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Annex 3
Requirements for measles, mumps and rubella vaccines and combined vaccine (live)
(Requirements for Biological Substances No. 47)

Introduction

Combined live vaccine for measles, mumps and rubella (MMR) is used widely for the immunization of children in certain regions of the world because of its advantages over the individual vaccines. Combined vaccine provokes an adequate immune response in children simultaneously for the three infections and facilitates the implementation of current immunization strategies.

Measles

Measles is an almost invariable clinical experience of childhood, and is often a severe disease, frequently complicated by middle-ear infection or bronchopneumonia. In some countries it is a major cause of illness and death. Encephalitis occurs in approximately one of every 2000 reported cases; survivors often have permanent brain damage and mental retardation. Death, predominantly from respiratory and neurological causes, occurs in one of every 3000 reported measles cases. The risk of death is greater for infants and adults than for children and adolescents.

Contracting measles during pregnancy increases fetal risk. Most commonly, this risk involves premature labour and moderately increased rates of spontaneous abortion and of low birth weight.

Subacute sclerosing panencephalitis, a slow virus infection of the central nervous system, is associated with measles virus. It is noteworthy that, in the United States of America, widespread use of measles vaccine has led to the virtual disappearance of this infection.

Immunization against measles has been of interest to WHO for many years, and especially since the Expanded Programme on Immunization

was launched in 1974 with measles as one of the principal diseases against which it was directed. In support of the immunization programme, reference materials for anti-measles serum and measles vaccine virus have been established. The Requirements for Measles Vaccine (Live) were adopted by the WHO Expert Committee on Biological Standardization in 1966 (*1*), with an Addendum in 1981 to include an accelerated test for stability (*2*). Since the original production of measles vaccine, the search for improved immunizing agents has continued.

The last revision of the Requirements for Measles Vaccine (Live) was adopted by the WHO Expert Committee on Biological Standardization in 1987 (*3*). In that revision, account was taken of the opinions of consultants, of the regulations and requirements for the manufacture and control of measles vaccine that had been formulated in a number of countries, and of information from published and unpublished sources. For the present revised Requirements, published together with the new Requirements for Combined Vaccine, some changes have been made to the previous revision.

Mumps

Mumps is an acute disease of children and young adults, caused by a paramyxovirus of which there is only a single serotype. Mumps virus produces no symptoms in about one-third of infected people. In those with a clinical response, glandular and nerve tissue are most often affected. The most common signs are fever and swelling of the parotid glands. Other complications, which may appear simultaneously with these signs or in any sequence, are epididymo-orchitis, meningo-encephalitis, cranial nerve involvement (especially eighth cranial nerve damage leading to hearing impairment), pancreatitis, oophoritis, mastitis and myocarditis. Frequent viruria and abnormal renal function suggest that mumps virus may infect the kidneys. In some instances, one or more of the other complications may be present in the absence of parotitis.

The most common complication of mumps in children is meningitis, sometimes associated with encephalitis, and in young adults orchitis. Most complications due to mumps infection resolve without permanent damage. Death following mumps is rare and is mostly due to mumps encephalitis. Many people reach adulthood without developing immunity and potential target populations for immunization therefore include both children and susceptible adults.

The Requirements for Mumps Vaccine (Live) were adopted by the WHO Expert Committee on Biological Standardization in 1987 (*4*), and have been included in this combined document with some changes.

Rubella

Rubella (German measles) gives rise to a mild exanthematous illness, accompanied by few constitutional symptoms, and occurs most commonly

in childhood. If the infection occurs in a woman in early pregnancy however, the virus may cross the placenta to reach the fetus, in which the infection can induce birth defects. These defects may be serious and permanent and include congenital heart disease, cataract formation, deafness and mental retardation. The prevention of fetal infection, therefore, is the primary purpose of rubella immunization.

To meet the need for an international standard for anti-rubella serum for use in the assay of rubella antibodies and in the control of specific (anti-rubella) immunoglobulins, the WHO Expert Committee on Biological Standardization established the second International Reference Preparation of Anti-Rubella Serum in 1970 (*5*). The Requirements for Rubella Vaccine (Live) were adopted by the Committee in 1977 (*6*), with an Addendum in 1980 (*7*), and have been included in this combined document with some changes.

Vaccine requirements

The combination of different strains of attenuated measles, mumps and rubella viruses in a single vaccine might interfere with the immune response to one or more of the components or provoke serious adverse reactions. Therefore, before use for routine immunization of children, every proposed combination of the different strains of measles, mumps and rubella viruses should be studied in clinical trials to determine the minimum effective virus dose, optimal balance of components and general safety profile.

In the present Requirements, no reference is made to the maximum permitted number of passages between the master seed and working seed; instead, it is recommended that manufacturers follow the routine production procedure employed to produce the clinical trial material that established the safety and efficacy of the vaccine. Reference to passage levels of virus may become irrelevant with the use of fermenters and of different multiplicities of infection and numbers of harvests. For each strain of virus, it is necessary to establish the relationship between the laboratory estimation of virus titre on the one hand and safety and efficacy for humans on the other. Where individual vaccines are to be used in combination with other vaccines, the dose-response curve for each component should be determined by administering it in the proposed combined form.

It is essential that every precaution be taken to exclude adventitious agents from vaccines for use in humans. In the Requirements formulated below, tests have been described for detecting adventitious agents that might be present in cell cultures used for vaccine production.

The systematic use of cells from birds maintained in closed colonies that have been subject to continuous and systematic veterinary and laboratory monitoring for the presence of infectious agents, or of cells derived from well characterized human diploid cell lines, has improved the quality of cell

substrates. However, although a group convened by WHO in 1980 (*8*) concluded that, for cell cultures, it was sufficient to conduct tests for extraneous agents on control cells, tests for the absence of such agents are still mandatory for individual harvests and/or virus pools in the present Requirements. On the other hand, tests for extraneous agents on small laboratory animals have been abandoned.

Experience has shown that the maintenance of a standard operating procedure, including a standard temperature of incubation and the use of a seed lot system, results in a consistent product that shows a regular pattern of attenuation and immunogenicity.

I. Requirements for measles vaccine

General considerations

Hundreds of millions of people have been vaccinated with live attenuated measles virus vaccines produced from a variety of strains, and there is ample evidence that these vaccines are safe and effective. Production of such vaccines requires the observance of certain rules, and it is thus important that international requirements for live attenuated measles vaccines should be available to manufacturers and national control authorities.

The antibody response in people inoculated with live measles vaccine can be accurately measured serologically, and a number of studies have established that the presence of detectable levels of antibody is correlated with protection against the disease. Immunity following the use of live measles vaccine appears to be of long duration, as indicated by the persistence of neutralizing antibodies in children several years after receiving vaccines prepared from further-attenuated strains derived from the Edmonston strain of measles virus. Although persistence of antibodies to the Edmonston strain has been demonstrated, it is important that studies should be undertaken to determine the duration of immunity induced by vaccines derived from approved strains.

The optimum age for the immunization of babies may differ from one country to another. If immunization is carried out too early in life, there may be no or poor protection, because of pre-existing maternal antibodies, especially if vaccine strains have been over-attenuated. On the other hand, the pattern of incidence of measles in some developing countries is such that it may be necessary to immunize babies as early as six months of age. The minimum and maximum virus titre and the general safety of the virus strain should be defined. The poorer seroconversion rate and antibody titre when measles vaccine is administered early in life can be partially corrected by a second injection later in life.

It is obviously important that the strains of virus used to prepare live measles vaccine should show no tendency to produce neurological

complications of the type encountered in some cases of natural measles. Present experience indicates that the live vaccines so far used are, indeed, safe in this respect. In the absence of a more satisfactory test, the intracerebral inoculation of monkeys has been used as a laboratory test to evaluate this property; the development of more reliable methods is desirable. Post-measles encephalopathy may be the result of an immunopathogenic reaction, but the underlying mechanism is not known.

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 health administration 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 included as Appendix 1.

Should individual countries wish to adopt these Requirements as the basis of their national regulations for measles vaccines, 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. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum morbillorum 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 morbillorum vivum" is a preparation of live attenuated measles virus grown in a suitable cell culture. The preparation shall satisfy all the requirements formulated below.

At present, live measles vaccines are blended with an appropriate stabilizer and lyophilized. They are available for distribution only in that form, either as monovalent vaccines or in combination with live mumps and/or live rubella vaccines.

A.1.3 *International reference materials*

The International Reference Reagent for the Assay of Measles Vaccine (Live) and the International Standard for Anti-Measles Serum are available on request from the National Institute for Biological Standards and Control, Potters Bar, England.

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

Working seed lot: A quantity of virus of uniform composition, fully characterized, 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, animal or other 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 number 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 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 measles virus used in the production of measles 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 was demonstrated by clinical evaluation.

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

The lowest immunizing dose of virus in the vaccine that induces seroconversion in susceptible individuals shall be established in a dose-response study. This dose shall serve as a basis for establishing parameters for stability and expiry of the vaccine.

A.3 General manufacturing requirements

The requirements of Good Manufacturing Practices for Pharmaceutical (*9*) and Biological (*10*) Products and the Requirements for Human Diploid Cells Used for the Production of Measles, Mumps and Rubella Vaccines (Live) (Appendix 2) shall apply to establishments manufacturing measles vaccine, with the addition of the following requirements.

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

Measles 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 measles. Production and control shall be organized as two separate units of the manufacturing establishment with independent responsibilities.

Only the virus seed lot and cell cultures approved by the national control authority for the production of measles 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 (*10*) 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 (*10*) shall apply to the manufacture of measles vaccine.

A.4.1 *Source materials*

A.4.1.1 Strain of measles virus

The strain of measles virus used in the production of live measles vaccine shall be certified according to the specifications of section A.2. The vaccine strain shall be approved by the national control authority. The seed lot or five consistent lots of vaccine derived from the seed lot shall have been shown to be non-neuropathogenic in monkeys (see section A.4.2.1) and to yield live measles vaccine of adequate immunogenicity and safety in human beings.

A.4.1.2 Cell cultures

Measles virus used in the production of measles vaccine shall be propagated in cell cultures approved by the national control authority. All information on the source and method of preparation of the cell-culture system used shall be available to the national control authority.

> In some countries biochemical tests for the detection of RNA viruses are applied to production cell cultures.

