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Meeting Report

WHO Working Group meeting on Standardization of Acellular Pertussis Vaccines: potency assay

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Abstract

At the meeting of the WHO Working Group on acellular pertussis vaccines held in March 2006, St. Albans, United Kingdom, it was noted that there are no globally agreed methods for evaluation and licensing of new acellular pertussis vaccines. New vaccines have recently been developed in China and India, as reported to the Working Group. In this context, potency testing and potential improvements of the protective

animal models with inclusion of a reference material were recognized as critical issues in the quality assessment of acellular pertussis vaccines. One of these models, namely Modified Intracerebral Challenge Assay (MICA), is currently used as a potency assay in Japan, China and Korea. At present, different whole cell pertussis vaccines are used as reference standards in these assays. However, the suitability of whole cell pertussis vaccines as reference preparations for acellular pertussis vaccines in the MICA had not been studied. Therefore, consideration of the establishment of an acellular pertussis vaccine derived International Standard and subsequent switch to secondary standards based on acellular pertussis vaccines was identified as an important step towards standardization of these vaccines as determined by MICA. To take this issue forward, a collaborative study comparing whole cell references, a candidate acellular pertussis vaccine reference (JN1H-3) and various acellular pertussis products was undertaken in 2006. Fourteen laboratories including NCLs and manufacturers from China, Japan and Korea were involved in the study, which was coordinated by NIBSC, NICPBP (China), NIID (Japan), KFDA (Korea).

This meeting was co-organized by the National Institute for Control of Pharmaceutical and Biological Products in Beijing and the Quality, Safety and Standards Team of the Immunization, Vaccines and Biologicals Department of the World Health Organization in Geneva. The aim of the meeting was to review the outcomes of the collaborative study with the study participants and coordinators and to develop a plan of action towards improved standardization in this area. Moreover, the meeting served as an opportunity to consider the use of protective assays in the future and to develop a plan for the revision of WHO guidelines for acellular pertussis vaccines (WHO TRS 878, annex 2).

The results of the collaborative study showed that MICA worked reliably and gave consistent results between laboratories. JN1H-3 was found to give similar dose-response lines to a variety of acellular pertussis vaccines and DTaP formulations, irrespective of the differences in acellular pertussis components. It was agreed that an important advantage of this assay is the expression of the potency of test vaccines in IU. The

Working Group agreed that once the study report is finalized and reviewed by the study participants, it will be submitted to the Expert Committee on Biological Standardization at its meeting in October 2008, with a recommendation that JN1H-3 be established as the First International Standard for acellular pertussis vaccine in MICA.

1. Background information

At the previous meeting of the WHO Working Group on Acellular Pertussis Vaccines in March 2006 (6), the value of protection animal models in the evaluation of new acellular pertussis vaccines was recognized and the need for a reference preparation was identified as a significant factor for standardization of these vaccines. One of these models, namely Modified Intracerebral Challenge Assay (MICA), is currently used as a potency assay in Japan, China and Korea with different whole cell pertussis vaccines as reference preparations. However, the suitability of whole cell pertussis vaccines as reference preparations for aP vaccines in the MICA had not been studied. Establishment of an acellular pertussis vaccine derived International Standard and subsequent switch to secondary standards based on acellular pertussis vaccines was considered an important step towards standardization of the MICA assay.

To take this issue forward, a collaborative study was undertaken in 2006 with the following objectives:

1. to evaluate the dose-response relationships of the 3rd International Standard for whole cell pertussis vaccines (3rd IS wP) as well as those for various in-house wP references currently used for control of acellular pertussis vaccines;
2. to compare JN1H-3 and various in-house wP references;
3. to review the consistency of potency estimates for acellular pertussis vaccines using 3rd IS for whole cell pertussis vaccine, in-house whole cell pertussis vaccine reference preparations and JN1H-3 as reference standards.

Vaccine samples used in the study included DTaP vaccines with various pertussis formulations including 2 component (purified); 2 component (co-purified); 3 components (purified) and 5 components (purified) DTaP.

2. Introduction and objectives of the meeting

On behalf of WHO, Dr Knezevic welcomed all participants and thanked them for their willingness to assist WHO in its effort towards standardization of acellular pertussis vaccines. She emphasized the role of the National Control Laboratories (NCLs) in setting up lot release systems at the national level and establishing appropriate testing for new vaccines. Dr Knezevic pointed out the importance of conducting regulatory research, highlighting the example of NIID, NICPBP and KFDA in the area of acellular pertussis vaccines and encouraged other NCLs to take a lead in the areas of their interest. Substantial progress in the development and approval of new acellular pertussis vaccines made in China was a reason for organizing this discussion in Beijing. All participants greatly appreciated the kind hospitality of NICPBP staff in Beijing who hosted this meeting.

Dr Knezevic reminded participants that the WHO guidelines for acellular pertussis vaccines were adopted in 1996 (1) with a number of unresolved issues in terms of quality control, due to the lack of knowledge at that time. To improve the situation, WHO set up a Working Group on pertussis vaccines in 1998 and since then discussions on quality, safety and efficacy of these vaccines were held as follows: Washington DC in 2000; Ferney-Voltaire 2003, Geneva 2005 and St. Albans 2006. In addition, several collaborative studies were conducted to resolve issues for which there was no data or no consensus. One of these issues is potency testing which is the focus of this meeting.

