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# Production and control of tetanus vaccine

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*A training curriculum*

**MODULE VIII**  
Documentation and release  
procedures



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**World Health Organization**  
Geneva

*in collaboration with*

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**National Public Health Institute**  
Helsinki

PRODUCTION AND CONTROL OF TETANUS VACCINE  
A TRAINING CURRICULUM

INTRODUCTION

- MODULE I: PRINCIPLES AND PRACTICE OF QUALITY CONTROL
- MODULE II: TETANUS - MICROBIOLOGY AND CLINICAL ASPECTS
- MODULE III: PRINCIPLES OF TETANUS VACCINE PRODUCTION
- MODULE IV: MICROBIOLOGICAL AND IMMUNOCHEMICAL TESTS
- MODULE V: CHEMICAL AND PHYSICAL TESTS
- MODULE VI: LABORATORY ANIMAL TESTS
- MODULE VII: INFECTIOUS DISEASE SURVEILLANCE SYSTEMS
- MODULE VIII: DOCUMENTATION AND RELEASE PROCEDURES
- MODULE IX: QUALITY AUDITS
- MODULE X: REFERENCE MATERIALS FOR QUALITY CONTROL

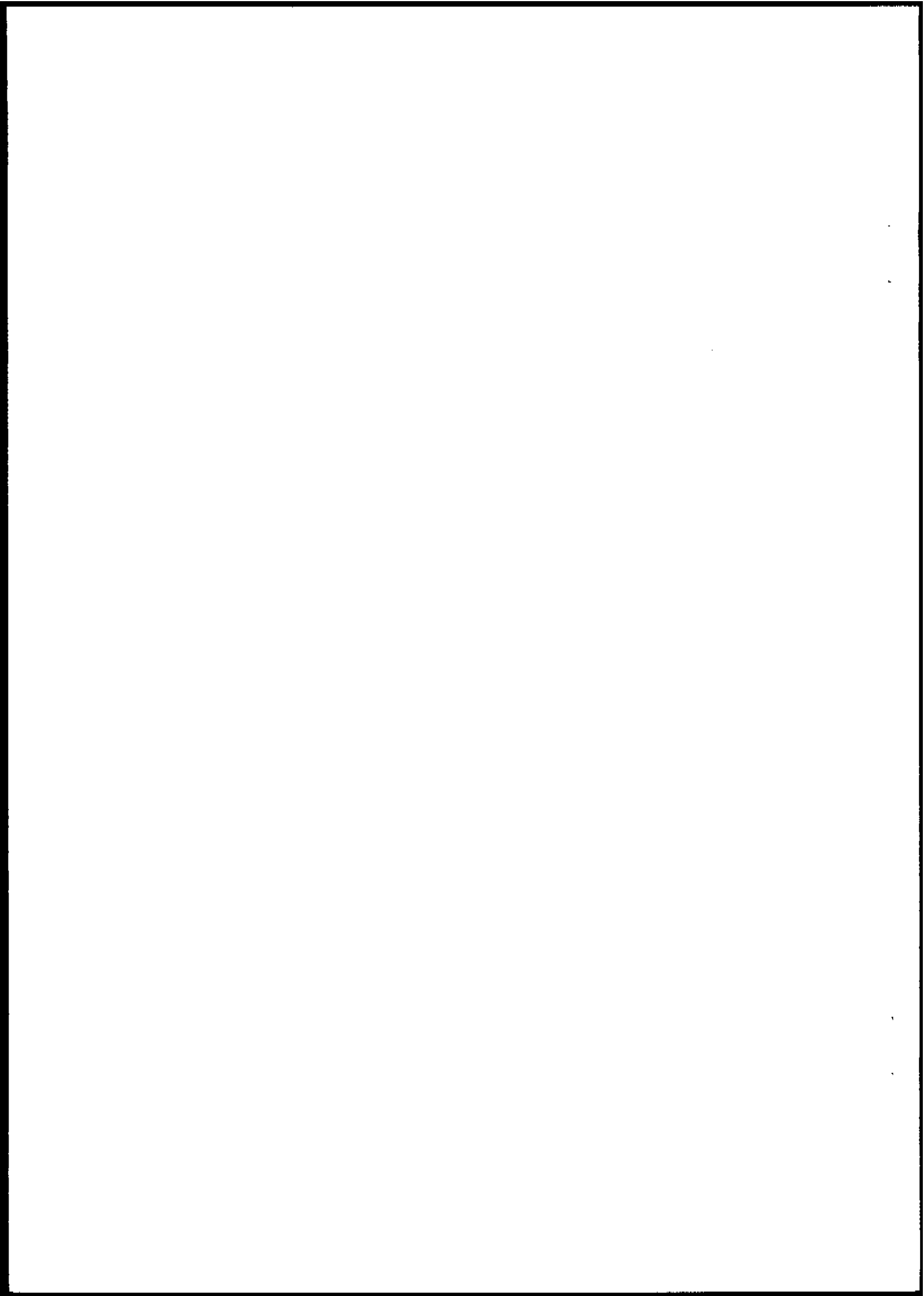
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## MODULE VIII

### DOCUMENTATION AND RELEASE PROCEDURES

#### CONTENTS:

1. Introduction	1	
2. WHO requirements for summary protocol	1	
3. National control requirements	1	
4. Critical issues	2	
5. Evaluating tetanus vaccine protocols	2	
6. Other documents needed	3	
7. Model protocol	4	
8. Model certificates	10	
9. References	13	
Annex 1	WHO Expert Committee on Biological Standardization Fortieth Report (1) Requirements for Tetanus Vaccine (adsorbed)	14
Annex 2	European Pharmacopoeia 1985: Monograph 452 Vaccinum Tetani Adsorbatum	29
Annex 3	European Pharmacopoeia 1982: Monograph 153 Vaccina Ad Usum Humanum	31



## Module VIII DOCUMENTATION AND RELEASE PROCEDURES

### 1. Introduction

Reliable evaluation of vaccine quality requires the review of the actual production process. The production process of any vaccine is complicated and includes innumerable details that need to be monitored, but could hardly be reported to NCA in full for every single batch produced. A compromise solution is the summary production protocol, which reports details of the most critical items in the production process, especially the results of quality control tests.

