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Annex 1
Requirements for varicella vaccine (live)
(Requirements for Biological Substances No. 36, revised 1993)

Introduction

Varicella, also known as chickenpox, is an acute and highly contagious disease of childhood caused by human (alpha) herpesvirus 3 (varicella-zoster virus). It is of widespread distribution, occurs in epidemic waves and affects most people by 20 years of age. The disease is characterized by a vesicular and papular rash that may be accompanied by fever, malaise, aches and arthralgia, and is usually more severe in adults than in children. Recovery from primary infection is commonly followed by the establishment of latent infection. Reactivation of latent virus may result in herpes zoster (shingles), in particular in patients with cancer, those receiving immunosuppressive chemotherapy and the elderly. Zoster is characterized by vesicular rash along the cutaneous distribution of the involved nerves, and by neuralgia with localized inflammation of the nerves and ganglia.

Because of associated complications, varicella virus infections may be of greater public health significance than is generally recognized. Varicella may be accompanied by severe complications, including pneumonia, encephalitis, acute cerebellar ataxia, Reye syndrome and hepatitis, in particular in immunosuppressed patients but also occasionally in normal children and adults. Zoster may also be associated with severe complications, including persistent neuralgia, paralysis, pneumonia, meningoencephalitis and ocular disease.

Available antiviral drugs are only partially effective in treating varicella and its complications, and hyperimmune globulin administered after known exposure is of limited value in aborting or attenuating infection. Vaccines therefore have a potentially important role in the control of varicella virus infections.

Several vaccine strains of attenuated varicella virus have been developed and compared in both normal and immunosuppressed people. The Oka strain has been shown to have the most desirable attribute of low virulence while inducing an adequate antibody response and protection against the disease.

Requirements for Varicella Vaccine (Live) were adopted by the WHO Expert Committee on Biological Standardization in 1984 and published in 1985 (*1*). Manufacturers have gained much experience with the vaccine since 1984 and a new revision of the Requirements has therefore been prepared.

General considerations

These Requirements have been formulated with reference to the Oka strain only, since no other strain of varicella virus is currently considered

suitable for vaccine production. The strain originated from a vesicle on a boy (Oka) who had chickenpox but who was otherwise healthy. The virus was isolated in human embryonic lung cells, and attenuated by serial passage in both human and guinea-pig embryo cells before further passages in human diploid cells. Clinical trials have been conducted in several countries. So far over 1 million people have been vaccinated with the virus, including both healthy children and adults and people with underlying disease, some with leukaemia. The vaccine has been shown to be safe, well tolerated and immunogenic in the doses used, which have ranged from 54 to 17000 plaque-forming units given by the subcutaneous route. There is evidence that some people who receive the Oka strain are protected from significant varicella disease in the absence of detectable seroconversion; this may be due to cell-mediated immunity.

Since varicella virus is very heat labile, special precautions need to be taken to maintain its viability throughout the manufacture and distribution procedures for the vaccine. The vaccine is currently available only in freeze-dried form.

Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements so that, if a national control authority so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, a summary protocol for recording the results of tests is provided as Appendix 1.

Should individual countries wish to adopt these Requirements as the basis for their national regulations concerning varicella vaccine, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the vaccine. It is desirable that the World Health Organization should be informed of any such changes.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum varicellae 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.

A.1.2 *Descriptive definition*

"Vaccinum varicellae vivum" is a freeze-dried preparation of live attenuated varicella virus grown in human diploid cell cultures. The preparation shall satisfy all the requirements formulated below.

The Oka strain is the only strain of varicella virus currently considered suitable for vaccine production.

A.1.3 *International reference materials*

No international reference materials are available.

A.1.4 *Terminology*

The following definitions are given for the purpose of these Requirements only.

Original vaccine: A vaccine prepared according to the manufacturer's specifications and shown on administration to humans to be safe and immunogenic.

Master seed lot: A quantity of virus or virus-infected cells derived from, or used to prepare, an original vaccine; the virus suspension has been processed as a single lot to ensure a uniform compositon and is fully characterized. The master seed lot is used for the preparation of working seed lots.

Working seed lot: A quantity of fully characterized virus of uniform composition, derived from a master seed lot. The working seed lot is used for the production of vaccines.

Cell seed: A quantity of fully characterized cells of human origin stored frozen at -70 °C or below in aliquots of uniform composition, one or more of which are used for the production of a manufacturer's working cell bank.

Manufacturer's working cell bank (MWCB): A quantity of cells of uniform composition, derived from one or more ampoules of the cell seed and stored frozen at -70 °C or below in aliquots, one or more of which are used for production purposes.

In normal practice a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national control authority. The cells are combined in a single pool, distributed into ampoules and preserved cryogenically to form the MWCB.

Production cell culture: A group of cell cultures derived from the same pool of cells and processed together.

Single harvest: A quantity of virus suspension derived from a batch of production cell cultures that were inoculated with the same working seed lot and processed together in a single production run.

Virus pool: A homogeneous pool of single harvests collected into a single vessel before clarification.