Dr Hadler from the WHO Office in China highlighted that China has achieved excellent success in its immunization program, vaccinating about 90% of its newborn children, eradicating polio, and ensuring effective control of other vaccine preventable diseases, notably diphtheria and pertussis. After many years with a relatively simple

national immunization schedule, which included 5 vaccines – DTP, BCG, OPV, measles, and hepatitis B. The Chinese government made a momentous decision in February 2007 to greatly expand national support for childhood immunization. Beginning in January 2008, the Chinese government will invest 2.5 billion RMB annually to purchase 12 vaccines, and autodisposable syringes, for all children in China. In addition to the 5 vaccines currently used, the program will now also include MMR, Japanese encephalitis, meningococcal polysaccharides A+C, and hepatitis A. Currently only Beijing utilizes DTaP for infant vaccination. With the introduction of the new program, the DTwP vaccine currently used in almost all provinces will be replaced by DTaP. This will be a big change affecting a large population and WHO involvement would be important.

On behalf of State Food and Drug Administration (SFDA), the National Regulatory Authority (NRA) in China, Professor Yin indicated that there is rapid social and economic progress in China, with 34 vaccine manufacturers producing 47 different vaccines against 26 diseases. SFDA faces a great challenge in the regulation of both domestically produced and imported products. Chinese Authorities appreciate the role that WHO plays in setting standards for biological products and facilitating communication among relevant experts at the global, regional and national level. Professor Yin expressed willingness to host future WHO activities in China in the area of biologicals, and in particular of vaccine products.

Dr. Jin briefly introduced the duties of the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). The Institute is the National Control Laboratory in charge of lot release of all vaccines and other biological products used in China, with the essential role of monitoring the quality, safety and efficacy of vaccines. The Institute is responsible for the establishment of national reference standards used for quality control of vaccines and is the key player in ensuring the quality of vaccines used for the national immunization programme. Dr Jin mentioned the importance of WHO activities in improving the health of Chinese people and highlighted that China welcomes the opportunity to collaborate with WHO and other organizations in regulatory and laboratory testing activities. One example is the recent establishment of a Memorandum

of Understanding with the National Institute for Biological Standards and Control (NIBSC) in the UK with the objective to exchange information and expertise in the area of vaccine quality control.

The Consultation appointed Dr Michael Corbel as Chairman and both Dr Maria Baca Estrada and Dr Dorothy Xing as Rapporteurs.

Dr. Corbel (NIBSC) reminded the participants that WHO has been involved in developing guidance for the assessment of acellular pertussis vaccines for 20 years. The first acellular pertussis vaccines were developed in Japan and subsequently different formulations have been developed by many manufacturers. There is no “generic” approach to the production process and many different formulations have been developed and used successfully. The establishment and standardization of a potency test for acellular pertussis vaccines has been a priority for the WHO for a number of years. The Japanese approach of using the MICA to assess the potency of acellular pertussis vaccines has been adopted by other countries including Korea and China. It appears that the MICA is a suitable model to assess the potency of acellular pertussis vaccines of different pertussis antigen compositions. The immunogenicity assay is used to assess the consistency of production of vaccines licensed in Europe and North America. It is important to note that these products have undergone clinical efficacy trials and consistency criteria were established using the data generated for the clinical trial batches. However, as effective vaccines are currently available, limiting the prevalence of the disease, there is great practical and ethical difficulty in carrying out clinical trials for licensing of new vaccines. Thus, there is a great need to standardize relevant animal protection models and to establish reference standards for the assessment of potency that will permit comparison between new and existing products.

The JN1H-3 reference standard is the only acellular pertussis preparation which has continuity with vaccine efficacy clinical studies, since a preparation from the same bulk (JN1H-6) was tested in Sweden in an efficacy trial and demonstrated 69% protection. The current collaborative study assessed whether the JN1H-3 preparation could be used as a

reference standard in the MICA potency assay. The use of JN1H-3 may provide a means of bridging new acellular pertussis vaccines to material tested in clinical trials and thereby facilitate characterization of these new formulations for licensing.

3. Revision of WHO guidelines for acellular pertussis vaccines in the context of the developments in the WHO biological standardization

Dr. Knezevic gave an overview of the WHO Biological Standardization Program, emphasizing the issues related to the standards currently under development or revision. WHO provides both written standards (guidelines and recommendations for production, quality control and evaluation of vaccines and other biologicals) and measurement standards (International Standards and International Reference Preparations).

Written standards are based on the translation of scientific developments into recommendations for issues that are relevant to global public health. One aim of this WHO program is to generate, analyse and disseminate timely and reliable evidence based standards, particularly for risk management purposes. It was recognized that the standardization of biological products in some cases should be initiated during the early stages of product development to ensure their availability throughout the evaluation process, from clinical trial stage, through licensing and post marketing surveillance. This approach would require more resources and well coordinated international effort in future. As an example of generic standards, Dr Knezevic highlighted revision of Good Manufacturing Practice for Biologicals, initiated in 2007, as one of the projects with broad implications for future production and evaluation of biologicals. Following recent developments of new vaccines, an increasing demand from Member States for technical assistance has been addressed to WHO and various options for responding to this challenge are now under discussion.

Another aspect that was highlighted is the expertise and functionality of the National Regulatory Authorities and their responsibility for regulatory oversight of

vaccines. Dr Knezevic explained that a vaccine of assured quality is one that consistently meets appropriate levels of purity, potency, safety and efficacy on a lot-to-lot basis, judged through an independent review system competent to take an evidence-based decision on the product for a specified population in a specific context (2).

