REVISED REQUIREMENTS FOR
INFLUENZA VACCINE (INACTIVATED)

(Requirements for Biological Substances No. 17)
(Revised)

INTRODUCTION

The only generally accepted means of influenza prophylaxis available at present is vaccination. The various types of vaccination procedure, with live attenuated or killed vaccines, have been reviewed by a WHO Scientific Group on Human Viral and Rickettsial Vaccines (1), which found that killed vaccines are preferred in most countries.

Since influenza vaccines were in use in many countries on a wide scale, in 1967 a group of experts formulated requirements for inactivated influenza vaccine and these were published as an annex to the twentieth report of the Expert Committee on Biological Standardization (2). During the next 5 years, technical developments in
the purification of the virus suspensions from which vaccines were made, as well as in the measurements of the virus content, were such that the potency of whole virus vaccines could be expressed in international units. Accordingly, an addendum to the requirements was annexed to the twenty-fifth report of the Expert Committee on Biological Standardization (3, pp. 15–17). Since then, however, technical developments have completely altered the method of measurement of the haemagglutinin content of the vaccines.

The WHO Expert Committee on Biological Standardization, in its twenty-ninth report (4), recognized that the International Reference Preparation of Influenza Virus Haemagglutinin (Type A) established in 1967 was no longer appropriate for the measurement of haemagglutinin content of virus strains appearing more recently. Accordingly the International Reference Preparation was withdrawn and, furthermore, the Committee recommended that the requirements for inactivated influenza vaccine should be completely revised.

The revised international requirements for inactivated influenza vaccines have been fitted into the framework adopted in the Requirements for Biological Substances No. 1–27, already published by WHO (4, page 142). A group of WHO consultants and staff members (listed on page 192) drafted them, taking into account the regulations and requirements for the manufacture and control of inactivated influenza vaccine in a number of countries, as well as information from published and unpublished reports. In addition, opinions and data have been received from a number of experts, whose assistance is acknowledged (see page 192).

GENERAL CONSIDERATIONS

Inactivated influenza vaccines have been in widespread use for about 35 years. The efficacy of immunization has varied according to circumstances, but protection rates of 75–90% have been reported. Differences in protective efficacy may result from continuing antigenic variation in the prevailing viruses.

Because of such variation the composition of inactivated influenza virus vaccine, unlike that of most viral vaccines, must be kept constantly under review. Accordingly, WHO publishes annually recommendations concerning the strains to be included in the vaccine.
When a new subtype of influenza A virus bearing new haemagglutinin (and neuraminidase) antigen(s) appears, it is likely that vaccine containing the antigens of the formerly prevalent influenza A subtype will be ineffective, and a vaccine containing the new pandemic virus will be required. In addition, during interpandemic periods, influenza A viruses undergo frequent and progressive antigenic drift in their haemagglutinin and neuraminidase antigens. Vaccines containing formerly prevalent viruses are expected to be less protective against virus variants showing antigenic 'drift' than against the homologous virus.

Influenza B strains do not vary in subtype. They undergo antigenic variation but with much less frequency than the A strains. Therefore, the influenza B component of bivalent vaccines requires less frequent change. Influenza vaccines usually contain one or more influenza A viruses of a single subtype and one influenza B virus. Occasionally, as in 1977–78, two influenza A subtypes may circulate concurrently. Vaccines containing both subtypes will then be required.

As a result of developments in technology, the WHO Expert Committee on Biological Standardization, in its twenty-ninth report (4), recommended that the potency of influenza vaccines should be expressed in micrograms of haemagglutinin per ml (or dose) as determined by suitable immunodiffusion methods. Appropriate techniques include single-radial-immunodiffusion and Laurell rocket immunoelectrophoresis. In order to standardize the methods, reference antigen (calibrated in micrograms of haemagglutinin per ml) and specific antihemagglutinin serum, suitable for use in the assay of the haemagglutinin content of each component of inactivated vaccines, are prepared and distributed by the National Institute for Biological Standards and Control, London. A new reference antigen and antiserum will be prepared each time it is necessary to introduce a new virus strain into the vaccines.

The formulation of a permanent and inflexible recommendation for the quantity of antigen of the A and B components of inactivated influenza vaccines is inappropriate. This is because of the antigenic variability of the viruses and the fact that, for new variants, differing amounts of antigen will be required to induce antibody levels consistent with immunity. The antibody response depends on the age of the vaccinated person, the presence or absence of prior immunological experience with viruses possessing haemagglutinin (and neuraminidase) antigens related to that of the vaccine strain, and the
number of doses and type of vaccine given (whole virus, disrupted virus antigen, purified surface antigen vaccines). Vaccine potency should be based on the results of clinical trials with various types of vaccine, performed at appropriate times, but particularly on the emergence of new variant strains in various age groups. The trials should include studies of antibody responses to vaccination, clinical reactions, and, where possible, the protective efficacy of the vaccine against infection by natural or artificial challenge.

Recent extensive clinical studies have provided a greater understanding of the parameters affecting the antibody responses and reactions to inactivated influenza vaccines in various age groups. Reactions, particularly in unprimed children, were noted mainly when whole-virus vaccines were given, whereas disruption of the virus decreased the reactogenicity. However, disruption of virus particles also diminished the ability of the vaccine to elicit antibody in unprimed subjects. On the other hand, both whole-virus and disrupted-virus vaccines were effective in inducing antibody responses in immunologically primed subjects. Modern vaccines prepared by suitable methods contain little nonviral protein and endotoxins. Adverse reactions were more closely associated with the content of intact virus particles.

Influenza virus vaccine had been produced in embryonated hens' eggs for many years before avian leukemia viruses, avian adenoviruses, and mycoplasma were known to be frequent contaminants of eggs. Even after the discovery of these contaminants, however, tests for their presence in inactivated virus suspensions have been consistently negative, indicating that the organisms have been absent or killed by the inactivation processes generally used. The present requirements, therefore, do not demand the use of eggs known to be free from avian leukemia viruses, avian adenoviruses, and mycoplasma, or the testing of vaccine for the presence of these organisms. It is important, however, that any new inactivation process used in influenza vaccine production should be shown to kill such organisms.

The present requirements are applicable to preparations consisting of whole or disrupted virus, grown in fertile eggs and intended to be given parenterally. If other tissue sources were to be used for vaccine virus cultivation, these requirements would need modification.

A number of adjuvants have been used as a means of enhancing the immune response to inactivated influenza vaccine. Until further
evidence of the safety and potency of adjuvant vaccines is available, requirements for their preparation and control cannot be formulated.

In emergencies, e.g., when an influenza epidemic is expected, the abnormal demand for vaccine may create difficulties in fulfilling these requirements. No attempt has been made to indicate in what respect these requirements may be modified, since under these circumstances each national health authority must exercise its own judgement.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in type of normal size have been written in the form of requirements, so that, if a health administration so desires, these parts as they appear may be used as definitive national requirements. The parts of each section that are printed in small type are comments and recommendations for guidance. In order to facilitate the international distribution of vaccine made in accordance with these requirements, a summary protocol for the recording of the results of the tests is included (see pages 165-170).

