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Annex 5
Recommendations for diphtheria, tetanus, pertussis and combined vaccines (Amendments 2003)

Introduction

These amendments should be read in conjunction with the introduction to the Requirements for diphtheria, tetanus, pertussis and combined vaccines published in WHO Technical Report Series, No. 800, 1990 (Annex 2).

Diphtheria and tetanus vaccines are among the most frequently used vaccines worldwide and have been remarkably successful products. Their use has resulted in a significant decrease in the incidence of these diseases in both the industrialized world and in developing countries. Nevertheless, some difficulties exist in the global harmonization of potency testing procedures, even when International Standards are used, and different approaches have been taken by different countries. Some follow WHO and *European Pharmacopoeia* procedures, whereas others follow the National Institutes of Health (NIH) procedures used in the USA, with or without modifications.

The approach taken by the *European Pharmacopoeia*, like that of WHO, is based on the determination of the immunizing potency of each final bulk by comparison with an appropriate reference material calibrated against the International Standard for Diphtheria Toxoid (adsorbed) or the International Standard for Tetanus Toxoid (adsorbed), as appropriate (*1*, *2*). There has been much activity in recent years aimed at simplifying the current tests, reducing the number of animals used and refining the end-point used in potency testing. Some studies have also considered the possibility of using the same animals to test the potency of several antigens.

The approach taken by the USA is based on the NIH assays (*3–6*) where the minimal acceptable potency is defined as the capacity of a test vaccine to induce an antibody response that reaches or surpasses the threshold of 2 units per ml. A suitable reference antitoxin, to which "units/ml" have been assigned, is used to express antibody concentration in relative terms, as measured by an in vivo toxin neutralization assay.

The inclusion of a control vaccine in the NIH test is being considered and would, in principle, improve control of the variations in the immune response induced in animals. The application of the Vero cell assay for the detection of anti-diphtheria toxin neutralizing antibodies is also being considered in the USA. Also, the expression of antibody levels in International Units could be achieved by calibration of the reference antitoxin against the International Standard for antiserum (see section A.1.3).

Despite many attempts to harmonize potency requirements globally, there are still no universally accepted methods. This leads to problems in international exchange of these vaccines arising from difficulties in the mutual recognition of the results of testing. The development of new combination vaccines, has led to an increased need for harmonization of the diphtheria and tetanus potency tests, creating a unique opportunity to resolve this long-standing issue.

The purpose of the potency test is to assess in a suitable animal model the capacity of the product being tested to induce a protective response analogous to that of toxoids shown to be efficacious in humans. The potency test has two stages. During the first stage a protective response is induced in mice or guinea-pigs, and during the second stage the protective response is measured by direct or indirect methods.

Considerable international consultation has identified the need to clarify the current WHO text relating to the introduction and use of simplified potency assays for the purpose of routine lot release. This should be seen as a first step towards the revision of the whole text of the current WHO Requirements (Recommendations) for Diphtheria, Tetanus, Pertussis and Combined Vaccines. The following amendments have thus been made to Annex 2, WHO Technical Report Series, No. 800, 1990. These include:

1. The updating of sections on International Reference Preparations for Diphtheria vaccine (adsorbed) and Tetanus vaccine (adsorbed);

2. The division of the sections on potency for Diphtheria vaccine (adsorbed) and for Tetanus vaccine (adsorbed) into two subsections to clearly distinguish the recommendations for licensing from those for routine batch release;

3. Simplification of the routine testing for batch release and use of fewer animals than used for licensing;

4. Amendment of the recommendations for diphtheria and tetanus potency testing in the diphtheria, tetanus, pertussis combined vaccine section to bring them in line with the changes outlined (in 2 and 3) above.

No changes have yet been made to the pertussis section of the Requirements for pertussis vaccines published in Technical Report Series, No 800, 1990 (annex 2).

Requirements for diphtheria vaccine (adsorbed)

Part A. Manufacturing recommendations

Replace section A.1.3, *International reference materials,* by the following:

A.1.3 *International reference materials*

The first International Reference Reagent of Diphtheria Toxoid for Flocculation Tests was established in 1988 (*7*).