Detailed descriptions of the actual production process, methods employed, procedures performed and control measures effected must be submitted to the NCA usually only once, when marketing approval or registration of the vaccine is sought. The daily adherence to these is the responsibility of the producer and the quality control unit of the manufacturer. All alterations in the production process must be reported to the NCA. This is also true for any protocol deviations that may occur during the processing of individual batches.

The NCA should review manufacturers regularly to assure their continued conformity to the described process and, especially, to the principles of GMP. For details of auditing techniques refer to Module IX.

### 2. WHO requirements for summary protocol

WHO has compiled detailed models for summary protocols for several vaccines, including tetanus vaccine. These can be used as reference examples when evaluating the protocols submitted by the manufacturers.

### 3. National control requirements

The national control procedures (principles of testing and release) should be defined in the national Quality Manual. At a minimum, the release of vaccines should always be based on an examination of the manufacturers' protocols. Set rules for this should be established. Usually, the production summary protocol is accompanied by a release document from manufacturer's quality control unit.

Usually, the head of NCA releases the vaccine lots on the basis of the tests performed on the samples and the summary protocols. A lot can be approved and released also on the basis of batch protocols and the certificate of the NCA of the country of origin provided that the NCA has

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

obtained satisfactory proof of the reliability of the manufacturer (and the NCA in country of origin). Usually such approval should be preceded by actual testing of at least some lots (three to five) with acceptable results. Statutory requirements of some countries mandate inspection and review of a complete licensing application as well.

For each vaccine, specifications should be clearly defined, written down and approved by the head of the NCA. Prior to release of testing results, summary protocols and the specifications should be compared. The lot can be released only if satisfactory concordance between these three aspects can be confirmed.

#### 4. Critical issues

Submitted protocols must be prepared in a logical structure and according to a standard format (which may vary from manufacturer to manufacturer). Should the submitted documents be different for any two lots it would be very difficult to compare their contents and acceptability.

Incomplete protocols should never be accepted, but the incompleteness need not necessarily lead to the rejection of a batch. Rather, the manufacturer should be given the opportunity to supplement the missing details.

All documents should be signed and dated and the identification of each lot (by lot numbers) should be unambiguous. Test results should also be presented in such a way that no doubts as to the significance of the result can arise (e.g. Sterility test: sterile/PASS). Dates for all tests should be shown.

An important requirement for the quality of the submitted documents is the manner in which corrections are entered into the protocols. This should follow the principles of GMP. Corrections must be made by striking out the erroneous markings with a line or a cross, so that the marking still remains legible. The correct marking is then added next to the erroneous marking, dated and signed by the person making the correction.

#### 5. Evaluating tetanus vaccine protocols

The most critical issues in evaluation of tetanus vaccine protocols are:

1. Identification of *C. tetani* strain and seed lot history.

## **PRODUCTION AND CONTROL OF TETANUS VACCINE**

### Training curriculum

2. Description of the production of single harvests, specifying in particular the inactivation details and yields.
3. Antigenic purity, irreversibility and specific toxicity of the bulk purified toxoid.
4. Sterility, potency of the final bulk and the concentration of residual free detoxifying agent.
5. Identity, sterility, potency, innocuity, adjuvant and preservative concentrations and stability of the final vaccine.

Annex 1 reproduces the current WHO requirements for tetanus vaccine. An example of Pharmacopoeia requirements is given in Annex 2 (European Pharmacopoeia) which should be read together with Annex 3, the general requirements for vaccines for human use.

### **6. Other documents needed**

A certificate from the responsible person in the quality control unit of the manufacturer stating that the product meets all relevant requirements (such as national or WHO requirements) is required.

If the vaccine in question is imported the documents should include a certificate from the National Control Authority of the country of origin.

## 7. Model protocol

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### SUMMARY PROTOCOL FOR TETANUS VACCINE (ADSORBED) PRODUCTION AND TESTING

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Summary information on final lot:

Name and address of manufacturer  
Lot no.  
Date of filling  
Volume of each recommended single human dose  
No. of doses per final container  
No. of final containers  
Expiry date

**Detailed information on manufacture and control**

#### **Strain**

Identity of *Cl. tetani* strain used for vaccine production  
Reference no. of seed lot  
Date(s) of reconstitution of ampoule(s) for manufacture

#### **Single harvests used for preparing the bulk purified toxoid**

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity, method of inactivation and yields.

#### **Bulk purified toxoid**

Reference no.  
Volume and Lf/ml  
Date and result of test for antigenic purity (Lf/mg of protein nitrogen)

#### *Test of irreversibility*

Lf/ml of test toxoid solution  
Temperature of incubation of test toxoid  
Dates of beginning and end of incubation

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

No. of guinea-pigs injected, route and date of injection

Date of end of observation

Result of test

### *Specific toxicity test*

No. of guinea-pigs injected and date of injection

No. of Lf per guinea-pig and route of injection

Date of end of observation

Result of test

### **Final bulk**

Identification

Volume

Lf/ml

### *Sterility test*

Date and result of test

### *Specific toxicity test (optional)*

No. of guinea-pigs injected and date of injection

Volume and route of injection

Date of end of observation

Result of test

*Potency test (Only one of the potency tests listed need be performed.)*

(1) Based on lethal or paralytic challenge

(i) Three-dilution assays

Species and weight of animals

Date of immunization and volume of dilutions administered

Date of challenge

Challenge dose (indicate whether lethal or paralytic)

Date of end of observation

Results

**PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

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<b>Dilution</b>	<b>Number of survivors or animals not paralysed)/Number of animals injected</b>	<b>Median effective dose (ED<sub>50</sub>)</b>
Reference vaccine ( ___ IU/ml)		ml
Test vaccine		ml

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Potency of test vaccine is \_\_\_ IU per single human dose.

Limits of 95% confidence interval (in %) are \_\_\_.

**(ii) One-dilution challenge test**

Date of performance of last satisfactory three-dilution test

Nature and reference No. of product tested  
(specify also whether it was a final bulk or a final product)

Provide relevant information validating the one-dilution assay system.

Identity and titre (IU/ml) of reference vaccine

Animal species and weight of animals

Date of immunization

Date of challenge

Challenge dose (specify whether lethal or paralytic)

Date of end of observation

Results

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

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	Reference vaccine	Test vaccine
Dilution used for immunization		
No. of survivors (or of animals not paralysed)/ No. animals injected		

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P value indicating the probability that the test vaccine contains more than 40 IU/ single human dose

(2) Test based on measuring antitoxin induction as an alternative to lethal or intradermal challenge

Provide separately all relevant information on other tests, such as competitive enzyme-linked immunosorbent assay (ELISA) or passive hemagglutination, including the data by which the method was validated.