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning inactivated influenza vaccine, it is recommended that a clause should be included permitting modifications of the manufacturing requirements on condition that it be demonstrated, to the satisfaction of the national control authority, that such modified requirements ensure that the degree of safety and the potency of the vaccine are at least equal to those provided by the requirements formulated below. The World Health Organization should then be informed of the action taken.

The terms “national control authority” and “national control laboratories”, as used in these requirements, always refer to the country in which the vaccine is manufactured.

PART A. MANUFACTURING REQUIREMENTS

1. DEFINITION

1.1 International name and proper name

The international name shall be “Vaccinum Influenzae Inactivatum”. The proper name shall be the equivalent of the international name in the language of the country of origin.
1.2 Descriptive definition

Vaccinum Influenzae Inactivatum shall consist of a suspension of the antigens of influenza virus types A and B, either individually or combined, grown in suitable embryonated hens' eggs and inactivated by a suitable method. The vaccines may contain whole virus or antigens obtained from the virus particles by partial or complete disruption by chemical means. Some vaccines may contain the purified surface antigens of the virus (haemagglutinin and neuraminidase). The preparation shall satisfy all the requirements formulated below. In some countries mineral carriers such as aluminium hydroxide, phosphate, or oxide or calcium phosphate have been added to the vaccine.

1.3 Reference materials for haemagglutinin

WHO influenza reference haemagglutinin antigens are prepared and distributed by the National Institute for Biological Standards and Control, London. They are preparations antigenically representative of the virus strains that are likely to be included in current vaccines. They are distributed on demand when a new influenza virus appears and the likelihood of its spread throughout the world makes desirable its inclusion in a vaccine.

The preparations contain a calibrated quantity of haemagglutinin of influenza virus measured in micrograms of haemagglutinin antigen per millilitre. The calibrations are performed at the National Institute for Biological Standards and Control, London, by single radial diffusion tests (3) with purified virus of known haemagglutinin antigen concentration. The reference preparations are used to calibrate the haemagglutinin content of national reference preparations for use in the manufacture and laboratory control of inactivated influenza vaccines by an in vitro immunodiffusion test.

These preparations, together with the specific antihemagglutinin sera, may be obtained from the National Institute for Biological Standards and Control for the purposes of such tests.

1.4 Terminology

Seed lot. A quantity of virus processed together, fully characterized, and of uniform composition. In each manufacturing
establishment a primary seed lot is that from which secondary seed lots are prepared. Vaccine is not more than two passages removed from the secondary seed lot.

Monovalent virus pool. A quantity of virus suspension derived from eggs that were inoculated with the same virus strain, incubated and harvested together or in successive sessions.

Monovalent bulk. A quantity of inactivated, concentrated, and purified material derived from one or more monovalent virus pools that have been processed under similar conditions.

Final bulk. The finished vaccine prepared from one or more monovalent bulk vaccines present in the container from which the final containers are filled. It may contain one or more virus strains.

Filling lot. A collection of sealed final containers that have been filled in one working session from a single final bulk. They are uniform with respect to the risk of contamination during filling and sealing.

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) (6, page 11) shall apply to establishments manufacturing inactivated influenza vaccine, with the addition of the following.

The areas where processing of inactivated influenza vaccine takes place shall be separate from those where work with live influenza virus is performed.

3. PRODUCTION CONTROL

3.1 Control of source materials

Strains of influenza virus used in the production of inactivated influenza vaccine shall be identified by historical records, which shall include information on the origin of the strains and their subsequent manipulation. The virus strain shall not have been passaged in continuous cell lines. If virus strains isolated in eggs are not available it may be necessary to use an isolate from suitable cell cultures. In this case the virus strain should be passaged in eggs using multiple
terminal dilution passages. The cell cultures permitted and the number of passages should be approved by the national control authority. Only strains that have been approved by the national control authority shall be used. It is now common practice to use recombinant strains giving high yields of the appropriate surface antigens. Where such strains are used, the parent high yield strain and the method of preparing the recombinant should be approved by the national control authority.

Suitable strains including recombinant strains for vaccine production are available from: Biologicals, World Health Organization, 1211 Geneva 27, Switzerland. Only recombinant strains, originating from an experienced, specialized laboratory should be used.

3.1.1 Identity of virus strain

Each manufacturer shall identify the haemagglutinin and neuraminidase antigens of the vaccine virus strains by suitable serological tests.

3.1.2 Virus propagation

Influenza virus used in the production of inactivated influenza vaccine shall be propagated in embryonated hens’ eggs. The eggs shall be derived from healthy flocks.

3.1.3 Seed lot system

The production of vaccine shall be based on the seed lot system. Each seed lot shall be identified as influenza virus of the appropriate strain by methods acceptable to the national control authority.

3.1.4 Tests on seed lots

The seed virus shall be shown to be free from demonstrable adventitious viable microorganisms by appropriate tests according to the requirements of Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances and other Sets of Recommendations No. 6 (General Requirements for the Sterility of Biological Substances) (3, pp. 49–52).

The seed virus should be prepared in specific-pathogen-free eggs and tested for the presence of avian leukosis virus and
3.2 Production precautions

The general production precautions formulated in Part A:A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (6, page 15) shall apply to the manufacture of inactivated influenza vaccine, with the addition of the following.

Only allantoic and amniotic fluids shall be harvested.

Penicillin or streptomycin shall not be used at any stage of manufacture of the vaccine.

Minimal concentrations of other suitable antibiotics may be used. It should be kept in mind, however, that cross-allergic reactions sometimes occur between cephalosporin, neomycin, and the penicillins.

In the case of vaccines that involve the splitting of the virus by chemical means, the splitting and the concentration of chemicals shall be carried out by methods approved by the national control authority.

3.3 Inactivation of monovalent virus pools

3.3.1 Time of inactivation

Inactivation of monovalent virus pools shall be initiated as soon as possible after their preparation.

However, if storage before inactivation is unavoidable, they should be held at a temperature of 5°C ± 3°C for not longer than 5 days.

Before inactivation of monovalent virus pools, samples should be taken and tested for bacterial and fungal contamination. Such tests serve to indicate the reliability of the source of eggs.

3.3.2 Inactivation procedure

The virus in the monovalent virus pools shall be inactivated by a method that has been demonstrated to be consistently effective in the hands of the manufacturer and has been approved by the national control authority. The inactivation process shall also have been
shown, to the satisfaction of the national control authority, to be capable of inactivating avian leukosis viruses and mycoplasma. If the virus pool is stored after inactivation, it shall be held at a temperature of 5°C±3°C. The inactivation procedure should cause minimum alteration of the haemagglutinin and neuraminidase.

If formalin (40% formaldehyde) or beta-propiolactone is used, the concentration should not exceed 1:2000 at any time during inactivation.

3.4 Concentration and purification procedures

The monovalent material shall be concentrated and purified by high-speed centrifugation or other suitable method approved by the national control authority, either before or after the inactivation procedure.

The aim is to separate the virus from other constituents in the allantoic fluids with the maximum efficiency. It is advisable to concentrate and purify the virus under optimum conditions to preserve its antigenic properties.

In some countries, electron microscopy is used to check the purity of the virus suspension. Another test for purity consists in injecting vaccine into mice and testing their sera for antibodies against egg protein.

