Distribution: General
English only

Report

Working Group on the standardization and control of Pertussis vaccines

World Health Organization
Geneva, Switzerland

6-7 May 2003

WORLD HEALTH ORGANIZATION
Quality Assurance and Safety of Biologicals

The meeting was opened by Dr D Wood, Acting Coordinator of Quality Assurance and Vaccines, WHO, Geneva who indicated that its primary purpose was to advise WHO on the upgrading of Guidelines for the production and quality assurance of acellular pertussis vaccines. These vaccines had been introduced against a background of a variety of formulations with no agreed standard and with no satisfactory animal model for the establishment of potency. WHO had established temporary recommendations but was aware of the need to keep the situation under review.It had initiated the Working Group in 1998 to review the position regarding the current status and need for improved potency assays. This had conducted an initial study to assess the potential of the currently available methods. On the basis of the results of this, at the last meeting in 2000, the decision had been taken to conduct a collaborative study focusing on the intra-nasal challenge assay (INCA) and also to examine the effect of active pertussis toxin on the modified intracerebral challenge assay (MICA). It was hoped that the outcome of these studies would enable the Group to advise WHO on the need for further action and whether the current recommendations on acellular pertussis vaccines need to be updated and if so, when. It was also recognized that the current recommendations in relation to whole cell pertussis vaccines needed to be considered, especially in relation to the interpretation of testing requirements. The conclusions and recommendations of the Group would form the basis of advice to WHO on future action.
The format of the meeting was in two parts; the bulk of the meeting was to be in open session involving all the participants but with a final closed session to finalize the conclusions and recommendations, restricted to those with no conflict of interest. A report of the meeting was to be prepared. This would be posted on the WHO web site and also published. The draft report would be presented to the ECBS meeting scheduled for Autumn 2003.

The meeting appointed Dr J H Kreeftenberg as Chairman and Dr M J Corbel as Rapporteur.

Dr E Griffiths, Biologics and Genetic Therapies, Canada then summarized the previous history of the Group. Following with the drafting of the current Guidelines in 1996 and their publication in 1998, the ECBS recognized the need for further discussion of unresolved issues and recommended the establishment of the Working Group. This issues included lack of consensus of antigenic composition of acellular pertussis vaccines, the use of antigens derived from different strains and purified by different methods and incomplete knowledge of mechanisms of protection. These factors complicated direct comparison of protective activity of different products by simple laboratory tests. The lack of unequivocal immunological correlates of human protection and the absence of a generally accepted animal model made it difficult to introduce new formulations as there were now few opportunities to undertake blinded efficacy and safety studies in humans. Currently lot release depended on characterization of antigens, determination of toxicity by specific assays for suspect components (histamine sensitization test (HST) and endotoxin assay), assay of potency by immunogenicity or INCA.
The Working Group met in 1998 to discuss assay methods. A collaborative study was set up to analyse a panel of coded samples of clinically effective vaccines in various protection models and in the immunogenicity assay (IA). The results showed that respiratory challenge models were promising but raised further questions including the relative role of different antigens in the various assays, the comparability of lung