Potency of test vaccine \_\_\_ IU per single human dose. Limits of 95% confidence interval (in %) are \_\_\_.

### *Test for residual free detoxifying agent*

Detoxifying agent (formaldehyde or glutaraldehyde)

Date of test

Result (in g/l)

pH

Date of measurement

Result

### **Final product**

#### *Identity test*

Date of test

Type of test and result

**PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

*Sterility test*

No. of times the test had to be performed  
No. of containers tested  
Media and temperatures of incubation  
Date of inoculation  
Date of end of observation  
Result of last test

*Potency test*

If the test was not performed on the final bulk, indicate this and report the data in the space provided for potency tests in the 'final bulk' section.

*Innocuity test*

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	Mice	Guinea-pigs
No. of animals		
Route of injection		
Volume of injection		
Date of end of test		
Results		

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*Test for adjuvant*

Date of test  
Nature and concentration of adjuvant per single human dose

*Test for preservative*

Date of test  
Nature and concentration of preservative

*pH*

Date of measurement  
Result

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

### *Inspection of final containers*

Date of inspection

Result

*Stability test* (Not required in summary protocols of every batch.)

Indicate separately all relevant details and (as a percentage) the calculated losses of potency per year at different temperatures as determined by accelerated degradation tests, and actual titers<sup>\*)</sup> (with limits of 95% confidence intervals) after storage for the maximum period claimed for the product at the recommended temperature.

<sup>\*)</sup> Needed only for three batches to validate the production method.

**8. Model certificates**

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**CERTIFICATION BY THE MANUFACTURER**

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Name of head of production (typed)

*Certification by person from the control laboratory of the manufacturing company taking overall responsibility for the production and control of vaccine.*

I certify that lot No \_\_\_\_ of tetanus vaccine (adsorbed), whose number appears on the label of the final containers, meets all national requirements<sup>\*)</sup> and satisfies Part A of the tetanus vaccine section of Requirements for Biological Substances Nos. 8 and 10, revised 1989 and (if applicable) addenda 19\_\_\_\_.

Signature

Name (typed)

Date

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**Certification by the National Control Authority**

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If the vaccine is to be exported, attach a certificate from the National Control Authority as shown on the next page, a label from a final container and an instruction leaflet for users.

<sup>\*)</sup> If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.

**PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

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**MODEL CERTIFICATE FOR THE RELEASE OF VACCINES**

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This certificate is to be provided by the National Control Authority of the country where the vaccines have been manufactured, upon request by the manufacturer

The following lots of \_\_\_\_\_<sup>1)</sup>  
vaccine produced by \_\_\_\_\_<sup>2)</sup>  
in \_\_\_\_\_<sup>3)</sup>

whose numbers appear on the labels of the final containers, meet all national requirements<sup>4)</sup> Part A of the \_\_\_\_\_<sup>1)</sup> section of the Requirements for Biological Substances Nos. 8 and 10 [Requirements for Diphtheria, Tetanus Pertussis and Combined Vaccines, revised 1989 and (if applicable) addendum 19\_\_]<sup>5)</sup> and the Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories, revised 1965).<sup>6)</sup>

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<b>Lot No.</b>	<b>Date of the last potency test by the manufacturer</b>	<b>Expiry date</b>
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As a minimum, this certificate is based on an examination of the manufacturing protocol.

The number of this certificate is

The Director of the National Control Laboratory (or Authority as appropriate)<sup>7)</sup>

Name (typed)

Signature

Date

**PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

- 1) Indicate type of vaccine (tetanus, diphtheria-tetanus. diphtheria--tetanus-pertussis).
- 2) Name of the manufacturer
- 3) Country
- 4) If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the National Control Authority.
- 5) With the exception of the provisions on shipping, which the National Control Authority may not be in a position to control.
- 6) Published in WHO Technical Report Series, no. 323, 1966.
- 7) Or his or her representative.

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

### 9. References

1. WHO Expert Committee on Biological Standardization Fortieth Report. WHO, Technical Report Series no 800, Annex 1, 1990.
2. European Pharmacopoeia. Vaccinum tetani adsorbatum (Tetanus Vaccine, Adsorbed). Monograph 452, 1982.
3. European Pharmacopoeia. Vaccina ad usum humanum (Vaccines for Human Use). Monograph 153, 1982.

**Annex 1**

**WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION  
Fortieth Report (1)**

**REQUIREMENTS FOR TETANUS VACCINE (ADSORBED)**

**PART A. MANUFACTURING REQUIREMENTS**

**A.1 Definitions**

**A.1.1 International name and proper name**

The international name shall be *Vaccinum tetani adsorbatum*. The proper name shall be the equivalent of the international name in the language of the country of use.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

**A.1.2 Descriptive definition**

*Vaccinum tetani adsorbatum* is a preparation of tetanus toxoid prepared by treating tetanus toxin by chemical means to render it nontoxic without losing its immunogenic potency. The toxoid is adsorbed on to a suitable adjuvant. The preparation shall satisfy the requirements formulated below.

The most common method of preparing toxoids from toxins is by means of formaldehyde.

**A.1.3 International reference materials**

The first International Reference Reagent of Tetanus Toxoid for Flocculation Tests was established in 1988 (6).

The second International Standard for Tetanus Toxoid, Adsorbed, was established in 1981 (11) for determining the potencies of vaccines containing tetanus toxoid. In view of the fact that different results may be obtained when potency tests are carried out in mice instead of guinea-pigs, tests in mice of vaccines containing tetanus toxoid should be performed using reference vaccines calibrated against the International

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

Standard by means of potency tests on guinea-pigs; however, this is not an entirely satisfactory procedure.

The second International Standard for Tetanus Antitoxin was established in 1969 (12); it has an *in vivo/in vitro* ratio of antitoxin activity of 1.4 and is made of purified hyperimmune horse serum.

The above-mentioned international reference materials are in the custody of the International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen. Samples are distributed free of charge on request to national control laboratories. The international reference materials are intended for the calibration of national reference materials for use in the manufacture and control of tetanus antitoxin and vaccine.