A.4.1.3 Avian-embryo cell cultures

If avian-embryo cell cultures are used for the propagation of measles vaccine virus, the eggs used as a source of cells shall be derived from a closed, specific-pathogen-free, healthy flock. This flock shall be monitored at regular intervals for *Mycobacterium avium*, fowlpox, avian retroviruses, Newcastle disease virus and other avian parainfluenza viruses, avian encephalomyelitis virus, infectious laryngotracheitis virus, avian reticuloendotheliosis virus, Marek's disease virus, infectious bursal disease virus, avian reovirus, avian adenoviruses, avian influenza virus, *Haemophilus paragallinarum, Salmonella gallinarum, Salmonella pullorum, Mycoplasma gallisepticum, Mycoplasma synoviae* and other agents pathogenic for birds.

> In some countries, all birds are bled when a colony is established, and thereafter 5% of the birds are bled each month. The serum samples are screened for antibodies to relevant pathogens. Any bird that dies is investigated to determine the cause of death.

A.4.1.4 Human diploid cells

If human diploid cells are used for the propagation of measles virus, 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 at least up to the population doubling level at which the cells are used to propagate the measles virus (production cell cultures) and identity. The cells shall not be used beyond the highest population doubling level shown to be capable of meeting the requirements of Appendix 2.

A.4.1.5 Other cells

In some countries, dog kidney cells are used for production. They should be obtained from four- to six-week-old dogs (usually Beagles) from a specific-pathogen-free colony that is monitored at regular intervals and shown to be free from rabies, distemper, canine hepatitis, listeriosis, leptospirosis, brucellosis, salmonellosis, tuberculosis, toxoplasmosis and mycoplasmas. Animals whose kidneys have been removed should undergo autopsy. Supernatants from their minced kidney and liver tissue should be used to inoculate medium capable of detecting *Mycobacterium tuberculosis* as well as guinea-pigs to be tested after 21 days for anti-leptospira antibodies and after 42 days for tuberculosis. Kidneys derived from animals belonging to one litter may be processed together to prepare primary cell cultures.

A.4.1.6 Serum used in cell-culture medium

Serum used for the propagation of cells for measles 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) (*11*) and to demonstrate freedom from viruses.

Suitable tests for detecting viruses in calf and newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*12*).

Serum of bovine origin must come from herds certified to be free of bovine spongiform encephalopathy and bovine leukosis.

Serum shall also be shown to be free from inhibitors of measles 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) (*13*).

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

A.4.1.7 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. The methods used to ensure this shall be approved by the national control authority.

A.4.2 *Virus seed*

The production of vaccine shall be based on the virus 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 measles 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.

The methods used to produce single harvests from the working seed lot shall be identical to those 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 and A.4.4 (with the exception of the tests for added substances and residual animal serum proteins in section A.4.4.5).

Tests for neurovirulence. Each master seed or working seed lot shall be shown to be free from neurovirulence by tests in measles-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 measles. The material under test should be given by injection of 0.5 ml into the thalamic region of each hemisphere. The total amount of measles 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, even from non-specific causes. At the end of the observation period each monkey is bled and its serum tested for anti-measles antibody. All test animals are anaesthetized and killed for autopsy; histopathological examinations of appropriate areas of the brain are made for evidence of central nervous system involvement.

As a check against the inadvertent introduction of wild measles virus, at least four measles-susceptible uninoculated monkeys should be maintained as a control, either as cage mates of, or within the same immediate area as, the inoculated test animals for the entire period of observation (17–21 days) plus an additional 10 days. Serum samples should be taken from the control monkeys at the time of inoculation of the test animals and again 10 days after the test animals are killed.

The virus seed lot passes the test if: (a) at least 80% of the inoculated monkeys are serologically positive for measles and all the serum samples from the control monkeys are shown to be free from anti-measles antibody; and (b) there is no clinical or histopathological evidence of involvement of the central nervous system attributable to the injected virus.

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 cell cultures*

From the cells used to prepare the cell cultures for growing attenuated measles virus, an amount of processed cell suspension at least equivalent to 5% of the total volume or 500 ml, whichever is the greater, shall be used to prepare control cultures of uninfected cells. These control cultures shall be observed microscopically for changes atrributable 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 the last virus harvest, whichever is the later. At the end of the observation period, fluids collected from the control cultures shall be pooled and tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at or below -60 °C.

In some countries, samples of fluid from each control vessel are collected at the same time as fluid is harvested from the corresponding production vessels. If several virus harvests are made from the same production cell batch, the control fluid taken at the time of each harvest is frozen and stored at or below -60 °C until the last virus harvest from that production cell batch is completed. The control fluids are then pooled in proportion to their amounts and submitted to the required tests.

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 a test 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. 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 IV 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 both human and simian cells.

Cell monolayers 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 Additional tests if chick cell cultures are used for production

If chicken cell cultures are used, a sample of fluids pooled from the control cultures shall be tested for adenoviruses and for avian retroviruses such as avian leukosis virus, by a method approved by the national control authority.

> Satisfactory procedures for testing for avian leukosis virus include tests for detecting the resistance-inducing factor (RIF), complement-fixation tests (CF) and enzyme-linked immunosorbent assays (ELISA).

The control cultures pass the test if there is no evidence of the presence of virus.

A certificate of freedom from avian leukosis virus and adenovirus provided by the supplier of the fertile eggs may satisfy the licensing authority.

A.4.3.4 Additional tests if human diploid cells are used for production

If human diploid cells are used for production, the cell cultures shall be identified as human by tests 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.

A.4.4 *Production and harvest of vaccine virus*

A.4.4.1 Cells used for vaccine production

On the day of inoculation with the seed lot virus, each production cell culture and control 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 medium for the cell cultures, the serum shall be removed from the cell cultures either before or after inoculation with the seed virus. Before the virus is harvested, the cell cultures shall be rinsed and the growth medium replaced with serum-free maintenance medium.

Penicillin or 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

Virus fluids shall be harvested by a method approved by the national control authority. A single harvest may be a combination of several consecutive harvests from one production cell culture. Single harvests are usually partly or fully stabilized and stored at or below -60 °C until pooling. No antibiotics shall be added at the time of harvesting nor at any later stage of manufacturing. Samples of single harvests shall be taken for testing for sterility and virus content; if not tested immediately, they shall be kept at or below -60 °C until testing is done.

Sterility tests. A volume of at least 20 ml of each single harvest 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) (*11*), 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.

Virus titration. The live virus content of each single harvest shall be determined by cell culture titration, using a reference preparation of live measles virus, the titre of which has been determined by comparison with the International Reference Reagent (see section A.1.3). Minimum acceptable titres should be established.

A.4.4.3 Virus pool

The virus pool shall be prepared from one or several single harvests 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.

In tests that require prior neutralization of measles virus, the antiserum used shall not be of human, simian or avian origin. The immunizing antigen used to prepare the antiserum shall be produced in cell cultures from a species different from that used for vaccine production. These cell cultures shall be free from extraneous microbial agents that might elicit antibodies inhibitory to the growth of any extraneous agents that may be present in the measles virus pool. They shall be tested for extraneous agents as specified in paragraphs 2 and 3 of section A.4.3.2.

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

Virus titration. The live virus content of each virus pool shall be determined by titration in cell culture against a reference preparation of live measles virus (see section A.1.3).

Tests of neutralized virus pool in cell cultures. A volume of each virus pool equivalent to at least 500 human doses or 50 ml, whichever represents the greater volume, shall be neutralized by specific antiserum and shall be tested for adventitious agents by inoculation of simian cell cultures. Similar volumes of the neutralized virus pool shall be tested likewise in human cell cultures and in cell cultures of the same type, but not of the same batch, as those used to prepare the virus pool. Uninoculated cell cultures shall be kept as a control. All cell cultures shall be observed for 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.

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.

Additional tests if chick cell cultures are used for production. A volume of each virus pool at least equivalent to 100 human doses of vaccine or 10 ml, whichever represents the greater volume, shall be tested in a group of embryos of fertilized chickens' eggs by the allantoic route of inoculation, and a similar sample shall be tested in a separate group of eggs by the yolk-sac route of inoculation. In both cases 0.5 ml of inoculum shall be used per egg.

The virus pool passes the test if, at the end of a three- to seven-day observation period, there is no evidence of the presence of any adventitious agents. If an adventitious agent is detected in the uninoculated controls, the test may be repeated.

A.4.4.4 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 from the pool for virus titration and sterility testing. If not tested immediately, the samples shall be kept at or below -60 °C until testing is done.

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 measles virus (see section A.1.3).

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

A.4.4.5 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 shall pass the tests specified in sections A.4.4.3 and A.4.4.4.

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

> The final bulk may be assayed for virus content.

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) (*11*), 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 given in Good Manufacturing Practices for Biological Products (*10*) 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, except for mass immunization campaigns.

A.6 Control tests on final product

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

A.6.1 *Identity tests*

The virus content of two or more individually labelled final containers, taken from the freeze-dried lot, shall be identified as measles 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) (*11*), or by acceptable methods approved by the national control authority.

A.6.3 *Virus concentration and thermostability*

The virus content in each of at least three containers selected at random from the freeze-dried lot shall be determined individually against a reference preparation of measles vaccine (see section A.1.3).

An additional three containers from the final freeze-dried vaccine lot shall be incubated at 37 °C for seven days. The geometric mean infectious virus titre of these vials shall be equal to or greater than the required minimum number of infective units per human dose, and the geometric mean virus titre of the vaccine shall not have decreased by more than 1.0 $\log_{10}$ infectious units during the period of incubation. Titration of non-exposed and exposed vials shall be made in parallel and results expressed in terms of PFU or $CCID_{50}$ per human dose. A reference reagent for measles virus, the titre of which has been determined by comparison with the International Reference Reagent (see section A.1.3), shall be included in each assay.

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 suitable test for virus content is described in Appendix 3.

The national control authority shall determine the minimum and maximum amount of vaccine virus that one human dose should contain.

The minimum quantity is generally considered to be 1000 viral infective units. Epidemiological studies have linked the use of high-titre vaccine with adverse events. In at least one country, however, the minimum dose has been set at 5000 infective units.

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 the 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*

Each container shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 27-28) shall apply.

A.8 Samples

The requirements of Good Manufacturing Practices for Biological Products (*10*, page 29, paragraph 9.5) shall apply.

A.9 Labelling

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 26-27) shall apply, with the addition of the following:

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

- a statement that the vaccine fulfils Part A of these Requirements;
- a statement of the nature of the preparation, specifying the strain of measles virus in the vaccine, the minimum number of infective units per human dose, and the origin of the substrate used to prepare the vaccine;
- a statement of the nature and quantity of any antibiotic present in the vaccine;
- a statement concerning the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;
- a statement indicating the volume and nature of diluent[1] to be added to reconstitute the vaccine, and specifying that the diluent should be supplied by the manufacturer;
- a statement that, after the vaccine is reconstituted, it should be used immediately or be stored between 0 °C and 8 °C in the dark for a period not exceeding eight hours.