colonisation produced by INCA or aerosol challenge (AC), the correlation with human protection and how to use respiratory challenge assays especially in relation to pass-fail criteria.
It seemed that respiratory challenge was too complex for routine lot release but might be useful for characterization of new products, in process development, stability studies, pre-licensing evaluation and post marketing evaluation especially in relation to the effects of emergence of new strains of *B pertussis*
The present meeting of the Group was to discuss the latest data and next steps in relation to potency testing of acellular vaccines and to consider updating the 1998 Guidelines. Uncertainty remains over the role of specific components in the pathogenesis of pertussis and the toxicity and reactogenicity of
pertussis vaccines. This has hampered development of rational limits for residual activities of such components. Some components were currently controlled by process validation, others such as endotoxin and PT are specifically monitored with limits based on clinical trials data. Current assays, the HST and CHO cell clustering assay (CCCA), had limitations and improvements were needed. An HPLC-based ADP ribosyl transferase assay developed in Canada, and examined in Europe looked promising but might need further development/ evaluation. The current meeting would consider this and make recommendations in the context of regulatory expectations.. The meeting also needed to consider International and other reference materials their development7replacement and applications. It also had to consider the need for revision of the 1979 WHO recommendations for whole cell pertussis vaccines, especially in relation to toxicity tests.
Dr R Dobbelaer (SIPH), Belgium) reminded the meeting that in the enlarged European Union more than 50% of the pertussis vaccines used would be whole cell, therefore revision of the 1979 Guidelines was not just relevant to developing countries.
Dr M J Corbel (NIBSC, UK) summarized the objectives, design and outcomes of the collaborative studies on the INCA set up in September 2001 and that on the effect of active PT on the MICA initiated in November 2001.
The INCA had been selected as the respiratory challenge method for further study because of its relative simplicity and lack of need for specialized equipment. A harmonized protocol had been developed by Dr N Guiso. The main objective of the study was to determine if the assay could be used in independent laboratories to distinguish active from inactive vaccines and to assign potency. The harmonized protocol used 3 week old Balb/c mice immunized with 125 ul volumes of vaccine at days 0 and 14 and challenged intranasally at day 28 with 50 ul of a *B.pertussis* strain 18323 suspension containing a dose in the range 10^6 to 10^9 cfu. Lung cfu counts were to be performed at day of challenge (0+2 hrs) and 5 and 8 days after challenge. The tests were top be performed twice on different days. Participants were free to perform their own in house procedures in parallel with the harmonized INCA.
Participants were from 11 laboratories in 8 countries (see appendix). The coded test samples were
A= a 3 component DTaP vaccine in alumininium hydroxide adjuvant, B= JNIH-3 reference 2 component acellular pertussis vaccine, C= a 1/10 dilution of A in aluminium hydroxide, D= aluminium hydroxide gel, E =heat denatured sample A.(see appendix). The data were returned to the statistician and log transformed values subjected to analysis of variance.
The conclusions of the study were that

- the harmonized INCA could be transferred between laboratories

- the INCA and other methods including in house INCAs, AC and MICA, differentiated the 5 samples
- All could distinguish active from inactive vaccine
- The INCA could distinguish samples A and C but clear evidence of a dose response was not obtained probably because the dilution of C was too great for a linear response
- Significant inter-laboratory variability was seen in absolute responses
- The procedure should be optimized to allow estimation of relative potencies
- JNIH-3 performed satisfactorily in this study but may not be an ideal reference for all types of acellular pertussis vaccines. JNIH-3 is the only reference currently available which is traceable to clinical trials but relates to a vaccine of much lower efficacy than those currently in use

Because of a prevailing view that early Japanese acellular pertussis vaccines had a significant content of active PT and that this was necessary for activity in the intracerebral challenge test, the effect of measured quantities of PT on the performance of the MICA was examined in a collaborative study. .The objectives of this were

- To determine if active PT influenced the results of the MICA
- To determine the influence of mouse strain on the MICA
- To compare the MICA with respiratory challenge tests
- To provide advice to WHO on the MICA

The design of the study was that participating laboratories were asked to evaluate five coded samples (A-E) comprising three separate samples of the same genetically detoxified DTaP vaccine in aluminium hydroxide adjuvant either unmodified (A) or supplemented with 5ng PT (B) or 50 ng PT (E). Sample C contained aluminium hydroxide gel alone and sample D was JNIH-3. The active PT contents of the samples were determined by the participants using either the conventional HST or the rectal temperature modification (RHST) using JNIH-5 as PT reference or the in house PT reference.

The data were returned to the statistician for analysis. The conclusions of the study were that

- All the test systems could distinguish the acellular vaccines from the negative control
- The MICA was effective for determining activity of genetically detoxified vaccines with or without active PT.
- Active PT increased the apparent potency in the MICA. A similar effect was seen on the respiratory challenge methods.
- The results of respiratory challenge paralleled those of the MICA although absolute values differed.
- Active PT increased the variability of results in the AMPT
- Mouse strains probably influenced the MICA results but this needs further study.
- JNIH-3 could be used as a reference in the MICA
- MICA is effective for assigning relative potency to acellular pertussis vaccines.

The statistical outcomes of these studies were analysed in detail by Dr R Gaines Das (NIBSC,UK).