### A.1.4 Terminology

*Seed lot:* A quantity of bacterial suspension that is derived from one strain, has been processed as a single lot and has a uniform composition. It is used for preparing the inoculum for the production medium.

*Single harvest:* The toxic filtrate or toxoid obtained from one batch of cultures inoculated, harvested and processed together.

*Bulk purified toxoid:* The processed purified material, prepared from either a single harvest or a pool of a number of single harvests. It is the parent material from which the final bulk is prepared.

*Final bulk:* The final homogeneous vaccine present in a single container from which the final containers are filled either directly or through one or more intermediate containers.

*Final lot:* A collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling. A final lot must therefore have been filled from a single container in one continuous working session.

### A.2 General manufacturing requirements

The general requirements for manufacturing establishments contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply to establishments manufacturing tetanus vaccine with the addition of the following:

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

All manufacturing processes up to and including the completion of detoxification shall be carried out in completely separate areas and by means of separate equipment.

Written descriptions of procedures for the preparation and testing of tetanus vaccine adopted by a manufacturer together with appropriate evidence that each production step has been validated shall be submitted for approval to the national control authority. Proposals for modifications of the manufacturing and/or control methods shall also be submitted for approval to the national control authority before such modifications are implemented.

### A.3 Production control

#### A.3.1 Control of source materials

##### A.3.1.1. *Strains of Clostridium tetani*

Strains of *Cl. tetani* used in preparing tetanus toxoid shall be identified by a record of their history and of all tests made periodically to verify strain characters. The strain shall be maintained as a freeze-dried culture.

A highly toxinogenic strain of *Cl. tetani* should be used. A strain that has proved satisfactory in many laboratories is the Harvard strain.

##### A.3.1.2 *Seed lot system*

The production of tetanus toxin shall be based on a seed lot system. Cultures of the working seed shall have the same characteristics as those of the strain from which the parent seed lot was derived. The preparation of the seed lot shall comply with the requirements of Part A, section A.3.2.

##### A.3.1.3 *Culture medium for production of toxin*

It is particularly important to ensure that the final product is free from substances likely to cause toxic or allergic reactions in humans.

The method of detecting these substances should be approved by the national control authority.

If the medium is prepared from a protein digest, e.g., casein hydrolysate or digested muscle, precautions should be taken to ensure that digestion has proceeded sufficiently. Established limits, if any, for mammalian

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

protein and human blood-group substances in the final vaccine should not be exceeded.

#### A.3.2 Production precautions

The general production precautions, as formulated in the requirements of Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7), shall apply to the manufacture of tetanus vaccine.

Suitable methods for the production of tetanus vaccine are given in the *Manual for the production and control of vaccines: tetanus toxoid (13)*.

Personnel employed in production and quality control must be adequately trained and immunized.

#### A.3.3 Control of single harvest

Consistency of production shall be demonstrated.

Consistency may be demonstrated by measuring, e.g., the bacterial growth rate, pH and rate of toxin production.

Any culture showing anomalous growth characteristics shall be investigated and shown to be satisfactory before being accepted as a single harvest.

##### A.3.3.1 *Control of bacterial purity*

Samples of cultures used for preparing single harvests of toxoid shall be tested for bacterial purity by microscopic examination of stained smears or by inoculation into appropriate culture media. Single harvests shall not be used for preparing bulk materials if contamination has occurred at any stage in their production.

##### A.3.3.2 *Filtration*

After having been sampled for control of purity, cultures shall be sterilized by means of filtration. A preservative may be added, but phenol shall not be used for this purpose.

Cultures should be filtered as soon as possible after the end of their incubation period. To facilitate filtration, cultures may be centrifuged,

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

provided that suitable precautions are taken to avoid the formation of potentially hazardous aerosols. A filter aid may be added beforehand. In some countries, no filter capable of shedding fibres may be used.

#### A.3.3.3 *Determination of antigen concentration*

The supernatant of the culture prior to inactivation shall be tested by a method approved by the national control authority.

It is advisable to determine the antigen content by measuring the toxin content. This is usually done *in vivo*.

Another suitable method for determining the antigen concentration is the flocculation test described in the *Manual for the production and control of vaccines: tetanus toxoid (13)*; it should be performed both on the supernatant and, for purposes of comparison, a reference material calibrated against the International Reference Reagent of Tetanus Toxoid for Flocculation Tests, or an equivalent reference material approved by the national control authority.

It is preferable for culture filtrates used in preparing purified toxoid to contain not less than 40 Lf/ml.

Antigen content is a good indicator of consistency of production.

#### A.3.3.4 *Detoxification and purification of toxin*

Purification may either precede or follow detoxification. Purification before detoxification results in a purer product, but particular care must be taken to avoid reversion to toxin, which may also occur when detoxification precedes purification. The method and agent used for detoxification and the method of purification shall be approved by the national control authority.

After detoxification has been completed, the detoxifying agent shall be removed or neutralized by a method approved by the national control authority.

The method of purification shall be such that no substances are incorporated into the final product that are likely to cause untoward reactions in humans. The rate of detoxification may vary and shall be monitored. Harvests shall not be transferred from the detoxification area until the detoxification has been shown to be complete.

### A.3.4 Control of bulk purified toxoid

#### A.3.4.1 *Preparation*

The bulk purified toxoid shall be prepared from either a single harvest or a pool of single harvests, and shall be sterile. Phenol shall not be used as a preservative.

It is advisable to sterilize the bulk purified toxoid by filtration. A preservative approved by the national control authority may be added to the bulk toxoid.

#### A.3.4.2 *Sterility*

Each bulk toxoid shall be tested for bacterial and mycotic sterility in accordance with the requirements of Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances (9)) or by a method approved by the national control authority. If a preservative has been added to the purified bulk, appropriate measures shall be taken to prevent any interference by it in the sterility test.

#### A.3.4.3 *Specific toxicity*

Each bulk purified toxoid shall be tested for the presence of tetanus toxin by injection into at least five guinea-pigs, each weighing 250-350 g. Each guinea-pig shall be given a subcutaneous injection of 1 ml of a dilution of purified toxoid containing at least 500 Lf of toxoid. Each guinea-pig shall be observed daily and closely examined weekly for signs of tetanic paralysis. Animals that die shall be examined by autopsy. The bulk purified toxoid shall pass the test if no guinea-pig shows symptoms of specific paralysis or any other signs of tetanus within 21 days of injection and if at least 80% of the animals survive the test period. The guinea-pigs shall not have been used previously for experimental purposes.