Final bulk: The homogeneous finished virus suspension prepared from one or more clarified virus pools in the vessel from which the final containers are filled.

Filling lot (final lot): A collection of sealed final containers of finished vaccine that are homogeneous with respect to the risk of contamination during filling and freeze-drying. All the final containers must, therefore, have been filled from a single vessel of final bulk in one working session and lyophilized under standardized conditions in a common chamber.

Cell-culture infective dose 50% ($CCID_{50}$): The quantity of a virus suspension that is estimated to infect 50% of sensitive cell cultures.

Plaque-forming unit (PFU): The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

A.2 Certification of the strain of virus for use in vaccine production

The strain of varicella virus used in the production of varicella vaccine shall be identified by historical records that include information on the origin of the strain, its method of attenuation and the passage level at which attenuation and efficacy were demonstrated by clinical evaluation.

The strain of varicella virus used in the production of vaccine shall have been shown to be safe and immunogenic by appropriate laboratory tests (see section A.4 of these Requirements) and by tests in susceptible humans. Only strains that are approved by the national control authority shall be used.

A.3 General manufacturing requirements

The general manufacturing requirements contained in Good Manufacturing Practices for Pharmaceutical (*2*) and Biological (*3*) Products and the Requirements for Human Diploid Cells Used for the Production of Varicella Vaccine (Live) (Appendix 2) shall apply to establishments manufacturing varicella vaccine. Particular attention should be paid to the following requirements.

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

Varicella vaccine shall be produced by staff who have not handled other infectious microorganisms or animals on the same working day. The staff shall consist of persons who are periodically examined medically and found to be healthy. Steps shall be taken to ensure that all personnel involved in the production areas are immune to varicella. Production and control shall be organized as two separate units of the manufacturing establishment with independent responsibilities.

Only the virus seed lots and cell cultures approved by the national control authority for the production of varicella vaccine shall be introduced or handled in the production area.

Persons not directly concerned with the production processes, other than official inspectors, shall not be permitted to enter the production area without valid reason and specific authorization.

Particular attention shall be given to the recommendations in Good Manufacturing Practices for Biological Products (*3*) 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.

A.4 Production control

The general production precautions formulated in Good Manufacturing Practices for Biological Products (*4*, sections 4 and 8) shall apply to the manufacture of varicella vaccine.

A.4.1 *Control of source materials*

A.4.1.1 Strain of varicella virus

The strain of varicella virus used in the production of live varicella vaccine shall be certified according to the specifications of section A.2. The vaccine strain shall be approved by the national control authority.

The master seed or each working seed lot of virus or virus-infected cells shall have been shown to be non-neuropathogenic in monkeys (see section A.4.2.1) and to yield live varicella vaccine of adequate immunogenicity and safety in human beings.

A.4.1.2 Cell cultures

The human diploid cells used for the propagation of varicella virus shall be approved by and registered with the national control authority.

A MWCB shall be established in conformity with the requirements of Appendix 2. The cell seed shall be derived from an early population doubling of the approved diploid cell strain, and the MWCB shall be prepared from it by serial subculture up to an approved population doubling level. Each manufacturer shall show to the satisfaction of the national control authority that the cell substrate propagated from the accepted cell strain and laid down as a MWCB conforms with the requirements of Appendix 2 concerning tests in cell cultures, animals and eggs for freedom from extraneous agents, lack of tumorigenicity, normal karyology and identity. The cells shall not be used beyond a number of population doublings equivalent to two-thirds of the total corresponding to the average lifetime of the cells.

A.4.1.3 Serum used in cell-culture medium

Serum used for the propagation of cells for varicella vaccine production shall be tested to demonstrate freedom from bacteria, fungi and mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*4*) and to demonstrate freedom from viruses. Serum of bovine origin must come from countries or herds certified to be free of bovine spongiform encephalopathy.

> Some countries require that bovine serum should come from herds that have not been given feed derived from ruminant protein.
>
> Suitable tests for detecting viruses in calf or newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*5*).

Serum shall also be shown to be free from inhibitors of varicella virus. Human serum shall not be used. If human albumin is used, it shall meet the revised Requirements for Biological Substances No. 27 (Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives) (*6*).

> In some countries sera are also examined for freedom from certain phages.

A.4.1.4 Trypsin used for preparing cell cultures

Trypsin used for preparing cell cultures shall be bacteriologically sterile and free from mycoplasmas and viruses, especially porcine parvoviruses. If bovine trypsin is used, it must come from countries or herds certified to be free of bovine spongiform encephalopathy. The methods used to ensure this shall be approved by the national control authority.

A.4.2 ***Control of virus seed***

The production of vaccine shall be based on the seed lot system. Seed lots shall be prepared in cells homologous to those used for production of the final vaccine.

> It is recommended that a large working seed lot be set aside for the preparation of batches of vaccine.

Each seed lot shall be identified as varicella virus by appropriate serological methods (see section A.6.1).

Virus seed lots shall be stored lyophilized in a dedicated temperature-monitored refrigerator at a temperature lower than -20 °C or, if not lyophilized, at or below -60 °C.