WHO has established a number of written and measurement standards for the production, control and evaluation of pertussis vaccines, the details of which are available at the WHO biological web site (<http://www.who.int/biologicals/en/>). In addition, several consultations of the WHO Working Group on pertussis vaccines were held to discuss developments in the evaluation of these vaccines and to advise WHO on the need for the improvements in this area (3-6). In March 2005, current understanding of the protection against *B. pertussis* infection in humans and elucidation of mechanisms of protection following immunization was considered as an important element for identifying suitable methods to assess vaccine efficacy during clinical evaluation of new vaccines. In line with this, appropriate quality control tests, for vaccine potency in particular, were identified as an issue that requires further considerations. Some of the difficulties in establishing quality control tests for acellular pertussis vaccines are due to the diversity of vaccine formulations, which contain different amounts and types of antigens. There are also differences in critical production steps such as detoxification and purification methods. This has led to the acceptance of product-specific reference standards and release criteria. Therefore, the key issues identified for the evaluation of acellular pertussis vaccine included the establishment of a protection assay to assess vaccine potency (MICA and intranasal challenge assay (INCA)), evaluation of JN1H-3 as reference preparation for MICA, guidance for toxicity testing, establishment of criteria for clinical evaluation of new acellular pertussis vaccines and other regulatory considerations for licensing and lot release.

At the WHO Working Group on standardization of acellular pertussis vaccines meeting in March 2006, the suitability of both the MICA and the INCA models to assess acellular pertussis vaccine potency was discussed. The value of these assays for licensing new acellular pertussis vaccines was considered, including the possibility of the

establishment of adequate validity criteria and potential optimization of the assays (6). The MICA was considered a suitable protection model since it can provide quantitative measurement of protective efficacy and it is already used as a potency test in Japan, China and Korea. However, it was recognized that the introduction of a common acellular pertussis vaccine reference preparation was important. The MICA could be used:

- To characterize new acellular pertussis vaccines in terms of protection in animals
- To licence new acellular pertussis vaccines
- To evaluate potential impact of manufacturing changes
- To serve as a potency test for lot release

At the meeting in March 2006 the group concluded that the inclusion of the INCA and MICA in the recommendations for acellular pertussis vaccines should be elaborated and the description of the testing procedure, value of the assays and assay validation criteria provided. In addition, there was an agreement that the evaluation of the JN1H-3 preparation as a candidate IS was warranted.

WHO guidelines and recommendations regarding acellular pertussis vaccines need to be revised in light of new information, in particular the establishment of a challenge model to assess potency of acellular pertussis vaccines and the evaluation of a suitable reference standard for this model. In 2006-2007 a collaborative study was carried out to address some of these issues. The aim of the current meeting was to gather the participants and coordinators of the collaborative study to review and discuss the outcomes of the study and to develop a plan towards standardization of acellular pertussis vaccines in 2008-2009. Several acellular pertussis vaccine experts who did not take part in the study were also invited to provide an independent view of the study results. Moreover, the meeting also served as an opportunity to consider the use of protection assays in future and to develop plans for the revision of WHO guidelines for acellular pertussis vaccines.

4. Current approaches in quality control of acellular pertussis vaccines

Dr M Corbel (NIBSC, UK) summarized the current situation in quality control of acellular pertussis vaccine and highlighted certain problems. There are many different acellular pertussis vaccine preparations on the market. They differ in the method of purification (i.e. co-purified and individually purified components), the type of antigen (e.g. 1 to 5 different components), the method of detoxification, the quantity of each antigen and the combination with other vaccine antigens (e.g. Hep B, Hib, IPV). There are no agreed release criteria. Currently the regulatory approaches are either to demonstrate consistency with clinical lots or an equivalent using an immunogenicity assay, or to use a potency assay based on protection.

Other tests include the assessment of identity, composition, safety (freedom from toxicity), potency and consistency of production. There are a number of safety tests used for acellular pertussis vaccines. However, there is no general agreement on choice of assay or acceptable limits for residual toxicity. Currently, the only generally accepted test for pertussis toxin (PT) in the final product is the histamine sensitization test (HIST). Other methods such as CHO cell assay may be used for in-process control at the level of the detoxified PT bulk but due to concerns regarding aggregation and interference from the aluminium adjuvant this test cannot be used for the final product. There are two different approaches to assess histamine sensitization, the pass-fail method and the Japanese quantitative assay. In regard to the pass-fail method, there are a number of outstanding issues such as definition of assay sensitivity (e.g. narrow range of permissible sensitivity), inclusion of a reference standard in the assay, expression of results in IU with traceability to IS, and establishment of “safe” limits for the maximum permissible amount of PT activity based on a panel of products with a known history of safety. Another important aspect is the potential interaction between PT and other components or factors in the combination products. These interactions can potentially affect the outcome of the HIST. Therefore, there was a need for replacement of the HIST test by the Japanese quantitative assay or possibly other assays under development such as the ADP-

ribosyl transferase HPLC assay together with the fetuin binding assay, or the human vascular endothelial cell permeability assay, subject to validation.

The immunogenicity assay is widely used as a routine release procedure in Europe and North America for the assessment of consistency of production. This assay measures the binding of antibodies to specific antigens of the vaccine and is a product-specific assay. The relationship between the induction of antibodies measured by this test and the protection against infection with *B. pertussis* is not known.