#### A.3.4.4 *Reversion to toxicity*

Each bulk purified toxoid shall be tested to ensure that reversion to toxin cannot take place on storage. The bulk toxoid shall be diluted in order to obtain the same concentration and chemical environment as those present in the final bulk vaccine, except for the presence of adjuvant.

To determine whether reversion has occurred, diluted toxoids which have been stored at +37 °C for six weeks shall be tested. The test employed

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

shall be approved by the national control authority and shall be sufficiently sensitive to detect very small amounts of toxin. No toxicity shall be detected.

In one country, the test is performed on toxoids that have been stored at +34 °C.

Similar dilutions of toxoid held at +2-8 °C during the same period of time as those held at +34 °C or +37 °C may be tested as controls.

Mice are not as sensitive to tetanus toxin as guinea-pigs but may be used for the test, subject to the approval of the national control authority.

#### A.3.4.5 *Antigenic purity*

Each bulk purified toxoid shall be tested for antigenic purity by determining the Lf value and the concentration of protein (nondialysable) nitrogen. The Lf value shall be determined by comparison with a reference material calibrated against the International Reference Reagent of Tetanus Toxoid for Flocculation Tests or an equivalent reference preparation approved by the national control authority. The method of testing shall be approved by the national control authority. The bulk purified toxoid shall pass the test if it contains no fewer than 1000 Lf per mg of protein (nondialysable) nitrogen.

Preparation of toxoid containing more than 1500 Lf per mg is both feasible and desirable.

An indication of the antigenic quality of the toxoid may be obtained by measuring the total combining power and expressing it in relation to the number of Lf units. A suitable method for measuring the total combining power is given in the *Manual for the production and control of vaccines: tetanus toxoid (13)*.

#### A.3.5 Control of final bulk

##### A.3.5.1 *Preparation*

The final bulk shall be prepared from bulk purified toxoid. The number of Lf in a single human dose shall be approved by the national control authority but shall not exceed 25 if more than one dose is recommended for primary immunization.

##### A.3.5.2 *Preservative*

If the vaccine is to be filled in multidose containers, a suitable antimicrobial preservative shall be added. The amount of preservative in

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

the final bulk shall have been shown to have no deleterious effect on the toxoid or on other vaccine components with which the toxoid may be combined, and to cause no unexpected adverse reactions in humans. The preservative and its concentration shall be approved by the national control authority.

Phenol shall not be used as a preservative.

### A.3.5.3 *Adjuvants*

The adjuvants used, their purity and their concentration shall be approved by the national control authority.

Aluminium or calcium compounds are generally used as mineral carriers.

The concentration of aluminium shall not exceed 1.25 mg and that of calcium 1.3 mg per single human dose.

In some countries, these upper limits for the concentration of mineral carriers are considered to be too high and the limits are set at about half those given above.

In some countries, the adsorbent is precipitated in the presence of the toxoid.

The formulation shall be such that the vaccine remains suspended for a reasonable time after shaking.

### A.3.5.4 *Sterility*

Each final bulk shall be tested for bacterial and mycotic sterility in accordance with the requirements of Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances, revised 1973) (9) or by a method approved by the national control authorities. If preservative has been added to the final bulk, adequate measures shall be taken to prevent any interference by it in the sterility test.

### A.3.5.5 *Specific toxicity*

In some countries, each final bulk is tested for specific toxicity in at least five guinea-pigs, each weighing 250-350 g. Each guinea-pig is given a subcutaneous injection of a quantity equivalent to at least five single human doses, and is then observed daily and examined closely every week for signs of tetanic paralysis. Animals that die are examined by

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

autopsy. The final bulk passes the test if no guinea-pig shows paralysis or any other signs of tetanus within 21 days of injection and if at least 80% of the animals survive the test period. The guinea-pigs must not have been used previously for experimental purposes.

#### A.3.5.6 Potency

The immunizing potency of each final bulk shall be determined by comparison with an appropriate reference material properly calibrated against the International Standard for Tetanus Toxoid, Adsorbed. The test shall involve the inoculation of guinea-pigs or mice with appropriate doses or dilutions of both the final product and the reference material. After immunization, the animals shall be bled or challenged by the subcutaneous route (10). If animals are bled, the antitoxin levels of the individual animals may be titrated by means of toxin neutralization tests performed using *in vivo* or *in vitro* serological methods that have been validated on vaccines of the type being tested. Appropriate statistical methods shall be used to calculate the potency of the final bulk. The method adopted and the interpretation of the results shall be approved by the national control authority.

Care should be taken to ensure that diluents are inert and not pyrogenic. Phosphates might interfere with the adsorption of toxoid.

When consistency of production and testing have been established, the numbers of animals injected with each dilution of product may be reduced to levels substantially lower than those originally needed for the three-dilution assays described in the *Manual of details of tests required on final vaccines used in the WHO Expanded Programme on Immunization (10)*, provided that the resulting assays are statistically valid. Test methods based on individual quantification of antitoxin allow the use of fewer animals than are needed in challenge tests.

Depending on the purpose, two types of potency assays may be considered.

Three-dilution assay may be used to test consistency of production and product stability, and to calibrate reference preparations.

One-dilution assays<sup>1)</sup> for evaluating the response based on the same principle as the three-dilution assays may be used at the discretion of the national control authority for the routine testing of vaccine lots of a given formulation as soon as the production process has been established and consistency in production and control has been demonstrated. The test involves the selection of a dose of reference vaccine, expressed as a

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

fraction of 40 IU (i.e., of the minimum potency of a single human dose) that elicits a minimal protective effect in mice or 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 latter is significantly greater than that to the former ( $P < 0.05$ ), the potency of the test vaccine is satisfactory. One-dilution tests offer advantages only when vaccine potencies are consistently and substantially in excess of 40 IU per single human dose.

The potency of the final bulk shall be approved by the national control authority. The potency of tetanus vaccine used for the immunization of children shall not be less than 40 IU per single human dose. For three-dilution assays, the limits of the 95% confidence intervals of the estimate of potency shall be within 50-200% of the estimated potency unless the lower limit of the 95% confidence interval of the estimated potency is greater than 40 IU per single human dose. When one-dilution tests are performed, the potency of the test vaccine shall be demonstrated to be significantly greater than 40 IU per human dose.

