Report

WHO Consultation on DT Potency Assay and Consistency Measurement

Bilthoven, The Netherlands
16-17 December 2002

WORLD HEALTH ORGANIZATION
Quality Assurance and Safety of Biologicals
Agenda

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Opening Remarks

Dr Hans Kreeftenberg of RIVM welcomed the participants to Bilthoven, and then turned the meeting over to Dr Knezevic. Dr Knezevic noted that the WHO convened two meetings of the Working Group on Harmonization of Antigen Content and Potency Measurements of the D- and T- components in Vaccines (Bilthoven, 15-16 July, 1999; Bethesda, February 3, 2000). The International Symposium on Tetanus Vaccines for Human Use, held in Strasbourg, 22-23 June 2000 was also an opportunity to discuss this issue. Each of these meetings had stressed the need for harmonization, and for a simple and robust assay for lot release to demonstrate consistency in immunogenicity of the D and T- components in vaccines. Subsequently a draft proposed amendment to the current WHO Requirements as well as a review of the situation were presented to the Expert Committee on Biological Standardization (ECBS) at its meetings held in October 2000 and November 2001 (1,2). The purpose of the present meeting is to describe developments since that time and to review the current position.

Dr Knezevic asked Dr Griffiths to chair the meeting. Drs Gaines Das and Knezevic acted as Rapporteurs. Dr Griffiths continued the meeting by thanking the host and then asked each participant to identify themselves and their affiliations (see Annex 1). He then presented an overview of the issues.

Overview of Issues

Dr. Griffiths noted that DT vaccines have been remarkably successful, but that there are recognized problems in standardization and control of potencies of D- and T-toxoids, even with International Standards. There is no globally accepted approach to potency measurement. In 1979 WHO requirements (3) included for the first time definite recommendations for minimum potency requirements of 30 IU for D and 40 IU for T, and in 1990 confidence limits were added to the Requirements (4). When consistency of production and testing has been established, the current WHO Requirements allow use of fewer animals and one-dilution assays with the approval of the national regulatory authority. Methods based on toxin neutralization in vivo or on validated in vitro methods are also allowed. Since 1990 there has been much work done on validation of in vitro assays and the potential effect of this work on requirements for vaccine batch release has been considered.
In the approach adopted by the EU and WHO, vaccines must meet minimum potency requirements in terms of International Units (IU) defined by the appropriate vaccine standard. A different approach used in the USA does not include a reference vaccine and therefore the potency cannot be expressed in International Units. Globally, countries follow one or the other approach. The situation and issues were reviewed in the 1999 WHO Consultation, and it was agreed that there were problems with both approaches and that there was a need to move forward in some way. It was further agreed that once the consistent manufacturing of clinically safe and effective toxoids had been well established, a single dose immunogenicity assay would be sufficient to demonstrate consistency in immunological characteristics for purposes of lot release. The ECBS strongly endorsed the development of simplified lot release as a first step toward harmonization at its meeting in 2000 (5).

As a result of previous meetings and discussions (1,2) a draft proposal for simplified lot release was prepared and has been widely circulated for comments. This has been well received in principle, but many technical issues need to be resolved and agreed.

The aims of this meeting were to review progress towards the development of robust and standardized in vitro assays for antitoxin measurement, to discuss potential solutions to outstanding technical issues related to the proposal for simplified lot release, and to consider steps which may advance the aims of assay simplification and harmonization.

**EU Future Direction**

Dr Winsnes reviewed the current Ph.Eur. position and summarized the results of recently completed and on-going collaborative studies on the characterization and validation of in vitro serological methods for the testing of D- and T- vaccines.

The EU requires estimates of vaccine potency in terms of IU, relative to an appropriate vaccine standard, and requirements are set in terms of the lower fiducial limit of the estimates. A procedure for one-dilution assays is also described in the European Pharmacopeia (EP) for release of well-characterized products with a history of consistent good quality.

