Annex 5

REQUIREMENTS FOR MEASLES VACCINE (LIVE)

(Requirements for Biological Substances No. 12) (Revised 1987)

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INTRODUCTION

Measles, an almost invariable clinical experience of childhood, is in some countries a major cause of illness and of death in children. Immunization against measles has been of interest to WHO for many years, and more especially since the Expanded Programme on Immunization was launched in 1974 with measles as one of the principal diseases against which it is directed. Since the original production of measles vaccine, many years ago, the search for improved immunizing agents continues.

In support of this, reference materials such as anti-measles serum and measles vaccine virus have been established, and Requirements for Measles Vaccine (Live) and Measles Vaccine (Inactivated) were adopted by the WHO Expert Committee on Biological Standardization in 1966 (1). Although new developments may be anticipated today, only live measles vaccines are used, and it has become necessary to update the requirements for these. In drafting this revision, account has been taken of the opinions of consultants, of the regulations and requirements for the manufacture and control of measles vaccines that have been formulated in a number of countries, and of information from published and unpublished sources. In addition, opinions and data have been received from a number of experts, whose assistance is gratefully acknowledged below.

GENERAL CONSIDERATIONS

Hundreds of millions of persons have been vaccinated with live attenuated measles virus vaccines, and there is ample evidence that measles vaccines which are safe and effective are produced throughout the world. Production of such vaccines requires the observance of certain rules, and it is thus important that up-to-date international requirements for live attenuated measles vaccines should be formulated and made available to manufacturers and national control authorities.

The antibody response in persons 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; this is indicated by the persistence of neutralizing antibodies in children who, a number of years ago, received vaccines prepared from several attenuated strains derived from the Edmonston strain of measles virus. Nevertheless, it is important that studies should be made to ascertain the exact duration of immunity induced by vaccines derived from various 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, 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 6 months of age. The poorer seroconversion rate when measles vaccine is administered early in life can be circumvented by a second injection later in life.

Measles vaccine can be combined with other live attenuated vaccines such as mumps and rubella vaccines, and such combinations are also highly effective.

While the basic immunogenic potential of each strain of measles vaccine can be assessed by the antibody response in man, this is not a practical method for the routine testing of the potency of batches of vaccines. Such testing is done by measuring infectivity when cell cultures are inoculated with various dilutions of vaccines. For each strain of measles vaccine, it is necessary to establish the relationship between the laboratory estimation of virus titre on the one hand and safety and efficacy for man on the other. Where measles vaccines are to be used in combination with other vaccines, dose–response curves for each component should be determined by administering it in the proposed combined form.

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 by which this property could be evaluated. The development of more reliable methods is desirable. The possibility exists that post-measles encephalopathy is the result of an immunopathogenic reaction; the underlying mechanism is not known.

It is essential that every precaution should be taken to exclude all 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 resulted in the production of cell substrates of better quality
than hitherto; however, in spite of the conclusion of a group
convened by WHO in 1980 (2) that it was sufficient to conduct the
tests for extraneous agents in cell cultures on control cells, tests for
the absence of such agents are still mandatory for individual harvests
and/or virus pools in these revised requirements. On the other hand,
tests for extraneous agents on small laboratory animals have been
abandoned, except for the testing of virus seeds and, when measles
vaccines are produced in human diploid cells, for the testing of
manufacturer’s working cell banks.

Each of the following sections constitutes a recommendation. The
parts of each section that are printed in large type have been written
in the form of requirements, so that, if a health administration so
desires, these parts as they appear may be used as definitive national
requirements. The parts of each section that are printed in small type
are comments or recommendations for guidance.

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

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

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.

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.

1.3 International reference materials

The International Reference Preparation of Anti-Measles Serum was established in 1964. It is intended for the calibration of national standards or reference preparations for use in the manufacture and laboratory control of anti-measles serum and of human immunoglobulin. It is also intended for assessing the antibody response to measles vaccines. The International Reference Preparation of Anti-Measles Serum is in the custody of the State Serum Institute, Copenhagen, and is available on request.

An International Reference Reagent for the Assay of Measles Vaccine (Live) is available on request from the National Institute for Biological Standards and Control, Potters Bar, England, and is suitable for estimating the *in vitro* infectivity of measles vaccines.

1.4 Terminology

The following definitions are given for the purposes of these Requirements only.
Master virus seed lot: a quantity of virus of uniform composition, processed at one time, and distributed into a number of containers. Master virus seed lots are derived from a seed virus used in the preparation of vaccines shown to be immunogenic and safe in man, and not more passages removed from it than a number approved by the national control authority. They are used for the preparation of working virus seed lots.