3.5 Control of monovalent bulk

3.5.1 Test for effective inactivation

The monovalent bulk shall be tested for the presence of viable influenza virus by inoculation of embryonated hens’ eggs, with a method approved by the national control authority.

A suitable method consists in inoculating 0.2 ml of undiluted monovalent bulk and 1:10 and 1:100 dilutions of the monovalent bulk into the allantoic cavities of fertile eggs (a group of 10 eggs in each case), and incubating at 33-37°C for 3 days. At least 8 of the 10 eggs should survive at each dosage level. Harvest 0.5 ml of the allantoic fluid from each surviving egg, pooling the fluid harvested from each group. Then 0.2 ml of the pooled fluid is inoculated, undiluted, into a further 3 groups each of 10 fertile eggs. Haemagglutinin activity should not be detected in these new groups of eggs.

In some countries, a survival proportion as high as 80% during incubation may not be achievable. The national control authority should decide in this case about the limits of acceptability.
3.5.2 Tests for the concentration of haemagglutinin antigen

The content of haemagglutinin in the monovalent bulk shall be determined by an immunodiffusion technique, such as single-radial-immunodiffusion or immunoelectrophoresis. The test shall be made in comparison with the WHO influenza reference haemagglutinin antigen or with a national preparation calibrated against it.

For certain types of vaccine, immunodiffusion techniques may not give reproducible results. In such cases, other known methods may be used.

3.5.3 Tests for the presence of neuraminidase

In some countries, a test is included for the presence of neuraminidase. The ratio of haemagglutinin to neuraminidase should be similar to that of the expected values for the virus strain and method of vaccine production used, neuraminidases of different strains varying markedly in their stability during processing.

3.6 Control of final bulk

3.6.1 Preservatives and other substances added

In preparing the final bulk, only preservatives or other substances, including diluents, approved by the national control authority shall be added. Such substances shall have been shown by appropriate tests not to impair the safety or effectiveness of the product in the concentrations used, and they shall not be added before samples have been taken for any tests that would be affected by the substances added.

If mercury-containing preservatives are used, the final bulk should be tested for mercury content.

3.6.2 Test for influenza virus

The final bulk shall be tested for the presence of viable virus by an approved method (see Part A, section 3.5.1).

3.6.3 Test for content of haemagglutinin antigen

The haemagglutinin concentration in the final bulk shall be determined as described in Part A, section 3.5.2, unless such a test is done on each filling lot.
In some countries, the concentrations of haemagglutinin contained in the vaccine, particularly with certain virus strains, are such that this test is difficult to include at this stage. It may be omitted on the advice of the national control authority and, in such cases, the measurement of haemagglutinin content is based on the test on the monovalent bulk (see Part A, section 3.5.2).

3.6.4 Test for identity of the virus antigens

The identity of the haemagglutinin antigen or of the haemagglutinins in the vaccine shall be determined by an immunological technique, such as immunodiffusion or haemagglutination inhibition, using the appropriate specific immune serum. For adsorbed vaccines, the presence of specific antibodies in sera from immunized animals may be used.

In some countries, a test to identify the specific neuraminidase antigen is also included.

3.6.5 Sterility tests

Each final bulk shall be tested for sterility according to the requirements given in Part A, section 5, of the revised Requirements for Biological Substances and other sets of Recommendations No. 6 (General Requirements for the Sterility of Biological Substances) (3, page 48).

3.6.6 Protein nitrogen content

The protein nitrogen content shall be determined, and the upper limit shall be no greater than that approved by the national control authority (see Part B, section 1).

The protein nitrogen content of the final bulk should be as low as possible in relation to the haemagglutinin content of the vaccine. Values of less than 25 μg of protein nitrogen per virus strain per single human dose are obtainable.

For the final bulk, the upper limit should be less than 50 μg of protein nitrogen per single human dose.

3.6.7 Test for endotoxin

In some countries, tests for endotoxins—the Limulus amoebocyte lysate test, the rabbit test, or the leukopenia test in
mice—are included. The endotoxin content is measured against a reference preparation, the permissible level of the endotoxin being determined by the national control authority.

3.6.8 Tests for chemicals used in production

The concentration of each detergent, organic solvent, inactivating agent, and preservative remaining in the final vaccine shall be determined by methods approved by the national control authority. These concentrations shall not exceed upper limits specified by the national control authority. For preservatives, the concentration shall not be less than a lower limit specified by the national control authority.

Tests for chemicals that are not used during preparation of the final bulk may also be performed on monovalent bulks.

4. FILLING AND CONTAINERS

The requirements concerning 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) (5, page 16) shall apply.

Single and multiple-dose containers may be used. If multiple-dose containers are used, a suitable preservative, approved by the national control authority, should be incorporated.

5. CONTROL TESTS ON FINAL PRODUCT

5.1 Identity test

An identity test shall be performed on at least one labelled container from each filling lot by a method approved by the national control authority.

5.2 Sterility tests

Each filling lot shall be tested for sterility according to the requirements given in Part A, section 5.2, of the revised Requirements for Biological Substances and other Sets of Recommendations No. 6 (General Requirements for the Sterility of Biological Substances) (3, page 49).
5.3 Test for content of virus antigen

A test shall be performed on each filling lot according to the requirements given in Part A, section 3.5.2, unless such a test has been performed on the final bulk (see Part A, section 3.6.3).

5.4. Inocuity tests

Each filling lot shall be tested for abnormal toxicity by appropriate tests by means of parenteral injections in mice and guinea-pigs. The test procedures shall be those approved by the national control authority.

5.5 Inspection of final containers

Each container in each filling lot shall be inspected, and those showing abnormalities shall be discarded.

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) (6, page 17) shall apply.

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) (6, page 18) shall apply.

8. LABELLING

The requirements given in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (6, page 18) shall apply, with the addition of the following.

If the product is issued in a multiple-dose container, the label on the container shall state the number of doses.
The label on the carton enclosing one or more final containers or the leaflet accompanying the container shall include the following additional information.

- A statement that the virus was propagated in hens' eggs;
- the method used for inactivating the virus;
- the name and maximum quantity of any antibiotic present in the vaccine;
- the strains included in the vaccine (for the nomenclature to be used for designating strains, see 7);
- the quantity of haemagglutinin per dose for each strain expressed as micrograms of haemagglutinin per dose; and
- the name and concentration of the preservative, if added.

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) (6, page 18) shall apply.

10. STORAGE AND EXPIRY DATE

The requirements given in Part A, section 10, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (6, page 19) shall apply.

10.1 Storage conditions

Inactivated influenza vaccine shall be stored at a temperature of 5°C±3°C.

10.2 Expiry date

The expiry date shall be fixed with the approval of the national control authority.

The expiry date should be not more than two years after the last satisfactory test for virus antigen content (see Part A, sections 3.6.3 and 5.3), provided that the vaccine has been stored continuously at a temperature of 5°C±3°C. It is desirable that the expiry date should not exceed one year from the date of issue by the manufacturer.
PART B. NATIONAL CONTROL REQUIREMENTS

1. GENERAL

The general requirements for control laboratories, contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (6, page 19), shall apply.

The national control authority shall give directions to manufacturers concerning the influenza virus strains to be used, the virus antigen content (haemagglutinin), and the recommended human dose.