Single harvests shall be produced from the working seed lot by a validated process that has been demonstrated to be equivalent to that employed to produce the vaccine used in the clinical trials to establish the safety and efficacy of the virus strain and the suitability of the master seed lot.

Particular attention should be given to the need to maintain consistency with respect to such factors as multiplicity of infection and cell-culture conditions, for example the duration and temperature of incubation.

Some national control authorities require manufacturers to demonstrate consistency of the product for several consecutive (e.g. five) production lots.

A.4.2.1 Tests on virus seed lots

The seed lot used for the production of vaccine shall be free from detectable extraneous agents, and shall be produced in conditions that satisfy the requirements of sections A.4.3, A.4.4 and A.4.5 (with the exception of the tests for added substances and residual animal serum proteins in section A.4.5.3).

Tests for neurovirulence. The master seed or each working seed lot shall be shown to be free from neurovirulence by tests in varicella-susceptible monkeys of a species approved by the national control authority. To avoid the unnecessary use of monkeys, virus seed lots should be prepared in large quantities.

Neurovirulence tests can be conducted as follows: At least 10 monkeys should be employed in each test. Immediately before inoculation, all monkeys should be shown to be serologically negative for varicella. The material under test should be given by injection of 0.5 ml into the thalamic region of each hemisphere. The total amount of varicella virus given to each monkey should be not less than the amount contained in the recommended single human dose of vaccine. The monkeys should be observed for 17-21 days for symptoms of paralysis and other evidence of neurological involvement. Animals that die within 48 hours of injection may be replaced. The test is invalid and should be repeated if more than 20% of the monkeys die from non-specific causes. At the end of the observation period all the monkeys are anaesthetized and killed for autopsy; histopathological examinations of appropriate areas of the brain are made for evidence of central nervous system involvement.

The material passes the test if there is no clinical or histopathological evidence of involvement of the central nervous system attributable to the injected virus.

Some national control authorities advise that monkeys inoculated with samples of the fluids collected from the cultures described in section A.4.3 can serve as controls.

In some countries the seed lot is not tested but vaccines are accepted provided that each of the first five undiluted clarified virus pools prepared from the same seed lot satisfies the requirements of the test for neurovirulence.

A.4.3 ***Control of cell cultures***

From the cells used to prepare the cell cultures for growing attenuated varicella virus, an amount of processed cell suspension equivalent to at least 5% of the total volume, but not more than 1000 ml, shall be used to prepare control cultures of uninfected cells. These control cultures shall be observed microscopically for changes attributable to the presence of

adventitious agents for at least 14 days after the day of inoculation of the production cultures or until the time of final virus harvest if this is later. At the end of the observation period, a sample of fluid shall be collected from each control culture, and the pooled fluids from each batch shall be tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at or below -60 °C.

If any tests show evidence of the presence of adventitious agents in control cultures, the corresponding virus harvest shall not be used for vaccine production.

For the tests to be valid, no more than 20% of the culture vessels shall have been discarded for non-specific reasons by the end of the test period.

A.4.3.1 Test for haemadsorbing viruses

At the end of the observation period, 25% of the control cell cultures shall be tested for the presence of haemadsorbing viruses, using guinea-pig red cells, and shown to be negative. If the red cells have been stored, the duration of storage shall not have exceeded seven days, and the temperature of storage shall have been in the range of 2-8 °C.

> In some countries, the national control authority requires that tests for haemadsorbing viruses should also be made on control cultures 3-5 days and 12 days after inoculation of the production cultures, and that other types of red cells, including cells from humans (blood group O), monkeys and chickens (or other avian species), should be used in addition to guinea-pig cells. In all tests readings should be taken after incubation for 30 minutes at 0-4 °C, and again after a further incubation for 30 minutes at 20-25 °C. For the test with monkey red cells readings should also be taken after a final incubation for 30 minutes at 34-37 °C.

A.4.3.2 Tests for non-haemadsorbing extraneous agents

Ten millilitres of the pooled cell-culture fluid collected at the end of the observation period shall be tested in the same cell substrate, but not the same batch, as that used for virus growth. Additional 10 ml samples of each pool shall be tested in simian cells.

Bottles of cell cultures shall be inoculated in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm^2 per ml of pooled fluid. At least one bottle of the cell cultures shall remain uninoculated as a control.

The inoculated cultures shall be incubated at a temperature of 35-37 °C and shall be examined for abnormal morphology for a period of at least 14 days.

> Some national control authorities require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least seven days. Furthermore, some national control authorities require that these cells should be tested for the presence of haemadsorbing viruses.

A.4.3.3 Identity test

The control cell cultures shall be shown to be of human origin by tests approved by the national control authority.

Suitable tests are isozyme analysis, human leukocyte antigen (HLA) and other immunological tests, and karyotyping of at least one metaphase spread of chromosomes.

A.4.4 *Control of single harvests*

A.4.4.1 Cells used for vaccine production

On the day of inoculation of production cell cultures with the virus seed lot, each control cell culture and production cell culture shall be examined for degeneration caused by infective agents. If such examination shows evidence of the presence in a cell culture of any adventitious agent, the whole group of cultures concerned shall not be used for vaccine production.