A.10 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*10*) 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

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required by Good Manufacturing Practices for Biological Products (*10*, pages 26-27), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

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

[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.

loss of virus titre. After distribution, live measles vaccine shall be stored at all times at a temperature below 8 °C. Storage below 0 °C is permitted.

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 contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*14*) shall apply.

The national control authority shall give directions to manufacturers concerning the measles 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.

In addition, the national control authority shall provide, for virus titration (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3), a reference preparation of live measles virus calibrated against the International Reference Reagent (see section A.1.3) and shall specify the virus content required to achieve adequate immunization of humans with the recommended human dose.

Protocols reporting the results of tests (Appendix 1) should be available for examination by the national control authority.

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 4.

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

II. Requirements for mumps vaccine

General considerations

Mumps vaccines have been shown to induce long-term protective immunity. In the United States of America, immunity induced by vaccines based on the Jeryl Lynn strain has apparently persisted for at least 20 years, while there is evidence from Japan that immunity of at least 10 years' duration is produced by vaccines containing the Urabe strain. In the United States of America widespread use of mumps vaccine since 1967 has dramatically reduced the reported incidence of mumps.

Several strains of attenuated mumps virus have been developed for use in vaccines. The Jeryl Lynn strain, which is grown in chick-embryo cell cultures, was licensed in the United States of America in 1967 and by 1992 had been given to approximately 135 million children and adults throughout the world. It induces seroconversion in at least 97% of children and at least 93% of adults, whether used singly or in combination with measles and rubella vaccines.

Vaccines based on the Leningrad-3 strain of attenuated mumps virus have been in use since 1974 in the former USSR, where they are produced in cell cultures of Japanese quail embryo, and have subsequently been introduced in other countries. The L-Zagreb vaccine produced in chick embryo cells has been used in the former area of Yugoslavia since 1976. About 8-11 million doses of vaccine based on the Leningrad-3 strain are produced annually as monovalent vaccine or in combination with measles and rubella vaccines.

The Urabe strain of attenuated live mumps vaccine was first licensed for production in 1979 in Japan and thereafter in Belgium, France and Italy. It is produced either in the amnion of embryonated hens' eggs or in chick-embryo cell cultures. By 1991, more than 60 million people had been immunized with the Urabe strain in Japan and other countries. Its immunogenic properties are similar to those of the Jeryl Lynn strain used as a monovalent product or in combination with measles and rubella vaccines.

The Rubini strain is the only mumps vaccine strain grown in human diploid cells. It was approved in Switzerland in 1985 and by 1990 more than four million people had been immunized with it throughout the world. Since the introduction of Rubini mumps vaccine in Switzerland, mumps cases have shown a downward trend.

Three additional strains of attenuated mumps vaccine have been approved for production in Japan: the Hoshino and Torii Miyahara strains, which are grown in chick-embryo cell culture, and the NK-M46 strain. These strains have been less extensively used than the Jeryl Lynn, Urabe, Leningrad-3, L-Zagreb and Rubini strains. Recent advances in molecular biology have permitted the characterization of different strains.

Lymphocytic meningitis has been associated with the administration of certain mumps vaccine strains (in particular the Urabe strain), as occasionally confirmed by sequencing of the genes of viral isolates from the cerebrospinal fluid. It is important to note that the prevalence is low and demonstrating the association of vaccination with meningitis is highly dependent on the numbers of subjects vaccinated, medical practices with regard to frequency of lumbar puncture, and the quality of follow-up observations. Lymphocytic meningitis after vaccination has often been difficult to detect and is benign in outcome. Active surveillance is necessary to establish the precise incidence of adverse reactions.

For the standardization of the infectious virus content of mumps vaccines, there is a need for an international reference preparation against which the titre can be assessed. A reference preparation of antibody to mumps virus is also needed.

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 health administration 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 included as Appendix 5.

Should individual countries wish to adopt these Requirements as the basis of their national regulations for mumps 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. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum parotitidis vivum". The proper name in the country's language shall be the equivalent of the international name.

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

A.1.2 *Descriptive definition*

"Vaccinum parotitidis vivum" is a preparation of live attenuated mumps virus grown in avian embryo cells or other suitable cells. The preparation shall satisfy all the requirements formulated below.

At present, live mumps vaccines are blended with an appropriate stabilizer and lyophilized. They are available as monovalent vaccines or in combination with live measles and live rubella vaccines.

A.1.3 *International reference materials*

Since no international reference materials have been established for live mumps vaccine, national control authorities should provide a reference preparation of live mumps virus for validating tests to determine virus content (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3).

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

Working seed lot: A quantity of virus of uniform composition, fully characterized, 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, animal or other 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 number 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 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 mumps virus used in the production of mumps 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 was demonstrated by clinical evaluation.

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

The lowest immunizing dose of vaccine that induces seroconversion in susceptible individuals shall be established in a dose-response study. This dose shall serve as a basis for establishing parameters for stability and expiry of the vaccine.

A.3 General manufacturing requirements

The requirements of Good Manufacturing Practices for Pharmaceutical (*9*) and Biological (*10*) Products and the Requirements for Human Diploid Cells Used for the Production of Measles, Mumps and Rubella Vaccines (Live) (Appendix 2) shall apply to establishments manufacturing live mumps vaccine, with the addition of the following requirements.

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

Mumps 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 mumps. Production and control shall be organized as two separate units of the manufacturing establishment with independent responsibilities.

Only the virus seed lot and substrates approved by the national control authority for the production of mumps 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 (*10*) 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 (*10*) shall apply to the manufacture of mumps vaccine.

A.4.1 *Source materials*

A.4.1.1 Strain of mumps virus

The strain of mumps virus used in the production of live mumps vaccine shall be certified according to the specifications of section A.2. The vaccine strain shall be approved by the national control authority. The virus shall at no time have been passaged in a continuous cell line. The seed lot or five consistent lots of vaccine derived from the seed lot shall have been shown to be non-neuropathogenic in monkeys (see section A.4.2.1) and to yield live mumps vaccine of adequate immunogenicity and safety in human beings.

A.4.1.2 Cell cultures

Mumps virus used in the production of mumps vaccine shall be propagated in cell cultures approved by the national control authority. All information on the source and method of preparation of the cell-culture system used shall be available to the national control authority.

> In some countries biochemical tests for the detection of RNA viruses are applied to production cell cultures.

A.4.1.3 Avian embryos and cell cultures

If avian embryos or cell cultures are used for the propagation of mumps vaccine virus, the eggs used shall be derived from a closed, specific-pathogen-free, healthy flock. This flock shall be monitored at regular intervals for *Mycobacterium avium*, fowlpox, avian retroviruses, Newcastle disease virus and other avian parainfluenza viruses, avian encephalomyelitis virus, infectious laryngotracheitis virus, avian reticu-

loendotheliosis virus, Marek's disease virus, infectious bursal disease virus, avian reovirus, avian adenoviruses, avian influenza virus, *Haemophilus paragallinarum, Salmonella gallinarum, Salmonella pullorum, Mycoplasma gallisepticum, Mycoplasma synoviae* and other agents pathogenic for birds.

In some countries, all birds are bled when a colony is established, and thereafter 5% of the birds are bled each month. The serum samples are screened for antibodies to relevant pathogens. Any bird that dies is investigated to determine the cause of death.

A.4.1.4 Human diploid cells

If human diploid cells are used for the propagation of mumps virus, 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 at least up to the population doubling level at which the cells are used to propagate the mumps virus (production cell cultures) and identity. The cells shall not be used beyond the highest population doubling level shown to be capable of meeting the requirements of Appendix 2.

A.4.1.5 Serum used in cell-culture medium

Serum used for the propagation of cells for mumps 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) (*11*) and to demonstrate freedom from viruses.

Suitable tests for detecting viruses in calf and newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*12*).

Serum of bovine origin must come from herds certified to be free of bovine spongiform encephalopathy and bovine leukosis.

Serum shall also be shown to be free from inhibitors of mumps 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) (*13*).

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

A.4.1.6 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. The methods used to ensure this shall be approved by the national control authority.

A.4.2 ***Virus seed***

The production of vaccine shall be based on the virus 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 mumps 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.

The methods used to produce single harvests from the working seed lot shall be identical to those 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. and A.4.4 (with the exception of the tests for added substances and residual animal serum proteins in section A.4.4.5).

Test for neurovirulence. Each master seed or working seed lot shall be shown to be free from neurovirulence by tests in mumps-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 mumps. The material under test should be given by injection of 0.5 ml into the thalamic region of each hemisphere. The total amount of mumps 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, even from non-specific causes. At the end of the observation period each monkey is bled and its serum tested for anti-mumps antibody. All test animals are anaesthetized and killed for autopsy; histopathological examinations of appropriate areas of the brain are made for evidence of central nervous system involvement.

The virus seed lot passes the test if: (a) at least 80% of the inoculated monkeys are serologically positive for mumps; and (b) there is no clinical or histopathological evidence of involvement of the central nervous system attributable to the injected virus.

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 cell cultures***

From the cells used to prepare the cell cultures for growing attenuated mumps virus, an amount of processed cell suspension at least equivalent to 5% of the total volume or 500 ml, whichever is the greater, 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 the last virus harvest, whichever is the later. At the end of the observation period, fluids collected from the control cultures shall be pooled and tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at or below -60 °C.

In some countries, samples of fluid from each control vessel are collected at the same time as fluid is harvested from the corresponding production vessels. If several virus harvests are made from the same production cell batch, the control fluid taken at the time of each harvest is frozen and stored at or below -60 °C until the last virus harvest from that production cell batch is completed. The control fluids are then pooled in proportion to their amounts and submitted to the required tests.

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

For a test 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. 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 IV 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 both human and simian cells.

Cell monolayers 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 Additional test if avian-embryo cell cultures are used for production

If avian-embryo cell cultures are used, a sample of fluids pooled from the control cultures shall be tested for adenoviruses and for avian retroviruses such as avian leukosis virus, by a method approved by the national control authority.

Satisfactory procedures for testing for avian leukosis virus include tests for detecting the resistance-inducing factor (RIF), complement-fixation tests (CF) and enzyme-linked immunosorbent assays (ELISA).

The control cultures pass the test if there is no evidence of the presence of virus.

A certificate of freedom from avian leukosis virus and adenovirus provided by the supplier of the fertile eggs may satisfy the licensing authority.

A.4.3.4 Additional tests if human diploid cells are used for production

If human diploid cells are used for production, the cell cultures shall be identified as human by tests 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.