Working virus seed lot: a quantity of virus suspension that has been processed together, is uniform with respect to composition, and is only one passage from a master seed lot produced on the same substrate. Material is drawn from working seed lots for inoculating cell cultures for the production of vaccines.

Cell substrate lot: a number of cell cultures derived from the same pool of cells, processed and prepared together.

Single harvest: a virus suspension derived from one cell substrate lot, all the cultures having been inoculated at the same time with the same inoculum. Single harvests may be derived from one cell substrate lot by repeated harvesting at intervals.

Virus pool: a pool of a number of single harvests before clarification.

Final bulk suspension: a quantity of vaccine after completion of preparations for filling and present in the container from which the final containers are filled. The final bulk may be prepared from one clarified bulk suspension, or from a blend of clarified bulk suspensions, or from a dilution thereof.

Final lot: a collection of sealed final containers that derive from the same final bulk and that are homogeneous with respect to the risk of contamination during filling and freeze-drying. A final lot therefore consists of finished material dispensed into containers during one working session and lyophilized together.

Tissue culture infective dose 50% (TCID$_{50}$): the quantity of a virus suspension that will 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.

2. Certification of the Strain of Virus for Use in Vaccine Production

2.1 The strain of measles virus used in the production of measles vaccine shall be identified by historical records, which shall include
information on the origin of the strain as well as on the method used in the attenuation of it.

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

2.3 A dose–response study should establish the minimal vaccine dose inducing seroconversion in susceptible individuals.

3. General Manufacturing Requirements

The general manufacturing requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (3) shall apply to establishments manufacturing measles vaccine, with the addition of the following directives.

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

The production of measles vaccine shall be conducted by staff who have not handled other infectious microorganisms or animals on the same working day. The staff shall consist of persons who shall be 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 Part A, section 1, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (3) regarding the training and experience of the persons in charge of production and testing, and of those assigned to various areas of responsibility in the manufacturing
establishment, as well as to the registration of such personnel with the national control authority.

4. Production Control

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

4.1 Substrate for virus propagation

4.1.1 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.

4.1.2 If chick-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. Monitoring shall be performed at regular intervals for *Mycobacterium avium*, fowl pox, avian retroviruses, Newcastle disease virus, avian encephalomyelitis virus, infectious laryngotracheitis virus, reticuloendotheliosis virus, Marek's disease virus, infectious bursal disease virus, avian reovirus, avian adenovirus, avian influenza virus, avian parainfluenza virus, *Haemophilus paragallinarum*, *Salmonella gallinarum*, *Salmonella pullorum*, *Mycoplasma gallisepticum*, and *Mycoplasma synoviae*.

4.1.3 If human diploid cells are used for the propagation of measles virus, a manufacturer's working cell bank (MWCB) shall be established in conformity with the provisions of Part C of these Requirements. 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 tests in animals and eggs for freedom
from extraneous agents, for lack of tumorigenicity, for 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 for identity, as indicated in Part C of these Requirements. The cells shall not be used beyond two-thirds of the total number of population doublings corresponding to the average finite life of the cells.

4.1.4 *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 according to the requirements in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for Sterility of Biological Substances) (4) and to demonstrate freedom from viruses.

Suitable tests for the detection of viruses in calf or newborn calf serum are given in Appendix 3 of the Requirements for Poliomyelitis Vaccine (Oral) (Requirements for Biological Substances No. 7) (5).

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 WHO Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products (Requirements for Biological Substances No. 27) (6).

In some countries sera are also examined for freedom from phage.

4.1.5 *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.

4.2 *Virus seed*

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

It is recommended that a large working virus vaccine seed lot be set aside as the basic material that the manufacturer should use for the preparation of each batch of vaccine.
Virus seed lots shall be stored lyophilized in a special refrigerator at a temperature lower than \(-20^\circ\text{C}\), or, if not lyophilized, at a temperature of \(-60^\circ\text{C}\) or lower.

The virus in the final vaccine shall not be more than 5 cell culture passages removed from the virus seed used in the preparation of a vaccine shown to be immunogenic and safe in man.

4.2.1 Tests on virus seed lots

The seed lot used for the production of vaccine shall be free from detectable extraneous agents, including those which might have contaminated the original human specimen from which the virus strain was initially isolated, the cell culture used during the initial passages, or the attenuation process.

The seed lot virus shall be produced in conditions which satisfy sections 4.3 and 4.4 (with the exception of 4.4.5.1 and 4.4.5.2) of Part A of these Requirements. Tests for extraneous agents on small laboratory animals and for neurovirulence shall be done on either the master or the working seed lot as indicated in sections 4.2.1.1 and 4.2.1.2 below.

