MANUAL FOR THE PRODUCTION AND CONTROL OF VACCINES

TETANUS TOXOID
FOREWORD

A number of countries are wishing to produce vaccines at the national level for which they require the necessary technical information. With this aim the present manual has been prepared.

It should be stressed that this manual is intended only to provide general information on methods - preferably the least complicated ones - for the production of a tetanus vaccine of acceptable quality meeting the WHO Requirements and to describe the necessary tests. There exist other production methods, which may lead to a similar result. The manual, therefore, does not necessarily express any preference for the methods chosen and should not be regarded as a description of those production methods preferred by WHO. This is true also for all (bio)chemical preparations, specified in the manual, as well as for the manufacturers and special equipment mentioned.

If clarification is required on any point, reference should be made to the WHO Secretariat (Chief, BiologicaIs).

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2 WHO Technical Report Series 1979 (to be published); Appendix T.27 of this manual.
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TETANUS TOXOID

(Requirements for Biological Substances No. 10 (revised 1978)) See Appendix T.27

T1. INTRODUCTION

T1.1 Description of the vaccine (Part A, Section 1.2, page 41) See Appendix T.27

Tetanus toxoid is prepared from the toxin produced by the growth of a highly toxigenic strain of Clostridium tetani in a suitable medium. The supernatant fluid, which contains the toxin, is separated from the organisms; the toxin so separated from the organisms is detoxified and purified.

T1.2 Efficacy of the vaccine

Tetanus toxoid is the immunizing antigen protecting against the adverse effects of infection by tetanus organisms. Although the toxoid induces the production of tetanus antitoxin and will thereby protect against the lethal effects of tetanus toxin, it does not necessarily prevent the growth of the organisms.

The toxoid is a very good antigen and even plain toxoid (a toxoid without the addition of an adjuvant) given in adequate dosage can be relied upon to protect when used for primary immunization. It is common, however, for the toxoid to be adsorbed on to an adjuvant (usually aluminium hydroxide or phosphate). Tetanus toxoid, either plain or adsorbed, is rarely given alone in children for whom it is much more commonly included in a combined vaccine together with diphtheria toxoid and pertussis vaccine.

Tetanus toxoid is used alone in pregnant women in order to prevent neonatal tetanus (tetanus neonatorum). It is given also to casualties and is used prophylactically in groups in which tetanus is an occupational hazard, e.g. those working with machines.
A tetanus toxoid complying with the WHO Requirements and given in three doses, suitably spaced for primary immunization, will protect against tetanus; a reinforcing dose should be given at school entry (about five years of age).

### T1.3 Suggested method of production

This manual describing the production and control of tetanus toxoid suggests methods of production that have been shown to give a satisfactory product. The method will not necessarily utilize the most refined, expensive and modern equipment that may be in use in some laboratories having many years' experience in the production of the toxoid, but the suggested method is known to yield toxoid of consistently good quality. It is only after a new production area has had a number of years of experience in the production of the toxoid by a relatively simple method that more refined methods should be considered.

The controls applied both during production and on the final product follow closely those included in the WHO Requirements (see Appendix T.27). A most important factor in vaccine manufacture is consistency of production of quality vaccine and additional controls to ensure this are suggested.

In attempting to make the manual as widely applicable as possible, it has been necessary in some instances to suggest alternative control tests. If a laboratory has no experience of any of the suggested tests the one of choice should be the one most easily carried out having regard to the availability of materials and resources.

In general the methods will comply with the requirements of most national authorities for the production and control of biological substances. If there is a divergence in any particular country, permission to use methods other than those recommended must be obtained from the national control authority.

### T1.4 WHO Requirements (Technical Report Series (1964), 293)

See Appendix T.27

The WHO Requirements for Tetanus Toxoid were first formulated in 1964; since then there have been several developments in vaccine production and the Requirements were therefore revised in 1978. The control procedures suggested in this manual follow them closely. Although the method of potency control suggested in the manual may not be identical with that used by many countries, the quality of the final toxoid will satisfy the requirements of all countries.

### T1.5 Glossary of terms

- **Kf** = Flocculation time (in minutes) as observed in the flocculation reaction.
- **Lf** = Limes flocculation; the amount of toxin or toxoid which when mixed with 1 International Unit of antitoxin gives a Ramon flocculation in the shortest time.
- **Lf/mg N** = Flocculation units per milligram of total nitrogen as determined by the Kjeldahl analysis.
- **Lf/ml FN** = Flocculation units per milligram of protein nitrogen (FN) which is usually determined by the precipitation of the proteins with trichloroacetic acid.
- **I.U.** = International Unit is the specific activity of a stated amount of the International Standard as defined by the WHO Expert Committee on Biological Standardization.
The minimum amount of toxin which when combined with 1 I.U. of antitoxin kills an animal of defined weight in four days (the \( L_+ \) is dependent upon the animal species used).