Animal models of protection can measure the induction of both cell-mediated immunity and specific antibodies. Several collaborative studies have been conducted to assess the suitability of protection models to evaluate acellular pertussis vaccines. Two models have been developed, the MICA and the intranasal challenge assay (INCA). The INCA has been used for the characterization of acellular pertussis vaccines; a collaborative study demonstrated that this assay can be transferred between laboratories and is capable of detecting differences between lots. This assay can be used to characterize new acellular pertussis vaccines for preclinical evaluation or for trend evaluation. The protection models can be used to assess different vaccine formulations. However, the need for a reference standard is clear. The JN1H-3 preparation is the only candidate that can be bridged to lots tested in clinical trials. However, it has to be assessed against different acellular pertussis antigen preparations and multi-component vaccines. Given the number of new acellular pertussis vaccines under development, protection assays are needed to bridge clinically tested products and new products. This requires the establishment of new reference materials to assess validation criteria and specifications for new products.

5. Quality control and postmarketing surveillance of acellular pertussis vaccines in China, Japan and Korea: regulatory perspective

5.1. Quality control and lot release of acellular pertussis vaccines in China

Dr. Zhang presented the history and current situation of pertussis vaccines in China. Whole cell pertussis vaccine has been produced and used in China since the 1950s. DTaP vaccine was first licensed in 1995. Currently, a total of six acellular pertussis vaccines from local manufacturers have been licensed in China and another five are under development. These vaccines are co-purified two component products (PT and FHA) and are used in combination with Diphtheria and Tetanus vaccines. In addition, two imported products are also licensed in the Chinese market. These vaccines, produced by European and North American manufacturers, contain purified pertussis antigens (2 & 3 components). All acellular pertussis vaccines licensed for the Chinese market must achieve a potency of ≥ 8.0 units/ ml as determined by MICA. From 2006 all acellular pertussis vaccines in China are subject to lot release which is conducted both by protocol review and independent testing. At present, the potency is expressed in terms of a national whole cell pertussis vaccine reference, and a need for an acellular pertussis based International Standard for this assay was emphasized.

The experience gained at the National Control Laboratory in China (NICPBP), as part of lot release, is that MICA assay provides valuable information on the protection activity in animal model for all the locally produced vaccines as well as for imported products that were originally licensed in Europe or North America. The potency and safety requirements for the approval of DTaP vaccines in China are outlined in the Chinese Pharmacopoeia 2005 edition. Other standards are also applied such as the Chinese GMP standards for pharmaceutical and biological products and WHO requirements. The specifications for the antigen content of DTaP vaccines are: 18 μg protein nitrogen/ ml for acellular pertussis components, 25 Lf/ ml of diphtheria toxoid and 7Lf/ ml of tetanus toxoid with aluminum hydroxide. The specification for potency (MICA) for the final product is ≥ 8.0 units/ ml. The safety evaluation is performed using the same methods as described in Japanese Pharmacopoeia with some modifications (ie mouse strain and age/ weight). Specifications are 10 BWDU/ml for the body weight

decrease, 0.5 LPU/ml for the leukocytosis promoting test and 0.8 HSU/ml for the histamine sensitization test respectively.

Vaccines are produced using a Master Seed of *B. pertussis* isolated in China. Gene fragments homology analysis and geno-typing of the five protective antigens had been undertaken on this seed lot. The NICPBP reviews the batch information for each lot and tests 10-20% of randomly selected lots for potency using MICA. Other tests such as identity and histamine sensitization tests are carried out for 100% of lots. The Chinese authorities also perform GMP inspection for all manufacturers. Since 2006, a total of 64 batches of DTaP vaccine were tested of which 2 lots were rejected. The MICA potency test is used for lot release of acellular pertussis vaccines in China and the results of the NICPBP demonstrate good correlation with those of the manufacturers. Similarly, the histamine sensitization units (HSU) of products tested at the NICPBP are consistent with those of the manufacturers.

Dr Zhang has initiated an epidemiological program to survey pertussis in China. Data from clinical trials have demonstrated the effectiveness of the vaccines. Vaccines that complied with the potency specification of ≥ 8.0 units/ ml, determined by MICA, showed seroconversion rates at ≥ 4 fold increase or ≥ 20 EU/ ml of anti-PT and anti-FHA in 88-98% and 93-97% of the population, respectively. In general, there was low rate for reactogenicity in the immunized population. However, the direct relationship of the result from each of the three toxicity tests to the clinical outcome is not clear. The NICPBP has been involved in the research on new potential acellular pertussis vaccine components. Using the MICA they showed that recombinant antigens, such as pertactin, can confer protection against challenge when compared with an in-house reference vaccine. In contrast, immunization of mice with recombinant Fim (Fim2, Fim3) antigens induced low partial protection when compared with the reference vaccine.

5.2. Potency testing and post-marketing surveillance of acellular pertussis vaccines in Japan

The MICA has been mandatory for lot release of acellular pertussis vaccines since their introduction in Japan in 1981. This was based on data which suggested correlation of MICA results with the results of the original Kendrick test of the acellular pertussis vaccines as well as whole cell pertussis vaccines. Japanese acellular pertussis vaccines contain mainly PT and FHA and comply with the specification in terms of MICA. They are shown to be clinically efficacious as pertussis incidence has been continuously declining in Japan (7).

In Japan, national surveillance is carried out regularly to assess the immune status of the population against infectious diseases for which vaccines are available (e.g. *B. pertussis*). The national surveillance program can provide useful information to identify population groups with low levels of antibodies and design immunization programs to protect these vulnerable groups. In addition, the surveillance assists in the evaluation of consistency and interchangeability of vaccines used in Japan. Dr. Y. Horiuchi highlighted that the residual toxicity of current acellular pertussis vaccines used in Japan is 10 times less than the residual toxicity in whole cell pertussis vaccines used in Japan before 1980s. Although it is clear that severe adverse events are rare, the levels of residual PT in acellular pertussis vaccines showed correlation with clinical adverse events. The frequency of local swelling at the immunization site after booster doses would decrease when acellular pertussis vaccines containing low PT activity were used for the primary immunization. He noted that the information currently available may not directly prove the relevance of the well known toxicity parameters to the adverse reactivity observed.