A.4.3.5 Embryonated eggs

Of each batch of eggs used for vaccine production, 2% (or 20 eggs, whichever is the larger quantity) 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 virus harvest, a sample of 0.25 ml of amniotic fluid taken from each control egg shall be tested for haemagglutinating agents by the addition of chick erythrocytes, both directly and after one passage through specific-pathogen-free eggs. The details of the test shall be approved by the national control authority. In addition, a pool of amniotic fluid shall be tested for adventitious agents, including avian leukosis virus, by the methods specified in section A.4.3.3.

A.4.4 *Production and harvest of vaccine virus*

A.4.4.1 Cells used for vaccine production

On the day of inoculation with the seed lot virus, each production cell culture and control 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 medium for the cell cultures, the serum shall be removed from the cell cultures either before or after inoculation with seed virus. Before the virus is harvested, the cell cultures shall be rinsed and the growth medium replaced with serum-free maintenance medium.

Penicillin or 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

Virus fluids shall be harvested by a method approved by the national control authority. A single harvest may be a combination of several consecutive harvests from one production cell culture. Single harvests are usually partly or fully stabilized and stored at or below -60 °C until pooling. No antibiotics shall be added at the time of harvesting nor at any later stage of manufacturing. Samples of single harvests shall be taken for testing for sterility and virus content; if not tested immediately, they shall be kept at or below -60 °C until testing is done.

Sterility tests. A volume of at least 20 ml of each single harvest 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) (*11*), 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.

Virus titration. The live virus content of each single harvest may be determined by cell culture titration, using a reference preparation of live mumps virus, the titre of which has been determined by comparison with a reference preparation approved by the national control authority (see section A.1.3). Minimum acceptable titres should be established.

A.4.4.3 Virus pool

The virus pool shall be prepared from one or several single harvests 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.

In tests that require prior neutralization of mumps virus, the antiserum used shall not be of human, simian or avian origin. The immunizing antigen used to prepare the antiserum shall be produced in cell cultures from a species different from that used for vaccine production. These cell cultures shall be free from extraneous microbial agents that might elicit antibodies inhibitory to the growth of any extraneous agents that may be present in the mumps virus pool. The tests shall be carried out as specified in paragraphs 2 and 3 of section A.4.3.2.

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

Virus titration. The live virus content of each virus pool shall be determined by titration in cell culture against a reference preparation of live mumps virus (see section A.1.3).

Tests of neutralized virus pool in cell cultures. A volume of each virus pool equivalent to at least 500 human doses or 50 ml, whichever represents the greater volume, shall be neutralized by specific antiserum and shall be tested for adventitious agents by inoculation of simian cell cultures. Similar volumes of the neutralized virus pool shall be tested likewise in human cell cultures and in cell cultures of the same type, but not of the same batch, as those used to prepare the virus pool. Uninoculated cell cultures shall be kept as a control. All cell cultures shall be observed for 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.

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

Additional tests if avian eggs or cell cultures are used for production. A volume of each virus pool at least equivalent to 100 human doses of vaccine or 10 ml, whichever is the greater volume, shall be tested in a group of embryos of fertilized avian eggs by the allantoic route of inoculation, and a similar sample shall be tested in a separate group of eggs by the yolk-sac route of inoculation. In both cases 0.5 ml of inoculum shall be used per egg.

The virus pool passes the test if, at the end of a three- to seven-day observation period, there is no evidence of the presence of any adventitious agents. If an adventitious agent is detected in the uninoculated controls, the test may be repeated.

A.4.4.4 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 sterility testing. If not tested immediately, the samples shall be kept at or below −60 °C until testing is done.

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 mumps virus (see section A.1.3).

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

A.4.4.5 Final bulk

The final bulk shall be prepared from one or more clarified virus pools obtained from substrates of which controls pass the tests specified in section A.4.3. The virus pools shall pass the tests specified in sections A.4.4.3 and A.4.4.4.

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

The final bulk may be assayed for virus content.

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) (*11*), 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 for filling and containers given in Good Manufacturing Practices for Biological Products (*10*) 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, except for mass immunization campaigns.

A.6 Control tests on final product

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

A.6.1 *Identity tests*

The virus content of two or more individually labelled final containers, taken from the freeze-dried lot, shall be identified as mumps 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) (*11*), or by acceptable methods appoved by the national control authority.

A.6.3 *Virus concentration and thermostability*

The virus content in each of at least three containers selected at random from the freeze-dried lot shall be determined individually against a reference preparation of mumps vaccine (see section A.1.3).

An additional three containers from the final freeze-dried vaccine lot shall be incubated at 37 °C for seven days. The geometric mean infectious virus titre of these vials shall be equal to or greater than the required minimum number of infective units per human dose, and the geometric mean virus titre of the vaccine shall not have decreased by more than 1.0 $\log_{10}$ infectious units during the period of incubation. Titration of non-exposed and exposed vials shall be made in parallel and results expressed in terms of PFU or $CCID_{50}$ per human dose. A reference preparation of mumps virus (see section A.1.3) shall be included in each assay.

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 suitable test for virus content is described in Appendix 6.

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

Most countries use at least 10^3 $CCID_{50}$ per dose.

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 the 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*

Each container shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 27-28) shall apply.

A.8 Samples

The requirements of Good Manufacturing Practices for Biological Products (*10*, page 29, paragraph 9.5) shall apply.

A.9 Labelling

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 26-27) shall apply, with the addition of the following:

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

- a statement that the vaccine fulfils Part A of these Requirements;
- a statement of the nature of the preparation, specifying the strain of mumps virus in the vaccine, the minimum number of infective units per human dose, and the origin of the substrate used to prepare the vaccine;
- a statement of the nature and quantity of any antibiotic present in the vaccine;
- a statement concerning the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;
- a statement indicating the volume and nature of the diluent[1] to be added to reconstitute the vaccine, and specifying that the diluent should be supplied by the manufacturer;
- the statement that, after the vaccine is reconstituted, it should be used immediately or be stored between 0 °C and 8 °C in the dark for a period not exceeding eight hours.

A.10 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*10*) 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

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required by Good Manufacturing Practices for Biological Products (*10*, pages 26-27), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.11.1 *Storage conditions*

Before distribution, the manufacturer shall store lyophilized vaccine at a temperature shown by the manufacturer to be compatible with minimal

[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.

loss of virus titre. After distribution, live mumps vaccine shall be stored at all times at a temperature below 8 °C. Storage below 0 °C is permitted.

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 contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*14*) shall apply.

The national control authority shall give directions to manufacturers concerning the mumps 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.

In addition, the national control authority shall provide a reference preparation of live mumps virus (see section A.1.3) for virus titration (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3), and shall specify the virus content required to achieve adequate immunization of humans with the recommended human dose.

Protocols reporting the results of tests (Appendix 5) should be available for examination by the national control authority.

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 5, 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 4.

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

III. Requirements for rubella vaccine

General considerations

Three virus strains, attenuated by repeated passage in cell culture, have been widely used for rubella vaccine production: the HPV-77 strain, originally recovered from a young adult with typical rubella; the Cendehill strain isolated from the urine of a child with rubella; and the RA-27/3 strain recovered from the explanted tissues of a fetus obtained at therapeutic abortion because of maternal rubella virus infection. Of these, only the RA-27/3 strain grown in human diploid cells is currently in wide use. In addition, the Takahashi, Matsuura and TO-336 attenuated strains, grown in primary cultures of rabbit kidney ephithelial cells or Japanese quail embryo fibroblasts, have been used on a large scale in Japan.

All vaccines are administered by the subcutaneous route. The vaccine viruses produce infection and an immune response in approximately 95% of recipients. These infections differ from those produced by the wild virus in the limited duration and quantity of virus excretion by the pharynx, the low incidence of detectable viraemia and, as with other attenuated vaccines, the lower antibody response.

Although communicability of the vaccine-induced infections was considered a possibility, there is considerable evidence from programmes of mass vaccination that this does not occur. The clinical consequences of the natural disease (e.g. fever, rash, lymphadenopathy) are uncommon after vaccination. However, vaccine recipients may show transient joint and peripheral nervous system involvement, manifested clinically as arthralgia or arthritis, and paraesthesia of the extremities, which occur commonly in the natural disease. Such symptoms are related to age and sex and have been noted most commonly in women. The rates observed vary widely depending on the method used for observation and follow-up, but are usually less than 1% in children and less than 10% in adults. The immunity produced by the vaccine appears to be long-lasting, and antibodies have been shown to persist for at least seven years, but continued observations of vaccinated individuals will be necessary to determine the exact duration of the protective effect.

Vaccination of pregnant women with rubella vaccine is not recommended. Although to date no case of a congenital defect has been attributed to the vaccine viruses, the attenuated viruses can cross the placenta and have been recovered from the fetus. Thus, while the evidence suggests a lower risk than with the natural virus, no assurance of safety can be given to the pregnant woman. In addition, vaccination is not recommended in the two-month period before conception.

The extensive use of rubella vaccine has produced a significant decline in the number of reported cases of both rubella and the congenital rubella syndrome. With this evidence of efficacy, the use of attenuated vaccines is becoming increasingly common throughout the world. For this reason,

requirements for the production and testing of live rubella vaccine were published by WHO in 1977 (*6*).

The Requirements formulated below do not include genetic marker tests because there is no definitive laboratory test for attenuation of rubella virus analogous to the neurovirulence tests for poliomyelitis virus vaccine (oral) and yellow fever virus vaccines. Experience has shown that the maintenance of a standard operating procedure, including a standard temperature of incubation and the use of a seed lot system, results in a consistent product that shows a regular pattern of attenuation and immunogenicity. Nevertheless the search for suitable marker tests continues.

Each of 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 health administration 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 included as Appendix 7.

Should individual countries wish to adopt these Requirements as the basis of their national regulations for rubella 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. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "Vaccinum rubellae 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 rubellae vivum" is a preparation of live attenuated rubella virus grown in a suitable cell culture. The preparation shall satisfy all the requirements formulated below.

At present, live rubella vaccines are blended with an appropriate stabilizer and lyophilized. They are available for distribution only in that form, either as monovalent vaccines or in combination with live measles and/or live mumps vaccines.

A.1.3 *International reference materials*

Since no international reference materials have been established for live rubella vaccine, national control authorities should provide a reference preparation of live rubella virus for use in tests to determine virus content (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3).

The second International Reference Preparation of Human Anti-Rubella Serum is in the custody of the International Laboratory for Biological Standards, State Serum Institute, Copenhagen, Denmark. Samples are distributed free of charge to national control laboratories on request. The International Reference Preparation is intended for the calibration of national standards and reference preparations.

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

Working seed lot: A quantity of virus of uniform composition, fully characterized, 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, animal or other 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 number 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 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 rubella virus used in the production of rubella 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 was demonstrated by clinical evaluation.