The Third International Standard of Diphtheria Toxoid Adsorbed was established in 1999 (*8*) for determining the potency of vaccines containing diphtheria toxoid. The assigned activity of 160 IU/ampoule is based on its calibration in guinea-pig challenge assays. Potencies calculated by other methods should not be assumed to be transferable without validation. When potency tests are carried out in mice instead of guinea-pigs, transferability should be demonstrated.

The International Standard for Diphtheria Antitoxin[1] was established in 1934. It is made from horse hyperimmune serum for use in toxin neutralization potency assays, in vivo.

The above-mentioned reference materials are in the custody of the National Institute for Biological Standards and Control, Potters Bar, Herts., England (web site: http://www.nibsc.ac.uk). The WHO catalogue of international biological standards should be consulted for the latest list of appropriate international standards and reference materials (http://www.who.int/biologicals). International reference materials are intended for the calibration of national reference materials for use in the manufacture and laboratory control of diphtheria antitoxin and vaccines.

[1] The original standard is a freeze-dried preparation and new standard is a liquid fill of 10 IU/ml, made every 2 years.

Replace section A.3.5.6, *Potency,* by the following:

A.3.5.6 ***Potency***

a) Potency test for licensing

The potency of the final bulk is determined by comparison with an appropriate reference material[1] calibrated against the International Standard for Diphtheria Toxoid, Adsorbed. A three-dilution assay should be used to evaluate consistency of production of the vaccine in question. Three-dilution assays should also be used to test product stability for the purpose of establishing shelf-life as well as to calibrate reference preparations.

Potency should be determined by the inoculation of guinea-pigs with appropriate doses or dilutions of both the tested product and the reference material. After immunization, guinea-pigs may be challenged either by the subcutaneous or the intradermal route, or bled to obtain sera for measurement of the antitoxin or antibody response. When guinea-pigs are bled, the antibody levels of the individual animals may be titrated by means of toxin neutralization tests in vivo or in vitro, such as the Vero cell assay.

> The ELISA assay (*9*) or another suitable in vitro method may be used to measure the antibody response to diphtheria toxoid provided these assays have been validated against the challenge assay or the toxin neutralization test, using the particular product in question. A minimum of three assays with a suitable dose–response range is likely to be required for validation.

Appropriate statistical methods should be used to calculate the potency of the final bulk (*9*). The national regulatory authority should approve the method and the interpretation of the results.

If mice are used for the potency assay, they should be bled and antibody levels of the individual animals titrated by means of toxin neutralization tests in vivo in guinea-pigs, or in vitro using the Vero cell assay. Because mice are not sensitive to diphtheria toxin, challenge with diphtheria toxin is not possible.

> The ELISA or toxoid-binding inhibition (ToBI) assay (*9*) or another suitable method may be used to measure the antibody response to diphtheria toxoid, provided these assays have been validated against the toxin neutralization test, using the particular product in question. A minimum of three assays with a suitable dose–response range is likely to be required for validation.

The potency of diphtheria vaccine used for the immunization of children should not be less than 30 IU per single human dose. The results

[1] Such material could be monocomponent or multicomponent.

of all statistically valid tests should be combined in a geometric mean estimate and the confidence limits calculated. If the lower limit of the 95% confidence interval of the estimated potency is less than 30 IU per single human dose, then the limits of the 95% confidence interval should be within 50–200% of the estimated potency.

The potency values mentioned above do not apply to diphtheria vaccine for use in adolescents or adults.

b) Potency test for routine lot release

Following licensing, and once consistency in production and quality control of the vaccine has been further confirmed on a continuous basis, then the determination of potency in routine lot release may, with the approval of the national regulatory authority, be based on the results of serological assays, or on a challenge assay, both involving a reduced number of animals and/or doses.

To further confirm consistency on a continuous basis, the potency of about ten recent batches of vaccine should be tested using the full three-dilution assay. If potency expressed in International Units is relatively uniform and if the expectations of linearity and parallelism are consistently satisfied, then fewer doses may be used and the assumptions of linearity and parallelism need not be tested in each assay. When vaccine lots consistently give a lower limit of the 95% confidence intervals for the estimated potency well in excess of 30 IU per single human dose, one-dilution tests may offer advantages. If one-dilution assays are not advantageous, a reduction in animal usage may, nevertheless, be achieved by use of two-dilution assays or another suitable design modification.