After virus inoculation, cultures for vaccine production shall be incubated under controlled temperature conditions approved by the national control authority.

If animal serum is used in the growth or maintenance medium for the cell cultures, the serum shall be removed from the cultures either before or after inoculation with the seed virus, but before harvest. Before the virus is harvested, the cell cultures shall be rinsed and the growth medium replaced with serum-free maintenance medium.

Penicillin and other β-lactam antibiotics shall not be used at any stage of manufacture.

Minimal concentrations of other suitable antibiotics may be used if approved by the national control authority.

A.4.4.2 Single harvests

The infected cells from which a single harvest will be derived shall be washed, released from the glass or other support surface, and pooled. The cell suspension shall be disrupted by sonication or another appropriate method and tested in cell cultures for sterility as specified below.

Sterility tests. A volume of at least 20 ml of each single harvest shall be tested for bacterial and mycotic sterility and for mycoplasmas according to Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*4*), or by a method approved by the national control authority.

In some countries, the sample of pooled fluid is ultracentrifuged and both the pellet and its supernatant fluid are tested for sterility.

Tests for mycoplasmas should be done in both solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas. At least 10 ml of single harvests should be

used for each group of tests. Certain nutritionally fastidious mycoplasmas are best detected by DNA fluorescent staining on the surface of cultured indicator cells. Approved non-culture methods including DNA probes may also be used.

A.4.5 ***Control of virus pool and final bulk***

A.4.5.1 Virus pool

The virus pool shall be prepared from one or several single harvests that satisfy the requirements of section A.4.4.2 and shall be submitted to the following tests, unless these tests have already been done on each single harvest; even in that event, the virus pool shall be tested for sterility. Samples that are not tested immediately shall be stored at or below -60 °C.

Sterility tests. Sterility tests shall be performed as indicated in section A.4.4.2.

Tests of neutralized virus pool in cell cultures. A volume of each virus pool equivalent to at least 500 human doses shall be neutralized by specific antiserum, which shall not be of human, simian or bovine origin. The immunizing antigen used to prepare the antiserum shall not be made using the vaccine strain. It shall be produced in cell cultures free from extraneous microbial agents that might elicit antibodies inhibitory to the growth of any extraneous agents that may be present in the varicella virus pool.

The neutralized sample of virus pool shall be tested in cell cultures of the same type, but not of the same batch, as those used to prepare the virus pool and in other human cell cultures. Uninoculated cell cultures shall be kept as a control. All cell cultures shall be observed for at least 14 days.

Suitable cells are HeLa and MRC5.

Some national control authorities require that, at the end of the observation period, a subculture is made in the same culture system.

The virus pool passes the test if there is no evidence of the presence of an adventitious agent, and no more than 20% of the culture vessels have been discarded for non-specific reasons by the end of the test period.

A.4.5.2 Clarification of the virus pool

The virus pool shall be clarified by a method that will maximize removal of cells and cell debris.

The clarified virus pool may be stabilized and stored at or below -60 °C before being used to prepare final bulk for freeze-drying.

Samples of the clarified bulk suspension shall be taken immediately after clarification to ensure that no microscopically observable cells or cell particles remain. Samples shall also be taken for virus titration and for sterility tests. If not tested immediately, the samples shall be kept at or below -60 °C until testing is done.

A suitable test for the presence of residual intact cells is as follows: a sample of 50 ml is centrifuged at 750*g* for 30 minutes and the sediment resuspended in 0.9 ml of an isotonic solution (to give about a 50-fold concentration of the original sample). The concentrated suspension is examined microscopically for the presence of intact cells.

Virus titration. The live virus content of the clarified virus pool shall be determined by titration in cell culture against a reference preparation of live varicella virus (see section A.6.3).

Sterility tests. Sterility tests shall be performed as indicated in section A.4.4.2.

A.4.5.3 Final bulk

The final bulk shall be prepared from one or more clarified virus pools obtained from substrates of which control cultures pass the tests specified in section A.4.3. The virus pools and final bulk shall pass the tests specified in section A.4.5.

Assays of the virus content of the final bulk may be required by some national control authorities.

Added substances. Any substance such as diluent or stabilizer that is added to the product during preparation of 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.

Residual animal serum proteins. If serum has been used in the cell-culture system, a sample of the final bulk shall be tested to verify that the residual amount of serum albumin is less than 50 ng per single human dose.

Alternatively the test may be performed on the clarified pools.

Sterility tests. Each final bulk shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*4*), or by a method approved by the national control authority.

Storage. Until it is distributed into containers and lyophilized, the final bulk shall be stored in conditions shown by the manufacturer to retain the activity of the vaccine.

A.5 Filling and containers

The requirements concerning filling and containers in Good Manufacturing Practices for Biological Products (*3*, section 4) shall apply.

Care shall be taken to ensure that the material of which the container and, if applicable, the closure are made does not adversely affect the virus content of the vaccine under the recommended conditions of storage.