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

The lowest dose of vaccine that induces seroconversion in susceptible individuals shall be established in a dose-response study. This dose shall serve as a basis for establishing parameters for stability and expiry of the vaccine.

A.3 General manufacturing requirements

The requirements of Good Manufacturing Practices for Pharmaceutical (*9*) and Biological (*10*) Products and the Requirements for Human Diploid Cells Used for the Production of Measles, Mumps and Rubella Vaccines (Live) (Appendix 2) shall apply to establishments manufacturing rubella vaccine with the addition of the following requirements.

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

Rubella 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 rubella. Production and control shall be organized as two separate units of the manufacturing establishment with independent responsibilities.

Only the virus seed lot and cell cultures approved by the national control authority for the production of rubella 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 (*10*) 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 (*10*) shall apply to the manufacture of rubella vaccine.

A.4.1. *Source materials*

A.4.1.1 Strain of rubella virus

The strain of rubella virus used in the production of live rubella vaccine shall be certified according to the specifications of section A.2. The vaccine strain shall be approved by the national control authority. The virus shall at no time have been passaged in a continuous cell line. The seed lot or five consistent lots of vaccine derived from the seed lot shall have been shown to be non-neuropathogenic in monkeys (see section A.4.2.1) and to yield live rubella vaccine of adequate immunogenicity and safety in human beings.

A.4.1.2 Cell cultures

Rubella virus used in the production of rubella vaccine shall be propagated in cell cultures approved by the national control authority. All information on the source and method of preparation of the cell-culture system used shall be available to the national control authority.

> In some countries, biochemical tests for the detection of RNA viruses are applied to production cell cultures.

A.4.1.3 Human diploid cells

If human diploid cells are used for the propagation of rubella virus, 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 at least up to the population doubling level at which the cells are used to propagate the rubella virus (production cell cultures) and identity. The cells shall not be used beyond the highest population doubling level shown to be capable of meeting the requirements of Appendix 2.

A.4.1.4 Rabbit kidney cell cultures

If rabbit kidney cell cultures are used for the propagation of rubella virus, the rabbits (*Oryctolagus cuniculus*) from which the kidneys are taken shall be from a closed colony continuously monitored for coccidiosis, myxomatosis, rabbitpox virus, fibromatosis, herpesvirus cuniculi, *Mycobacterium tuberculosis, Nosema cuniculi, Toxoplasma gondii,* rabbit kidney vacuolating virus, syncytium virus of rabbits, and other pathogenic microorganisms and viral agents naturally occurring in rabbits.

A.4.1.5 Serum used in cell-culture medium

Serum used for the propagation of cells for rubella 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) (*11*) and to demonstrate freedom from viruses.

> Suitable tests for detecting viruses in calf and newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*12*).

Serum of bovine origin must come from herds certified to be free from bovine spongiform encephalopathy and bovine leukosis.

Serum shall also be shown to be free from inhibitors of rubella 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) (*13*).

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

A.4.1.6 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. The method used to ensure this shall be approved by the national control authority.

A.4.2 *Virus seed*

The production of vaccine shall be based on the virus 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 rubella 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.

The methods used to produce single harvests from the working seed lot shall be identical to those 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 and A.4.4 (with the exception of the tests for added substances and residual animal proteins in section A.4.4.5).

Tests for neurovirulence. Each master seed or working seed lot shall be shown to be free from neurovirulence by tests in rubella-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 rubella. The material under test should be given by injection of 0.5 ml into the thalamic region of each hemisphere. The total amount of rubella 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, even from non-specific causes. At the end of the observation period each monkey is bled and its serum tested for anti-rubella antibody. All

test animals are anaesthetized and killed for autopsy; histopathological examinations of appropriate areas of the brain are made for evidence of central nervous system involvement.

The virus seed lot passes the test if: (a) at least 80% of the inoculated monkeys are serologically positive for rubella; and (b) there is no clinical or histopathological evidence of involvement of the central nervous system attributable to the injected virus.

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 cell cultures*

From the cells used to prepare the cell cultures for growing attenuated rubella virus, an amount of processed cell suspension at least equivalent to 5% of the total volume or 500 ml, whichever is the greater, 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 culture or until the time of the last virus harvest, whichever is the later. At the end of the observation period, fluids collected from the control cultures shall be pooled and tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at or below -60 °C.

In some countries, samples of fluid from each control vessel are collected at the same time as fluid is harvested from the corresponding production vessels. If several virus harvests are made from the same production cell batch, the control fluid taken at the time of each harvest is frozen and stored at or below -60 °C until the last virus harvest from that production cell batch is completed. The control fluids are then pooled in proportion to their amounts and submitted to the required tests.

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

For a test 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 cells shall be tested for the presence of haemadsorbing viruses, using guinea-pig red cells. 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 IV 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 both human and simian cells.

Cell monolayers 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 Additional test if rabbit kidney cells are used for production

A sample of control culture taken at the time of the last virus harvest shall be stained by appropriate methods for the detection of *Nosema cuniculi.*

In some countries the Giemsa stain is used, but in others the immunofluorescent technique is preferred because it appears to be more sensitive for the detection of low levels of *Nosema cuniculi* contamination.

Only if the cells are shown to be free from contamination may the corresponding harvest be used for production.

A.4.3.4 Additional tests if human diploid cells are used for production

If human diploid cells are used for production, the cell cultures shall be identified as human by tests 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.

A.4.4 ***Production and harvest of vaccine virus***

A.4.4.1 Cells used for vaccine production

On the day of inoculation with the seed lot virus, each production cell culture and control 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, cell 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 medium for cell cultures, the serum shall be removed from the cell cultures either before or after inoculation with seed virus. 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

Virus fluids shall be harvested by a method approved by the national control authority. A single harvest may be a combination of several consecutive harvests from one production cell culture. Single harvests are usually partly or fully stabilized and stored at or below -60 °C until pooling. No antibiotics shall be added at the time of harvesting nor at any later stage of manufacturing. Samples of single harvests shall be taken for testing for sterility and virus content; if not tested immediately, they shall be kept at or below -60 °C until testing is done.

Sterility tests. A volume of at least 20 ml of each single harvest 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) (*11*), 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.

Virus titration. The live virus content of each single harvest shall be determined by cell culture titration, using a reference preparation of live rubella virus, the titre of which has been determined by comparison with a reference preparation approved by the national control authority (see section A.1.3). Minimum acceptable titres should be established.

A.4.4.3 Virus pool

The virus pool shall be prepared from one or several single harvests 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.

In tests that require prior neutralization of rubella virus, the antiserum used shall not be of human, simian or avian origin. The immunizing antigen used to prepare the antiserum shall be produced in cell cultures from a species different from that used for vaccine production. These cell cultures shall be free from extraneous microbial agents that might elicit antibodies inhibitory to the growth of any extraneous agents that may be present in the rubella virus pool. The tests shall be carried out as specified in paragraphs 2 and 3 of section A.4.3.2.

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

Virus titration. The live virus content of each virus pool shall be determined by titration in cell culture against a reference preparation of live rubella virus (see section A.1.3).

Tests of neutralized virus pool in cell cultures. A volume of each virus pool equivalent to at least 500 human doses or 50 ml, whichever represents the greater volume, shall be neutralized by specific antiserum and shall be tested for adventitious agents by inoculation into simian cell cultures. Similar volumes of the neutralized virus pool shall be tested likewise in human cell cultures and in cell cultures of the same type, but not of the same batch, as those used to prepare the virus pool. Uninoculated cell cultures shall be kept as a control. All cell cultures shall be observed for 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.

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

Additional test if rabbit kidney cell cultures are used for production. A minimum of 15 ml of each virus pool shall be tested by inoculation of at least five healthy rabbits, each weighing 1500-2500 g. Each rabbit shall receive intradermal injections in multiple sites of a total of 1.0 ml of the virus pool and a subcutaneous injection of 2.0 ml of the virus pool, and the animals shall be observed for at least 30 days. Any rabbit that dies after the first 24 hours of the test or is killed because of illness shall undergo autopsy and the brain and organs shall be removed and examined.

The virus pool is satisfactory if at least 80% of the rabbits remain healthy and survive the observation period, and provided that none of the rabbits used in the test show lesions of any kind at the sites of inoculation or evidence of any viral infection.

A.4.4.4 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 sterility testing. If not tested immediately, the samples shall be kept at or below -60 °C until testing is done.

Virus titration. The live virus content of the clarified virus pool shall be determined by titration in cell cultures against a reference preparation of live rubella virus (see section A.1.3).

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

A.4.4.5 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 shall pass the tests specified in sections A.4.4.3 and A.4.4.4.

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

The final bulk may be assayed for virus content.

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, section 5.2 and 5.3 of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (*11*), 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 given in Good Manufacturing Practices for Biological Products (*10*) 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, except for mass immunization campaigns.

A.6 Control tests on final product

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

A.6.1 *Identity tests*

The virus content of two or more individually labelled final containers, taken from the freeze-dried lot, shall be identified individually as rubella 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 (General Requirements for the Sterility of Biological Substances) (*11*), or by methods approved by the national control authority.

A.6.3 *Virus concentration and thermostability*

The virus content in each of at least three ampoules selected at random from the freeze-dried lot shall be determined individually against a reference preparation of rubella vaccine (see section A.1.3).

An additional three containers from the final freeze-dried vaccine lot shall be incubated at 37 °C for seven days. The geometric mean infectious virus titre of these vials shall be equal to or greater than the required minimum number of infective units per human dose, and the geometric mean virus titre of the vaccine shall not have decreased by more than 1.0 $\log_{10}$ infectious units during the period of incubation. Titration of non-exposed and exposed vials shall be made in parallel and results expressed in terms of PFU or $CCID_{50}$ per human dose. A reference preparation of rubella virus (see section A.1.3) shall be included in each assay.

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

A suitable test for virus content is described in Appendix 8.

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

Most countries use at least 10^3 $CCID_{50}$ per dose.

A.6.4 *General safety tests*

Each final lot shall be tested for 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 the 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*

Each container shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 27-28) shall apply.

A.8 Samples

The requirements of Good Manufacturing Practices for Biological Products (*10*, page 29, paragraph 9.5) shall apply.

A.9 Labelling

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 26-27) shall apply, with the addition of the following:

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

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

- a statement indicating the volume and nature of diluent[1] to be added in order to reconstitute the vaccine, and specifying that the diluent should be supplied by the manufacturer;
- a statement that, after the vaccine is reconstituted, it should be used immediately or be stored between 0 °C and 8 °C in the dark for a period not exceeding eight hours;
- a statement that the vaccine must not be given to a pregnant woman and that a woman must not become pregnant within two months of receiving the vaccine.