\( L_+/10 \) The minimum amount of toxin which when combined with 0.1 I.U. of antitoxin kills a mouse of a defined weight in four days. (For tetanus)

\( L_r \) The minimum amount of toxin which when combined with a fixed amount of antitoxin (usually 0.002 I.U. of antitoxin) in the volume of 0.2 ml causes a local skin reaction that is just visible. (Only for diphtheria)

\( L_{50} \) The amount of toxin that kills 50% of a group of animals within four days (the \( L_{50} \) differs for different animal species).

\( M.L.D. \) Minimal lethal dose, the amount of toxin which kills animals within four days (\( M.L.D. \) is different for different animal species). In general the \( M.L.D. \) has been replaced by the \( L_{50} \).

\( A.B.V. \) Antitoxin binding value, a value which defines the toxin plus toxoid in a mixture (determined in animals).

\( O.U. \) Opacity Unit is the measure of opacity as determined in comparison with the International Reference Preparation of Opacity.

\( T.C.P. \) Total combining power, the reciprocal of the volume of toxoid which when diluted is found to be equivalent to 1 International Unit.

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The dose of a vaccine which protects 50% of the immunized animals against a challenge dose of virulent bacteria or toxin.

**Single harvest.** The toxic filtrate 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 toxoid present in a single container from which the final containers are filled either directly or through one or more intermediate containers.

**Final lot or filling lot.** A collection of sealed final containers that is homogenous with respect to the risk of contamination during filling. A final lot must, therefore, be filled in one working session.

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**T2. PREMISES**

(Part A, Section, page 13) See Appendix T.29 For more details see also: Manual for the Design, Equipping and Staffing of Facilities for Production and Quality Control of Bacterial Vaccines (BLG/UNDP/78.1)

**T2.1 Design of premises**

Tetanus toxin must be produced in separate premises because *Clostridium tetani* is a spore bearing organism. The most important requirements for the premises in which toxin is to be produced are that they should be clean, comfortable to work in, have control of temperature and humidity, include all the
essential facilities, and have built-in safety measures against hazards such as contamination. The lighting should be such that all equipment can be seen and operated without difficulty. It is advisable that the laboratories should be supplied with filtered air or that work should be done in laminar-flow cabinets that are monitored regularly to ensure that they are operating correctly. It should be stressed, however, that the use of laminar-flow cabinets does not obviate the need for a high standard of microbiological technique.

T2.2 Quality of materials

The main objective in the choice of building materials for a laboratory is that the shedding or collection of dust shall be avoided. This applies to all surfaces including the floor, which should be covered with an impervious epoxy resin surface. Where there is heavy traffic, quarry tiles or terrazzo floors are to be preferred. The walls and ceiling should be of a high quality hard plaster sealed by painting with a washable material that does not flake. Ledges, that inevitably collect dust, should be avoided and benches should be impervious and without cracks or crevices. Suitable construction materials for benches include laminated plastics, metal and wood covered with an epoxy resin. They should be able to withstand frequent swabbing and disinfection without deterioration. It must be possible to spray the whole area with a disinfectant without damage to any of the materials.

T2.3 Changing facilities

Modern practices in the production of virus vaccines made from attenuated strains of living organisms demand that, for entry into a production area, all outdoor clothing be removed, and that the operators be cocooned in sterile clothing. This is because there are no purification or inactivation steps involved in the production process. Although such stringent precautions are less important in the production of a toxoid or bacterial vaccine in which the organisms are killed by a chemical or in which purification is involved, it is essential to have some barrier between the outside dusty contaminated atmosphere and the production area. The areas set aside for the culture of organisms, processing, blending and filling of final vaccine must be approachable only through an anteroom equipped for washing, change of footwear, and the donning of protective clothing such as clean gowns that do not shed dust or fibres. Where open operations are carried out, either a cap and mask or a total head hood should be worn. Access to this anteroom must be from other parts of the production area and not directly from the exterior.

A production unit and its staff can be used for the production of more than one vaccine in a sequential production programme. The only vaccine requiring a separate facility is tetanus.

T2.4 Premises for breeding and keeping animals

It is a commonly mistaken view that animals can be kept anywhere and under any conditions. If reliance is to be placed upon the results of animal experiments, the animals must be housed under conditions in which they will remain healthy and will not be subjected to cross infection.