Single-dose containers are recommended because the product is thermolabile.

A.6 Control tests on final product

Samples shall be taken from each final lot for the following tests.

A.6.1 *Identity test*

The virus in one or more individually labelled final containers shall be identified as varicella virus by appropriate methods.

Methods such as seroneutralization in cell culture with specific antiserum are suitable.

A.6.2 *Sterility tests*

Reconstituted vaccine shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances) (*4*), or by a method approved by the national control authority.

A.6.3 *Virus content*

The virus content in each of at least three ampoules selected at random from the final lot shall be determined individually against a reference preparation of varicella vaccine. Since no international reference materials have been established for live varicella vaccine, no requirements for potency based on such materials can be formulated. The national control authority shall provide or approve a reference preparation of live varicella virus for use in tests to determine virus concentration.

It is desirable to measure the virus activity in each of five or more separate vaccine containers in comparison with a reference preparation of live varicella virus.

The national control authority shall specify the minimum amount of vaccine virus that one human dose may contain.

An accelerated stability test should be considered by the national control authority as an element in establishing consistency of production.

The detailed procedures for carrying out this test and for interpreting the results should be approved by the national control authority, which should also specify the acceptable confidence limits.

A.6.4 *General safety tests*

Each final lot shall be tested for the absence of abnormal toxicity in mice and guinea-pigs by appropriate tests approved by the national control authority.

A.6.5 *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. The upper limit of the moisture content shall be specified by the national control authority.

Generally, moisture levels of less than 2% are considered satisfactory.

A.6.6 *Inspection of final containers*

Every container in each final lot shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records

The requirements in section 8 of Good Manufacturing Practices for Biological Products (*3*, pages 27–28) shall apply.

A.8 Samples

The requirements in section 9 of Good Manufacturing Practices for Biological Products (*3*, page 29) shall apply.

A.9 Labelling

The requirements in section 7 of Good Manufacturing Practices for Biological Products (*3*, pages 26–27) shall apply, with the addition of the following:

The label on the carton or the leaflet accompanying each container shall:

- state that the vaccine fulfils Part A of these Requirements;
- state the nature of the preparation, specifying the strain of varicella virus in the vaccine, the minimum number of infective units per human dose, and the origin of the substrate used to prepare the vaccine;
- state the nature and quantity of any residual antibiotic present in the vaccine;
- draw attention to the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;

- indicate the volume and nature of diluent[1] to be added to reconstitute the vaccine, and specify that the diluent should be supplied by the manufacturer;
- state that the vaccine should be administered immediately after reconstitution;
- warn that the vaccine is not to be administered to pregnant women.

A.10 Distribution and shipping

The requirements in section 8 of Good Manufacturing Practices for Biological Products (*3*) shall apply.

Shipments should be at temperatures of 8 °C or below and parcels should contain cold-chain monitors.

A.11 Storage and expiry date

A.11.1 *Storage conditions*

Before distribution, the manufacturer shall store lyophilized vaccines at a temperature shown by the manufacturer to be compatible with minimal loss of virus titre. After distribution, live varicella vaccine shall be stored at all times at a temperature below 8 °C.

A.11.2 *Validity period*

The validity period is a defined period of time at prescribed conditions of storage. It shall be based upon the stability of the vaccine, as determined experimentally, and shall be approved by the national control authority.

Part B. National control requirements

B.1 General

The general requirements for control laboratories in the Guidelines for National Authorities on Quality Assurance for Biological Products (*7*) shall apply.

The national control authority shall give directions to manufacturers concerning the varicella virus strains to be used in vaccine production and concerning the recommended human dose.

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

[1] No preservative or any substance that has a deleterious effect on the virus should be present in the diluent used to reconstitute the vaccine.

In addition, the national control authority shall provide or approve a reference preparation of live varicella virus for virus titration (see sections A.4.5.2 and A.6.3), and shall specify the virus content required to achieve adequate immunization of humans with the recommended human dose.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or part A of the present Requirements. A protocol based on the model given in Appendix 1, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 3.

The purpose of the certificate is to facilitate the exchange of live varicella virus vaccines among countries.

Authors

The draft of these revised Requirements was prepared by Dr V. Grachev, Scientist, Biologicals, and Dr D. Magrath, Chief, Biologicals, World Health Organization, Geneva, Switzerland.

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Appendix 1

Summary protocol for the production and testing of varicella vaccine (live)[1]

The following protocol is intended *for guidance*, and indicates the information that should be provided as a minimum.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a certificate from the national control authority of the country in which the vaccine was produced stating that the product meets national requirements as well as the Requirements published by WHO (see Appendix 3).

Control of source materials (A.4.1)

Strain of varicella virus (A.4.1.1) _____

Cell cultures (A.4.1.2)

Provide information on the source of the manufacturer's working cell bank (MWCB).

Serum used in cell-culture medium (A.4.1.3)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Tests for adventitious agents

Methods _____

Date of inoculation _____

Results _____

[1] Based on Requirements for Varicella Vaccine (Live) (Requirements for Biological Substances No. 36, revised 1993). In: *WHO Expert Committee on Biological Standardization. Forty-fourth Report.* Geneva, World Health Organization, 1994, Annex 2 (WHO Technical Report Series, No. 848).