A.10 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*10*) 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

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required by Good Manufacturing Practices for Biological Products (*10*, pages 26-27), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

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 rubella vaccine shall be stored at all times at a temperature below 8 °C. Storage below 0 °C is permitted.

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 contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*14*) shall apply.

[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.

The national control authority shall give directions to manufacturers concerning the rubella 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.

In addition, the national control authority shall provide a reference preparation of live rubella virus (see section A.1.3) for virus titration (see sections A.4.4.2, A.4.4.3, A.4.4.4 and A.6.3), and shall specify the virus content required to achieve adequate immunization of humans with the recommended human dose.

Protocols reporting the results of tests (Appendix 7) should be available for approval by the national control authority.

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 7, 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 4.

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

IV. Requirements for measles–mumps–rubella (MMR) combined vaccine

General considerations

Requirements for combined vaccines against measles, mumps and rubella (MMR) must include the tests applicable to the monovalent final bulks incorporated into the final product. However, further tests are required after blending, because of the possible effects that both diluents and stabilizers may have on the potency and stability of the final product. The tests to be carried out on combined vaccines are on the whole the same as those specified for the individual components, so that cross-references to the Requirements for these components would suffice in many cases.

No international reference materials exist for the combined vaccine. For each component, the live virus concentration is determined by titration

against a reference preparation in a suitable cell-culture system, after selective neutralization, as necessary, of the other two components with appropriate antisera.

The requirements for filling, sampling, labelling, transportation, distribution and storage are the same for the combined vaccine as for the individual (measles, mumps and rubella) vaccines. The validity period of combined vaccines is determined by the component with the shortest shelf-life.

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 health administration 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 included as Appendix 9.

Should individual countries wish to adopt these Requirements as the basis of their national regulations for combined 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. The World Health Organization should then be informed of the action taken.

Part A. Manufacturing requirements

A.1 Descriptive definition

Measles, mumps and rubella combined vaccine (live) is a mixed preparation containing suitable attenuated strains of live measles, mumps and rubella viruses. The combined vaccine shall satisfy all the requirements formulated below.

A.2 Manufacturing requirements for MMR vaccine

A.2.1 *Final bulks of individual components*

Final bulks of the individual components shall be prepared and tested as follows: for measles components the Requirements for Measles Vaccine shall apply; for mumps components the Requirements for Mumps Vaccine shall apply; for rubella components, the Requirements for Rubella Vaccine shall apply.

A.2.2 *Final bulk of combined components*

The final bulk of combined virus suspension shall be prepared from final bulks of the individual components that satisfy the requirements of section A.4.4.5 of the Requirements for each of the individual vaccines.

Only stabilizers, diluents and other substances approved by the national control authority shall be added to the vaccine. Any such substances shall have been shown by appropriate tests to have, in the amounts used, no deleterious effects on the product.

The final bulk may be assayed for virus content.

A.2.2.1 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.

A.2.2.2 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) (*11*), or by a method approved by the national control authority.

A.2.2.3 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.3 Filling and containers

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (*10*) 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, except for mass immunization campaigns.

A.4 Control tests on final product

The following tests shall be carried out on the final product.

A.4.1 *Sterility test*

Each final lot 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) (*11*), or by a method approved by the national control authority.

A.4.2 *Virus concentration, thermostability and identity*

The live virus concentration in each freeze-dried final lot shall be determined by titration in suitable cell-culture systems against appropriate reference preparations of individual vaccine components. The titration of each individual component shall be carried out after selective neutralization, as necessary, of the other two components with specific antisera. The virus content of at least three containers selected at random from each freeze-dried lot shall be determined individually. The minimum acceptable virus titre per human dose shall be approved by the national control authority.

> The other virus vaccine components have been observed to interfere with mumps virus growth in individuals immunized with MMR vaccines who have shown a suboptimal immune response to mumps virus; the mumps virus concentration in the combined vaccine may therefore have to be higher than that in the monovalent mumps vaccine.
>
> A suitable virus titration assay is described in Appendix 10.

The thermostability of MMR vaccine shall be tested by incubation of at least three containers of each final freeze-dried vaccine lot at 37 °C for seven days. At the end of this period, each individual component shall be titrated as outlined above after selective neutralization, as necessary, of the other two components.

The requirements for virus concentration and thermostability in section A.6.3 of the Requirements for each of the individual components shall apply.

The successful neutralization of individual vaccine components during the virus titration shall serve as an identity test.

A.4.3 *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.4.4 *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.

> Moisture levels of less than 2% are usually considered satisfactory.

A.4.5 *Inspection of final containers*

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

A.5 Records

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 27-28) shall apply.

A.6 Samples

The requirements of Good Manufacturing Practices for Biological Products (*10*, page 29, paragraph 9.5) shall apply.

A.7 Labelling

The requirements of Good Manufacturing Practices for Biological Products (*10*, pages 26-27) shall apply, with the addition of the following:

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

- a statement that the vaccine fulfils Part A of these Requirements;
- a statement of the nature of the preparation, specifying the strains of measles, mumps and rubella viruses in the vaccine, the minimum number of infective units of each virus per human dose, and the origin of the substrates used to prepare the vaccine;
- a statement of the nature and quantity of any antibiotic present in the vaccine;
- a statement concerning the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;
- a statement indicating the volume and nature of diluent[1] to be added in order to reconstitute the vaccine, and specifying that the diluent should be supplied by the manufacturer;
- a statement that, after the vaccine is reconstituted, it should be used immediately or be stored between 0 °C and 8 °C in the dark for a period not exceeding eight hours;
- a statement that the vaccine must not be given to a pregnant woman and that a woman must not become pregnant within two months of receiving the vaccine.

A.8 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (*10*) shall apply.

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

[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.

A.9 Storage and expiry date

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required by Good Manufacturing Practices for Biological Products (*10*, pages 26-27), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

A.9.1 *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 MMR vaccine shall be stored at all times at a temperature below 8 °C. Storage below 0 °C is permitted.

A.9.2 *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 contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (*14*) shall apply.

The national control authority shall give directions to manufacturers concerning the measles, mumps and rubella 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.

In addition, the national control authority shall provide a reference preparation of live measles, mumps and rubella vaccine for virus titration (see section A.4.2) 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 9, 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 4.

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

Authors

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

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References

1. Requirements for Measles Vaccine (Live) and Measles Vaccine (Inactivated) (Requirements for Biological Substances No. 12). In: *WHO Expert Committee on Biological Standardization. Eighteenth Report.* Geneva, World Health Organization, 1966, Annex 2 (WHO Technical Report Series, No. 329).

2. Requirements for Measles Vaccine (Live) (Requirements for Biological Substances No. 12), Addendum 1981. In: *WHO Expert Committee on Biological Standardization. Thirty-second Report.* Geneva, World Health Organization, 1982, Annex 6 (WHO Technical Report Series, No. 673).

3. Requirements for Measles Vaccine (Live) (Requirements for Biological Substances No. 12, revised 1987). In: *WHO Expert Committee on Biological Standardization. Thirty-eighth Report.* Geneva, World Health Organization, 1988, Annex 5 (WHO Technical Report Series, No. 771).

4. Requirements for Mumps Vaccine (Live) (Requirements for Biological Substances No. 38). In: *WHO Expert Committee on Biological Standardization. Thirty-seventh Report.* Geneva, World Health Organization, 1987, Annex 7 (WHO Technical Report Series, No. 760).

5. *WHO Expert Committee on Biological Standardization. Twenty-third Report.* Geneva, World Health Organization, 1971: 18 (WHO Technical Report Series, No. 463).

6. Requirements for Rubella Vaccine (Live) (Requirements for Biological Substances No. 24). In: *WHO Expert Committee on Biological Standardization. Twenty-eighth Report.* Geneva, World Health Organization, 1977, Annex 3 (WHO Technical Report Series, No. 610).

7. Requirements for Rubella Vaccine (Live) (Requirements for Biological Substances No. 24), Addendum 1980. In: *WHO Expert Committee on Biological Standardization. Thirty-first Report.* Geneva, World Health Organization, 1981, Annex 12 (WHO Technical Report Series, No. 658).

8. A review of tests on virus vaccines. Report of a Group of Consultants, Geneva, 17–19 November 1980. In: *WHO Expert Committee on Biological Standardization. Thirty-second Report.* Geneva, World Health Organization, 1982, Annex 3 (WHO Technical Report Series, No. 673).

9. 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).

10. 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).

11. 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).

12. Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7, revised 1989). In: *WHO Expert Committee on Biological Standardization. Fortieth Report.* Geneva, World Health Organization, 1990, Annex 1 (WHO Technical Report Series, No. 800).

13. Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (Requirements for Biological Substances No. 27, revised 1992). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 2 (WHO Technical Report Series, No. 840).

14. Guidelines for national authorities on quality assurance for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second Report.* Geneva, World Health Organization, 1992, Annex 2 (WHO Technical Report Series, No. 822).

Appendix 1

Summary protocol for production and testing of measles 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, a copy of the leaflet that accompanies the vaccine container, and 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 4).

Source materials (A.4.1)

Strain of measles virus (A.4.1.1) _____

Cell cultures (A.4.1.2)

Provide information on the source and method of preparation of the cell cultures.

Avian-embryo cell cultures (A.4.1.3)

Provide information on the source of the closed, specific-pathogen-free, healthy flock.

Types of test for infections _____ Results _____

Certified satisfactory _____ Date _____

Signature of head of laboratory _____

Human diploid cells (A.4.1.4)

Provide information on the source of the manufacturer's working cell bank (MWCB) (see Appendix 2).

Other cells (A.4.1.5) _____

Serum used in cell-culture medium (A.4.1.6)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

[1] Based on Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No. 840).

Tests for adventitious agents

Methods _____

Date of inoculation _____

Results _____

Trypsin used for preparing cell cultures (A.4.1.7)

Sterility tests

	bacteria	*mycoplasmas*
Date of inoculation	_____	_____
Results	_____	_____

Tests for adventitious agents (including porcine parvoviruses)

Methods _____

Date of inoculation _____

Results _____

Production of the working seed lot (A.4.2)

Summary information

Name and address of manufacturer _____

_____

Virus strain _____

Reference no. of virus seed used to prepare manufacturer's original measles 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 _____

History of vaccine strain

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

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 measles vaccine virus no. ___ satisfies Part A, sections 2 to 4.4.5, of the Requirements for Measles Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Control 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)

Cell substrate used for virus growth

Type of cells _____

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Human cells

Type of cells _____

Date of inoculation _____

Results _____

Additional tests if chick cell cultures are used for production (A.4.3.3)

Test for avian adenoviruses

Method _____

Date _____

Results _____

Test for avian leukosis virus

Method _____

Date _____

Results _____

Additional tests if human diploid cells are used for production (A.4.3.4)

Identity test

Method _____

Date _____

Results _____

Production and harvest of vaccine virus (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.