In some countries, potency testing is not carried out on each final bulk but on each final lot.

<sup>1)</sup> Information on one-dilution assay methods is given in document BS/89.1618, available on request from Biologicals, World Health Organization, Geneva, Switzerland.

#### *A.3.5.7 Amount of residual free detoxifying agent*

The amount of residual free detoxifying agent in each final bulk shall be determined by a method approved by the national control authority and, if formaldehyde has been used, the residual content shall be not more than 0.2 g/l.

The colorimetric determination of the reaction product of formaldehyde and fuchsin-sulphurous acid is a suitable method.

In some countries, the amount of residual free detoxifying agent is determined in the purified bulk.

If applicable, appropriate tests for the presence of other detoxifying agents (e.g., glutaraldehyde) shall be performed. The tests used and the maximum permissible concentrations of such chemicals shall be approved by the national control authority.

## **PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

### **A.3.5.8 pH**

The pH of the final bulk shall be measured.

The pH should be between 6.0 and 7.0.

### **A.4 Filling and containers**

The requirements applicable to filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

Single-dose or multiple-dose containers may be used. Vaccines in multidose containers shall contain a suitable antimicrobial preservative.

### **A.5 Control of final product**

#### **A.5.1 Identity**

An identity test shall be performed on at least one labelled container from each final lot.

Flocculation in solution, immunoprecipitation of the toxoid in gels or any other specific interaction between the vaccine and tetanus antitoxin may serve as an identity test. Tests on toxoids adsorbed on to aluminium or calcium carriers may be performed after the carrier has been dissolved, or the adsorbed toxoid wholly or partially eluted by sodium citrate at pH 9.

If adequate quantities of toxoid cannot be recovered from the adsorbed vaccine, specific antitoxin may be sought in the sera of animals used in the innocuity test.

#### **A.5.2 Sterility**

Final containers shall be tested for bacterial and mycotic sterility by a method approved by the national control authority.

Many countries have regulations governing the sterility testing of the final product. Where these do not exist, the requirements published by WHO shall be met (9). If a preservative has been added to the vaccine, appropriate measures shall be taken to prevent any interference by it in the sterility test.

## **PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

### **A.5.3 Potency**

A potency test shall be carried out as provided in Part A, section A.3.5.6, on each final lot, if such a test has not been performed on the final bulk.

### **A.5.4 Innocuity**

Each final lot shall be tested for abnormal toxicity by the injection by the intraperitoneal route of one human dose, but not more than 1 ml, into each of five mice (weighing 17-22 g) and at least one human dose, but not more than 1 ml, into each of two guinea-pigs (weighing 250-350 g). The tests shall be approved by the national control authority. The final product shall be considered innocuous if the animals survive for at least seven days without showing significant signs of toxicity.

### **A.5.5 Adjuvant content**

The adjuvant content of each final lot shall be determined by a method approved by the national control authority (see Part A, section A.3.5.3).

In some countries, this test is used to verify the homogeneity of filling.

### **A.5.6 Preservative content**

The preservative content of each final lot shall be determined (see Part A, section A.3.5.2). The method used shall be approved by the national control authority.

In some countries, this test is applied to the final bulk only

### **A.5.7 pH**

The pH of each final lot shall be measured.

The pH should be between 6.0 and 7.0.

### **A.5.8 Inspection of final containers**

Each container in each final lot shall be inspected visually, and those showing abnormalities - such as improper sealing, lack of integrity, clumping or the presence of particles - shall be discarded.

### A.6 Records

The requirements given in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

Written records shall be kept of all tests, irrespective of their results. The records shall be of a type approved by the national control authority.

A model of a suitable summary protocol to be used for tetanus vaccines is given in Appendix 3.

### A.7 Samples

The requirements given in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

### A.8 Labelling

The label printed on or affixed to each container and the label on the carton enclosing one or more containers shall show as a minimum:

- the words *Vaccinum tetani adsorbatum* and/or the proper name of the product,
- the name and address of the manufacturer,
- the number of the final lot,
- the recommended storage temperature and the expiry date if kept at that temperature, and
- the recommended single human dose and route of administration.

In addition, the label printed on or affixed to the container, or the label on the cartons, or the leaflet accompanying the container shall contain the following:

- a statement that the vaccine satisfies the requirements of this document,
- the nature and amount of any preservative present in the vaccine,
- the nature and amount of the adsorbing agent,
- the recommended temperature for storage and transport,
- a warning that the adsorbed vaccine should not be frozen,
- a warning that the adsorbed vaccine should be shaken before use, and
- instructions for the use of the vaccine and information on contraindications and the reactions that may follow vaccination.

## **PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

### **A.9 Distribution and transport**

The requirements given in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

### **A.10 Stability, storage and expiry date**

#### **A.10.1 Stability**

Tests shall be conducted to determine the loss of potency to be expected during storage. The stability of the vaccine shall be demonstrated to the satisfaction of the national control authority; final containers from at least three lots derived from different lots of purified bulk toxoid shall be tested on the expiry date to demonstrate stability during storage. The vaccine shall meet the requirements for the final product (see Part A, sections A.5.3, A.5.4, A.5.7 and A.5.8) up to the expiry date, provided that it has been stored at the recommended temperature. When any changes are made in the production procedure that may affect the stability of the product, the vaccine produced by the new method shall be shown to be stable. The statements concerning storage temperature and expiry date appearing on the label, as required in Part A, section A.8, shall be based on experimental evidence and shall be submitted for approval the national control authority.

#### **A.10.2 Storage conditions**

Storage at a temperature of  $5 \pm 3^{\circ}\text{C}$  has been found to be satisfactory.

Adsorbed vaccines shall not be frozen.

#### **A.10.3 Expiry date**

The expiry date shall be approved by the national control authority based on the stability studies referred to in section A.10.1 shall relate to the date of the last satisfactory potency determination, performed in accordance with Part A, section A.5.3, i.e., date on which the test animals were immunized with the vaccine.

## **PART B. NATIONAL CONTROL REQUIREMENTS**

### **B.1 General**

The general requirements for control laboratories contained in part B of the revised Requirements for Biological Substances No. 1 General

## **PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

Requirements for Manufacturing Establishments and Control Laboratories) (7) shall apply.