Trypsin used for preparing cell cultures (A.4.1.4)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Media used			
Observation period			
Results			

Tests for adventitious viruses (including porcine parvoviruses)

Methods _____

Date of inoculation _____

Results _____

Production of the working seed lot (virus seed) (A.4.2)

Summary information

Name and address of manufacturer _____

_____

Virus strain _____

Reference no. of virus seed used to prepare manufacturer's first varicella vaccine that was safe and immunogenic in humans _____

Reference no. of master seed lot _____

No. of passages between the two above seeds _____

Working seed lot

Date of preparation _____

No. of containers prepared _____

Reference no. _____

Conditions of storage _____

Identity test

Date _____

Method _____

Results _____

History of vaccine strain

Provide a brief account, indicating how the vaccine strain was acquired, outlining its history up to production of master seed lot, and specifying the criteria on which acceptability for virus production is based.

Tests on virus seed lots (A.4.2.1)

Complete sections A.4.3, A.4.4 and A.4.5 of the summary protocol, with the exception of the tests for added substances and residual animal serum proteins in section A.4.5.3.

Tests for neurovirulence[1]

No. of monkeys in test _____

Species _____

Volume injected _____

No. of monkeys surviving without specific symptoms _____

Results of serological tests _____

Results of histopathological examination (specify findings) _____

Certification of working seed lot

Name (typed) and signature of head of production laboratory _____

_____

Certification by the head of the control laboratory of the manufacturer taking overall responsibility for production and control of the working seed lot:

I certify that the working seed lot of varicella virus no. _____ satisfies Part A, sections 2 to 4.5, of the Requirements for Biological Substances No. 36 (Requirements for Varicella Vaccine (Live)), revised 1993.

Signature _____

Name (typed) _____

Date _____

[1] Either the master seed or each working seed lot should be tested.

Control of cell cultures (A. 4.3)

Provide information on the control cell cultures corresponding to each single harvest, using extra pages if necessary.

Cell substrate used for production of virus _____

Reference no. of control cell cultures _____

Quantity of cell cultures used as control cultures _____

Period of observation of control cells _____

Test for haemadsorbing viruses (A.4.3.1)

Type of red blood cells _____

Date of test _____

Results _____

Tests for non-haemadsorbing extraneous agents (A. 4.3.2)

Human diploid cells of the type used for virus growth

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Identity test (A.4.3.3)

Method _____

Date _____

Results _____

Control of single harvests (A. 4.4)

Cells used for vaccine production (A. 4.4.1)

Observation of cell cultures before inoculation

Methods _____

Results _____

Antibiotics added (if used) _____

Concentration _____

Single harvests (A.4.4.2)

Report the results of tests on each single harvest, using extra pages if necessary.

Method of cell disruption _____

Reference no. of single harvest _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Control of virus pool and final bulk (A.4.5)

Virus pool (A.4.5.1)

If any test had to be repeated or any abnormal result was observed, this must be specified.

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Tests of neutralized virus pool in cell cultures

Species in which neutralizing serum was prepared and cell substrate in which immunogen was produced _____

Human diploid cells of the type for virus growth

Date of inoculation _____

Results _____

Second type of human cells

Type of cells _____

Date of inoculation _____

Results _____

Clarification of the virus pool (A. 4.5.2)

Date of clarification _____

Results of clarification _____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Final bulk (A. 4.5.3)

Reference no. of final bulk _____

Total volume of final bulk _____

Added substances (diluent, stabilizer) and final concentration _____

Residual animal serum proteins

Date _____

Method _____

Results (indicate amount and nature of serum protein(s) present per human dose) _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Filling and containers (A. 5)

Name and address of manufacturer _____

_____

Proprietary name of vaccine _____

Reference no. of final lot _____

Expiry date _____

No. of containers in the lot _____

No. of doses per container _____

Lot no. of final bulk _____

Date of filling of final containers _____

Control tests on final product (A.6)

Identity test (A.6.1)

Date _____

Method _____

Results _____

Sterility tests (A.6.2)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Media used	_____	_____	_____
Observation period	_____	_____	_____
Results	_____	_____	_____

Virus content (A.6.3)

Date of inoculation _____

Type of cell cultures _____

Reference preparation used _____

	Vaccine samples			*Reference samples*
	1	*2*	*3*	
Virus concentration in each container (in PFU or $CCID_{50}$)	____	____	____	_____
Mean virus titre per human dose, with 95% fiducial limits	_____			_____

General safety tests (A.6.4)

Test in mice

Date of inoculation _____

No. of mice tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Test in guinea-pigs

Date of inoculation _____

No. of guinea-pigs tested _____

Volume and route of injection _____

Observation period _____

Results (give details of deaths) _____

Residual moisture (A.6.5)

Date _____

Method _____

Size of sample _____

Moisture content (%) _____

Inspection of final containers (A.6.6)

Date and result _____

Submission addressed to national control authority for batch release

Name (typed) and signature of head of production laboratory _____

_____

Date _____

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot no._____ of varicella vaccine (live) satisfies national requirements and/or Part A of the Requirements for Biological Substances No. 36 (Requirements for Varicella Vaccine (Live)), revised 1993.