No. of passages from the primary seed _____

Reference no. of single harvest _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Virus pool (A.4.4.3)

Reference no. of virus pool _____

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

Tests for neurovirulence[1] (A.4.2.1)

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) _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

[1] Only for master seed or working seed lot.

Tests of neutralized virus pool in cell cultures

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

Cells used for virus growth
- Type of cells _____
- Date of inoculation _____
- Results _____

Simian cells
- Type of cells _____
- Date of inoculation _____
- Results _____

Human cells
- Type of cells _____
- Date of inoculation _____
- Results _____

Additional tests if chick cell cultures are used for production

Test in embryonated eggs inoculated by allantoic route
- No. and age of eggs inoculated _____
- Date _____
- Results _____

Test in embryonated eggs inoculated by yolk-sac route
- No. and age of eggs inoculated _____
- Date _____
- Results _____

Clarification of the virus pool (A.4.4.4)

Date of clarification _____

Results of clarification _____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Final bulk (A.4.4.5)

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			
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			
Results			

Virus concentration and thermostability (A.6.3)

Date of inoculation _____

Type of cell cultures _____

	Control (unheated) samples			*Samples incubated at 37°C for 7 days*		
	1	*2*	*3*	*1*	*2*	*3*
Virus concentration in each container (in PFU or $CCID_{50}$)						

Mean virus titre per human dose, with 95% fiducial limits _____ _____

Mean loss in titre due to heat exposure (in $\log_{10}$ units) _____

Reference preparation

Identification _____

Theoretical titre _____

Actual titre _____

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 measles vaccine (live) satisfies national requirements and/or Part A of the Requirements for Measles Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Appendix 2

Requirements for human diploid cells used for the production of measles, mumps and rubella vaccines (live)

The following requirements concern the testing of the cell substrate for the production of measles, mumps and rubella vaccines if human diploid cells are used; they should therefore be added to or substituted for the corresponding sections in Part A of the individual Requirements, as appropriate. All the other sections in Parts A and B of the individual Requirements remain applicable.

1. Terminology

Production cell culture: 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 *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, 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 and 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. The test is not valid unless at least 80% of the animals remain healthy and survive the observation period.

> 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, which shall be examined after not less than five days. The allantoic fluids of the fertile eggs 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 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 those to be 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 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 from 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.

For vaccine production, examination of the cells is usually made between the 27th and 33rd population doubling. The national control authority should determine the permissible level of cell population doubling.

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

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 virus vaccines with the addition of the following.

[1] These upper limits are based on extensive experience with the examination of WI-38 and MRC5 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.

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 life of the cells, as determined under the particular conditions of the production establishment (see section 2.1.4).

It is advisable to ensure that both the trypsin and the animal serum used in the preparation or growth of the cell suspensions are free from extraneous agents.

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 as specified in Part A, section 5.2, of the revised 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 Commitee on Biological Standardization. Twenty-fifth Report.* Geneva, World Health Organization, 1973, Annex 4 (WHO Technical Report Series, No. 530).

Appendix 3

Proposed assay method for determining the virus content of live measles vaccine

1. Cell-culture infective dose method

Three vials of the preparation to be assayed and at least one container of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps of 0.5 $\log_{10}$ units selected so that the dilution range encompasses at least three dilutions that will infect between 10% and 90% of the cultures inoculated.

For each vaccine dilution, a volume of 0.1 ml is dispensed into each of 8-12 flat-bottomed wells in a microtitre plate, followed by 0.1 ml of a suitable cell suspension of the Vero cell line. The plates are incubated at the optimal growth temperature for the tested measles virus strain.

The presence or absence of a cytopathic effect is recorded after four days, with the final reading at 10 days. The titre is calculated as a multiple of the median cell-culture infective dose ($CCID_{50}$) by an appropriate method. If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

2. Plaque method

Three vials of the preparation to be assayed and at least one of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps selected to give at least 50% of wells with between 10 and 80 plaques per well.

For each vaccine dilution, a volume of 0.05 ml is dispensed into each of three wells in a 24-well tissue-culture plate. A separate plate is used for each of the three test samples and the reference preparation is included in each plate. The wells are seeded with 1.0 ml of an appropriate cell suspension of the Vero cell line and the plates are incubated for three hours, under 5% carbon dioxide, at the optimum growth temperature for the tested measles virus strain. The medium is then removed from each well and replaced with 1.0 ml of medium containing 0.8% carboxymethyl-cellulose or other appropriate overlay. The plates are then incubated as before for 7-10 days depending on the strain tested.

Plaques are visualized by fixing and staining the cell sheets. A convenient reagent for both purposes is 0.5% crystal violet in 20% ethanol. The medium is first removed from each well and the cell sheet gently washed

with buffer. Crystal violet in ethanol is added and the plates are left at room temperature for 20 minutes. The stain is then removed, the cells are washed gently with water and the plates are dried. The plaques are counted and wells with numbers between 10 and 80 are considered valid. The titre is calculated in plaque-forming units (PFU) per dose from the direct counts.

If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

3. Combined vaccines

For assays of the measles component in multivalent vaccines, the heterologous component(s) must first be neutralized. Reconstituted vaccine is incubated with appropriate polyclonal or monoclonal antibodies and the vaccine is then assayed in the normal way. Allowance must be made for the initial dilution effect of the neutralizing serum. (See also Appendix 10, page 201.)

Appendix 4

Model certificate for the release of measles, mumps, rubella or combined vaccines[1]

The following lots of _____[2] vaccine produced by _____[3] in _____,[4] whose numbers appear on the labels of the final containers, meet all national requirements[5] and comply with Part A[6] of Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) [if applicable, revised 19___, addendum 19___]),[7] Good Manufacturing Practices for Pharmaceutical Products[8] and the Good Manufacturing Practices for Biological Products.[9]

Lot no.	Expiry date	Lot no.	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):[10]

Signature _____

Name (typed) _____

Date _____

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

[2] Indicate type of vaccine.

[3] Name of manufacturer.

[4] Country.

[5] 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.

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

[7] WHO Technical Report Series, No. 840, 1994, Annex 3.

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

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

[10] Or his or her representative.

Appendix 5

Summary protocol for production and testing of mumps 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, a copy of the leaflet that accompanies the vaccine container, and 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 4).

Source materials (A.4.1)

Strain of mumps virus (A.4.1.1) _____

Cell cultures (A.4.1.2)

Provide information on the source and method of preparation of the cell cultures.

Avian embryos and cell cultures (A.4.1.3)

Provide information on the source of the closed, specific-pathogen-free, healthy flock.

Types of test for infections _____ Results _____

Certified satisfactory _____ Date _____

Signature of head of laboratory _____

Human diploid cells (A.4.1.4)

Provide information on the source of the manufacturer's working cell bank (MWCB) (see Appendix 2).

Serum used in cell-culture medium (A.4.1.5)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

[1] Based on Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No. 840).

Tests for adventitious agents

Methods _____

Date of inoculation _____

Results _____

Trypsin used for preparing cell cultures (A.4.1.6)

Sterility tests

	bacteria	*mycoplasmas*
Date of inoculation	_____	_____
Results	_____	_____

Tests for adventitious agents (including porcine parvoviruses)

Methods _____

Date of inoculation _____

Results _____

Production of the working seed lot (A.4.2)

Summary information

Name and adress of manufacturer _____

_____

Virus strain _____

Reference no. of virus seed used to prepare manufacturer's original mumps 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 _____

History of vaccine strain

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

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 mumps vaccine virus no. ___ satisfies Part A, sections 2 to 4.4.5, of the Requirements for Mumps Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Control 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)

Cell substrate used for virus growth

Type of cells _____

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Human cells

Type of cells _____

Date of inoculation _____

Results _____

Additional tests if avian-embryo cell cultures are used for production (A.4.3.3)

Test for avian adenoviruses

Method _____

Date _____

Results _____

Test for avian leukosis virus

Method _____

Date _____

Results _____

Additional tests if human diploid cells are used for production (A.4.3.4)

Identity test

Method _____

Date _____

Results _____

Embryonated eggs (A.4.3.5)
Provide details of test methods and results.

Production and harvest of vaccine virus (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.

No. of passages from the primary seed _____

Reference no. of single harvest _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Virus pool (A.4.4.3)

Reference no. of virus pool _____

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

Tests for neurovirulence[1] (A.4.2.1)

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) _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

[1] Only for master seed or working seed lot.

Tests of neutralized virus pool in cell cultures

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

Cells used for virus growth
- Type of cells _____
- Date of inoculation _____
- Results _____

Simian cells
- Type of cells _____
- Date of inoculation _____
- Results _____

Human cells
- Type of cells _____
- Date of inoculation _____
- Results _____

Additional tests if avian eggs or cell cultures are used for production

Test in embryonated eggs inoculated by allantoic route
- No. and age of eggs inoculated _____
- Date _____
- Results _____

Test in embryonated eggs inoculated by yolk-sac route
- No. and age of eggs inoculated _____
- Date _____
- Results _____

Clarification of the virus pool (A.4.4.4)

Date of clarification _____

Results of clarification _____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation			
Results			

Final bulk (A.4.4.5)

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			
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			
Results			

Virus concentration and thermostability (A.6.3)

Date of inoculation _____

Type of cell cultures _____

	Control (unheated) samples			*Samples incubated at 37°C for 7 days*		
	1	*2*	*3*	*1*	*2*	*3*
Virus concentration in each container (in PFU or $CCID_{50}$)						
Mean virus titre per human dose, with 95% fiducial limits						

Mean loss in titre due to heat exposure (in $\log_{10}$ units) _____

Reference preparation

Identification _____

Theoretical titre _____

Actual titre _____

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 mumps vaccine (live) satisfies national requirements and/or Part A of the Requirements for Mumps Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Appendix 6

Proposed assay method for determining the virus content of live mumps vaccine

1. Cell-culture infective dose method

Three vials of the preparation to be assayed and at least one of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps of 0.5 $\log_{10}$ units, selected so that the dilution range encompasses at least three dilutions that will infect between 10% and 90% of the cultures inoculated.

For each vaccine dilution, a volume of 0.1 ml is dispensed into each of 8–12 flat-bottomed wells in a microtitre plate, followed by 0.1 ml of a suitable cell suspension of the Vero cell line. The plates are incubated at the optimal growth temperature for the tested mumps virus strain.