5.3. Quality control of acellular pertussis vaccines in Korea

Dr. Y. Kim described the history and the role of the Korea Food and Drug Administration (KFDA). The Biologics Headquarters within the KFDA is the National Regulatory Authority and the National Control Laboratory responsible for the licensing

and the laboratory testing of all biologics including vaccines. There is a close cooperation between the KFDA (national and regional authorities) and the Korea Center for Disease Control. The regulatory activities are coordinated through the life-cycle of the product including the licensing and control laboratory activities, the clinical evaluation and surveillance studies which are used to further assess effectiveness of the product.

Whole cell pertussis vaccine was introduced in the Korean national immunization programme in 1956. In 1984 acellular pertussis vaccine was licensed and has been used since 1986. Bults of DTaP product from Japan and Europe are imported and the final product is manufactured in Korea. Final product is also imported from Europe and Asia. The NCL in Korea uses the MICA to assess the potency of pertussis components although some European products are assessed for consistency only by the immunogenicity test. Assessment of safety is carried out by inoculating mice with one human dose of test sample or a reference preparation and 3 different tests are performed with the same group of animals. On day 1 the body weight of each animal is recorded to determine the weight loss as a measure of toxicity. For the body weight decreasing (BWD) test, the body weight of each mouse is measured before and 16 hours after the vaccine inoculation. The body weight is statistically analysed. The specification for this test is 10 BWDU/ml. Day 3 following inoculation the leukocytosis promoting test is performed by determining the number of leukocytes in blood, the specification of this test is: 0.5 LPU/ml. The histamine sensitization test is carried out on day 4 using the Japanese temperature test and the specification is: ≤ 0.4 HSU/ml.

6. Quality control of acellular pertussis vaccines - manufacturers' perspective

6.1. Japanese manufacturers

Dr Watanabe highlighted that five manufacturers produce acellular pertussis vaccines in Japan, using a similar manufacturing process but with differences in the antigen content. All vaccines contain PT and FHA. However, the quantity of other

antigens (e.g. pertactin) differs. All manufacturers use the MICA to assess potency of the acellular pertussis components and although the vaccines differ in antigen content they all confer protection in the MICA model. Training of personnel is critical to ensure reproducibility of the MICA.

6.2. Chinese manufacturers

Dr Xu provided a summary of the manufacturers' view on the quality control of acellular pertussis vaccines in China. Reactogenicity observed following immunization with whole cell pertussis vaccines has led to the development of acellular pertussis vaccines all over the world, including China. Based on the understanding of pathogenesis of *B. pertussis* and the knowledge regarding protective antigens, manufacturers in China developed acellular pertussis vaccines that contain PT and FHA antigens. These vaccines were tested in clinical trials and were found to be safe and immunogenic. Quality control testing of acellular pertussis vaccines manufactured by Beijing Tiantan Biologics demonstrated consistency of production, including potency as assessed by the MICA and very low levels of residual PT. Acceptance limits for the reference standard used in the MICA should be established by each laboratory. These limits might be expressed in terms of the ED₅₀ of the standard. Beijing Tiantan Biologics is expected to supply not less than 8 million doses of DTaP vaccines to the Chinese government in 2008.

6.3. Korean manufacturers

An outline of the situation in Korea was provided by Dr Lee. Korea Vaccine Co. LTD has been producing a number of viral and bacterial vaccines since 1956. The potency of the acellular pertussis vaccine is determined using the MICA assay with ICR mouse strain, 3-4 weeks old, body weight 16-20 g. The interval between immunization and challenge is 21 days. Details of the assay were described with particular emphasis on the in-house reference which is a whole cell freeze-dried vaccine preparation with 112 Units per vial. Results of the assay are analysed by the bioassay assist program, version 2.0.0 (probit method). Validity criteria of the assay include deaths within three days of

challenge, paralysis after two weeks, LD₅₀ of challenge preparation and the percentage of viability of the total cell count obtained by the opacity assay.

7. Collaborative study: JN1H-3 as a candidate for International Standard for MICA assay

7.1. Study design, testing methods and samples

Dr D. Xing (NIBSC, UK) reviewed the background to the international collaborative study and its purpose, objectives and design. Acellular pertussis vaccines were 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. However, the difficulties involved in assessment of new acellular pertussis vaccines and the problem of licensing them in the face of ethical and practical constraints on efficacy studies have been recognised. 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.

Currently, the MICA is used in Japan, Korea and China and possibly other Asian countries. It has been proved to work reliably and is able to determine protective efficacy in animals. However, different whole cell pertussis vaccine standards are used in this test for definition of the 'unit' in terms of which the potency is expressed. Establishment of an international vaccine standard for MICA may lead to improved agreement of estimates between countries and this might also benefit development of new products. The two-component freeze-dried acellular pertussis vaccine preparation JN1H-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 from these can provide some continuity. However, following previous collaborative studies, there are still issues

that need to be addressed. The relationship of JN1H-3 with other Pa formulations in the MICA needs to be investigated. Re-calculation of vaccine potencies in terms of JN1H-3, in comparison with the calculation against whole cell vaccine reference needs to be carried out. Establishment of assay validity criteria need to be considered. Careful consideration should also be given to the impact of switching from current whole cell vaccine standards to an acellular pertussis vaccine based standard by countries who use MICA. To take these issues forward, an international collaborative study involving 14 laboratories in three countries was undertaken in 2006/ 2007. The objectives of the study were:

1. To investigate the relationship of the current International Standard for Whole Cell Pertussis Vaccine (PwIS) to in house whole cell pertussis standards (PwIHR), currently used for control of acellular pertussis vaccines.
2. To investigate the relationship of JN1H-3 to the PwIS and the various PwIHR currently used for control of acellular pertussis vaccines and, if possible, to assign a unitage to JN1H-3.
3. To investigate the consistency of estimates of acellular pertussis vaccines using as standard each of PwIS, PwIHR, JN1H-3.