In 1996 a study of serological methods for potency testing of tetanus toxoid-containing vaccines was initiated. One phase of the study showed that estimates of vaccine potency based on the measurement of toxin neutralizing activity of serum correlated with those obtained through the use of challenge methods, and it was further shown that validated serological assays (Elisa and Tobi) gave results that correlated with the toxin neutralization activity. Following the success of this project (6), a study of diphtheria toxoid-containing vaccines has been initiated, and preliminary results are promising. One finding of these series of studies has been to highlight the need for in-house validation of serological assays, and the critical nature of the reagents used for these assays.

On the basis of the results for tetanus vaccines, amendments to the EP requirements for Tetanus Vaccine, adsorbed, have been proposed and are currently available for comment (7). These proposals include the use of serological responses in
guinea pigs to reference and test vaccines as the basis of determination of vaccine potency as an alternative to the challenge test.

Dr Behr-Gross of EDQM noted that the policy of Ph. Eur. Commission, with the backing of the Biological Standardisation Programme, supports on-going review of tests with the aim of both simplifying lot release and reducing and refining animal testing. The programme includes projects related to the validation and use of serological testing of D- and T- vaccines, and development of required reagents.

**USA Approach for the Future**

Dr Arciniega prefaced his presentation by stating that he was relaying the results of discussions among scientists at CBER, and that those remarks should not be construed as FDA policy. Harmonization is in the best interest of regulators, manufacturers, and most importantly vaccine users; however, compromises should not be made exclusively for the sake of abolishing the use of animals. Dr Arciniega enlisted among the incentives for harmonization the mutual acceptance of outcomes resulting from the use of common methods and a better acceptance of vaccine control activities by society. He also suggested that change should be incremental. The use of non-functional antibody tests for D and T serologic potency testing might be premature.

Since 1947, potency testing of D- and T- toxoids in the USA has been carried out, essentially without changes, as described in the NIH Minimum Requirements. NIH test design has been subject of some criticism. The test involves the immunization of guinea pigs with a relatively high dose of undiluted vaccine (no more than one half the total human dose, based on the convention that a total human dose consists of three single human doses of 0.5 ml each). The high dose of vaccine may make the test insensitive to deleterious changes in toxoid quality and/ or quantity. In addition, as presently carried out, the test does not include a control vaccine, and the serum neutralizing activity is determined in a pool of serum of the animals injected. The neutralizing activity of the pooled guinea pig serum is expressed in Units of Antitoxin/mL, relative to the US Standard of equine antitoxin in use. Some concern was expressed that such Units might not be equivalent to International Units. Dr Winsnes asked if CBER would provide samples of US Diphtheria Antitoxin to be included in a collaborative study being organized by EP. Dr Arciniega answered the request positively.

To achieve harmonization, CBER scientists suggested the possibility of including a control vaccine, although some opposition to the adoption of this practice comes from the fact that it would increase the number of animals required per test. Moreover, control vaccine is expected to be used to validate assay performance and not as a comparator. A careful consideration should be also given to the composition of the control vaccine, in view of the multiplicity of D and T-containing clinical formulations. Analysis of individual sera may also be possible if an *in vitro* antibody assay is adopted. However, any criterion for acceptance other than the current minimum of 2 Units of Diphtheria Antitoxin per ml of guinea pig serum would require adequate justification, and probably some clinical studies. Nevertheless, any proposal of a modified potency test for toxoids will be considered under the Equivalent Methods and Processes Section of the CFR. Regarding the immunizing
dose, the use of lower doses of undiluted vaccine are permitted by the Minimum Requirements, without exceeding 0.75 mL. A slide summarizing the D and T contents (in Lf) of DTP vaccines licensed in the US was shown, indicating that at least for one product 0.75 mL was not an excessive immunizing dose.