They require:

(a) clean living conditions with plenty of light and good ventilation. Animals generate a great deal of body heat as well as humidity and overcrowding can be disastrous.

(b) a good balanced diet with some variety. It is important that contamination of food and bedding, which usually is caused by wild rodents, should be prevented;

(c) living quarters that can be cleaned daily and disinfected between batches of animals.
It is important to control the bacterial, fungal and helminth population in the breeding stock. Many intercurrent infections are able to affect the results of animal experiments. Such infections should be kept to a minimum by segregation of animals from different sources, selective breeding from healthy stock and as a last resort by suitable treatment with therapeutic agents.

The quality control of tetanus toxoid requires a large supply of healthy animals, and such control should not be attempted unless good animals are available either from one's own facility or from a regular supplier.

Such an animal facility should be capable of housing and if necessary breeding the animals used for tests on a number of vaccines such as diphtheria, pertussis and tetanus, and may be used also for the testing of cholera and typhoid vaccines. For the testing of these vaccines only mice and guinea-pigs are needed in appreciable numbers.

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T3. EQUIPMENT FOR TOXIN PRODUCTION

(Part A, Section 2, page 42) See Appendix T.27

T3.1 Equipment for production in static culture

The equipment used for the production of toxin will depend upon the system chosen for cultivation of the bacteria, but in principle no expensive equipment is needed. It can be carried out in wide-mouthed bottles, tall beakers or, for larger scale production, in stainless-steel vessels.

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Convenient sizes of containers range from 5-litre beakers containing 3.5 litres of medium to 20-litre stainless-steel containers containing 15 litres of medium. The beakers are covered with a thin layer of non-absorbent cotton-wool 1-2 cm thick held between two layers of hydrophil gauze. An injection needle or a glass tube of about 15 mm diameter, open at both ends, is passed through the thick covering and held in position by rubber washers which fit snugly to the needle or tube on both sides of the cotton cover. The upper end of the needle or glass tube is covered either with an aluminium foil or plugged with cotton.

Mueller & Miller used Erlenmeyer flasks of 2 litres capacity containing 1700 ml of medium.

T3.2 Fermenter production

For large-scale production stainless-steel fermenters can be used of any size from 20 litres upwards. They need not be highly instrumented since the growth of tetanus requires only a control on impeller speed, air supply and temperature, and the fermenters may be purchased from commercial manufacturers or made to order by a good fabricator of stainless-steel. Although the initial capital investment may seem high they have an assured working life of at least 10 years and are likely to present the minimum in demands on servicing if carefully designed.

The equipment used for the production of tetanus toxin must be kept separate at all times from all other equipment.

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T4. STAFF

T4.1 Qualifications and numbers

The number of staff involved in the production of tetanus toxoid depends to some extent upon the volume of toxoid to be produced and the number of batches required.
It is not advisable to employ staff who have no knowledge of microbiology but a well-trained nucleus of staff can train other staff quite quickly. The production of the media would require one graduate scientist and one technician.

Maintenance of seed culture, preparation of inoculum, growth of organisms, separation, purification and detoxification of toxin, can be carried out by a graduate microbiologist with some years' experience and three technicians.

Quality control of the bulk toxoid and the final product will require also a graduate scientist and one technician.

The animal facility should be supervised by a senior technician with adequate experience and training in the maintenance of small animals. Provision should be made for professional veterinary consultation when required.

Finally, it is important that in every laboratory for vaccine production at least one mechanical engineer should be available to be responsible for the equipment.

T4.2 Experience

The technical staff should have had an education beyond normal schooling. In further education they should have had an opportunity of studying microbiology and have a working knowledge of sterile techniques and the handling of bacteria. The scientific staff should have spent at least three to six months in a production unit actively engaged in the manufacture and control of bacterial vaccines.

T4.3 Health

All staff should be in good health and should be immunized against or known to have natural immunity against any infectious organisms that they may be required to handle. They should be immunized against tuberculosis and not be suffering from any other respiratory or diarrhoeal disease. Any staff members having a septic wound should not enter the production area.

The technical staff looking after the breeding colony or experimental animals should also be in good health and free from any latent infection.

T4.4 Organization of activity

Although the same staff can be used also for the production of all vaccines they must not move from the tetanus toxin production area into the other production areas on the same working day. The staff may go into the tetanus area, however, after working in the other areas.