2. RELEASE AND CERTIFICATION

A vaccine lot shall be released only if it fulfils Part A of these requirements.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishments and shall certify that the lot of vaccine in question meets all national requirements as well as Part A of these requirements. Furthermore, this certificate shall state the date of the last satisfactory determination of content of virus antigen, the lot number, the number under which the lot was released, and the number appearing on the labels of containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of inactivated influenza vaccine between countries.

3. CLINICAL EVALUATION OF INFLUENZA VACCINES

In the case of a new manufacturer or of a significant change in the manufacturing process, the national control authority shall ascertain that the vaccine is safe and effective by arranging for studies in man of some of the lots of vaccine that have satisfied the above-mentioned requirements. Such studies shall include the assessment of the immune responses and reactions in various age groups. Studies to demonstrate the safety and efficacy of the vaccine shall be carried out and evaluated before approval for release of any lots of the vaccine for general use.
SUMMARY PROTOCOLS FOR INFLUENZA VACCINE (INACTIVATED)

Primary/Secondary Seed Lot Type A

Name and address of manufacturer
Laboratory reference No. of lot
Date when the processing was completed

Information on manufacture

1. Virus used to inoculate eggs for the manufacture of the lot
   (a) strain and substrain
   (b) passage level
   (c) source and reference No.
   (d) remarks

2. Results of sterility tests

3. Conditions of storage

Primary/Secondary Seed Lot Type B

Name and address of manufacturer
Laboratory reference No. of lot
Date when the processing was completed

Information on manufacture

1. Virus used to inoculate eggs for the manufacture of the lot
   (a) strain and substrain
   (b) passage level
   (c) source and reference No.
   (d) remarks

2. Results of sterility tests
3. Conditions of storage

**Monovalent Virus Pool Type A**

Name and address of manufacturer

Laboratory reference number of virus pool

1. Virus used to inoculate eggs*
   (a) primary seed strain and source
   (b) passage level of primary seed
   (c) secondary seed lot, reference No., and source

2. Date of inoculation

3. Date of harvesting allantoic fluids

4. Conditions of storage before inactivation

5. Date of inactivation

6. Method of inactivation

7. Concentration of inactivating agent

8. Conditions of storage after inactivation

9. Concentration/purification procedure

10. Antibiotics used during preparation, if any

**Tests on monovalent bulk**

1. Test for absence of viable influenza virus
   No. of eggs inoculated

* If there are more than four virus pools in the monovalent bulk, the relevant data should be given on a separate sheet.
<table>
<thead>
<tr>
<th>Incubation time and temperature</th>
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<tbody>
<tr>
<td>Results</td>
<td></td>
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2. Test for presence of neuraminidase (if performed)

<table>
<thead>
<tr>
<th>Method</th>
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<tbody>
<tr>
<td>Results</td>
<td></td>
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**Monovalent Virus Pool Type B**

Name and address of manufacturer |          |
Laboratory reference number of virus pool |          |

1. Virus used to inoculate eggs
   (a) primary seed strain and source |          |
   (b) passage level of primary seed |          |
   (c) secondary seed lot, reference No., and source |          |

2. Date of inoculation |          |
3. Date of harvesting allantoic fluids |          |
4. Conditions of storage before inactivation |          |
5. Date of inactivation |          |
6. Method of inactivation |          |

7. Concentration of inactivating agent |          |
8. Conditions of storage after inactivation |          |
9. Concentration/purification procedure |          |

10. Antibiotics used during preparation, if any |          |
Tests on monovalent bulk

1. Test for absence of viable influenza virus
   No. of eggs inoculated
   Incubation time and temperature
   Results

2. Test for presence of neuraminidase (if performed)
   Method
   Results

Final Bulk

1. Name and address of manufacturer
2. Identification of final bulk
3. Identification of monovalent bulk vaccines used to prepare final bulk
4. Date of manufacture

Control of final bulk

1. Preservative(s) added and concentration
2. Any other substances added and concentration
3. Test for viable virus
   No. of eggs inoculated
   Dilution and dose per egg
   Incubation time and temperature
   Results
4. Test for content of haemagglutinin antigen
   Method
   Results

5. Test for identity of virus antigens
   Method
   Results
   (Result of test for specific neuraminidase, if performed)

6. Test for sterility
   Results

7. Protein nitrogen content
   ug per single human dose

8. Test for endotoxin
   Method
   Results

9. Tests for chemicals used
   Results

Final Product

1. Identity tests
   Method
   Results
2. Sterility tests
   Results

3. Test for virus antigen content (unless done on final bulk)
   Method
   Results

4. Innocuity tests
   No. and species of animals
   Doses injected
   Period of observation
   Results

5. Inspection of final container
   Results

6. Other tests

7. Remarks

Signature of head of laboratory

Certification by person taking overall responsibility for production of the vaccine:
I certify that lot No. ______ of vaccine satisfies Part A of the WHO Requirements for Inactivated Influenza Vaccine (Inactivated).
   Signature
   Name typed

The protocol must be accompanied by a sample of the label and a copy of the leaflet.
REQUIREMENTS FOR INFLUENZA VACCINE (LIVE)
(Requirements for Biological Substances No. 28)

INTRODUCTION

The possibility of controlling epidemic influenza by the use of live attenuated vaccine given by the intranasal route has been investigated. For some years, the principle has had a sound basis, having been tested in infectious diseases, notably poliomyelitis, in which an attenuated virus given by the natural route of infection has been shown to give protection without disease. By analogy, it should be possible to initiate a benign infection by influenza virus with an attenuated strain that would give protection against the prevalent infectious wild strain. This approach has been tried on a number of occasions with mixed success. Some trials showed great promise, whereas others gave results indicating potential dangers that might arise from the use of these products because of insufficient knowledge of the fundamental principles involved.

The aim is to produce from a wild strain an attenuated virus that will be stable and not cause influenza when given to susceptible individuals on a wide scale. Thanks to modern technology, there are now tests that can answer many of the questions unresolved for decades.

An attenuated influenza vaccine made from a live attenuated strain of virus is now used in so many countries that it is appropriate to formulate WHO Requirements for such a vaccine.

The international requirements for influenza vaccine (live) have been fitted into the framework adopted in the Requirements for Biological Substances No. 1-27, already published by WHO (4, page 142). A group of WHO consultants and staff members (listed on page 192) drafted them, taking into account the regulations and requirements for the manufacture and control of influenza vaccine (live) in a number of countries, as well as information from published and unpublished reports. In addition, opinions and data have been received from a number of experts, whose assistance is acknowledged (see page 192).

GENERAL CONSIDERATIONS

There are many potential problems related to the development of a safe and effective live influenza vaccine. It is important, therefore,
to characterize any candidate vaccine in carefully designed laboratory and clinical studies before its widespread use is considered. The studies should be designed so as to confirm that the vaccine virus is attenuated, is not transmitted to susceptible contacts, is genetically stable, is immunogenic, and has suitable laboratory markers of attenuation. In addition, the appropriate nature of the vaccine strain will need to be reviewed continuously to ensure the development of vaccines that contain the currently prevalent antigens and have been demonstrated to be safe and effective. Therefore, the development and control of live influenza vaccines require a long-term commitment to laboratory and clinical studies, and it is recommended that only countries with adequate resources for these studies should consider the manufacture and control of these products.