**Review of Progress Towards Simplified Lot Release Assay**

Dr Sesardic of NIBSC said that batch release is essential to ensure that vaccines are safe and have consistent relevant activity. Progress has been made with the use of the one-dilution test after validation, and the development of serological methods to replace direct challenge. Methods in use in the Ph.Eur. require the use of vaccine standards. Data from the recent collaborative studies of the IS for Tetanus Toxoid, adsorbed (8) and of the IS for Diphtheria Toxoid, adsorbed (9) were presented. These show that the mono-component standards can give consistent results between laboratories in the classical guinea pig assays. Replacement of these assays with serological assays is supported by data from European studies, although not all issues regarding validation and essential critical reagents have been completely resolved. In particular, additional reference reagents including species-specific antisera may be helpful for the validation of serological methods. Further work is ongoing for use of the same animal model for testing of multiple antigens, and for the development of alternative assays for antigen detection.

Dr Di Fabio from the WHO Regional Office for the Americas / Pan American Health Organization said that most Latin American countries have adopted an approach based on the USA test. Guinea pigs were difficult to obtain and maintain in some areas. For this reason initiatives had been undertaken to validate tests in mice and *in vitro* tests. There were issues needing resolution before such tests could be implemented, and many reagents required for valid *in vitro* tests were not readily available. There was a need for clear direction from WHO on test specifications and validation.

Dr Jadhav from the Serum Institute of India noted that some previous comments had appeared to distinguish between developed and developing countries, and considered that this should not be relevant for the WHO Guidelines, which should apply to all countries. The Indian Pharmacopoeia allows two methods, antibody induction (derived from the British Pharmacopoeia 1963) and challenge (derived from the British Pharmacopoeia 1980). When carried out without simplification the challenge method requires the use of large numbers of animals. Data supporting the agreement between test results for vaccines based on antibody induction and based on challenge assay were presented. Data supporting the possible use of serological results from the same guinea pigs for testing of both the D and T components of vaccines were also presented. The doses used for immunization and testing may require local optimisation, and this should be reflected in the Guidelines. Where there is experience of consistency of manufacturing, then simplified assays would facilitate lot release, but some national control authorities may be reluctant to approve this without a clear statement in the Guidelines about when and how simplified methods may be used. Harmonization would help to resolve difficulties caused to vaccine suppliers by the different requirements of different countries.
Dr Kaligis of BioFarma, Indonesia, noted that the number and size of batches of vaccines produced yearly have an important influence on the frequency of testing. Documented evidence of consistency of manufacturing and of potency measured in IU is based on the tests performed on each batch. Potency of vaccines produced by BioFarma, expressed in IU, and tested in different laboratories, showed different results, although the minimum requirements were exceeded for all tested vaccines. Alternatives to this approach, such as an assay based on a comparison with a lot of ‘proven clinical efficacy’ appeared promising, but it was not clear whether such an alternative would be feasible or how it could be implemented.

Dr Takahashi from the National Institute of Infectious Diseases, Japan, said that current requirements in Japan had resulted in control of disease and provided evidence of effective protection. Vaccines differ from other medical products, since safety and efficacy may be affected by the combination of products used in the series of immunizations. Thus, interchangeability of vaccines from different manufacturers is an important issue, because use of a single brand for the series of injections cannot be ensured. Unified regulation and appropriately validated test methods applicable to the range of available products are essential for safe and effective vaccination. In Japan, a particular mouse strain had been successfully validated against the guinea pig assay and potency estimates obtained with that strain agreed with those obtained in guinea pigs. Data were presented showing a relationship between the potency of tetanus toxoid, measured in the guinea pig model and the antitoxin titre induced in infants (10).

Dr Sabouraud of Aventis Pasteur, representing the IFPMA, presented a view from industry. Very large numbers of animals were required for the quality control testing necessary to meet the regulatory requirements. Their data on the reliability of EP and USA methods showed that a proportion of the EP assays were invalid (10% for T and 30% for D), but that results obtained with the USA method were not well reproduced (a test vaccine with a known anti-toxin response range gave results outside the range in 75% of tests). Harmonization of WHO and EP Guidelines, and use of one-dilution tests would lead to substantial simplification and reduction in the number of animals required. Many vaccines are distributed globally and harmonization of WHO/EP and USA requirements is also desirable. Ideally industry would want a test for each vaccine component which was easy to carry out on a routine basis, was reliable and consistent between laboratories, required few animals, had a globally harmonized design and specification and used a single reference vaccine for all product combinations. For batch release, validated assays for antigen content might be considered in the future.