4.2.1.1 Tests in small laboratory animals

4.2.1.1.1 Test in adult mice

Each of at least 10 adult mice, of 15–20 g weight, shall be inoculated intracerebrally with 0.03 ml and intraperitoneally with at least 0.5 ml of the virus seed. The mice shall be observed for at least 21 days. All mice that die after the first 24 hours of the test or that show signs of illness shall be autopsied and examined for evidence of viral infection, both by direct macroscopical observation and by subinoculation of appropriate tissue suspensions by the intracerebral and intraperitoneal routes into at least 5 additional mice, which shall be observed for 21 days.

The virus seed passes the test if at least 80% of the original inoculated mice survive the observation period and if no mouse shows evidence of infection with adventitious transmissible agents attributable to the virus seed.

In some countries the national control authority permits a 60% survival of the original inoculated animals.
4.2.1.1.2 Tests in suckling mice

Each of at least 20 mice, less than 24 hours old, shall be inoculated intracerebrally with 0.01 ml and intraperitoneally with at least 0.1 ml of the virus seed. The mice shall be observed daily for at least 14 days. All mice that die after the first 24 hours of the test or that show signs of illness shall be autopsied and examined for evidence of viral infection, both by direct macroscopical observation and by subinoculation of appropriate tissue suspensions by the intracerebral and intraperitoneal routes into at least 5 additional suckling mice, which shall be observed daily for 14 days.

In addition, in some countries a blind passage of a suspension of the pooled emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test is made.

The virus seed passes the test if at least 80% of the original inoculated mice survive the observation period and if no mouse shows evidence of infection with adventitious transmissible agents attributable to the virus seed.

4.2.1.1.3 Tests in guinea-pigs

The virus seed shall be tested for adventitious agents by the intraperitoneal inoculation of 5.0 ml of the virus seed into each of at least 5 guinea-pigs of 350–450 g weight. The animals shall be observed for at least 42 days for signs of disease. All guinea-pigs that die after the first 24 hours of the test or that show signs of illness shall be autopsied and examined both microscopically and by tissue culture for evidence of infection. Animals that survive the observation period shall be sacrificed and examined in a similar manner.

The virus seed passes the test if at least 80% of the guinea-pigs survive the observation period and if no guinea-pig shows evidence of infection with adventitious transmissible agents attributable to the virus seed.

4.2.1.2 Tests for neurovirulence

Each master or working virus vaccine seed lot shall be shown to be free from neurovirulence by tests in measles-susceptible monkeys.

Such tests can be conducted as follows:
Immediately prior to the inoculation each monkey should be shown to be serologically negative for measles. At least 10 monkeys should be employed in each test. The material under
test should be given to each monkey by inoculation of 0.5 ml into the thalamic region of each hemisphere. The total amount of measles virus inoculated into each monkey should be not less than the amount contained in the recommended single human dose of vaccine. Monkeys should be observed for 17–21 days for symptoms of paralysis and other evidence of neurological involvement. Animals that die within 48 hours after injection may be replaced. The test is invalid and should be repeated if more than 20% of the monkeys die even from nonspecific causes. At the end of the observation period each monkey is (a) bled and the serum tested for measles antibody and (b) anaesthetized, sacrificed and autopsied; 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 4 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 material passes the test if: (a) at least 80% of the inoculated monkeys are serologically positive for measles with an adequate level of specific antibody (0.2 IU/ml or greater) and all the serum samples from the control monkeys are shown to be free from measles antibody; and (b) there is no clinical or histopathological evidence of involvement of the central nervous system attributable to the inoculated virus.

In some countries the seed lot itself is not tested but vaccines are accepted provided each of the first 5 undiluted clarified virus pools prepared from the same seed lot satisfies the requirements of the test for neurovirulence.

4.3 Control of cell cultures

Either 5% or not less than 500 ml of the cell suspension at the concentration employed for vaccine production cultures shall be used to prepare control cultures.

Cells set aside as control material shall be treated in a similar manner to the production cell cultures, but shall remain uninoculated as control cultures for the detection of extraneous viruses; they shall be incubated under the same conditions as the inoculated cultures for at least 2 weeks, or until the time of the last harvest of the production cultures, whichever is the later, and shall be observed microscopically for changes attributable to the presence of adventitious agents. At the end of the observation period, fluids
collected from the control cultures as well as cell sheets from a proportion of the control vessels shall be tested for the presence of adventitious agents by the tests described in this section. If any such tests show evidence of the presence in a control culture of any adventitious agent, the measles vaccine virus grown in the corresponding batch of production cultures shall not be used for vaccine production.