The development of a suitable vaccine comprises a number of essential stages:

1. Primary investigations into the attenuation are carried out in the laboratory on animals (ferrets). The results, compared with those of inoculating wild virus, make it possible to decide whether limited trials in human beings are acceptable. There are also a number of tests in vitro (genotypical characterization by RNA-RNA hybridization, culture of tracheal epithelium) that may provide indications on attenuation. The results of these tests should confirm those of animal tests.

2. Limited trials in human beings, performed in an environment offering the maximum guarantee of isolation and discipline. These trials are to make sure that the virus is not excreted at too high a titre and too long, that the virus is not transmitted, that the reactions are acceptable, and that a sufficient antibody level is achieved.

3. Human trials on a larger scale, with subjects in good health and of different ages, if the previous trials were satisfactory. These trials serve to specify the necessary dose and to prove the innocuity of the vaccine for different age groups and the absence of transmission. A placebo group is essential to evaluate the incidence and intensity of reactions. Pulmonary function should be evaluated in vaccinated subjects.

4. Innocuity tests in the population at high risk, for which the vaccine is specially intended (subjects with chronic bronchitis and asthma).

In developing a new vaccine strain, methods of attenuation that have been shown to produce safe and immunogenic vaccine should
be used. Over the last few years several new methods of attenuation have been developed, involving host-range infectivity, temperature sensitivity, or cold-adapted mutant strains. Candidate viruses may be derived by the selection from wild virus populations of mutants possessing suitable characteristics or by the genetic transfer of the characteristics from standard parent attenuated strains to wild strains by recombination (gene reassortment). Techniques of gene analysis of candidate viruses should be employed for their selection and laboratory characterization. Genetic stability is an extremely important characteristic for the selection of vaccine strains and should be determined in order to ensure that reversion to virulence does not occur during passage in man.

Recombination between attenuated parent strains and the new wild virus followed by careful selection has been shown to be an effective and rapid method of producing attenuated strains with the surface antigens of the wild parent. Vaccine strains should be adequately cloned by multiple terminal dilution passages in specific pathogen-free eggs or by other acceptable methods.

The availability of reliable markers of attenuation is important. It may be desirable to select vaccine strains that, in addition to markers correlating with human virulence, also possess other stable markers that do not co-vary with virulence for man and, therefore, might be used to identify vaccine strains in epidemiological surveillance.

Wide-scale use of live influenza vaccines in large field trials or in routine immunization has demonstrated that satisfactory products can be obtained. However, mild upper respiratory reactions in some persons and occasional benign systemic reactions have been observed. The vaccines are usually given intranasally and the virus replicates in the nasopharyngeal mucosa. The virus is shed by some vaccinated persons, but excretion is usually less in quantity and shorter in duration than is the case during infections caused by virulent influenza viruses. The possibility of virus transmission to susceptible contacts, therefore, is much smaller than it is for wild virus.

Influenza virus is unique from the viewpoint of antigenic change, although live vaccines appear to confer a degree of cross-protection against related strains within a common haemagglutinin. The use of a vaccine strain antigenically identical to the naturally prevalent virus strain is expected to provide optimum protective efficacy. Since the interval between the appearance of a new influenza virus variant and its spread throughout the world may be a matter of months, the development of an appropriate vaccine strain must be rapid.
The testing of the lots of experimental vaccine that may be established as the seed from which vaccine is prepared should take into account the limited period in which a new strain has to be developed. Therefore, some tests included for routine production lots may have to be amended for these experimental vaccine lots. The subsequent testing in man should also be designed to allow the most informative evaluation of the safety and immunogenicity of the strain in the shortest possible time. Vaccine strains should be evaluated in clinical studies including representative population groups for which vaccine may be recommended, e.g., children and high-risk adults.

Where appropriate, the technology and experience acquired over the last two decades in the production and control of all live virus vaccines should be applied to live influenza vaccines. As for all live vaccines, the substrate on which the virus is propagated is important. Since influenza virus is usually produced in embryonated hens' eggs, the eggs should be obtained from closed flocks that are continuously monitored for known pathogenic agents. These flocks are now available in many countries, and the eggs — or cell cultures derived from them — have been widely used in the production of measles vaccine and other vaccines produced in avian substrates. Furthermore, stability after lyophilization is comparable to that of other live virus vaccines.

In addition to the many technical problems associated with the rapid development of suitable attenuated vaccine strains, consideration should be given also to the need for special administrative arrangements for licensing, which involve the submission of data and their review by national control authorities. The usual periods of time required for licensing procedures will need to be shortened considerably if appropriate live vaccines are to be made available when needed soon after the emergence of a new variant. Close collaboration between manufacturers and the national control authorities is required, particularly during the phases of development, production, and testing of the initial batches of live vaccines.

In emergencies, such as an influenza pandemic, the abnormal demands for vaccine may create difficulties if all the tests in these requirements are to be carried out. No attempt has been made to indicate in what respect these requirements may be modified, since such decisions are the responsibility of national control authorities.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in type of normal size have been written in the form of requirements so that, if a health administration
so desires, these parts as they appear may be used as definitive national requirements. The parts of each section that are printed in small type are comments and recommendations for guidance. In order to facilitate the international distribution of vaccine made in accordance with these requirements, a summary protocol for the recording of the results of the tests is included (see pages 187-192).

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning live influenza vaccine, it is recommended that a clause should be included permitting modifications of the manufacturing requirements on condition that it be demonstrated, to the satisfaction of the national control authority, that such modified requirements ensure that the degree of safety and the potency of the vaccine are at least equal to those provided by the requirements formulated below. The World Health Organization should then be informed of the action taken.

The terms "national control authority" and "national control laboratory", as used in these requirements, always refer to the country in which the vaccine is manufactured.

PART A. MANUFACTURING REQUIREMENTS

1. DEFINITION

1.1 International name and proper name

The international name shall be "Vaccinum Influenzae Vivum". The proper name shall be the equivalent of the international name in the language of the country of origin.

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

1.2 Descriptive definition

Vaccinum Influenzae Vivum is a preparation of live attenuated influenza virus grown in suitable embryonated hens' eggs.

1.3 International standards and international reference preparations

Since no international reference preparations of live attenuated influenza virus are available, no requirements based
on comparisons with such preparations can be formulated at present.
National control authorities should provide a reference preparation of live attenuated influenza virus for use in tests of virus infectivity (see Part A, sections 3.5.3 and 5.3).

1.4 Terminology

Vaccine strain. An attenuated influenza virus that has been shown to be immunogenic and safe during clinical trials in human beings.

Seed lot. A quantity of virus derived from the vaccine strain processed together, fully characterized, and of uniform composition. Seed lots are not more than two passages removed from the original vaccine strain.

Single harvest. A quantity of virus suspension harvested from eggs from the same source that were inoculated with the same virus strain and incubated and harvested together or in successive batches.

Bulk suspension. Material prepared from one or more single harvests and before filling into final containers.

Final bulk. The finished preparation present in the container from which the final containers are filled. The final bulk may be prepared from one clarified bulk suspension, or from a blend of clarified bulk suspensions, or from a dilution thereof.

Filling lot (final lot). A collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling and drying. A filling lot must, therefore, have been filled in one working session and have been dried together.