Discussion and Conclusions

Lively discussions resulted from the presentations from the participants. Several participants provided references indicating that the potency of vaccines determined in IU correlated to human serological response in clinical studies (10, 11,12,13,14,15). The choice of doses used for the serological assays, and the marked difference in doses used in the Ph.Eur. and in the USA, was discussed. Some participants commented that there was some evidence that vaccines passing the USA criteria failed the EP criteria. From a practical point of view, the immunizing dose will influence the design of the antibody-measuring system; for example, a relatively
low dose will induce a low antibody response that will require a sensitive detection method. Differences in detection methods may drive the choice of reference materials. The role of dose size in vaccine acceptance criteria was discussed. The need to relate test criteria to immunization schedule received brief comment. Participants related various experiences with the use of guinea pigs and/or mice and what they saw as the advantages and disadvantages with the different animal models. The suggestion that the same guinea pigs could be simultaneously used to test the D- and T-components of combined vaccines appeared promising, but representatives of industry said that the implications of the longer time required for this test may not be acceptable. All participants supported the principles of reduction and refinement, and agreed that this was a relevant factor but not one that should override the need for sound scientific data. There was general agreement on the acceptability of the one-dilution test for lot release of a product with a history of consistent manufacture, but a disadvantage of this test is the loss of quantitative information about the vaccine potency, and the need to carry out full assays for stability studies. Different possible reference preparations and the purposes they might serve were discussed. Dr Knight commented that a ‘generic DTP vaccine’ might be more useful as the reference for some combined vaccines than the monocomponent reference preparations (D and T). A generic DTP reference preparation can be more readily reconstituted than the separate D- and T-components and presence of the P component may overcome difficulty of the interpretation of the potency results of tested DTP vaccines.

Dr Hunolstein presented results of a European serological study (16) in which the clinical relevance of factors such as vaccine dose and immunization schedule were emphasized. One finding was the circulation in some populations of non-toxigenic strains of bacteria. Possible causes for this, and whether this might reflect vaccines used for immunization and immunization schedule were discussed.

The proposed amendment to WHO Guidelines was then considered in detail. Participants agreed that the need to carry out a potency test on each final bulk, as presently specified in the Guidelines A.3.5.6 should incorporate in large print a paragraph similar to that in the present proposals.

However, participants considered that the amendment should not be based on a comparison with ‘a lot of the same product shown to be clinically effective’. Doubts were expressed about the feasibility of defining, obtaining and maintaining such a lot. Furthermore, the provision of a separate control preparation for each individual product was not considered practical, especially by representatives of the manufacturers. The loss of any comparability between different products, if the international unit was not the basis for potency, was viewed as a problem with possible implications for clinical use of the vaccines. Maintenance of continuity, both in terms of vaccine potency, and of serological measures was considered important, highlighting the need for appropriate reference preparations with broad applicability.

The majority of participants considered that estimation of vaccine potency in terms of IU as defined by the appropriate generic standard, and of lower limits of that potency as the basis for product acceptance had on the whole performed well in ensuring vaccine efficacy. It was recognized that there might be problems in specific cases. Nevertheless, generic reference preparations appear to work well overall, and
where they do not, provision for exceptions may be made. This is consistent with the
approach adopted by the Ph.Eur., and different from the approach in the USA.

Simplified lot release may require periodic review to ensure that validity of all
procedures is maintained. One disadvantage of simplified lot release based on one-
dilution tests is that strictly quantitative estimates of vaccine potency will be lost and
this may have implications for epidemiological studies. It was noted that there is a
need to support the data generated by simplified potency assay with physical /
chemical methods in order to ensure consistency of production.

In concluding discussions the participants agreed on the following items:

- The key principles of the current requirements, minimum potency and direct
  comparison to a standard calibrated in IU, should be retained.