Participating laboratories were asked to carry out at least two independent assays on different occasions for evaluation of the 3rd IS; JN1H-3 and the in-house reference together with the five coded samples (P1-P5) comprising two 2 component, one 3 component and one 5 component acellular pertussis vaccine in combination with D and T antigens plus the candidate Chinese Reference Preparation for acellular pertussis vaccine. Laboratories were asked to use their own methodology, reagents and calculation methods, include their controls and to use assay runs that met their internal control criteria. The results were analysed by an independent statistician to ensure consistent treatment of all data. It is expected that the results of the study should provide a consensus on the suitability of JN1H-3 as an IS for MICA and if possible define a unitage which maintains continuity with currently used values.

7.2. Statistical analysis of the results

Dr R E Gaines Das (NIBSC, UK) presented the statistical analysis of the collaborative study results. The statistical approach used the linear parallel line model with probit transformed responses and lines were fitted using iterative maximum likelihood methods. The dose-response lines for challenge showed no significant deviations from linearity and valid challenge LD₅₀ were calculated. The majority of assay analyses were statistically valid. Analysis of dose-response lines showed no detectable consistent differences among slopes for the acellular pertussis vaccines and JN1H-3. However, there may be differences between the whole cell and acellular vaccines in their responses in the MICA. The results showed that the laboratory geometric mean estimates for the various I_hR_s in terms of IS3 are significantly non-homogeneous, and there are also significant differences in estimates made using the different I_hR/ mouse strains.

Comparisons of JN1H-3 with the different I_hR/ mouse strains are highly significantly non-homogeneous ($p < 0.0001$). In contrast, comparisons of JN1H-3 with IS3 are non-homogeneous ($p \sim 0.01$) but the inter-laboratory variability is markedly less. Unweighted analysis of variance does not show significant inter-laboratory differences and there is no evidence of differences among estimates from the different mouse strains. The GCVs reflect this with the overall between laboratories GCV for JN1H-3 in terms of the various I_hR/mouse strains of 70% compared with overall between laboratory GCV for JN1H-3 in terms of IS3 of 43%.

Estimates of potency of DTaP vaccine products in terms of the various reference preparations showed that for each vaccine preparation the GCV for estimates in terms of JN1H-3 is less than the GCV for estimates in terms of the common Pw IS3 which in turn is less than the GCV for estimates in terms of the various I_hR Pw. This is consistent with the observed chi-squared values for homogeneity (as reflected in the probability levels for homogeneity), which are generally smaller for estimates in terms of JN1H-3 than for estimates in terms of IS3, which in turn are less than those for estimates in terms of the

various IhR. It is also noted that estimates in terms of the various IhR show significant or marginally significant differences among the IhR/mouse strains. Estimates in terms of JN1H-3 do not show significant differences among the different mouse strains although there is a general tendency for estimates in terms of JN1H-3 to be more consistent within the same mouse strain than overall.

7.3. Main outcomes of the collaborative study

Dr D Xing summarized the conclusions of the study as follows:

- In all participating laboratories the MICA performs as a valid assay. Reliability of MICA is supported by the study results.
- The MICA is able to distinguish protective efficacy in animals and make comparisons between products, irrespective of number of acellular pertussis components in the formulations.
- There are significant differences between estimates in terms of the various IhRs currently in use, which indicates the need for standardisation of MICA to improve agreement of estimates between countries.
- Slope differences observed in some laboratories in this study for whole cell vaccine compared with the acellular vaccine, suggest that acellular products may not behave exactly the same in the MICA as whole cell vaccine.
- Estimates of DTaP products in terms of JN1H-3 showed less inter-laboratory variation than when whole cell international standard was used. This suggests that an acellular vaccine reference is more suitable than a whole cell reference for acellular products in the MICA.
- JN1H-3 is shown to be a suitable candidate for International Standard for MICA. Dose-response relations for a variety of acellular pertussis and DTaP formulations are similar, irrespective of different numbers of acellular pertussis components.
- Evidence from current and previous collaborative studies indicates that JN1H-3 has maintained its biological activity, supporting its stability.

- Potency estimates in terms of JN1H-3 show improvement in inter-laboratory agreement compared with 1hR and Pw IS3.
- On the basis of these results, it is proposed that JN1H-3 should be established as an International Standard for acellular pertussis vaccine for use in MICA and other protective bioassays.
- To maintain broad continuity of units (expressed in terms of in house references of whole cell pertussis vaccines) currently used to describe the activity of acellular vaccine, it is suggested that JN1H-3 be assigned an activity of 34 IU (25-46) per ampoule.