Egg infectivity dose 50% (EID₅₀). The quantity of a virus suspension that will infect 50% of embryonated eggs.

2. GENERAL MANUFACTURING REQUIREMENTS

The general manufacturing requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (5, pp. 11–22) shall apply to establishments manufacturing live influenza vaccine, with the addition of the following.

Production areas shall be decontaminated before they are used for the manufacture of live influenza vaccine.

The production of live influenza vaccine shall be conducted by staff who have not handled other infectious microorganisms or
animals on the same working day. The staff shall consist of persons who shall have been examined medically and found to be healthy. Staff with respiratory infections shall be excluded.

Only eggs obtained from specific pathogen-free flocks approved by the national control authority for the production of live influenza vaccine shall be introduced into or handled in the production area.

Visitors and persons not directly concerned with the production process shall not be permitted to enter the production area.

Particular attention shall be paid to the recommendations given in Part A, section 1, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (6, page 13) regarding the training and experience of the persons in charge of production and testing and of those assigned to various areas of responsibility in the manufacturing establishment, as well as to the registration of such personnel with the national control authority.

3. PRODUCTION CONTROL

The general production precautions 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) (6, page 15) shall apply to the manufacture of live influenza vaccine.

3.1 Control of source materials

3.1.1 Virus strains

The strain of influenza virus used in the production of live influenza vaccine shall be identified by historical records, which shall include information on the origin of the strain and on the method used to attenuate it. The strain shall be shown to be immunogenic and safe, and only strains that are approved by the national control authority shall be used.

3.1.2 Substrate for virus propagation

Influenza virus used in the production of live influenza vaccine shall be propagated in specific pathogen-free eggs approved by the
national control authority. All information on the flock used as the source of eggs shall be available to the national control authority.

The eggs shall be derived from closed flocks continuously monitored for the absence of avian encephalomyelitis, avian adenovirus, avian reticuloendotheliosis virus, Newcastle disease, fowlpox, avian infectious bronchitis, infectious bursal agent, laryngotracheitis, influenza type A infection, leukemia virus, Marek disease herpetovirus, avian reovirus, *Mycoplasma* species—e.g., *M. gallisepticum* and *M. synoviae*, *Salmonella* species—e.g., *S. pullorum*, EDS67, and *Mycobacterium tuberculosis* (of avian origin). The flock shall be free from these infectious agents.

3.1.3 *Seed lot system*

The production of vaccine shall be based on a seed lot system.

Each seed lot shall be identified as containing the appropriate virus. If not lyophilized, the seed lot shall be stored at a temperature lower than \(-60^\circ\text{C}\); if lyophilized, the storage temperature shall be lower than \(-20^\circ\text{C}\).

All single harvests shall contain influenza virus derived only from eggs inoculated with the same seed lot. The virus in the final vaccine shall be not more than three passages removed from the vaccine strain used in the preparation of a vaccine shown to be immunogenic and safe in man.

3.1.4 *Tests on seed lots*

The seed lot used for the production of vaccine shall be free from extraneous agents. The eggs used shall conform to the tests specified in Part A, section 3.2. The seed lot shall be produced on the substrate specified in Part A, section 3.1.2, and shall be controlled by means of the tests mentioned in Part A, section 3.4.

If the vaccine strain has been passaged in substrates other than specific pathogen-free eggs, the national control authority should specify the additional tests for the detection of the adventitious agents that may have been present in the substrate used.

3.2 *Tests on control eggs*

Of each batch of eggs used for propagation of the virus 2% (or at least 20) shall be held as uninoculated controls and incubated for the
same time and at the same temperature as the inoculated eggs. At the
time of harvesting the virus from the inoculated eggs, allantoic fluids
shall be taken from the unoinoculated eggs and examined for haemag-
glutinating agents (see Part A, section 3.2.1).

To ensure freedom from other avian infectious agents, the
greatest reliance is placed on the continuous monitoring of the
flock from which the eggs are obtained. However, national
control authorities should include additional tests for micro-
organisms if necessary. Tests for avian leukosis virus and inoc-
ulation of the control fluids on chorioallantoic membranes are
essential.

3.2.1 Tests for haemagglutinating agents

A sample of 0.25 ml of allantoic fluid taken from each egg shall
be tested by the addition of chick erythrocytes for haemagglutinating
agents both directly and after one passage through specific pathogen-
free eggs. The details of the test shall be approved by the national
control authority.

3.3 Harvesting

Only the allantoic fluids from the inoculated eggs shall be
harvested. No penicillin or streptomycin shall be present in the
harvested fluids or added at the time of harvesting or subse-
quently.

If antibiotics are used, it should be kept in mind that allergic
cross-reactions sometimes occur between cephalosporin,
neomycin, and the penicillins.

It is a wise precaution to pool the allantoic fluids from a
limited number of eggs (e.g., 30-50) and to test these small pools
for sterility and virus titre before blending into a single harvest.
Only small pools that are free from bacteria and fungi should be
blended into the harvest.

The pools should be stored at a temperature of 5°C-3°C.
Small quantities of other antibiotics may be added, with the
approval of the national control authority. However, if anti-
biotics are added, samples for sterility testing should be taken
before the antibiotic is added, and all small pools shown to be
contaminated should be discarded.

3.4 Tests on single harvest

At the time of pooling the allantoic fluids and before clarification,
samples shall be set aside for examination for extraneous agents. If
the samples are not tested immediately, they shall be stored at 
-60°C or below.

For the purposes of the tests required on neutralized virus 
harvests, the hyperimmune serum shall be of non-avian, non-simian, 
non-human origin. The virus used for the production of the hyper-
immune serum shall be grown either in non-avian cell cultures or in 
specific-pathogen-free eggs. If eggs are used, they shall be obtained 
from a flock different from that used to supply the production 
eggs.

The virus pool passes the test if there is no evidence of the 
presence of any adventitious agents attributable to the virus pool 
when tested according to the requirements of this section and its 
subsections.

3.4.1 Tests in animals

After neutralization of the influenza virus by the hyperimmune 
serum, the virus pool sample shall be inoculated into animals as 
described in sections 3.4.1.1 and 3.4.1.2 below.

3.4.1.1 Tests in adult mice. A total volume of at least 10 ml of the 
virus pool shall be tested by inoculating 0.5 ml of the virus-serum 
mixture into each of 20 or more adult mice by the intraperitoneal 
route. A total volume of at least 0.6 ml of the virus shall be tested in 
20 or more mice by inoculating 0.03 ml into each mouse by the intra-
cerebral route. The same mice may be used for both routes of inocu-
lation.

The mice shall be observed for at least 21 days. All mice that die 
after the first 24 h of the test, or that have been sacrificed after 
showing signs of illness, shall be autopsied and examined for 
evidence of viral infection, by macroscopic observation and by 
subinoculation of appropriate tissue suspensions by the intracerebral 
and intraperitoneal routes into at least 5 additional mice, which shall 
be observed for 21 days.

The virus pool shall be considered to have passed the test if at 
least 80% of the original inoculated mice survive the observation 
period and if no mice show evidence of infection with adventitious 
transmissible agents attributable to the virus pool.