Quality control and production should be administratively separate but it is important that quality control staff have first-hand knowledge and experience of production. There is no need for animal testing to be duplicated, but it is important that the detailed results of all "in process" and final tests should be available to both production and quality control staff.

In any event, accurate and clear permanent records signed legibly and dated must be kept, and must be made available at all times to the production and quality control staff as well as to the appropriate inspectors from local health authorities. Examples of forms suitable for keeping such records are shown in Appendices T.25 and T.26. Though the production facilities for tetanus toxin cannot be used for the manufacture of other bacterial vaccines, the staff should be trained in the production of a number of other bacterial vaccines as it can be used for this purpose in other areas at times when tetanus production is not in progress. Similarly, the quality control staff should be capable of the control of all bacterial vaccines.
T5. MEDIA

The medium most commonly used for the cultivation of Cl. tetani is the medium originally described by Mueller & Miller (J. Immunol. (1947) 56, 143).

Some modifications in the formulation of the original medium have been made and these are also described in Appendix T.1.

The main constituent of the medium is a tryptic digest of casein. This may be prepared in the laboratory or bought commercially.

T6. STRAINS OF CL. TETANI

T6.1 Obtaining the strain

In the production of tetanus toxin, the strain plays an important role. The strain which has been most commonly used in combination with the media described originates from the New York State Department of Health and should be obtained from a laboratory which is experienced in toxin production. The strain is a highly toxigenic strain and differs considerably from normal Cl. tetani strains.

Although all tetanus toxoids are made from a few highly toxigenic strains, it is not possible to obtain the same yields of toxin by a strain if the growth medium is markedly changed in its composition. A new production laboratory, therefore, will need to obtain a strain and undergo a period of adaptation of the organism to the medium selected for growth before maximum yields may be expected.

1 New manufacturers may obtain data concerning a suitable strain from the WHO Secretariat (Chief, Biologicals).

T6.2 Maintenance of the strain

The stock cultures prepared from the original strain have to be maintained in the freeze-dried form to preserve viability. The working seed culture prepared from this can be maintained by daily subculture in glucose broth until the culture shows a fall in toxin production. The daily subcultures are grown in an anaerobic jar.

T6.3 Preparation for production

The containers of the culture medium that have been autoclaved and rapidly cooled in running tapwater or in ice cold water are transferred to the inoculation room where the 24-hour-old seed cultures and the required number of sterile syringes or Pasteur pipettes are kept. The purity of each seed culture is checked by the examination of Gram stained smears and only those cultures found to be pure are used for the inoculation of the freshly prepared and cooled medium.

T7. PRODUCTION OF VACCINE

In order to follow more easily the production of purified tetanus toxoid suggested flow charts are shown in Appendix T.26. These indicate at each step in the production where materials are added as well as where samples are taken for testing. The tests applied are also specified.

T7.1 Production of toxin

The containers of the medium are inoculated aseptically with a glass syringe or Pasteur pipettes using one seed culture tube for the inoculation of a single container. The containers are then incubated at 35°C - 37°C. Such cultivation cannot be considered to be performed initially under anaerobic circumstances, but the highly reducing properties of the medium are sufficient to promote good growth and toxin production. The toxin is harvested after five to seven days' incubation: the average yield of toxin varies between 60 and 80Lf/ml.
T7.2 Harvesting of toxin

At the end of the incubation period, 10 ml samples are withdrawn from each container and each sample is centrifuged at 3000 rpm for 10 minutes. The supernatant in each sample is checked for toxin (Lf) content. Smears are prepared from the deposit, Gram stained and examined for purity. The contents of the pure-wet containers are filtered through a thick pad of absorbent cotton wool and pooled together in a stainless-steel pressure tank.

T7.3 Separation of toxin from culture

Separation of the toxin may be carried out before or after detoxification. The yield of toxin from toxin is similar by either method, but when detoxification takes place in the presence of the organisms more formaldehyde (0.6% v/v) is required. The reason for detoxifying the whole culture is a safety measure to avoid handling large quantities of potent toxin and viable organisms which is dangerous even though the staff may be specially trained in such procedures.

When the toxin is separated from the culture before toxoiding a Seitz filter containing the required number of clarifying and sterilizing pads is sterilized by autoclaving at 126°C for 45 minutes. The filter press must not be fully tightened before sterilization and all valves kept open to allow the free passage of steam during sterilization. The harvest is filtered through the sterile filter under positive pressure not exceeding 10 pounds per square inch (69 kPa). The filtrate is collected in sterile glass or stainless-steel containers of an appropriate capacity.