For the tests to be valid, at least 80% of the culture vessels shall be available and suitable for evaluation at the end of the respective test periods.

**Test for haemadsorbing viruses**

At the end of the observation period, cells comprising 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 7 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 additional tests for haemadsorbing viruses should be made on control cultures between 3 and 5 days, and again at 12 days using also other types of red cells, including those from humans (blood group IV, O), monkeys, and chickens (or other avian species). All tests should be read after incubation for 30 minutes at 0–4 °C, and again after a further incubation for 30 minutes at 20–25 °C. The test with monkey red cells should be read once more after yet another incubation for 30 minutes at 34–37 °C.

**Tests for non-haemadsorbing extraneous agents**

At 14 days after the day of inoculation of the production cultures, or at the time of final virus harvest, if this is later, a sample of cell culture fluid shall be taken from each control culture and pooled. Ten millilitres of the pool shall be tested in the same substrate, but not the same batch as that used for the production of virus growth, and additional 10-ml samples of each pool shall be tested in both human and simian cells.

Each sample shall be inoculated into bottles of cell cultures 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 cm² 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 7 days. Furthermore, some national control authorities require that these cells should be tested for the presence of haemadsorbing viruses.

For the tests to be valid, at least 80% of the culture vessels shall be available and suitable for evaluation at the end of the respective test periods.

If any cytopathic changes occur in any of the cultures, the virus harvests produced from the batch of cells from which the control cells were taken shall be discarded.

4.3.3 Additional test if chicken-cell cultures are used

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, using 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 (COFAL), 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.

4.3.4 Additional tests on control cells 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, as specified in Part C, section 2.2.2, of these Requirements.
4.4 Production and harvest of virus vaccines

4.4.1 Cells used for vaccine production

If human diploid cells are used as the substrate for the multiplication of the vaccine virus, they shall satisfy the conditions specified in Part C, section 2.2.1, of these Requirements.

On the day of inoculation with the seed lot virus, each 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 for the maintenance of cell cultures before the harvesting of virus, the medium shall be removed and replaced with serum-free maintenance medium, the cells being rinsed before being added to the new medium.

Beta-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 authorities.

4.4.2 Single harvests

Harvesting of virus fluids shall be carried out by a method approved by the national control authority. A single harvest may be a combination of several consecutive harvests from one production cell lot. Single harvests are stored at a suitable temperature 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, samples shall be kept at a temperature below $-50 ^\circ C$ until testing is done.

4.4.2.1 Sterility tests

A volume of at least 10 ml of each single harvest shall be tested for bacterial and mycotic sterility according to the requirements in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for Sterility of Biological
Substances) (4), as well as for mycoplasmas by a method approved by the national control authority.

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

4.4.2.2 Virus titration

The live virus content of each single harvest may be determined by cell culture titration, using a reference material of live measles virus, the titre of which has been determined by comparison with the International Reference Reagent (see Part A, section 1.3, of these Requirements).

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; however, even in that event, sterility tests shall be done on the virus pool.

The virus pool may be clarified, stabilized and stored at \(-50 \, ^\circ\text{C}\) or lower before being used to prepare final bulk for freeze-drying.

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 for the preparation of the antiserum shall be produced in cell culture 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.

4.4.3.1 Sterility tests

Sterility tests shall be performed as indicated in Part A, section 4.4.2.1, of these Requirements.

4.4.3.2 Virus titration

Virus titration shall be performed as indicated in Part A, section 6.3, of these Requirements.
4.4.3.3 Test for mycobacteria

Regardless of which substrate is used for producing the virus vaccine, tests for detecting the presence of mycobacteria pathogenic for man shall be performed. Tests for mycobacteria shall be done on the pellet obtained after centrifugation of 20 ml of the virus pool.

4.4.3.4 Tests in cell cultures of neutralized virus pool

A volume of each virus pool equivalent to at least 500 human doses 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 likewise be tested in human cell cultures and in cell cultures of the same type but not the same batch of cells as that used in the preparation of 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 tests if none of the inoculated cell cultures shows evidence of the presence of any adventitious agents.

4.4.3.5 Additional tests if chick-embryo tissue is used as substrate for production

If chick-embryo tissue is used for vaccine production, the following additional tests shall be made.

A volume of each virus pool, equivalent to at least 100 human doses of vaccine, or 10 ml, shall be tested in a group of embryos of fertilized chicken’s 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, using 0.5 ml of inoculum per egg in both cases.

The virus pool passes the test if there is no evidence of the presence of any adventitious agents. If, however, an adventitious agent is detected in the uninoculated controls, the test may be repeated.