3.4.1.2 Tests in suckling mice. A total volume of at least 2 ml of 
virus shall be tested by inoculating each of 20 or more suckling mice
by the intraperitoneal route with 0.1 ml of the virus/serum mixtures.
A total volume of at least 0.1 ml of virus shall be tested in 10 or more
newborn mice by inoculating 0.01 ml into each mouse by the intra-
cerebral route.

The mice shall be observed daily for at least 21 days. All mice that
die after the first 24 hrs. of the test or that have been sacrificed after
showing signs of illness shall be autopsied and examined to
determine the cause of death or illness.

In some countries a subinoculation is appropriate, and in
others an additional blind passage is made of a suspension of the
pooled emulsified tissue (minus skin and visera) of all mice
surviving the original 21-day test.

The virus pool shall be considered to have passed the test if at
least 80% of the original inoculated mice survive the observation
period and if no mice show evidence of infection with adventitious
transmissible agents attributable to the virus pool.

3.4.2 Tests in cell cultures

After neutralization of the influenza virus by the hyperimmune
serum, the virus pool sample shall be tested in cell cultures as
described below (sections 3.4.2.1-3.4.2.3).

3.4.2.1 Tests in human cells. A sample of at least 5 ml of the virus
pool after neutralization shall be inoculated on cell cultures of human
origin. The cell cultures and the period of observation shall be
approved by the national control authority. None of the cell cultures
shall show evidence of the presence of any adventitious agent.

3.4.2.2 Tests in simian cells. A sample of at least 5 ml of the virus
pool after neutralization shall be inoculated on simian cell cultures.
The cell cultures and the period of observation shall be approved by
the national control authority. None of the cell cultures shall show
evidence of the presence of any adventitious agent.

3.4.2.3 Test in chicken cells. A sample of at least 5 ml of the virus
pool after neutralization shall be inoculated on chicken cell cultures.
The cell cultures and the period of observation shall be approved by
the national control authority. None of the cell cultures shall show
evidence of the presence of any adventitious agent. The absence of
avian leukemia viruses shall be ascertained by testing. The chicken cell
cultures may be used also for this purpose.

3.4.3 Tests in embryonated eggs

A sample of at least 10 ml of each single harvest after neutraliza-
tion shall be tested in a group of embryonated hens' eggs by the
allantoic route of inoculation, and a similar sample in a separate
group of eggs by the yolk sac route of inoculation, using at least
0.25 ml of the virus/serum mixture per egg for each route of inocu-
lation. The incubation of the eggs and the observation time shall be
approved by the national control authority.

The virus pool passes the test if there is no evidence of the
presence of any adventitious agents attributable to the virus pool.

3.4.4 Tests for bacteria, fungi, and mycoplasma

The single harvest shall be tested for the presence of bacteria,
fungi, and mycoplasmas according to the requirements given in
Part A, section 5, of the revised Requirements for Biological
Substances and other sets of Recommendations (General
Requirements for the Sterility of Biological Substances) (3, page 48).

3.4.4.1 Test for mycobacteria. Each single harvest shall be tested for
the presence of mycobacteria by culture methods appropriate for the
detection of the organisms most likely to be found in the eggs used.

It is common practice to concentrate the virus harvest by
centrifugation and to inoculate the pellet into guinea-pigs or on
to solid media shown to be suitable for the detection of myco-
bacteria.

3.5 Control of bulk suspension after clarification

3.5.1 Clarification of bulk suspension

The bulk suspension shall be clarified by a method that will
remove intact tissue cells.

3.5.2 Sampling

Samples of the clarified bulk suspension shall be taken
immediately after clarification. If not tested immediately, the samples
shall be kept at a temperature below -60°C until the test for virus titration (see section 3.5.3 below) is made.

3.5.3 Virus titration

The influenza virus content in the clarified bulk suspension shall be determined by titrations in eggs, using as a control a reference preparation of influenza virus of known infectivity (see Part B, section 1).

3.6 The final bulk

The operations necessary for preparing the final bulk shall be conducted in such a manner as to avoid contamination of the product.

The dilution and mixing procedures involved in preparing the final bulk should be those approved by the national control authority.

3.6.1 Added substances

Any substance that is added to the product in preparing the final bulk shall have been shown, to the satisfaction of the national control authority, not to impair the safety and efficacy of the vaccine in the concentration used.

4. FILLING AND CONTAINERS

The requirements concerning 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) (6, page 16) shall apply.

Care should be taken to ensure that the material of which the container is made does not adversely affect the virus content of the vaccine under the recommended conditions of storage.

5. CONTROL TESTS ON FINAL PRODUCT

Samples shall be taken from each filling lot for the tests mentioned in this section and its subsections.
5.1 Identity test

The virus in the final containers shall be identified by appropriate immunological methods to identify the haemagglutinin, neuraminidase, and any specific marker of attenuation of the seed virus.

The specific marker tests may also be performed on the final bulk instead of on the final product.

5.2 Tests for bacteria and fungi

Reconstituted vaccine shall be tested for bacterial and mycotic sterility according to the requirements given in Part A, section 5, of the Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances) (A, page 48).

5.3 Virus titration

The virus content of each of at least three ampoules selected at random from each drying lot shall be determined individually. The details of the test shall be approved by the national control authority.

This determination shall be made in terms of EID₉₀ in parallel with the determination of the virus concentration of a reference preparation of known virus titre (see Part B, section 1). The determination of the EID₉₀ per dose shall be based on at least five inoculated eggs per dilution, using tenfold dilutions.

The number of EID₉₀ per human dose of vaccine shall be determined. The requirements for virus content per human dose shall be based on clinical trials in man.

5.4 Innocuity tests

The innocuity of the product shall be tested by appropriate tests in mice and guinea-pigs by means of parenteral injections. The tests shall be those approved by the national control authority.

5.5 Residual moisture

The residual moisture in a representative sample of each freeze-dried lot shall be determined by a method approved by the national control authority.
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) (6, page 17) shall apply.

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) (6, page 18) shall apply.

8. LABELLING

The requirements given in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (6, page 18) shall apply, with the addition of the following.

The label on the container shall include the following information:

— the name(s) of the strain(s) of influenza virus (7) contained in the vaccine.

The leaflet accompanying the package shall include the following information:

— the name(s) of the strain(s) of influenza virus (7) contained in the vaccine;
— the nature and amount of any preservative or stabilizer present in the vaccine;
— the amount of virus contained in one recommended human dose for adults and children;
— the name and maximum quantity of any antibiotic present in the vaccine;
— a statement that after the vaccine has been reconstituted it should be used without delay, or, if not used immediately, stored between 2 °C and 8 °C for a period not exceeding 8 hrs; and
— a statement that the vaccine has been prepared in hens’ eggs.
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) (6, page 18) shall apply.

10. STORAGE AND EXPIRY DATE

The requirements given in Part A, section 10 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (6, page 19) shall apply.

10.1 Storage conditions and stability

After labelling, packaging, and distribution or issue, the vaccine shall be stored at a temperature not exceeding 8°C. The vaccines shall have been shown to maintain the virus content of the human dose for a period equal to that between the date of issue and the expiry date. The manufacturers shall have shown by accelerated degradation tests that the vaccine in the freeze-dried form is stable.