Discussion on the collaborative study outcomes

Difficulties in introducing new formulations and new acellular pertussis products into the market are recognized, as there are limited opportunities to undertake new blinded efficacy studies in human. The MICA provides a quantitative measurement of the protective efficacy in animals. Potency of test vaccines expressed in IU is an important advantage of this assay in comparison with other assays currently in use. This makes it possible to compare the protective efficacy between products, irrespective of the number of acellular pertussis antigen components in the formulation. The MICA could thus prove of value in licensing and lot release of acellular pertussis vaccines. At present there is no recognized acellular pertussis reference standard. This creates problems in standardization of assays for acellular pertussis and in development of new acellular pertussis products. A stable reference that can be related to vaccines of known efficacy is needed for protection assays. In the previous and current studies, JN1H-3 has been shown to have biological activity with dose-response relations comparable and similar to those of a variety of current acellular pertussis and DTaP formulations with different numbers of components. The group agreed that JN1H-3 could have value as a reference preparation for MICA for acellular pertussis products. However, JN1H-3 would not be suitable as a standard for immunogenicity tests because such tests need product specific references. Discussion also focused on the unitage assignment for JN1H-3. The group agreed that to maintain a broad continuity of units currently in use, an activity of 34 IU

(25-46) per ampoule should be proposed for JN1H-3 based on the results of overall mean of laboratory mean estimates.

The implication of switching from current whole cell pertussis references to JN1H-3 was reviewed by the group. In comparing the use of whole cell pertussis IHRs and JN1H-3 as references in the assays, it was noted that, in general, the results for the four products (P1-P4) tested in this study there was agreement in terms of pass/ fail. However, a few discrepancies (~7%) in results were found for products that have borderline potency activity in some of the laboratories. It was recommended that communication between manufacturers and NRA/ NCL should be encouraged. Further evaluation of the impact of this change should be carried out in each country. It should be noted that the direct correlation of results determined in MICA and clinical efficacy is still unknown, although vaccines which meet release criteria by the MICA are shown to be clinically effective.

The group agreed that the use of JN1H-3 as reference in MICA in comparison with whole cell pertussis IS could have several advantages. JN1H-3 can be related to a vaccine for which clinical efficacy was demonstrated in clinical trial; use of JN1H-3 gives improved inter-laboratory consistency of results; the mechanism of action of JN1H-3 in MICA may be more similar to test acellular pertussis products than whole cell pertussis IS; standardisation of MICA can be improved. The proposed use of JN1H-3 as IS for acellular pertussis could be for:

- assessment of biological activity for new products in comparison with current/ or historic products known to be effective;
- calibration of national/ in house standards (secondary standards) for MICA;
- potency assignment during development of other protective assays e.g. intranasal challenge model for acellular pertussis vaccines.

A need for guidance on how to conduct calibration of secondary standards in terms of the JN1H-3 reference preparation was recognized.

8. Review of the recommendations for immunogenicity and potency testing of acellular pertussis vaccines in WHO guidelines and National Pharmacopoeias

Dr. D. Lei outlined the current requirements on immunogenicity and potency assays for acellular pertussis vaccines that are implemented in different countries. There are two widely used methods. The immunogenicity test is used to assess consistency of production in vaccines that have been tested for efficacy in clinical trials. In addition, a MICA is the model of choice in some countries to assess potency of acellular pertussis vaccines in routine lot release. One important advantage of the MICA is that it is a protection model that can be used with different vaccine formulations and with the same reference standard.

The current WHO guidelines for acellular pertussis vaccines describe two different immunogenicity methods that may be used. One is based on the measurement of the geometric mean antibody titre induced by one pre-selected (within the linear-response) dose of vaccine. The other method is based on the immunization of mice with serial dilutions of test and reference vaccine and the calculation of the ED₅₀ for each antigen. The WHO guideline stresses the importance of careful consideration of the mouse strain and the ELISA antibody detection system used. Regardless of the method used, immunogenicity as measured by this test is not an index of clinical efficacy. The European Pharmacopoeia accepts the immunogenicity test and the specifications are based on the comparison of the antibody responses induced by the test vaccine against a reference preparation. The reference vaccine is a batch of similar vaccine shown to be effective in clinical trials or a batch representative thereof.

The Japanese requirements are based on the assessment of vaccine potency using the MICA. This assay is designed to assess the protective capacity of a test vaccine compared to that of a reference preparation to protect mice against an intracerebral challenge with a *B. pertussis* strain 18 323. This assay is not included in the current WHO recommendations for acellular pertussis vaccines. However based on the wide use of this

assay in many Asian countries and the results from the recent collaborative study, inclusion of this test in the WHO recommendations is warranted.

9. Different approaches in assessing acellular pertussis vaccines for licensing and lot release

The requirements for assessing acellular pertussis vaccines vary depending on the country. In China, the Chinese Pharmacopoeia requires that all pertussis vaccines be tested using the MICA. Therefore, local manufacturers and the Chinese NCL use the MICA to assess the potency of both locally produced and imported vaccines. However, for some imported vaccines, China accepts the immunogenicity assay as a release test by the manufacturer subject to the requirement that the vaccine lot complies with the specifications of the MICA carried out by the Chinese NCL. So far vaccine lots released by the manufacturer on the basis of immunogenicity tests have complied with the Chinese requirements for MICA. Further investigations to assess the correlation between these assays might be of interest.

The MICA is mandatory for lot release in Japan since initial implementation of DTaP in 1981. This is based on the fact that the first acellular pertussis antigen preparation, Pillemer's stromata protective antigen, comprising bacterial components with haemagglutination activity was shown to be strongly protective in both the laboratory animals (Kendrick test) and also clinically in the MRC trial (8).