- The use of single dilution assay for the purpose of batch release is already allowed
  by WHO. However, WHO Guidelines should clearly indicate in which
  circumstances the simplified assay could be used and in which circumstances full
  assay is required.

1. Reference preparations:

- Use of generic reference preparations with potency calibrated in International
  Units should continue, since generic reference preparations are satisfactory if not
  ideal in most cases.

- Variations in potency due to discrepancies in bioassay of heterogeneous materials
  may be less than the variability inherent in the assay system and may be taken into
  account by the way in which specifications are framed. If the discrepancies due to
  use of a generic reference preparation are unacceptably large under the conditions
  of assay, a more closely homologous reference preparation might be used,
  provided it has a potency traceable to the generic reference.

- Traceability and continuity of testing are important considerations for the
  interpretation of results.

- Comparability between products, produced by different manufacturers, allows
  interchange of safe and effective vaccines within an immunization programme.

- Proposal by the CBER scientists to consider the introduction of the use of a
  control vaccine in the NIH test were welcomed by the participants, and this,
  together with the greater reliance on serology in the WHO/EP Guidelines were
  promising moves towards harmonization.

2. Animal models:

- Reduction and refinement of animal based assays should be emphasized, although
  not at the expense of valid scientific information. The proposed amendment
  should suggest approaches towards reduction of a number of animals per group.
The guinea pig appeared to be suitable as a model, especially when more than one antigen is tested, although use of a specified strain of mice with appropriate validation was also acceptable (e.g., ddY strain validated and used for tetanus assay, in Japan); Some guidance provided by WHO will be useful in this context.

3. Correlation between \textit{in vivo} and a number of \textit{in vitro} methods had been demonstrated and replacement of challenge by serology was thus possible;

4. The measurement of potency for the purpose of \textbf{stability study} should be based on the full potency assay. The use of simplified assay for stability study will need further consideration. In practice, this should be considered on a case-by-case basis, but some WHO guidance may be helpful.

Summary and Future Work

The need for simplified requirements for lot release was agreed to be an achievable goal, but it was noted that such requirements would need to be clearly set out. It was recognized that there may be some problems with the current International Standards for Diphtheria Vaccine, adsorbed, and for Tetanus Vaccine, adsorbed. The International Standards for both D and T vaccine were calibrated using challenge assays in guinea pigs. However, each study report included information from assays using mice (8,9) and the effect of using the T standard in mouse assays was assessed in Europe (8). More information and guidance on use of the mouse assay would be helpful. Nevertheless, data from these recent collaborative studies indicate that the International Standards may be sufficient to meet many needs, subject to recognition that there may be occasional exceptions. The consensus was that there is a need for comparability between different products, and that requirements should continue to be expressed in terms of IU. Immediate harmonization of the diverse approaches used by the USA and WHO/EP is not possible, but there are promising moves toward agreement in the use of serology by WHO/EP and the possible adoption of a control vaccine by the USA.

Dr. Griffiths summarized the principal points addressed at the meeting. It was agreed that the Report of the Meeting would be circulated in January before submission as an information document to the February 2003 meeting of ECBS.

Dr. Griffiths thanked Drs Knezevic and Kreefenberg for the organization of the meeting, and all of the participants for their contributions.

References:

1. ECBS 2000 info doc 7, Proposed amendment to the requirements for diphtheria, tetanus, and combined vaccines
2. ECBS 2001, info doc 13, Current situation on Diphtheria and Tetanus potency testing
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12. Huang et al., Responses to primary and a booster dose of acellular, component, and whole-cell pertussis vaccines initiated at 2 months of age. Vaccine 1996;14, 916


14. Bartels et al., Immunogenicity and reactogenicity of a single dose of a diphtheria-tetanus-acellular pertussis component vaccine (DTaP) compared to a diphtheria-tetanus toxoid (Td) and a diphtheria toxoid vaccine (d) in adults. Vaccine 2001, 19, 3137-3145


Annex 1

List of Participants

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