The presence or absence of a cytopathic effect is recorded after four days, with the final reading at 7–10 days; if necessary, the medium should be changed after five days of incubation. The presence or absence of tissue-culture infection can also be determined by immunofluorescence. The titre is calculated as a multiple of the median cell-culture infective dose ($CCID_{50}$) by an appropriate method. If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

2. Plaque method

Three vials of the preparation to be assayed and at least one of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps selected to give at least 50% of wells with between 10 and 80 plaques per well.

For each vaccine dilution, a volume of 0.05 ml is dispensed into each of three wells in a 24-well tissue-culture plate. A separate plate is used for each of the three test samples and the reference preparation is included in each plate. The wells are seeded with 1.0 ml of an appropriate cell suspension of the Vero cell line and the plates are incubated for three hours, under 5% carbon dioxide, at the optimum growth temperature for the tested mumps virus strain. The medium is then removed from each well and replaced with 1.0 ml of medium containing 0.8% carboxymethyl-cellulose or other appropriate overlay. The plates are then incubated as before for 7–10 days depending on the strain tested.

Plaques are visualized by fixing and staining the cell sheets. A convenient reagent for both purposes is 0.5% crystal violet in 20% ethanol. The medium is first removed from each well and the cell sheet gently washed with buffer. Crystal violet in ethanol is added and the plates are left at room temperature for 20 minutes. The stain is then removed, the cells are washed gently with water and the plates are dried. The plaques are counted and wells with numbers between 10 and 80 are considered valid. The titre is calculated in plaque-forming units (PFU) per dose from the direct counts.

If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

3. Combined vaccines

For assays of the mumps component in multivalent vaccines, the heterologous component(s) must first be neutralized. Reconstituted vaccine is incubated with appropriate polyclonal or monoclonal antibodies and the vaccine is then assayed in the normal way. Allowance must be made for the initial dilution effect of the neutralizing serum. (See also Appendix 10, page 201.)

Appendix 7

Summary protocol for production and testing of rubella 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, a copy of the leaflet that accompanies the vaccine container, and 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 4).

Source materials (A.4.1)

Strain of rubella virus (A.4.1.1) _____

Cell cultures (A.4.1.2)

Provide information on the source and method of preparation of the cell cultures.

Human diploid cells (A.4.1.3)

Provide information on the source of the manufacturer's working cell bank (MWCB) (see Appendix 2).

Rabbit kidney cell cultures (A.4.1.4)

Provide information on the source and method of preparation of the cell cultures.

Serum used in cell-culture medium (A.4.1.5)

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

[1] Based on Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No. 840).

Tests for adventitious agents

Methods _____

Date of inoculation _____

Results _____

Trypsin used for preparing cell cultures (A.4.1.6)

Sterility tests

	bacteria	*mycoplasmas*
Date of inoculation	_____	_____
Results	_____	_____

Tests for adventitious agents (including porcine parvoviruses)

Methods _____

Date of inoculation _____

Results _____

Production of the working seed lot (A.4.2)

Summary information

Name and address of manufacturer _____

_____

Virus strain _____

Reference no. of virus seed used to prepare manufacturer's original rubella 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 _____

History of vaccine strain

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

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 rubella vaccine virus no. ___ satisfies Part A, sections 2 to 4.4.5, of the Requirements for Rubella Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Control 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)
Cell substrate used for virus growth

Type of cells _____

Date of inoculation _____

Results _____

Simian cells

Type of cells _____

Date of inoculation _____

Results _____

Human cells

Type of cells _____

Date of inoculation _____

Results _____

Additional test if rabbit kidney cells are used for production (A.4.3.3)

Method for detection of *Nosema cuniculi* _____

Date _____

Results _____

Additional tests if human diploid cells are used for production (A.4.3.4)

Identity test

Method _____

Date _____

Results _____

Production and harvest of vaccine virus (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.

No. of passages from the primary seed _____

Reference no. of single harvest _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Virus pool (A.4.4.3)

Reference no. of virus pool _____

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

Tests for neurovirulence[1] (A.4.2.1)

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) _____

Sterility tests	*bacteria*	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

[1] Only for master seed or working seed lot.

Tests of neutralized virus pool in cell cultures

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

Cells used for virus growth
- Type of cells _____
- Date of inoculation _____
- Results _____

Simian cells
- Type of cells _____
- Date of inoculation _____
- Results _____

Human cells
- Type of cells _____
- Date of inoculation _____
- Results _____

Additional test if rabbit kidney cell cultures are used for production

Volume tested _____

No. of rabbits inoculated _____

No. that survived test _____

Results _____

Clarification of the virus pool (A.4.4.4)

Date of clarification _____

Results of clarification _____

Virus titration

Cells used for titration _____

Date of inoculation _____

Results _____

Sterility tests

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Final bulk (A.4.4.5)

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	_____	_____	_____
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	_____	_____	_____
Results	_____	_____	_____

Virus concentration and thermostability (A.6.3)

Date of inoculation _____

Type of cell cultures _____

	Control (unheated) samples			*Samples incubated at 37 °C for 7 days*		
	1	*2*	*3*	*1*	*2*	*3*
Virus concentration in each container (in PFU or $CCID_{50}$)	___	___	___	___	___	___
Mean virus titre per human dose, with 95% fiducial limits	_____			_____		
Mean loss in titre due to heat exposure (in $\log_{10}$ units)	_____					

Reference preparation

Identification _____

Theoretical titre _____

Actual titre _____

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 rubella vaccine (live) satisfies national requirements and/or Part A of the Requirements for Rubella Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Appendix 8

Proposed assay method for determining the virus content of live rubella vaccine

1. Cell-culture infective dose method

Three vials of the preparation to be assayed and at least one of the reference preparation are reconstituted and diluted in an appropriate medium. It is convenient to use dilution steps of 0.5 $\log_{10}$ units, selected so that the dilution range encompasses at least three dilutions that will infect between 10% and 90% of the cultures inoculated.

For each vaccine dilution, a volume of 0.1 ml is dispensed into each of 8-12 flat-bottomed wells in a microtitre plate, followed by 0.1 ml of a suitable cell suspension of the RK-13 cell line. The plates are incubated at the optimal growth temperature for the tested rubella virus strain.

The presence or absence of a cytopathic effect is recorded after five days, with the final reading at 10-12 days. The titre is calculated as a multiple of the median cell-culture infective dose ($CCID_{50}$) by an appropriate method. If the potency is below the expected value, the assay can be repeated and the mean of all assays calculated. The assay is not considered valid when:

- the mean titre of the reference vaccine deviates by more than 0.5 $\log_{10}$ units from the established titre; and/or
- the difference in titre between any two vials of the test vaccine or between the vials of the reference vaccine is >0.5 $\log_{10}$ units.

Care must be taken to protect all virus samples from direct light.

2. Combined vaccines

For assays of the rubella component in multivalent vaccines, the mumps component must first be neutralized. Reconstituted vaccine is incubated with appropriate polyclonal or monoclonal antibodies and the vaccine is then assayed in the normal way. Allowance must be made for the initial dilution effect of the neutralizing serum. (See also Appendix 10, page 201.)

Appendix 9

Summary protocol for production and testing of measles–mumps–rubella combined 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, a copy of the leaflet that accompanies the vaccine container, and 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 4).

Information on blending

Measles component

Reference no. _____

$CCID_{50}$ or PFU/ml _____

Volume _____

Mumps component

Reference no. _____

$CCID_{50}$ or PFU/ml _____

Volume _____

Rubella component

Reference no. _____

$CCID_{50}$ or PFU/ml _____

Volume _____

Final bulk of combined components (A.2.2)

Reference no. of final bulk _____

Date of completion _____

Total volume of final bulk _____

Added substances (diluent, stabilizer) _____

[1] Based on Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live) (Requirements for Biological Substances No. 47). In: *WHO Expert Committee on Biological Standardization. Forty-third Report.* Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No. 840).

Residual animal serum proteins (A.2.2.1)

Date _____

Method _____

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

Sterility tests (A.2.2.2)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Filling and containers (A.3)

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.4)

Sterility tests (A.4.1)

	bacteria	*fungi*	*mycoplasmas*
Date of inoculation	_____	_____	_____
Results	_____	_____	_____

Virus concentration, thermostability and identity (A.4.2)

Record the results of virus titration and the thermostability test in the table opposite.

Identity test

	measles	*mumps*	*rubella*
Date of test	_____	_____	_____
Methods	_____	_____	_____
Results	_____	_____	_____

Virus concentration and thermostability test (A.4.2)

	Control (unheated) samples									Samples incubated at 37 °C for 7 days								
	Measles			Mumps			Rubella			Measles			Mumps			Rubella		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Virus concentration in each container (in PFU or $CCID_{50}$)																		
Mean virus titre per human dose, with 95% fiducial limits																		
Mean loss in titre due to heat exposure (in $\log_{10}$ units)																		
Reference preparation																		
Identification																		
Theoretical titre																		
Actual titre																		

General safety tests (A.4.3)

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.4.4)

Date _____

Method _____

Size of sample _____

Moisture content (%) _____

Inspection of final containers (A.4.5)

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 measles-mumps-rubella combined vaccine (live) satisfies national requirements and/or Part A of the Requirements for Measles-Mumps-Rubella Combined Vaccine in Requirements for Biological Substances No. 47 (Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)).

Signature _____

Name (typed) _____

Date _____

Appendix 10

Proposed assay method for determining the virus content of measles–mumps–rubella combined live vaccine

Before titration of each individual virus in combined vaccine, the other components should be selectively neutralized, as necessary, with specific antisera: for measles virus determination, neutralize the mumps and rubella components; for rubella virus, neutralize the mumps component (it is not necessary to neutralize measles virus as it will not grow in RK-13 cells); and for mumps virus, neutralize the measles and rubella components.

Antisera

All antisera should be heated for 30 minutes at 56 °C before use. Antisera against measles, mumps and rubella viruses should have a titre of at least 1:320 against 500 $CCID_{50}$ of the respective viruses as determined in the test described in Appendices 3, 6 and 8.

The suitability of each antiserum must be demonstrated, at the appropriate concentration, and the antiserum must be shown to neutralize the homologous virus after 1.5 hours at 20 °C and not to reduce the titre of the heterologous viruses under the same conditions.

Procedures

Dilute the antisera as appropriate to the type of virus, the diluent and the titre required.

Reconstitute the vaccine samples and the references with an appropriate diluent and make dilutions with the antisera as required. Prepare the reference vaccines (if monovalent) by dilution both in diluent alone and in combination with antisera. Take precautions to protect all samples from direct light.

Incubate all dilutions at 20 °C for 1.5 hours, and titrate each virus according to the methods described for the monovalent vaccines (Appendices 3, 6 and 8).