In Korea most acellular pertussis vaccines are assessed using the MICA. However, for some imported vaccines, which are licensed based on the immunogenicity test, the Korean NCL also releases these vaccines using the immunogenicity test.

The situation in other countries is different. The European Pharmacopoeia requires that the acellular pertussis vaccines be assessed for consistency using the immunogenicity

assay. In North America the use of immunogenicity assay is also the method of choice for evaluation of consistency of acellular pertussis vaccines for lot release.

In many countries the NCL is responsible for testing many different acellular pertussis vaccines. Thus different ELISAs and immunogenicity assays are required for each particular vaccine. Moreover, for vaccines based on co-purification process there is a significant percentage of unidentified components that may affect the interpretation of the ELISA results. It was noted that ultimately the NRA/NCL will have the responsibility for deciding on the type of assay (i.e. MICA or immunogenicity) used for licensing and for lot release. This decision should be based on the product characteristics, manufacturing history, clinical performance and all other available information. The WHO relies on the information given by NRAs/ NCLs and manufacturers regarding the needs for the development of standards and is willing to provide assistance in the implementation of recently established standards through regional and national workshops.

10. Other issues to be addressed in the revision of WHO guidelines on acellular pertussis vaccines: discussion

10.1. Toxicity tests

The histamine sensitization test (HIST) remains the test currently in use for toxicity assessment of acellular pertussis vaccines for active pertussis toxin (PT). There is at present no globally acceptable upper limit for PT content, except in countries using the temperature method. The issues regarding the variability of the assay based on the lethal end point and the difficulties for some complex combination vaccines to comply with the specifications set for DTaP vaccines have not yet been resolved. Many current HIST based on the lethal endpoint are not optimized and do not include a reference standard. The inclusion of a suitable reference in the assay, which addresses the assay sensitivity, would improve the interpretation of the results.

It was recognized that the Japanese method based on measurement of mouse temperature provides certain advantages in terms of assessing active PT. This method provides a quantitative assessment of PT content and has been adopted in other countries including Korea and China for routine lot release. For this test, a pharmacopoeial limit has been set. Japanese data were presented by Dr. Horiuchi on the effects of PT and endotoxin content on clinical reactogenicity. The Japanese pharmacopoeial requirement for PT is shown by Japanese data to reduce vaccine reactogenicity. However, the interaction of PT with other vaccine components and its relevance to clinical safety is not yet established. The European Directorate for the Quality of Medicines (EDQM) had proposed a collaborative study planned to address many of these concerns. This study has been unfortunately delayed and the group encouraged European colleagues to move forward on this important study. If this is not feasible, the Working Group recommended that WHO might consider setting up a collaborative study to explore potential improvements of this assay. It is important to liaise with the EDQM to ensure best possible use of available resources.

10.2. Challenges in conducting clinical evaluation of new acellular pertussis vaccines

The Working Group was informed by Dr Mago that Phase 3 clinical trials could be conducted in India since there are areas of the country where pertussis is endemic. However, there are ethical issues regarding the design of efficacy trials when there are effective and safe vaccines available. Therefore, it is likely that new vaccines would be evaluated by their ability to induce antibody titres against the vaccine components. In addition, post-marketing studies are critical to assess efficacy of new acellular pertussis vaccines, especially in situations where there will be a transition from the use of whole cell pertussis to acellular pertussis vaccines. One important aspect that may need to be monitored when whole cell pertussis vaccines are replaced by acellular pertussis vaccines is the potential increase in the incidence of *B. parapertussis* infections since whole cell pertussis vaccines have been reported to have better cross-protection against *B. parapertussis* than the acellular pertussis vaccines (9, 10).

11. Conclusions and way forward

The Working Group made the following conclusions:

11.1. MICA assay and JN1H-3 candidate material

1. Ability of MICA assay to provide quantitative measure of the protective efficacy in animals was re-affirmed in the collaborative study.
2. MICA assay with the potency of tested vaccines expressed in International Units is an important advantage of this assay in the context of licensing and lot release of acellular pertussis vaccines. This assay is appropriate for both National Control Laboratories and manufacturers since good inter-laboratory agreement is achievable.
3. On the basis of the results of the collaborative study, JN1H-3 is suitable as a reference for assessing a variety of different formulations of acellular pertussis vaccines, irrespective of the number and different components, by MICA assay.
4. Report of the collaborative study should be finalized taking into account comments provided by the participants of the study and will be submitted to the Expert Committee on Biological Standardization at its meeting in 2008.
5. Testing procedure and assay validity criteria for MICA should be described in the revised WHO recommendations for acellular pertussis vaccines with a variety of options for its use.

11.2. Toxicity testing

Collaborative study to explore potential improvements of the HIST assay should be considered and any outcomes of the proposed collaborative study should be reviewed by the Working Group in the context of the revision of the WHO recommendations for acellular pertussis vaccines.

11.3. Revision of current WHO guidelines for acellular pertussis vaccines - points to be addressed:

1. Evaluation of currently licensed vaccines should be distinguished from the new ones;
2. Developments since adoption of TRS 878 (e.g. protection assays, reference preparation and collaborative studies) are to be described;
3. Recommendations for production and control need to be updated;
4. Testing procedures for conduct of the protection assays and assay validity criteria should be provided;
5. Immunogenicity assays and their validity criteria require an update;
6. Testing procedures and analysis of the results for toxicity assessment need to be updated;
7. Reference preparations should be elaborated;
8. Recommendations for nonclinical and clinical evaluation of new acellular pertussis vaccines should be added;
9. Issues to be addressed in PMS (e.g. safety monitoring) should be described.

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