T7.4 Production of bulk toxoid

Detoxification: When the toxin has been separated from the culture, detoxification is effected by the addition of formaldehyde and incubation of the product at 37°C for some weeks.

The formalin to be used for toxoiding should be as pure as can be obtained, preferably Analar grade, or that conforming to the requirements of the International Pharmacopoeia or other national pharmacopoeias. It should be checked for its formaldehyde content before use (see Appendix T.18).

The procedure adopted is to measure the volume of the toxin by a suitable method and to add sufficient formalin (40% formaldehyde solution) to give a final concentration of about 0.5% v/v. The pH is adjusted to 7.6 with sterile sodium carbonate or bicarbonate solution and the containers incubated at 37°C. The pH is checked after four and seven days, and adjusted again if necessary to 7.6. Incubation is continued for four weeks.

At the end of the incubation period a sample should be taken and the bulk toxoid stored in the cold until the specific toxicity test has been completed (see Appendix T.14). Control tests should be carried out on samples as given under T7.5.

If the sample shows residual specific toxicity, add to the lot an additional quantity of 0.1% formalin and incubate for a further period of two weeks at 37°C, at which time a retest for specific toxicity should be carried out. When toxoiding is complete a sample is taken and the control tests as in T7.5(ii) are applied.

T7.5 "In process" tests or production control tests

When the whole culture is toxoided before purification a sample of the toxin is taken before detoxification for the determination of Lf/ml, but in this case none of the other tests included are T7.5(i) are applicable. After detoxification the tests listed in T7.5(ii) are applicable.

A sterility test is carried out at this time. Experience in the production of the toxoid may allow assessment of this test at a time earlier than the full 14 days incubation period, making it possible to proceed with production after incubation of the sterility samples for 48 to 72 hours. In any event the sterility test must be allowed to remain for the full 14 days.
Where a number of tests are applied at the same point in the production process it is advisable to carry out the tests in such an order that those making the least demands on resources and giving the quickest results should be performed first. These should be shown to be satisfactory before the more demanding and time-consuming tests are undertaken.

T7.5(i) "In process" tests for toxin

The following "in process" tests shall be carried out on the toxin:

I Sterility Appendix T.28
II Determination of LF content Appendix T.6
III Determination of M.L.D. (minimum lethal dose) Appendix T.7
IV Determination of M.T.V. (maximum toxin value) Appendix T.9
V Determination of LF/10 dose Appendix T.8

T7.5(ii) "In process" tests for toxoid

I Specific toxicity test Appendix T.14
II Sterility Appendices T.20 and T.28
III Determination of LF/ml Appendix T.6
IV Total combining power Appendix T.10

T7.6 Purification of toxoid

Although ultrafiltration of toxoid removes some impurities it is nevertheless advisable to apply a further purification procedure to tetanus toxoids.

The simplest and most commonly used procedure involves salting out of the toxoid with ammonium sulfate and the method described in Appendix T.5 is satisfactory. At low ammonium sulfate concentrations relatively impure toxoid is precipitated compared with that precipitated at higher ammonium sulfate concentrations (van Ramshorst, Antonie van Leeuwenhoek (1957) 22, 97). The immunizing potency of the toxoid precipitated by high ammonium sulfate concentrations appears to be very variable. Between these two extremes, toxoid of high purity (usually 1500-2000 LF/mgN) is obtained. As the optimum concentration of ammonium sulfate varies from batch to batch it is necessary to determine a precipitation curve for each batch (see Appendix T.5). The curve is S-shaped with the purest toxoid precipitated where the curve has the steepest slope. Usually the first precipitation is carried out at 12-15% of ammonium sulfate (the percentage of ammonium sulfate is expressed as grams of solid ammonium sulfate added to 100 ml of the toxoid), and this precipitate which consists of non-specific impurities is discarded. The ammonium sulfate concentration of the supernatant is then raised to approximately 25% or to the concentration as indicated by the precipitation curve in order to precipitate the purest toxoid. This precipitate is processed further as described in Appendix T.5.

Toxoid recovered by this procedure will contain about 60-70% of the original toxoid content with an average purity varying between 1000 and 1200 LF/mg protein N. Such a toxoid is considered acceptable.

T8. POOLING, STORAGE AND TESTING OF BULK TOXOID

T8.1 Pooling

The volume of an individual batch of toxin or toxoid is usually about 50-100 litres. Before proceeding to ultrafiltration and/or purification it is convenient to pool several
such batches to make about 500 litres (or more, if the facilities permit). If by ultrafiltration or salting out the volume is reduced approximately 1:50, then the resultant concentrated toxoid would be about 10 litres, a convenient quantity to handle for purification by fractional salting out. After purification, the volume of the concentrated and purified bulk toxoid would be still less (6-8 litres), again a convenient volume for the preparation of final vaccine.