> A one-dilution assay is based on the same principles for evaluating the response as the three-dilution assays. The assay involves the selection of a dose of the reference vaccine, expressed as a fraction of 30 IU (i.e. of the minimum potency of a single human dose), that elicits a minimum protective effect in guinea-pigs, and comparing its effect with the response elicited by the same fraction of a human dose of the test vaccine. If the response to the test vaccine is significantly greater than the response to the reference vaccine ($P \leq 0.05$), the potency of the test vaccine is satisfactory.

One-dilution assays provide assurance that the lower limit of the estimated potency is in excess of the minimum requirement. A disadvantage of such an approach is that strictly quantitative estimates of vaccine potency will not be possible.

If in vitro serological assays are used, they should show that the product induces an appropriate antibody response in animals in comparison with a reference material calibrated against the International Standard for Diphtheria Toxoid, Adsorbed.

The ELISA assay (*9*) or another suitable in vitro method may be used to measure the antibody response to diphtheria toxoid, provided these assays have been validated against the challenge assay or the toxin neutralization test, using the particular product in question. A minimum of three assays with a suitable dose–response range is likely to be required for validation of a particular product in a particular laboratory. These methods will require precise definition of the characteristics of reagents critical for successful performance of the testing method which may include positive and negative control sera, antigen and others.

There is a need to support the data generated by a simplified potency assay with physicochemical methods to ensure overall consistency of production.

Lot release based on a simplified approach will require periodic review to ensure that the validity of all procedures is maintained. The timing of the review should be decided on a case-by-case basis depending on the number of batches of vaccine produced annually and/or by time (at least every 2 years), as agreed by the national regulatory authority.

Recommendations for tetanus vaccine (adsorbed)

Part A. Manufacturing recommendations

Replace section A.1.3, *International reference materials*, by the following:

A.1.3 *International reference materials*

The first International Reference Reagent of Tetanus Toxoid for Flocculation Tests was established in 1988 (*7*).

The third International Standard of Tetanus Toxoid, Adsorbed, was established in 2000 (*10*) for determining the potency of vaccines containing tetanus toxoid. The assigned value of 469 IU/ampoule is based on its calibration in guinea-pig challenge assays. Potencies calculated by other methods should not be assumed to be transferable without validation. When potency tests are carried out in mice instead of guinea-pigs, transferability should be demonstrated.

The first International Standard for Tetanus Immunoglobulin, human was established in 1992 (*11*) for use in toxin neutralization potency tests.

The above-mentioned international standards are in the custody of the National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG. England (web site: http://www.nibsc.ac.uk). The WHO catalogue of international biological standards should be consulted for the latest list of appropriate international standards and reference materials (http://www.who.int/

biologicals). The international reference materials are intended for the calibration of national reference materials for use in the manufacture and laboratory control of tetanus antitoxin and vaccines.

Replace section A.3.5.6, *Potency,* by the following:

A.3.5.6 *Potency*

a) Potency test for licensing

The potency of the final bulk should be determined by comparison with an appropriate reference material[1] calibrated against the International Standard for Tetanus Toxoid, Adsorbed. A three-dilution assay should be used to evaluate consistency of production of the vaccine in question. Three-dilution assays should also be used to test product stability for the purpose of establishing shelf-life as well as to calibrate reference preparations.

The potency should be determined by the inoculation of guinea-pigs or mice with appropriate doses or dilutions of both the tested product and the reference material. After immunization, animals may be challenged by the subcutaneous route, or bled to obtain sera for measurement of the antitoxin response. When animals are bled, the antibody levels of the individual animals may be titrated by means of toxin neutralization tests in vivo.

> The ELISA or ToBI assay (*9*) or another suitable method may be used to measure the antibody response to tetanus toxoid, provided these assays have been validated against the challenge assay or the toxin neutralization test, using the particular product in question. A minimum of three assays with a suitable dose-response range is likely to be required for validation.

Appropriate statistical methods should be used to calculate the potency of the final bulk (*9*). The national regulatory authority should approve the method and the interpretation of the results.

The potency of tetanus vaccine used for the immunization of children should not be less than 40 IU per single human dose. The results of all statistically valid tests must be combined in a geometric mean estimate and its confidence limits should be calculated. If the lower limit of the 95% confidence interval of the estimated potency is less than 40 IU per single human dose, then the limits of the 95% confidence interval should be within 50–200% of the estimated potency.