Signature _____

Name (typed) _____

Date _____

Appendix 2

Requirements for human diploid cells used for the production of varicella vaccine (live)

The following requirements concern the testing of human diploid cells for the production of varicella vaccine; they should be read in conjunction with Part A of the main Requirements.

1. Terminology

Cell seed: A quantity of fully characterized cells of human origin stored frozen at -70 °C or below in aliquots of uniform composition, one or more of which are used for the production of a manufacturer's working cell bank.

Manufacturer's working cell bank (MWCB): A quantity of cells of uniform composition derived from one or more ampoules of the cell seed and stored frozen at -70 °C or below in aliquots, one or more of which are used for production purposes.

> In normal practice a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national control authority. The cells are combined in a single pool, distributed into ampoules and preserved cryogenically to form the MWCB.

Cell substrate: A collection of cell cultures at the population doubling level used for virus growth that have been prepared together from one or more ampoules of the manufacturer's working cell bank (MWCB).

2. Production control

2.1 *Control of source materials*

The cell seed and MWCB shall be approved by and registered with the national control authority. The cells shall have been characterized with respect to their genealogy, growth characteristics, life span, genetic markers (HLA), viability during storage and karyology, and have been shown to be free from bacteria, mycoplasmas, fungi and haemadsorbing and other viruses by the relevant tests in these Requirements. In addition, the cells of the MWCB shall have been shown to be diploid and stable with respect to karyology and morphology by the tests outlined in this section.

The MWCB shall also have been shown to yield cell cultures capable of producing vaccine that is both safe and immunogenic in humans.

Each production cell culture shall consist of cells at a passage level of up to two-thirds of the life span of the accepted cell strain and shall be tested for identity. It shall comply with the tests outlined in sections 2.1.1, 2.1.2 and 2.1.4 for normal karyology and freedom from adventitious agents.

2.1.1 *Tests in animals and eggs for extraneous agents*

The cells of the MWCB are suitable if at least 80% of the inoculated animals or eggs remain healthy and survive the observation period, and none of the animals or eggs shows evidence of the presence in the cells of any extraneous agent.

Tests in animals. The following groups of animals shall be inoculated with MWCB cells by the intramuscular route, at least 10^7 cells being divided equally between the animals in each group:

- two litters of suckling mice, totalling at least ten animals, less than 24 hours old
- ten adult mice weighing 15-20 g
- five guinea-pigs weighing 350-450 g
- five rabbits weighing 1.5-2.5 kg.

The animals shall be observed for at least four weeks. Any animals that are sick or show any abnormality shall be investigated to establish the cause of illness.

In some countries, the suckling and adult mice are also inoculated by the intracerebral route.

Tests in eggs. At least 10^6 viable MWCB cells shall be injected into the allantoic cavity of ten embryonated chickens' eggs, 9-11 days old, obtained from a healthy flock. The eggs shall be examined after not less than five days and the allantoic fluids shall be tested with erythrocytes from guinea-pigs and chickens (or other avian species) and human group O cells for the presence of haemagglutinins.

2.1.2 *Other tests for extraneous agents*

Suitable tests approved by the national control authority shall be performed in order to exclude the presence of retroviruses and the integration of nucleic acid of viral origin (hepatitis B virus and human immunodeficiency virus) in the genome of MWCB cells.

In some countries, the cells are also examined in ultra-thin sections and by negative staining under the electron microscope.

2.1.3 *Freedom from tumorigenicity*

The cells of the MWCB shall be shown to be free from potential tumorigenicity by appropriate animal tests, including positive controls, approved by the national control authority.

Suitable tests in immunosuppressed animals are as follows. Approximately 10^6 cells obtained from cultures at the same passage levels as the cultures used for vaccine production are injected into: newborn mice or hamsters treated with antilymphocyte serum; or athymic mice (nude *nu/nu* genotype); or thymectomized, irradiated mice with reconstituted bone marrow (T-B+). Some of the same group of animals should be inoculated with a similar dose of HeLa or KB cells as positive controls. The animals should be observed for not less than three weeks. Other tests in animals treated with immunosuppressive agents and with equal sensitivity to neoplastic cells may also be used.

The test is valid if the positive control animals develop tumours.

The cells are suitable for vaccine production if at least 80% of the inoculated animals remain healthy and survive the observation period, and none of the animals shows evidence of tumour formation attributable to the cells.

2.1.4 *Chromosomal characterization of the cell seed*

At least four samples of the cell seed shall be examined as described in section 2.1.5 at approximately equal intervals over the life span of the cell line during serial cultivation. Each sample shall consist of 1000 metaphase cells.