T8.2 Storage

As the concentrated and purified toxoids are very stable they can be stored in a refrigerator (5°C - 3°C) for some years before further processing but after a long storage period it is advisable to check the Lf content before further processing.

T8.3 Testing of bulk toxoid

Each bulk toxoid after purification shall be subjected to the following tests:

I Potency test  
II Specific toxicity  
III Sterility  
IV Test for antigenic concentration:  
(a) Determination of Lf/ml  
(b) Determination of total combining power (TCP)  
V Purity, Lf/mg protein N  
VI Thiomersal content  
VII Free (residual) formaldehyde content  
VIII Test for irreversibility

T8.4 Potency test

There are several methods of measuring the potency of tetanus toxoid, but for each the principle is the same. Groups of animals (guinea-pigs or mice) are immunized with graded doses of the toxoid and their responses measured either by determining the antibody titre in the sera or by challenging the animals with a paralytic or lethal dose of toxoid.

In order to rule out the variabilities that are inevitably involved in an animal test, similar groups of animals are immunized with a reference preparation that has been calibrated in International Units. By comparison of the responses elicited by the test and reference toxoid the potency of the test preparation may be recorded in International Units.

The suggested method for measuring the potency of tetanus toxoid is described in Appendix T.13.

T9. DILUTING AND FILLING

In some countries, tetanus toxoid is used both as a plain and as an adsorbed toxoid; it is advisable to use the adsorbed toxoid because of its superior immunogenicity.

T9.1 Preparation and testing of fluid toxoid

Fluid tetanus toxoid is prepared by diluting the concentrated bulk which has passed all statutory requirements: specific toxicity, sterility, potency, etc. The final bulk should contain at least 10 Lf/ml (5 Lf per human dose). Where fluid toxoid is used alone the concentration used should be not less than 10 Lf/human dose and in some countries be as high as 20 Lf. Dilution can be made in either normal physiological saline or buffer solutions such as phosphate or borate buffer; in order to maintain the pH of the final product through the storage period buffered saline is to be preferred. Fluid tetanus toxoid in phosphate buffered saline is prepared by the following method.
The Lf content of the bulk concentrated tetanus toxoid is determined and the quantity of the toxoid required for the final bulk is measured out aseptically. If a final bulk of 100 litres is to be prepared, the sodium chloride required to make 100 litres of 0.7% sodium chloride (700 g per 100 litres) is weighed out and dissolved in 20 litres of distilled water and 1 litre of one molar phosphate buffer is added. One litre of 1% thiomersal is also added, the volume is made up to about 70 litres with distilled water and the solution is sterilized by filtration (e.g. through a Sartorius or 0.22 micron membrane). At this stage the required volume of toxoid is added aseptically and the volume made up to 100 litres with sterile distilled water. The pH should be between 6.5 and 6.8 and is adjusted with sterile phosphate buffer solution if necessary. The final bulk is then thoroughly mixed.

T9.2 Adjuvants

Adjuvants such as aluminium phosphate or aluminium hydroxide are used in the preparation of adsorbed toxoids. Adjuvants may be prepared locally or obtained from a commercial source. The latter is especially advisable for aluminium hydroxide gel which is difficult to prepare, and preparations such as "Alhydrogel" are commercially available. Two methods for the preparation of aluminium phosphate are given in Appendix T.20. Calcium phosphate is also used as an adsorbant.

The concentration of aluminium shall not exceed 1.25 mg per single human dose.

T9.3 Preparation and testing of adsorbed toxoid

In the preparation of the final bulk the toxoids are added to the aluminium phosphate, the pH of the mixture is adjusted to 6.0. The mixture is held at this pH for 48 hours, then the pH is readjusted to 6.6 to 7.0. Adjuvant is added to the pertussis component and the pH adjusted to 6.6 to 7.0. The requisite quantities of adsorbed toxoids and pertussis are then pooled, mixed and the pH checked (see Appendix T.23). The pH may be directly adjusted to 6.8 when aluminium hydroxide is used. (The other two components, viz. diphtheria toxoid and pertussis vaccine are omitted when tetanus toxoid (adsorbed) alone is to be prepared.)

The vaccine is merthiolated 1:10 000, taking into account the quantity of merthiolate which entered through the various components.