> In some countries these potency values may not apply to tetanus vaccine for adolescent or adult use.[2]

[1] Such material could be monocomponent or multicomponent.
[2] Further guidance will be developed.

b) Potency test for routine lot release

Following licensing, and once consistency in production and quality control of the vaccine has been further confirmed on a continuous basis, then the determination of potency in routine lot release may, with the approval of the national regulatory authority, be based on the results of serological assays, or on a challenge assay, both involving a reduced number of animals and/or doses.

To further confirm consistency on a continuous basis, the potency of about ten recent batches of vaccine should be tested using the full three-dilution assay. If potency expressed in International Units is relatively uniform and if the expectations of linearity and parallelism are consistently satisfied, then fewer doses may be used and the assumptions of linearity and parallelism need not be tested in each assay. When vaccine potencies consistently give a lower limit of the 95% confidence intervals for the estimated potency in excess of 40 IU per single human dose, one-dilution tests may offer advantages. If one-dilution assays are not advantageous, a reduction in animal usage may, nevertheless, be achieved by use of two-dilution assays or another suitable design modification.

> A one-dilution assay is based on the same principles for evaluating the response as the three-dilution assays. The assay involves the selection of a dose of the reference vaccine, expressed as a fraction of 40 IU (i.e. of the minimum potency of a single human dose), that elicits a minimal protective effect, and comparing its effect with the response elicited by the same fraction of a human dose of the test vaccine. If the response to the test vaccine is significantly greater than the response to the reference vaccine ($P \leq 0.05$), the potency of the test vaccine is satisfactory.

One-dilution assays provide assurance that the lower limit of the estimated potency is in excess of the minimum requirement. A disadvantage of such an approach is that strictly quantitative estimates of vaccine potency cannot be obtained.

In vitro serological assays should show that the product induces an appropriate antibody response in animals in comparison with a reference material calibrated against the International Standard for Tetanus Toxoid, Adsorbed.

> The ELISA or ToBI assay (*9*) or another suitable method may be used to measure the antibody response to tetanus toxoid, provided these assays have been validated against the challenge assay or the toxin neutralization test, using the particular product in question. A minimum of three assays with a suitable dose–response range is likely to be required for validation for a particular product in a particular laboratory. These methods will require precise definition of the characteristics of reagents critical for successful performance of the testing method which may include positive and negative control sera, antigen and others.

There is a need to support the data generated by a simplified potency assay with physicochemical methods to ensure overall consistency of production.

Lot release based on a simplified approach will require periodic review to ensure that validity of all procedures is maintained. The timing of the review should be decided on a case-by-case basis depending on the number of batches of vaccine produced annually, or by time (e.g. every 2 years), as agreed by the national regulatory authority.

Recommendations for combined vaccines (adsorbed)

Part A. Manufacturing recommendations

A.2 *Special tests for DTP vaccines*

A.2.1 *Final bulk*

Replace section A.2.1.1, *Potency test*, by the following:

The following tests should be carried out on the final bulk vaccine.

A.2.1.1 *Potency test*

For the Diphtheria component, the recommendations for the licensing and routine lot release of Diphtheria vaccine (adsorbed) should apply (section A.3.5.6).

For the Tetanus component, the potency of which is tested in guinea-pigs, the recommendations for licensing and for routine lot release of Tetanus vaccine (adsorbed) should apply (section A.3.5.6). However, when tetanus toxoid is in combination with whole-cell pertussis vaccine and when the potency test for licensing is performed in mice, the estimated potency of tetanus vaccine used for immunization of children should be not less than 60 IU per single human dose. The same potency criteria should also apply when carrying out the routine lot release test.

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The first draft of this amendment was prepared at a WHO Informal Consultation (meeting of drafting group) held in Geneva, 30 June–2 July 2003 and attended by the following participants: Dr J. Arciniega, Office of Vaccines Review and Research, Center for Biologics Evaluation and Research, Bethesda, MD, USA; Dr M. Corbel, Division of Bacteriology, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Dr R. Gaines Das, Statistics Department, National Institute for Biological Standards and Control, Potters Bar, Herts.,

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The final draft was prepared by the drafting group, taking into account comments made by the reviewers of the document.

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