4.4.4 Clarification of the vaccine virus pool

The vaccine virus pool suspension shall be clarified by a method that will remove cells and cell debris. Samples of the clarified bulk
suspension shall be taken immediately after clarification to ensure that no cell or cell debris is left. Samples shall also be taken to control the identity and infectious virus content of the pool. If not tested immediately, the samples shall be kept at a temperature below $-50^\circ\text{C}$ until testing is done.

4.4.4.1 *Test for clarification*

Microscopical observation of a smear of a concentrated sample is suitable for ensuring that no cells or cell debris are detected after clarification.

4.4.4.2 *Test for virus content*

The viable virus content of samples from clarified bulk suspension shall be tested, using for comparison a reference preparation of live measles virus (see Part A, section 1.3, of these Requirements).

4.4.5 *Final bulk suspension*

The final bulk suspension shall be prepared from one or more clarified virus pools that are obtained from substrates of which control cultures pass the test specified in Part A, section 4.3 of these Requirements. They shall satisfy the tests specified in Part A, sections 4.4.3 and 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.

Assays of virus content may be done on the final bulk suspension.

4.4.5.1 *Added substances*

In preparing the final bulk, any substance such as diluent or stabilizer that is added to the product shall have been shown to the satisfaction of the national control authority not to impair the safety and efficacy of the vaccine in the concentration used.

4.4.5.2 *Residual animal serum proteins*

A sample of the final bulk shall be tested to verify that the level of contamination by heterologous serum is less than 1 part per million of the final reconstituted vaccine; that is, less than 0.5 $\mu$g if the human dose is 0.5 ml. Alternatively the test may be performed on the clarified bulk.

Serological tests such as ELISA are suitable for this purpose.
4.4.5.3 Storage

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

5. Filling and Containers

The requirements concerning filling and containers in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (3) shall apply.

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

Single-dose containers are recommended, except in the case of mass immunization campaigns.

6. Control Tests on Final Product

Samples shall be taken from each freeze-dried lot for the tests in the following sections.

Identity tests

The virus in 2 or more individually labelled final containers shall be identified as measles virus by appropriate methods, such as seroneutralization and then inoculation of cells.

6.2 Tests for bacteria and fungi

Reconstituted vaccine shall be tested for bacterial and mycotic sterility according to the requirements in Part A, section 5.2, of the Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances) (4), or by acceptable methods approved by the national control authority.

Virus concentration

The virus content in each of at least 3 ampoules selected at random from each drying lot shall be determined individually.
The national control authority shall determine the minimum content of the vaccine virus that should be contained in one human dose.

The minimum quantity of the vaccine virus that should be contained in one human dose is generally considered to be 1000 viral infective units. In at least one country, however, the minimum dose has been set at 5000 infective units.

An additional 3 ampoules of the final freeze-dried vaccine shall be exposed at 37 °C for 7 days. The geometric mean infectious virus titre of the vials that have been exposed shall be equal to or greater than the required minimum numbers of infective units per human dose, and the geometric mean virus titre of the vaccine shall not have been decreased by more than 1.0 log_{10} during the period of exposure. Titration of non-exposed and exposed vials shall be made in parallel and results expressed in terms of PFU and/or TCID_{50} per human dose. A reference reagent of measles virus, the titre of which has been determined by comparison with the International Reference Reagent (see Part A, section 1.3, of these Requirements), shall be included in each assay.

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

6.4 General safety tests

Innocuity shall be tested by appropriate tests in mice and guinea-pigs, using parenteral injections. The tests shall be those approved by the national control authority.

6.5 Residual moisture

The residual moisture in a representative sample of each freeze-dried lot shall be determined by a method approved by the national control authority. The upper limit for the moisture content shall be specified by the national control authority.

Moisture levels of less than 2% are usually considered satisfactory.
6.6 Inspection of final containers

Each container in each filling lot shall be inspected visually and any that show an abnormality shall be discarded.

7. Records

The requirements in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (3) shall apply.

8. Samples

The requirements in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (3) shall apply.