It is recommended that photographic reconstruction should be employed to prepare chromosome-banded karyotypes of 50 metaphase cells per 1000-cell sample, by either G-banding or Q-banding techniques. The incidence of karyotypic abnormalities (pseudodiploidy, inversions, translocations, etc.) that are detectable with the greater resolution provided by banding should not exceed that approved by the national control laboratory.

2.1.5 *Chromosomal characterization of the MWCB*

For the determination of the general character of the MWCB, a minimum of 500 cells in metaphase shall be examined at the production level, or at any passage thereafter, for frequency of polyploidy and for exact counts of chromosomes, frequency of breaks, structural abnormalities, and other abnormalities such as despiralization or marked attenuations of the primary or secondary constriction. The cells of the MWCB shall have normal karyology.

The manufacturer shall propose and the national control authority shall approve the permissible level of cell population doubling for vaccine production.

For WI-38 and MRC5 cells examined in metaphase, the generally accepted upper limits for abnormalities in 1000- and 500-cell samples are:[1]

Abnormality	*1000 cells*	*500 cells*
Chromatid and chromosome breaks	47/1000	26/500
Structural abnormalities	17/1000	10/500
Hyperploidy	8/1000	5/500
Hypoploidy	180/1000	90/500
Polyploidy	30/1000	17/500

[1] These upper limits are based on extensive experience with the examination of WI-38 and MCR5 cells reported to and examined by the ad hoc Committee on Karyological Controls of Human Substrates, which met in 1978 at Lake Placid, NY, USA. These values will not necessarily be applicable if other human cell strains are used.

All cells showing abnormalities shall be subjected to detailed examination and records shall be maintained of the detailed criteria applied to particular abnormalities in the karyotype analysis.

Stained slide preparations of the chromosomal monitoring of the working cell bank, or photographs of these, shall be maintained permanently as part of the record of the MWCB.

2.2 *Production precautions*

The general production precautions formulated in Good Manufacturing Practices for Pharmaceutical (*1*) and Biological (*2*) Products shall apply to the manufacture of varicella vaccine with the addition of the following.

2.2.1 *Cell cultures used for vaccine production*

Only human diploid cell cultures derived from a MWCB approved by the national control authority shall be used for vaccine production. The production of each single harvest shall be initiated from one or more new ampoules of the MWCB. All processing of the MWCB and subsequent cell cultures shall be done in an area in which no other cells are handled during the entire period of vaccine production. The cell cultures shall be used only if: (a) no changes have occurred in their growth characteristics, and (b) no abnormal karyotypic changes have been found to occur up to a number of population doublings that corresponds to the average finite lifetime of the cells, as determined under the particular conditions of the production establishment (see section 2.1.4).

2.2.2 *Identity test*

An identity test shall be performed on the control cell cultures by methods approved by the national control authority.

Suitable tests are isozyme analysis, HLA and other immunological tests, and karyotyping of at least one metaphase spread of chromosomes.

The cells shall be shown to be of human origin.

2.2.3 *Tests for bacteria, fungi and mycoplasmas*

A volume of 20 ml of the pooled supernatant fluids from the production cell cultures shall be tested for bacterial and mycotic sterility according to Part A, section 5.2, of the Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances (*3*), as well as for mycoplasmas by a method approved by the national control authority.

The tests for mycoplasmas should be done in both solid and liquid media that have been shown to be capable of supporting the growth of sterol-requiring and non-sterol-requiring mycoplasmas. At least 10 ml should be used for each group of tests. Approved non-culture methods may also be used.

References

1. Good manufacturing practices for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second Report.* Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 823).

2. Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second Report.* Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 822).

3. General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6, revised 1973). In: *WHO Expert Committee on Biological Standardization. Twenty-fifth Report.* Geneva, World Health Organization, 1973. Annex 4 (WHO Technical Report Series, No. 530).

Appendix 3

Model certificate for the release of varicella vaccine by national control authorities[1]

The following final lots of varicella vaccine produced by _____[2] in _____,[3] whose numbers appear on the labels of the final containers, meet all national requirements[4] and Part A[5] of Requirements for Biological Substances No. 36 (Requirements for Varicella Vaccine (Live)), revised 1993,[6] and comply with Good Manufacturing Practices for Pharmaceutical Products[7] and Good Manufacturing Practices for Biological Products.[8]

Final lot no.	Date of last potency test by manufacturer	Expiry date	Final lot no.	Date of last potency test by manufacturer	Expiry date
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____

As a minimum, this certificate is based on examination of the manufacturing protocol.

The Director of the National Control Laboratory (or Authority as appropriate):[9]

Signature _____

Name (typed) _____

Date _____

[1] To be completed by the national control authority of the country where the vaccine has been manufactured, and to be provided by the vaccine manufacturer to importers.

[2] Name of manufacturer.

[3] Country.

[4] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national control authority.

[5] With the exception of provisions on distribution and shipping, which the national control authority may not be in a position to assess.

[6] WHO Technical Report Series, No. 848, 1994, Annex 1.

[7] WHO Technical Report Series, No. 823, 1992, Annex 1.

[8] WHO Technical Report Series, No. 822, 1992, Annex 1.

[9] Or his or her representative.