
Production and control of tetanus vaccine

A training curriculum

MODULE X
Reference materials for
quality control



World Health Organization
Geneva

in collaboration with

National Public Health Institute
Helsinki

**PRODUCTION AND CONTROL OF TETANUS VACCINE
A TRAINING CURRICULUM**

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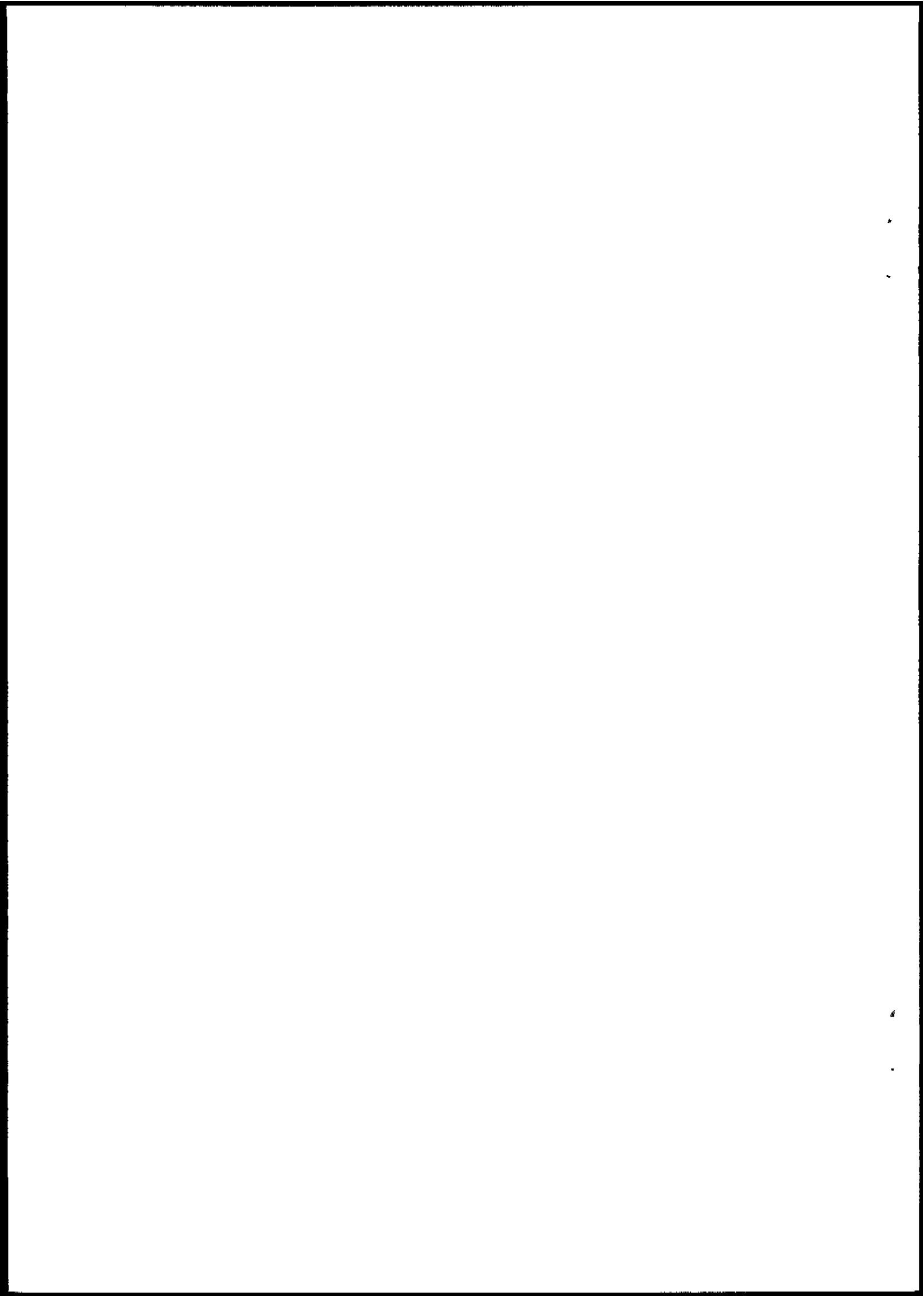
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MODULE X

REFERENCE MATERIALS FOR QUALITY CONTROL

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MODULE X REFERENCE MATERIALS FOR QUALITY CONTROL

1. Introduction

Biological standards and reference reagents are needed to measure the concentration of the effective constituents of substances which cannot be characterized adequately by chemical or physical methods. However, an in-house standard is of little use when inter-assay comparisons between different laboratories are needed, to determine protective doses for a biological preparation, for example, unless the standards used are of same origin and contain a fixed amount of units.