9. Labelling

The requirements regarding labels of individual containers and labels of packages in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (3) shall apply with the addition of the following:

The label on the carton enclosing one or more final containers, or the leaflet accompanying the container, shall contain the following additional information:

(a) a statement that the vaccine fulfils these Requirements;

(b) a statement of the nature of the preparation, specifying the designation of the strain of measles virus contained in the live attenuated virus vaccine, the minimum number of infective units per human dose, and the origin of the substrate used in the preparation of the vaccine;

(c) a statement of the nature and quantity of any antibiotic present in the vaccine;

(d) a statement concerning the photosensitivity of the vaccine, cautioning that both lyophilized and reconstituted vaccine should be protected from light;
(e) a statement indicating the volume and nature of diluent\(^1\) to be added in order to reconstitute the vaccine, and specifying that the diluent to be used is that supplied by the manufacturer;

(f) a statement that after the vaccine is reconstituted, it should be used without delay, or if not used immediately, stored between 0 °C and 10 °C and in the dark for a period not exceeding 8 hours.

10. Distribution and Shipping

The requirements in Part A, section 9, of the revised Requirements for Biological Substances No.1 (Requirements for Manufacturing Establishments and Control Laboratories) (3) shall apply.

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

11. Storage and Expiry Date

The statements concerning storage temperature and expiry date appearing on the label or the leaflet, as specified in Part A, section 9, of these Requirements, shall be based on experimental evidence and shall be submitted for approval to the national control authority.

11.1 Storage conditions

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

Expiry date

The expiry date shall be fixed with the approval of the national control authority and shall relate to the date of the last satisfactory test for virus concentration, this date being that on which the test-system was inoculated. It shall be based on experimental evidence

\(^1\) No preservative or any substance that has a deleterious effect on the virus should be present in the diluent used to reconstitute the vaccine.
and shall not be less than 2 years for a temperature of storage not higher than 8°C.

In some countries, manufacturers and control authorities have observed that measles vaccines continuously stored in the lyophilized state at temperatures not higher than -20°C do not lose potency over a period of several years. In such cases, national control authorities allow the dating period to start at the time the vaccine is taken out of the frozen state, provided that a satisfactory potency test has been successfully carried out within 12 months preceding the start of the dating period.

PART B. NATIONAL CONTROL REQUIREMENTS

1. General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (3) shall apply.

The national control authority shall provide a reference material of live measles virus (see Part A, section 1.3, of the present Requirements) for tests for virus concentration (see Part A, sections 4.4.2.2, 4.4.3.2, 4.4.4.2, and 6.3) and shall specify the requirement for virus content that shall be fulfilled, in order to achieve adequate immunization of humans when the recommended human dose is used.

2. Release and Certification

A vaccine shall be released only if it fulfils Part A of the present Requirements. A statement along the lines of that contained in Appendix 2 and signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether the lot of vaccine in question meets all national requirements, as well as Part A of the present Requirements. The certificate shall state the date of the last satisfactory test for virus concentration by the manufacturer and the final lot number which must appear on the labels of the containers.

The purpose of the certificate is to facilitate the exchange of live measles vaccines among countries.
PART C. REQUIREMENTS FOR HUMAN DIPLOID CELLS TO BE USED FOR THE PRODUCTION OF MEASLES VACCINE (LIVE)¹

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

1. Definitions

1.1 Terminology

Cell seed: a quantity of cells derived from a single human tissue and of uniform composition, stored frozen at −70 °C or below in aliquots, one or more of which would be used for the production of a manufacturer’s working cell bank.

Manufacturer’s working cell bank (MWCB): a quantity of cells derived from one or more aliquots of the cell seed, of uniform composition, stored frozen at −70 °C or below in aliquots, one or more of which would be used for the production of each single harvest.

In normal practice a cell seed is issued to manufacturers at or near the eighth population doubling level (PDL). It is expanded by serial subculture up to a PDL selected by the manufacturer, at which point the cells are combined into one or more pools and preserved cryogenically to form the MWCB. One or more of such ampoules from a pool would be used to prepare the production cell culture.

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 MWCB.

¹ Part C is based largely on Part C of the Requirements for Poliomyelitis Vaccine (Oral) (5, pp. 140–150).
2. Production Control

2.1 Control of source materials

The cell seed and the manufacturer's working cell bank shall be those 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 they shall have been shown to be free from bacteria, mycoplasma, fungi, and haemadsorbing and other viruses by the relevant tests in Part A of 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 man.

2.1.1 Tests in animals and eggs for extraneous agents

The cells of the MWCB are suitable if at least 80% of the animals or eggs which are inoculated with them 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.

2.1.1.1 Tests in animals

The tests in animals for adventitious agents in the MWCB shall include the inoculation of each of the following groups of animals with the cells by the intramuscular route, using at least $10^7$ cells divided equally between the animals in each group:

- 2 litters of suckling mice, totalling at least 10 animals, less than 24 hours old,
- 10 adult mice of 15–20 g weight,
- 5 guinea-pigs of 350–450 g weight, and
- 5 rabbits.