10.2 Expiry date

The expiry date shall be fixed with the approval of the national control authority and shall relate to the date of the last satisfactory test for virus concentration, the date of this test being that on which the test system was inoculated. The expiry date shall be not more than one year after the date of the last satisfactory test for virus concentration.

If experience shows that the vaccine remains stable longer, the national control authority may extend the expiry date.

PART B. NATIONAL CONTROL REQUIREMENTS

1. GENERAL

The general requirements for control laboratories, contained in Part B of the revised Requirements for Biological Substances No. 1
(General Requirements for Manufacturing Establishments and Control Laboratories) (6, page 19), shall apply.

The national control authority shall give directions to manufacturers concerning the influenza virus strains to be used for vaccine production.

The national control authority should take into consideration all information available on strains before deciding on those permitted for vaccine production.

In addition, the national control authority shall provide a reference preparation of live influenza virus to be used for checking the normal susceptibility of the titration system, and shall specify the requirements for virus content to be fulfilled in order to achieve adequate immunization of human beings with the recommended human dose.

2. RELEASE AND CERTIFICATION

A vaccine shall be released only if it fulfils Part A of the present requirements.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify that the lot of vaccine in question meets all national requirements as well as Part A of the present requirements. The certificate shall state the date of the last satisfactory test for virus concentration, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of live influenza vaccine between countries.

SUMMARY PROTOCOL FOR INFLUENZA VACCINE (LIVE)

Based on the Requirements for Influenza Vaccine (Live)
(Requirements for Biological Substances No. 29)

Name and address of manufacturer

Lot No. of vaccine
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<td>No. of eggs used for control</td>
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<td>Date</td>
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<tr>
<td>Test for haemagglutinating agents</td>
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<td>Result after one passage</td>
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<td><strong>Single harvests</strong></td>
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</tbody>
</table>
Test in embryonated eggs

Volume tested
No. of eggs inoculated by the allantoic route
No. of inoculated yolk sacs
Results

Sterility test
Results

Test for mycobacteria
Method
Results

Bulk Suspension

Method of clarification
Date of preparation
Storage conditions of samples
Virus content
Date of test
Results of egg titration

Final Bulk

Date of preparation
Substances added

Final product

Identity test
Date
Method
Results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemagglutinin</td>
<td></td>
</tr>
<tr>
<td>Neuraminidase</td>
<td></td>
</tr>
<tr>
<td>Markers of attenuation</td>
<td></td>
</tr>
</tbody>
</table>

Sterility test

Date

Results

Virus content

Date

No. of ampoules tested

Type A, EID<sub>50</sub>/ml found in each ampoule

Type B, EID<sub>50</sub>/ml found in each ampoule

Inocuity tests

Date

No. of mice given injections

Volume and route

Observation period

Results

No. of guinea-pigs

Volume and route

Observation period

Results

Residual moisture

Size of sample

Moisture content (%)

Signature of head of laboratory
Certification by person taking overall responsibility for production of the vaccine:
I certify that lot No. ______ of vaccine satisfies Part A of the WHO Requirements for Influenza Vaccine (Live).

Signature

Name typed

The protocol must be accompanied by a sample of the label and a copy of the leaflet.

AUTHORS

The Revised Requirements for Influenza Vaccine (Inactivated) and the Requirements for Influenza Vaccine (Live) were prepared by the following WHO consultants and staff members:
Dr F. Ennis, Division of Virology, Bureau of Biologics, Bethesda, MD, USA (Consultant)
Dr Ljerka Higy-Mandić, Biologicals, World Health Organization, Geneva, Switzerland
Dr C. Huygelen, Director of Research, RIT, Rixensart, Belgium (Consultant)
Dr F. T. Perkins, Chief, Biologicals, World Health Organization, Geneva, Switzerland
Dr J. D. van Ramshorst, Biologicals, World Health Organization, Geneva, Switzerland
Dr G. Schilt, Head, Division of Viral Products, National Institute for Biological Standards and Control, London, England (Consultant)
Dr C. Smith, Evans Biologicals, Liverpool, England (Consultant)
Dr R. Weisz-Maleček, Institute of Immunology, Zagreb, Yugoslavia (Consultant)

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Dr S. C. Arya, Deputy Director, National Institute of Communicable Diseases, Delhi, India
Dr M. Balasubramanyan, Central Research Institute, Kasauli, India
Dr V. F. Davey, Technical Director, Commonwealth Serum Laboratories, Parkville, Victoria, Australia

— 192 —
Mr J. Davidson, Head, Biological Products and Standards Department, Central Veterinary Laboratory, New Haw, Weybridge, England
Professor S. G. Dzagurov, Director, Tarasevič State Institute for the Standardization and Control of Medical Biological Preparations, Moscow, USSR
Dr F. Fenyes, Paul-Ehrlich Institute, Frankfurt am Main, Federal Republic of Germany
Dr S. S. Gandhi, National Biological Standards Laboratory, Parkville, Australia
Dr Ch. B. Gerichter, Director, Division of Laboratories, Ministry of Health, Jerusalem, Israel
Dr Graschkau, Behring Company, Marburg an der Lahn, Federal Republic of Germany
Dr C. Guthrie, Production Director, Commonwealth Serum Laboratories, Parkville, Victoria, Australia
Dr J. W. F. Hampton, National Biological Standards Laboratory, Parkville, Victoria, Australia
Dr M. R. Hilleman, Director, Virus and Cell Biology Research, Merck Institute for Therapeutic Research, Merck, Sharp & Dohme, West Point, PA, USA
Dr C. Huygelen, General Manager, Biological Division, RIT, Genval, Belgium
Professor L. Jännes, Chief, Production Laboratory, Central Public Health Laboratory, Helsinki, Finland
Dr M. Jegathesan, Division of Bacteriology, Institute for Medical Research, Kuala Lumpur, Malaysia
Professor W. Kurylewicz, Director, National Institute of Hygiene, Warsaw, Poland
Dr H. P. Lansberg, National Institute of Public Health, Bilthoven, Netherlands
Dr P. E. Lemoine, Chief, Vaccine Control Section, Institute of Hygiene and Epidemiology, Brussels, Belgium
Dr C. Lerche, Director, National Institute of Public Health, Oslo, Norway
Dr H. M. Meyer, Jr, Director, Bureau of Biologics, Food and Drug Administration, Bethesda, MD, USA
Dr R. Nettet, Director-General, National Health Laboratory, Paris, France
Dr F. Oberdoenster, State Central Institute for Seru and Vaccines, Berlin, German Democratic Republic
Dr F. Pacchiarini, Director General, Higher Institute of Health, Rome, Italy
Dr C. H. Smith, Glaxo Operation UK Ltd, Speke, Liverpool, England
Dr J. W. G. Smith, Director, National Institute for Biological Standards and Control, London, England
Dr J. Spanander, Director-General, National Institute of Public Health, Bilthoven, Netherlands
Mr J. R. Thayer, Chief, Inspector, National Biological Standards Laboratory, Canberra, Australia
Dr D. H. Thornton, Central Veterinary Laboratory, New Haw, Weybridge, England
Dr W. Aeg. Timmerman, De Bilt, Netherlands
Dr J. Withell, Chief, Viral Products Branch, National Biological Standards Laboratory, Parkville, Victoria, Australia

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REFERENCES