Where only low grade (type II or III) glass is available it is advisable to add a buffer to the final bulk to prevent the pH rising about 7.0 during the storage period of the vaccine in the final containers.

There are a number of formulations of vaccines. The commonest in use are as follows:

1. Diphtheria toxoid - at least 25 Lf per dose for adsorbed or combined vaccine. It is advisable not to use plain (unadsorbed) diphtheria toxoid alone.

2. Tetanus toxoid (plain toxoid) should contain at least 10 Lf per single human dose; in combination with pertussis the combined vaccine should contain at least 5 Lf of tetanus toxoid per single human dose.

3. Pertussis must contain not more than 20 Opacity Units (O.U.) with a potency of not less than 4 I.U. per single human dose.

The majority of control tests are carried out at this stage when all the ingredients of the vaccine, toxoids (diphtheria and tetanus), pertussis suspension, adjuvant (where applicable) and diluent, have been blended. These tests are:

I Potency test
II Innocuity test
III Specific toxicity test
IV Sterility test
The filling of biologicals must be carried out under strict aseptic conditions as the product once contaminated cannot be recovered nor terminally resterilized. It is, therefore, preferable that filling should be carried out by a completely automated process with least interference by the filling staff. Such completely automatic filling, stoppering and sealing equipment for multidose containers is commercially available (see Appendix T.24). Similarly, automatic ampoule filling and sealing equipment is also available (see Appendix T.24) for filling single dose ampoules. Semi-automatic machines for filling vials followed by manual stoppering and capping or manual stoppering and mechanical capping can also be used. It is preferable, however, to use completely automatic equipment so as to maintain aseptic conditions throughout the operation. Such machines necessarily require the use of vials properly standardized with respect to body diameter, height and dimensions of the mouth so as to be compatible with standard rubber closures. Similar precautions are also required for the dimensions of ampoules to be used in automatic filling.

The quality of glass containers influences the keeping quality of the final product. It is necessary, therefore, to use neutral borosilicate U.S.P. type 1 containers in the interest of quality and safety of the product though U.S.P. type 2 glass may be permissible in some countries.

The quality of rubber closures is also important. Rubber is known to absorb phenols and mercurial preservatives and good quality or coated rubber is needed to prevent this. Sources of rubber should be checked, therefore, for compatibility with the vaccine and its preservative. Butyl rubber stoppers treated with such preservatives as thiomersal or phenol prior to use in the final product are found to absorb a minimum quantity of preservative during storage of the vaccine. The use of butyl or other rubber stoppers which have been shown to be satisfactory is recommended.
The advantage of fully automatic filling and capping equipment is not that it saves labour but that it reduces contamination and thereby prevents unnecessary discarding of valuable vaccine. Stress should be placed on the need for regular maintenance of such equipment.

Prior to filling the vials and stoppers must be washed and sterilized. Many types of equipment are available for this purpose, and a list of some manufacturers is given in Appendix T.24, item 10.

The filling must be done in specially designed rooms or done using a large vertical laminar-flow system which gives good ("Class 100") conditions for sterile work. The names of some manufacturers of such equipment are given in Appendix T.24, item 11. Such laminar-flow systems should be tested from time to time to ensure that they are delivering sterile air. This may be done by exposing nutrient media in Petri-dishes and thereafter incubating them to test for sterility. Alternatively, the contents of the Petri-dishes may be transferred to more easily transportable containers for further incubation.

It is important to check the filling process regularly by filling at least 500 ampoules or vials with a suitable nutrient medium, preferably at the end of the working day. The filled ampoules should be incubated and if more than 1% of them are shown to be contaminated, the filling area, equipment and operating procedure must be investigated and improved.

T9.5 Inspection

Every single or multidose container should be inspected visually under proper illumination to detect the presence of foreign particles. A simple arrangement for this purpose is a fluorescent tube fitted at the top of a black and white background screen and illuminating the background. The fluorescent tube is covered with a shade in order to protect the eyes of the inspection staff from any direct glare. Vials are examined against both white and black background, and vials or ampoules showing foreign particles and defects in closures should be rejected.