The animals shall be observed for at least 4 weeks. Any animals that are sick or show any abnormality shall be investigated to establish the cause of illness.

In some countries the suckling and adult mice are also inoculated by the intracerebral route.
2.1.1.2 Tests in eggs

At least $10^6$ viable cells shall be injected into the allantoic cavity of 10 embryonated chicken’s eggs, 9–11 days old, which shall be examined after not less than 5 days. The allantoic fluids of the fertile eggs shall be tested with erythrocytes from guinea-pigs and chick or other avian species for the presence of haemagglutinins.

2.1.2 Other tests for extraneous agents

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

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

2.1.3 Freedom from tumorigenicity

The cells shall also be shown to be free from potential tumorigenicity by appropriate animal tests approved by the national control authority.

Suitable tests using immunosuppressed animals may be made 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 (nu/nu genotype); or thymectomized mice irradiated and bone marrow reconstituted (T−B+). Some of the same group of animals should be inoculated with a similar dose of HeLa or KB cells, and it should be shown that tumour formation is caused by the inoculation of the neoplastic tissue, thus demonstrating the ability of the strain of animals to give rise to tumours. The animals should be observed for not less than 3 weeks. Any other test using animals treated with immunosuppressive agents and with equal sensitivity to neoplastic cells may be used.

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

At least 4 samples from the cell seed shall be examined as described in Part C, section 2.1.5, of these Requirements 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 also recommended that photographic reconstruction should be employed in the preparation of chromosome-banded karyotypes of 50 metaphase cells per 1000-cell sample, using 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 be approved by the national control laboratory.

2.1.5 Chromosomal monitoring—preparation and testing

For the determination of the general character of the manufacturer’s working cell bank, 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.

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

For WI-38 and MRC5 cells examined in metaphase, the upper limits of acceptability (upper fiducial limits at 95% (Poisson)) for abnormalities for a 1000- and 500-cell sample are as follows:

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>1000 cells</th>
<th>500 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatid and chromosome breaks</td>
<td>47/1000</td>
<td>26/500</td>
</tr>
<tr>
<td>Structural abnormalities</td>
<td>17/1000</td>
<td>10/500</td>
</tr>
<tr>
<td>Hyperploidy</td>
<td>8/1000</td>
<td>5/500</td>
</tr>
<tr>
<td>Hypoploidy</td>
<td>180/1000</td>
<td>90/500</td>
</tr>
<tr>
<td>Polyploidy</td>
<td>30/1000</td>
<td>17/500</td>
</tr>
</tbody>
</table>

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

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

Only the cell pools of the MWCB that have normal karyology shall be used for vaccine production.

2.2 Production precautions

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

2.2.1 Cell cultures used for vaccine production

Only human diploid cell cultures derived from a MWCB approved by the national control authority shall be used for vaccine production. The production of each single harvest shall be initiated from one or more new ampoules of the cell seed. All processing of the cell seed 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 no changes have occurred in their growth characteristics, and if no changes from the normal karyology have been shown to occur within the total number of population doublings that correspond to the average finite life of the cells as determined under the particular conditions of the production establishment (see Part C, section 2.1.4, of these Requirements).

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.

3.2.2 Identity test

An identity test shall be performed on the control cell cultures by methods approved by the national control authority.
Suitable tests are isozymes, HLA or other immunological tests or karyotype of at least one metaphase spread of chromosomes.

The cells shall be shown to be of human origin.

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REFERENCES

Appendix 1

SUMMARY PROTOCOLS FOR MEASLES VACCINE (LIVE)

Based on Requirements for Biological Substances No. 12 (Requirements for Measles Vaccine (Live)) (Revised 1987)

The following protocols are intended for guidance and indicate the minimum of information to be provided. The Protocol for Final Lot must be accompanied by a sample of the label and a copy of the leaflet accompanying the vaccine container. It must also be accompanied by a certificate from the national control authority of the country in which the vaccine was produced stating that the product meets the national as well as the WHO Requirements (see Appendix 2).