Therefore, international biological standard preparations and reference reagents have been established to enable the relative potency of biologicals to be expressed in the same way, generally in International Units (I.U.). The standard preparations provide means of ensuring uniformity throughout the world in the designation of the relative potency of preparations used.

2. International standard preparations and reference reagents from WHO

The international standard preparations are intended for use in the calibration of the activity of national or in-house standard preparations and for the expression of their relative biological activity in International Units.

The *international standard preparations* are usually distributed in freeze-dried (lyophilized) form with the unitage defined on the basis of the total contents of the ampoule. The standard preparation is the complete material as it exists in the ampoules; the 'material' thus comprises the active ingredients together with all the other constituents that may be present (moisture, carrier, buffer salts, etc.) according to the form in which the standard preparation is available.

An *international reference preparation* to which an activity has been assigned in the form of International Units should also be considered functionally to be international standard.

International biological reference reagents are established for the purpose of providing biological diagnostic reagents of high specificity for the identification of microorganisms or their products, as well as other reagents used to calibrate certain reference materials used in the assay of a variety of biological substances. International Units are not assigned to these.

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The World Health Assembly has recommended that Member States of the Organization give official recognition to existing international standards and units.

Requests for the international reference materials should be addressed directly to the custodian laboratories, together with a statement of their intended use. The custodian laboratory for the international standards and reference reagents needed in the quality control of tetanus vaccine is:

International Laboratory for Biological Standards, Statens Seruminstitut, 80 Amager Boulevard, 2300 Copenhagen S, Denmark. (Tel. +45-32-683266; Fax +45-32-683868; Telex 31316 SERUM DK).

The standard preparations are distributed free of charge, in limited amounts, to national control laboratories for biological products and, for a small handling charge, to other laboratories or manufacturers.

The international standard preparations and reference reagents needed in the assays described in this curriculum are listed below.

2.1. Standard preparations

Tetanus antitoxin, human, 120 IU of Tetanus Antitoxin/ampoule.

- The ampoule contains 85 mg of freeze-dried human antitetanus immunoglobulin.

Tetanus toxoid, adsorbed, 2nd Standard 1981.

- The ampoule contains 27.5 mg of a dried mixture of tetanus toxoid (90 Lf/ampoule) adsorbed to aluminum hydroxide (1 mg Al³⁺/ampoule) and 22.5 mg of hemacel. This standard is used to calibrate the activity of the national or in-house standard used in the potency test. The potency assigned, 340 IU/ampoule, is, however, only for potency tests in guinea-pigs. The corresponding potency for tests in mice is only assumed to be 170 IU/ampoule.

Endotoxin for Limulus gelation, 1st Standard 1986, 14.000 IU/ampoule.

- This standard is distributed by the National Institute for Biological Standards and Control (NIBSC), P.O. Box 1193, Potters Bar EN6 3QG, United Kingdom (Tel +44-707-646977; Fax +44-707-646977).

2.2. Reference reagents

Tetanus toxoid for flocculation test, 1st Reference Reagent 1988.

- The ampoule contains 1000 Lf units of tetanus toxoid.

3. National standards and reference reagents

National standard preparations or reference reagents, hereafter both called reference materials, are preparations of biological substances to which a unitage has been assigned by the laboratory that is responsible for the calibration.

The calibration and the preparation of national reference material needs the approval of the National Control Authority. They are intended for national use only.

Whenever possible, countries in a given region should jointly prepare *regional reference materials* (RRM) which can also be used as national reference materials. By doing so countries are likely to produce and use references that are better characterized and of a higher quality than would otherwise be the case. An example of a RRM is the European Standard of Tetanus Vaccine (adsorbed), distributed by the European Pharmacopoeia Commission.

The guidelines for the preparation, characterization and calibration of national or laboratory working standards and reference reagents for biological substances are given in the WHO Technical Report Series 800 (3). These guidelines should be studied carefully before commencing any production of standard material. Some of the most important parts of the procedures are summarized below.