T10. Tests on final products

T10.1 Tests

The final product after filling shall be subjected to the following tests:

I. Potency test (if not done on final bulk)  Appendix T.13
II. Innocuity test  Appendix T.21
III. Identity test  Appendix T.12
IV. Sterility test  Appendix T.20
V. Thiomersal content  Appendix T.16
VI. pH  Appendix T.17
VII. Aluminium content (where applicable)  Appendix T.19

T10.2 Reporting of data

For each lot of final vaccine a written protocol should be assembled containing all the essential information on the production and testing of the vaccine. It should contain the data of all tests that have been performed and be signed legibly by a responsible member of staff. Apart from these final protocols careful records shall be kept in the laboratory indicating all steps of processing, testing, filling and distribution. All records shall be retained throughout the storage period of the vaccine. The record shall be available for inspection at all times by the national control authorities.
TII. LABELLING AND PACKAGING

TII.1 Labelling

The labelling of tetanus toxoid on the container and package shall comply with the Requirements of Section 8 or Part A of the Requirements for Diphtheria and Tetanus Toxoid (see Appendix T.27).

TII.2 Packaging

The size and type of container and package shall be that most convenient for the conditions of use. It is common to have 10-dose vials, but under some circumstances in which a large number of children may be assembled at a clinic, 25- or 50-dose vials may be more appropriate especially if a jet-injection gun is used. Experiences with jet-injection guns, however, in which adsorbed vaccines are used have given variable results.

Careful consideration must be given to the period over which the contents of a vial may be used. If a physician is giving vaccine to individual children in his surgery and entering the vial on a number of separate occasions, the bacteriostatic agent present in the vaccine must be shown to be capable of suppressing the growth of any organisms introduced into the vaccine during the removal of a single dose of vaccine. It is recommended that a vial once opened should be used on the same day and not be reused on subsequent days. If storage and reuse are absolutely unavoidable, the vial must be kept in a refrigerator at 5° - 10°C and never for more than one working week.

TII.3 RETAINED SAMPLES

At the time of release of a lot of vaccine the manufacturer must set aside and refrigerate enough samples to serve two essential purposes:

(i) to enable an investigation to be carried out in the event of a complaint from a user concerning the particular lot. It is advisable, therefore, to set aside sufficient samples to allow for a full test of the final vaccine to be repeated;

(ii) to ensure that the product has been stored correctly and that the potency has been maintained throughout the storage period.

T12. RELEASE

The mechanism for the release of vaccines should be determined by the national control authority. Where there is no national control authority permission to manufacture vaccines and the mechanism for release should be specified by the national health authority.

It is important that only those vaccines that fulfil Part A of the WHO Requirements for Tetanus Toxoid are released for use.

Where the producer is solely responsible for quality control and there is no national control authority, then it is essential that the chief of the quality control laboratory of the producer reports to the director of the institute independently of those involved in the production of the vaccine. The decision concerning the release of a batch of vaccines must be made by the chief of the control laboratory.
The storage of retained samples occupies refrigeration space that may be limited, but only if it is known that the complete lot has been distributed, used or destroyed at the end of the expiry period should such samples be discarded. It will be useful from time to time to keep some samples beyond the expiry date, and to check on the keeping properties, particularly for potency.

T14. SURVEILLANCE OF VACCINES

The surveillance of the use of vaccines is the responsibility of the National Government Health Authority. Such surveillance falls into two categories:

T14.1 Safety

Although every attempt is made in the laboratory to ensure that vaccines are safe by subjecting all lots to tests for freedom from abnormal toxicity, a constant watch should nevertheless be kept for adverse reactions in children.

There are contraindications to the use of all vaccines, and an investigation of all adverse reactions should be made particularly if they appear to be lot-related.

T14.2 Efficacy

Different populations differ in their antibody responses to vaccines, and it is essential to ensure that the community is giving adequate antibody responses to all components of the vaccines used. Many factors such as nutritional status, intercurrent infections and the age of the children have an effect upon the responses. A paired blood sample before and after vaccination should be taken from a number of the vaccinees and titrated to measure the antibody responses in order to have some idea of the effectiveness of the vaccination campaign. Furthermore, it is advisable to obtain blood samples from the same children two to five years later to determine how much antibody (and thereby protection) has been maintained.

T15. WHO REQUIREMENTS

Throughout the manual reference has been made to the requirements specified in the Requirements for Diphtheria Toxoid, Pertussis vaccine, Tetanus Toxoid and Combined Vaccines (1978). The part of these Requirements which refers to Tetanus Toxoid is reproduced in Appendix T.27 of this manual.

T16. SUMMARY PROTOCOLS

A summary protocol for recording the results of tests on a particular final lot of toxoid is included in the manual (see Appendix T.25). The tests specified follow closely those included in the WHO Requirements and the protocol is intended for the reporting of data to the national control authority or to the authorities in other countries to whom the vaccine may be exported. The tests specified are in such detail that inspection of the protocols should give an indication of whether the lot meets the WHO Requirements in every particular.