaccine for which the aP antigen composition is not the same as that of an already licensed aP vaccine that has been found suitably efficacious in a clinical efficacy trial. In this case it is also possible that some or all of the antigens in the vaccine are made by a different manufacturer and/or using new manufacturing processes.

7.2. Clinical Development Strategy

The group agreed that it is no longer/or may not be feasible to generate protective efficacy data for novel acellular pertussis vaccines. Therefore, pre-licensure assessments would have to be based on safety and immunogenicity studies. With no consensus on immunological correlates of protection, the immune responses to each antigen in a candidate acellular pertussis vaccine will have to be compared to responses to antigens in a control vaccine that is the same as or as close as possible (in terms of range of antigens and other features of composition) to a licensed vaccine that was previously shown to provide adequate protection against pertussis. Serological assays should be the same as or validated against those used in the immunogenicity studies performed during the protective efficacy study most relevant to the chosen control product.

The immunogenicity data should be described using GMCs and the percentages of subjects reaching assay cut-offs, the percentages of subjects achieving 4-fold increments in antibody concentrations. In addition, reverse cumulative distribution curves should be drawn up.

The aim of these immunological comparisons would be to demonstrate non-inferiority of the candidate acellular pertussis vaccine and the chosen control product with respect to selected parameters. The selection of the primary parameter for the assessment, the pre-defined margin of non-inferiority and hence the total sample size for a comparative study will need careful justification. Although it is considered very important that studies should compare immune responses between candidate and licensed acellular pertussis vaccines, comparisons to historical data that were generated during previous protective efficacy studies using similar assays may be used to provide supportive evidence.

In addition, in the case of vaccines for which the aP content is novel (in terms of range of antigens and/or manufacturing processes for one or more antigen) an

extensive pre-clinical evaluation should be undertaken. In particular, the response to the PT component should be fully assessed in animal models and by estimating the functional antibody to PT (using the CHO cell neutralization assay). Every effort should be made to evaluate and document the functional immune responses to each antigen. It was agreed that thorough product characterisation, e.g. production strains, purified antigens, and other in process and final product materials should be included as part of a licensing application. Characterisation should evaluate purity, integrity, and functional activity using a variety of approaches including physical-chemical evaluation, measurement of residual toxicity, and assessment of immunogenicity as evaluated by both binding and functional assays and protective effect in relevant animal models. There was consensus that a section on regulatory expectations for new acellular pertussis vaccines should be incorporated into the revised recommendations.

The Group noted that clinical trials are already under way in India and China with novel acellular pertussis vaccines. A review of the design of these trials would provide valuable insight into the feasibility of the above recommendations for clinical assessments. In this regard, Dr SS Jadhav described the development of new acellular pertussis vaccine at the Serum Institute of India. This new vaccine consists of individually prepared and purified antigenic components of *Bordetella pertussis* and *Bordetella bronchiseptica* adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. Non-clinical testing of this vaccine is underway and a clinical study is planned for 2007. The selection of antigens was discussed. The consultation found that the inclusion of any antigen other than those that have been tested before in previous efficacy trials (ie, PT, FHA, pertactin, fimbriae) would require additional considerations in clinical evaluation of such vaccine. For instance, the inclusion of the PRN antigen purified from *B. bronchiseptica* requires careful consideration since it is not the same antigen as PRN from *B.pertussis.*

7.3. Post-Licensure Requirements

In all the scenarios described, post-licensure surveillance programs aiming at measuring the effectiveness of aP vaccines against pertussis disease should be instituted. These data are required to provide reassurance regarding protection against disease and may also indicate when boosters should be given. Because of the complexities of such programs and the infrastructure needed to generate reliable data on disease, such data are most likely to come from public health bodies.

If the aP-containing vaccines contain a previously unused antigen, a specific post-licensure study of safety might also be required. The extent and duration of such a study would have to be decided on a case by case basis.

National regulatory agencies (NRAs) should give consideration to the country's ability to monitor the post-licensure safety and effectiveness of the vaccine before making a licensing decision.

Laboratories involved in evaluation of the clinical samples are encouraged to participate in the international networks aimed at developing standardized reagents and methods.

8. Conclusions and proposed next steps

The consultation concluded that sufficient evidence for the development of recommendations for acellular pertussis vaccines has been generated. The following proposals were made:

8.1. The inclusion of INCA and MICA in the recommendations for acellular pertussis vaccines

The group reached agreement on inclusion of sections on INCA and MICA into the recommendations. Description of the assay procedures, as well as the value of the assay and assay validity criteria should be included in the section. Potential improvements of these assays have been identified.

For the above purpose a detailed review of the data generated in previous collaborative studies as well as in development, licensing and lot release of existing vaccines is to be undertaken. Moreover, an additional investigation of JNIH-3 as a reference preparation was deemed necessary. In this context, the following actions were proposed:

- For the INCA model, further analysis of data generated in collaborative studies, to assess the relationship between JNIH-3 and other vaccine samples included in the study and to set up assay validity criteria. A draft section on the assay method will be sent to the group for comments.
- For the MICA model, revisiting collaborative study data to explore JNIH-3 as a standard. Evaluation of the relationship between JNIH-3 and the co-purified type of acellular pertussis vaccine. A draft section on the assay method will be sent to the group for comments.
- For the immunogenicity assay, to prepare an updated section on immunogenicity testing and include encouragement to establish stable reference material to avoid risk of drifting when using consecutive batches of in-house reference; describe single dilution assay and validation needed for establishing a single dilution model procedure
- For HIST, review outcomes of EDQM study which should give useful information for the revision of WHO recommendations, in consideration of establishing a limit/specification and encouragement of calibration of reference pertussis toxin preparations versus the International Standard
- Consider establishing an appropriate limit reflecting actual endotoxin levels by collecting more data e.g. from other countries such as China, India
- A number of different assays addressing different biological activities of pertussis toxin have been developed or are under development: e.g: enzymatic activity as measured by HPLC, fetuin binding, CHO cell clustering. WHO is supportive of the work on development of alternatives to animal tests.

8.2. The inclusion of a section on clinical evaluation in the recommendations for acellular pertussis vaccines

Recommendations for clinical evaluation of acellular pertussis vaccines with different product scenarios will be incorporated into the revised guidelines. It was also agreed that sections on regulatory expectations for new acellular pertussis vaccines and the current scope for post-marketing surveillance would be important and these will be also included in the revised guideline.

Draft recommendations for clinical evaluation of acellular pertussis vaccine is to be prepared and included in the first draft recommendations for evaluation of acellular pertussis vaccines.

Participants in the meeting:

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