3.1. Composition

The national reference material must have the same composition as the biological product of which the potency has to be assigned. Vaccine standards shall be made from a single homogenous bulk, from the same source material that is used in the normal vaccine production. Deviations may be accepted only to improve the stability of the reference preparation.

3.2. Quantity

It is recommended that each container have enough freeze-dried material for one and not more than one assay. If however, the amount in one container is sufficient for one or two additional assays, the stability of the reconstituted material (in +4 - +8 °C) must be checked by comparing it to a freshly prepared standard at appropriate intervals before adopting this practice into routine. In the case of liquids the amount should be such that an exact amount can be taken from it. The number of filled containers should meet the need for several years.

3.3. Stability

If the bulk material of a reference preparation has to be stored before being distributed into containers, it should be held under conditions known to be suitable for maintaining the specific activity without changing its physical or biological properties. The content in the final container must have a stability which enables its use for several years when stored under proper conditions. Calibration checks might be advisable with intervals of perhaps two to five years.

3.4. Filling of the containers

The bulk material of a reference preparation is filled in sterile containers in facilities reserved for this purpose only. An important requirement to be met by any batch of a reference material is that the material in every ampoule in the batch should be identical in terms of composition, quantity, potency and stability. If possible the reference material is stored in freeze-dried condition.

3.5. Calibration

If an international reference material authorized by the WHO is available, the national reference material must be calibrated against this international reference material. If such reference material is not available the reference material must either be a part of a lot used successfully in a clinical trial or must be calibrated against a lot used successfully in a clinical trial.

The calibration (estimation of relative potency) of the reference material must be submitted to a statistical evaluation and both the unitage and standard deviation must be shown on the label of the reference preparation.

The accuracy with which a reference material has to be calibrated is related to the accuracy of the test in which the reference material is used. The error of the calibration must always be insignificant compared with the error of a single estimate by the method in question.

The following procedure described by the National Institute of Public Health and Environmental Protection (RIVM), The Netherlands, for the calibration of a standard can be recommended:

The calibration tests are repeated until the cumulative 95% confidence interval of the weighted geometric mean value is 1/4 of the 95% confidence interval of an individual test.

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For the calibration of a new standard for the potency test the following approach is used:

The mean 95% confidence limits (lower and upper) of ten different, routinely performed potency assays are calculated. The standard preparation is tested until the cumulative value of the lower confidence limit is more than $1/4$, and the upper confidence limit less than $1/4$ of the corresponding mean values calculated from the assays mentioned above. When this accuracy is met the standard can be accepted for use.

The absolute value of the reference material in each potency test must be statistically evaluated in view of earlier results. This can preferably be done by the use of a Shewhart control chart: the average, plus two and three times, minus two and three times the standard deviation of the number of tests performed so far, are calculated.

Each time the results of the reference material exceeds the area confined by the plus and minus two times standard deviation, the test shall be repeated twice. Each time the result of the reference material exceeds the area confined by plus and minus three times the standard deviation, the test shall be repeated four times.

3.6. Stability

The stability of the reference preparation should be closely monitored. The following method, again suggested by the National Institute of Public Health and Environmental Protection, The Netherlands, can be recommended but may be substituted by another procedure shown to be suitable:

If the mean of the last ten absolute values or of the absolute values collected in one year (whichever is reached first) of the reference material in potency tests is less than the mean of the first ten values minus $1/4$ or $1/2$ of the standard deviation of the former ten values, the reference preparation shall be downgraded or discarded, respectively.

4. In-house standards and reference reagents

A quality control laboratory undertaking the assay of a large number of batches of a biological substance is usually expected to establish an in-house reference material, also called *laboratory working reference material*. This is to avoid making excessive demands on the supplies of the national reference material.

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The in-house reference material should be prepared and calibrated as mentioned above for the national reference material. The activity of the in-house reference material should be calibrated in International Units by comparison with the national reference material or, where this is not possible, by direct comparison with the international reference material.

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5. References

1. **Biological Substances: International Standards and Reference Reagents, 1990, WHO, Geneva.**
(The catalogue is available on request from WHO, Geneva. There is a small charge.)
2. **RIVM Course Book on the Quality Control of Bacterial Vaccines, 1990. National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands.**
3. **WHO. Guidelines for the preparation, characterization and establishment of international and other standards and reference reagents for biological substances (Revised 1989). Fortieth WHO Expert Committee on Biological Standardization. WHO TRS no 800, Annex 4, pp. 181-214.**

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