The detailed production and control procedures and any significant changes in them shall be discussed with and approved by national control authority, which shall obtain the International Standard for Tetanus Toxoid, Adsorbed and establish a national working reference preparation by comparison with it.

### **B.2 Official release and certification by the national control authority**

A vaccine shall be released only if it satisfies Part A of the present Requirements.

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify that the lot of vaccine in question satisfies all national requirements as well as Part A of the present Requirements. The certificate shall state the number under which the lot was released by the national control authority, and the number appearing on the labels of the containers. The official national release document shall be provided to importers of tetanus vaccines.

The purpose of the certificate is to facilitate the exchange of tetanus vaccines between countries. A model of a suitable certificate is given in Chapter 8 of this Module.

Annex 2

European Pharmacopoeia 1985:

Monograph 452

**VACCINUM TETANI ADSORBATUM**

Tetanus Vaccine (Adsorbed)

Tetanus vaccine (adsorbed) is a preparation of tetanus formol toxoid adsorbed on a mineral carrier. The formol toxoid is prepared from the toxin, produced by the growth in a suitable medium of *Clostridium tetani*, by a method that avoids reversion of toxoid to toxin, particularly on exposure to heat.

The vaccine is prepared by the addition of toxoid containing not less than 1000 flocculation equivalents (1000 Lf) per milligram of protein nitrogen to a suspension of hydrated aluminium phosphate, aluminium hydroxide or calcium phosphate in a 0.9 per cent *m/V* solution of sodium chloride or other suitable solution isotonic with blood. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and should not be added to the vaccine.

**IDENTIFICATION**

Dissolve in the vaccine to be examined sufficient sodium citrate R to give a 10 per cent *m/V* solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable tetanus antitoxin, giving a precipitate.

**TESTS**

**Specific toxicity** Inject subcutaneously five times the human dose stated on the label into each of five healthy guinea-pigs, each weighing 250 g to 350 g, that have not previously been treated with any material that will interfere with the test. If within 21 days of the injection any of the animals shows signs of or dies from tetanus the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test. If an animal dies in the second test, the vaccine does not comply with the test.

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

**Aluminium** When hydrated aluminium phosphate or aluminium hydroxide is used as the adsorbent, the vaccine complies with the test prescribed in the monograph on *Vaccina ad Usum Humanum*.

**Calcium** When calcium phosphate is used as the adsorbent, the vaccine complies with the test prescribed in the monograph on *Vaccina ad Usum Humanum*.

**Free formaldehyde** The vaccine complies with the test prescribed in the monograph on *Vaccina ad Usum Humanum*.

**Sterility** (V.2.1.1) The vaccine complies with the test for sterility.

**Abnormal toxicity** (V.2.1.5) The vaccine complies with the test for abnormal toxicity for immunosera and vaccines for human use.

## POTENCY

Carry out the assay of tetanus vaccine (adsorbed) (V.2.2.9) by one of the prescribed methods.

The lower fiducial limit ( $P = 0.95$ ) of the estimated potency is not less than 40 I.U. per human dose.

## STORAGE

See the monograph on *Vaccina ad Usum Humanum*.

*Period of validity* The period of validity is decided by the national authority in the light of experimental results. When stored in the prescribed conditions, the vaccine may be expected to retain its potency for not less than 5 years.

## LABELLING

See the monograph on *Vaccina ad Usum Humanum*.

The label on the *container* or the label on the *package* states in particular:

- the minimum number of International Units per human dose,
- the name of the adsorbent.

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

### Annex 3

European Pharmacopoeia 1982:

### Monograph 153

## VACCINA AD USUM HUMANUM

### Vaccines for Human Use

*The statements in this monograph are intended to be read in conjunction with the monographs on vaccines for human use in the Pharmacopoeia. The requirements do not necessarily apply to vaccines which are not the subject of such monographs.*

Vaccines for human use are preparations containing antigenic substances capable of inducing a specific and active immunity against the infecting agent or the toxin or the antigen elaborated by it. They shall have been shown to be active in man.

Vaccines for human use may consist either of the inactivated pathogenic organisms or of living organisms treated appropriately, if necessary, to attenuate their virulence without destroying their antigenic potency, or they may consist of antigenic fractions or substances produced by the same pathogenic organisms and rendered harmless whilst retaining their antigenic properties.

The methods of preparation vary according to the type of vaccine, as described below or in the individual monographs, and are designed to maintain the appropriate antigenic properties and to ensure as far as possible freedom from contamination with extraneous agents.

During preparation, suitable additives, including adjuvants, may be incorporated, but penicillin may not be used at any stage or added to the final product. Except where stated in a monograph, streptomycin may not be used in the production of vaccines; where its addition to cell cultures to be used in the production of viral vaccines is permitted it must not be detectable when the cultures are inoculated with the virus. A suitable antimicrobial preservative may be added to sterile and inactivated vaccines and is invariably added if these preparations are issued in multidose containers, unless otherwise prescribed in the monograph. The final product is distributed aseptically into sterile tamper-proof containers which are then closed so as to exclude contamination.

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

The vaccines may be adsorbed on aluminium hydroxide, aluminium phosphate, calcium phosphate or other adsorbent prescribed in the monograph. The adsorbents are prepared in special conditions which confer the appropriate physical form and adsorptive properties. The adsorbed products contain not more than 1.25 mg of aluminium (Al) (V.3.5.7) or not more than 1.3 mg of calcium (Ca) (V.3.5.8) per human dose, unless otherwise prescribed in the monograph.

For freeze-dried vaccines the method of freeze-drying is such as to reduce the water content to not more than 2.0 per cent m/m, unless otherwise stated in the monograph.

When phenol has been used in the preparation of the vaccine, not more than 0.25 per cent m/V is present in the final product (V.3.5.9) unless otherwise stated in the monograph.

When formaldehyde has been used in the preparation of the vaccine, not more than 0.02 per cent m/V of free formaldehyde is present in the final product (V.3.3.1).

### Bacterial Vaccines

Bacterial vaccines are prepared from cultures of suitable strains grown on solid or liquid media and contain inactivated or live bacteria or their antigenic components. They are suspensions of various degrees of opacity in colourless or almost colourless liquids, or may be freeze-dried.

The whole culture or the micro-organisms or fractions of them may be used in preparing the vaccine. Bacterial vaccines containing inactivated organisms may be prepared by inactivating the organisms by chemical or physical procedures without destroying their immunising property. Bacterial vaccines containing living bacteria are prepared from attenuated strains capable of producing immunity against the pathogenic strains of the same species or an antigenically related species. The concentration of living or inactivated bacteria is expressed in terms of International Units of Opacity or, where appropriate, is determined by direct cell count or, for living bacteria, by viable count.

### Bacterial Toxoids

Bacterial toxoids are prepared from toxins by diminishing their toxicity to a non-detectable level or by completely eliminating it by physical or chemical procedures without destroying their immunising property. The method of production is such that the toxoid does not revert to toxin.

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

The toxins are obtained from selected strains of specific micro-organisms, grown on media free as far as possible from ingredients known to cause toxic, allergic or other undesirable reactions in man.

The toxoids may be liquid or freeze-dried. They may be purified and adsorbed.

Adsorbed toxoids are suspensions of white or grey particles dispersed in colourless or pale yellow liquids and may form a sediment at the bottom of the container.

### Viral Vaccines

Viral vaccines are prepared using a seed-lot system from viruses grown in animals, in avian embryos, in suitable cell cultures or in suitable tissues. Viral vaccines consist of suspensions of live or inactivated viruses or of fractions of them. Live vaccines are usually prepared using attenuated strains. Inactivated vaccines may be prepared by suitable chemical or physical procedures.

Viral vaccines may vary in opacity according to the type of preparation. They may be coloured if they contain a pH indicator such as phenol red.

### STORAGE

Store protected from light. Unless otherwise stated in the monograph, vaccines should be stored at a temperature of  $5 \pm 3$  °C and liquid and adsorbed vaccines should not be allowed to freeze.

*Expiry date.* The expiry date is calculated from the beginning of the test for potency or the determination of virus titre as appropriate. It applies to vaccines stored in the prescribed conditions. Monographs indicate the period of validity.

### LABELLING

The labelling of vaccines complies with the relevant national legislation and international agreements. Unless otherwise stated in the monograph, the label on the *container* and the label on the *package* state:

- the name of the preparation,
- the batch number or other reference,
- the recommended human dose and route of administration,
- the storage conditions,

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

the expiry date, except that for containers of less than 1 ml which are individually packed, the expiry date may be omitted from the label on the container, provided it is shown on the package and the label on the package states that the container must be kept in the package until required for use.

In addition, the label on the *package* states:

- the name and amount of any antimicrobial preservative or other substance added to the vaccine, the name of any constituent that may cause adverse reactions and any contra-indications to the use of the vaccine, unless the relevant information is stated in a leaflet included in the package,
  
- for freeze-dried vaccines: - the name or composition and the volume of the reconstituting liquid to be added, - that the vaccine should be used immediately after reconstitution
  
- the name and address of the manufacturer.

## PRODUCTION AND CONTROL OF TETANUS VACCINE

Training curriculum

### INDEX FOR MODULE VIII

Abnormal toxicity . . . . .	30
Adjuvant . . . . .	3
Adjuvant content . . . . .	25
Adjuvants . . . . .	21
Aluminium . . . . .	30
Antigen concentration . . . . .	18
Determination of . . . . .	18
Antigenic purity . . . . .	3, 20
Bacterial purity . . . . .	17
Bulk purified toxoid . . . . .	3, 15, 19, 20
Calcium . . . . .	30
Certification . . . . .	3
Of vaccines . . . . .	28
Clostridium tetani	
Strains of . . . . .	16
Consistency	
Of production . . . . .	17, 18
Control of final bulk . . . . .	20
Control of final product . . . . .	24
Control of source materials . . . . .	16
Detoxification . . . . .	18
Expiry date . . . . .	27, 33
Filtration . . . . .	17
Final bulk . . . . .	3, 15, 21
Final containers	
Inspection of . . . . .	25
Final lot . . . . .	15
Flocculation . . . . .	24
Flocculation test . . . . .	18
Formaldehyde . . . . .	30
Identification	
Of tetanus strain . . . . .	2
Identity . . . . .	3, 24, 29
Inactivation . . . . .	3
Incomplete protocols . . . . .	2
Innocuity . . . . .	3, 25
International Laboratory for Biological Standards . . . . .	15
International reference materials . . . . .	14
Irreversibility . . . . .	3
Labelling . . . . .	26, 30, 33
Lf . . . . .	20

## PRODUCTION AND CONTROL OF TETANUS VACCINE

### Training curriculum

Media	
In production process . . . . .	16
Model certificates . . . . .	10
National control requirements . . . . .	1, 27
Period of validity . . . . .	30
pH . . . . .	24, 25
Phenol . . . . .	17, 21
Potency . . . . .	3, 22, 30
Potency test . . . . .	25
Precautions	
For production . . . . .	17
Preservative . . . . .	17, 20
Preservative concentration . . . . .	3
Preservative content . . . . .	25
Production control . . . . .	16
Production protocol . . . . .	1
Purification . . . . .	18
Quality Manual . . . . .	1
Records . . . . .	26
Release	
Of vaccines . . . . .	28
Requirements	
For manufacturing . . . . .	15
For tetanus vaccine (adsorbed) . . . . .	14
Reversion to toxicity . . . . .	19
Seed lot . . . . .	15
History . . . . .	2
Seed lot system . . . . .	16
Single harvest . . . . .	15
Single harvests . . . . .	3
Specific toxicity . . . . .	3, 19, 21, 29
Specifications	
For vaccines . . . . .	2
Stability . . . . .	27
Statens Seruminstitut . . . . .	15
Sterility . . . . .	3, 21, 24, 30
Sterility test	
Sterility . . . . .	19
Storage . . . . .	27, 30, 33
Strains	
Of Clostridium tetani . . . . .	16
Summary protocol	
WHO requirements . . . . .	1
Summary protocol for Tetanus Vaccine (adsorbed) . . . . .	4
Summary protocols . . . . .	2

**PRODUCTION AND CONTROL OF TETANUS VACCINE**

Training curriculum

Tetanus vaccine

European Pharmacopoeia Monograph . . . . .	29
Evaluation of protocols . . . . .	2

Vaccine protocols

For tetanus vaccine . . . . .	2
-------------------------------	---

Vaccines for Human Use

European Pharmacopoeia Monograph . . . . .	31
--	----

Vaccinum tetani adsorbatum . . . . .	14
--------------------------------------	----