I. PROTOCOL FOR WORKING VIRUS SEED LOT

A. Summary Information

<table>
<thead>
<tr>
<th>Name and address of manufacturer</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus strain</td>
<td></td>
</tr>
<tr>
<td>Reference no. of virus seed used to prepare your first measles vaccine that was safe and immunogenic in man</td>
<td></td>
</tr>
<tr>
<td>Reference no. of master seed virus</td>
<td></td>
</tr>
<tr>
<td>Number of passages between the two above seeds</td>
<td></td>
</tr>
<tr>
<td>Date of preparation of working seed virus</td>
<td></td>
</tr>
<tr>
<td>No. of containers of working seed virus prepared</td>
<td></td>
</tr>
<tr>
<td>Reference no. of working seed virus prepared</td>
<td></td>
</tr>
<tr>
<td>Conditions of storage of seed virus prepared</td>
<td></td>
</tr>
</tbody>
</table>

1 If product does not meet the national requirements, the reason why it does not should be stated.
History of vaccine strain

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

B. Control of Cell Cultures (A.4.3)\(^1\)

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

<table>
<thead>
<tr>
<th>Substrate used for production of virus seed lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference no. of control cell cultures</td>
</tr>
<tr>
<td>Quantity of overall cell cultures used as control cultures</td>
</tr>
<tr>
<td>Period of observation of uninoculated control cells</td>
</tr>
</tbody>
</table>

B.1 Tests for haemadsorbing viruses (A.4.3.1)

<table>
<thead>
<tr>
<th>Type of red blood cells</th>
<th>Date of test</th>
<th>Result of test</th>
</tr>
</thead>
</table>

B.2 Test on cell cultures for non-haemadsorbing extraneous agents (A.4.3.2)

<table>
<thead>
<tr>
<th>Simian cells</th>
<th>Type of cells</th>
<th>Date of inoculation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cells</td>
<td>Type of cells</td>
<td>Date of inoculation</td>
<td>Result</td>
</tr>
</tbody>
</table>

B.3 Other tests for non-haemadsorbing viruses

1 Case where substrate is chicken fibroblast (A.4.3.3)

<table>
<thead>
<tr>
<th>Test for avian leukosis virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
</tr>
<tr>
<td>Date</td>
</tr>
<tr>
<td>Results</td>
</tr>
</tbody>
</table>

\(^1\) Letters and numbers in parentheses refer to the corresponding parts and sections in the text of the Requirements for Measles Vaccine (Live).
Test for avian adenovirus
Method
Date
Results

2. Case where cell substrate is human diploid cell (HDC) (A.4.3.4)
Include the manufacturing protocol of the manufacturer's working cell bank (prepared along the lines indicated in Part C of the Requirements)
HDC cultures used for testing the particular control cell cultures
Reference no. of batch
Date of inoculation
Results

<table>
<thead>
<tr>
<th>C. Single Harvests Used in Preparation of Seed Lot (A.4.4.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report results of tests for each single harvest, using extra pages if necessary.</td>
</tr>
<tr>
<td>Name and concentration of antibiotics used in cell culture medium</td>
</tr>
<tr>
<td>No. and reference no. of single harvests</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tests on single harvests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of sterility test (A.4.4.2.1)</td>
</tr>
<tr>
<td>Result of sterility test</td>
</tr>
<tr>
<td>Date of test for mycoplasmas</td>
</tr>
<tr>
<td>Result of test for mycoplasmas</td>
</tr>
<tr>
<td>Date of test on virus concentration (A.4.4.2.2)</td>
</tr>
<tr>
<td>Result of test on virus concentration</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D. Virus Pool (A.4.4.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>If any test had to be repeated or any abnormal result was observed, this must be specified.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D.1 Tests for extraneous agents in small laboratory animals¹ (A.4.2.1.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report on separate pages the details of tests in suckling mice, adult mice, guinea-pigs, and chicken embryos, giving all relevant information, such as number of animals, weight (suckling mice), date and route of inoculation, quantity injected, route of inoculation, results (survival numbers).</td>
</tr>
</tbody>
</table>

¹ Not mandatory if already performed on the primary virus seed
### D.2 Tests for neurovirulence

<table>
<thead>
<tr>
<th>No. of monkeys in test</th>
<th>Volume injected</th>
<th>No. of monkeys surviving without specific symptoms</th>
<th>Result of serological tests</th>
<th>Result of histopathological examination (specify findings)</th>
</tr>
</thead>
</table>

### D.3 Tests for bacterial and mycotic sterility

<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th>Media used</th>
<th>Results</th>
</tr>
</thead>
</table>

### D.4 Tests for mycoplasmas

<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th>Media used</th>
<th>Results</th>
</tr>
</thead>
</table>

### D.5 Virus titration

<table>
<thead>
<tr>
<th>Cells used for titration</th>
<th>Date of inoculation</th>
<th>Results</th>
</tr>
</thead>
</table>

### D.6 Tests for mycobacteria

<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th>Media used</th>
<th>Period of observation</th>
<th>Results</th>
</tr>
</thead>
</table>

### D.7 Tests on cell cultures after seroneutralization

<table>
<thead>
<tr>
<th>Species in which neutralizing serum was prepared and cell substrate on which immunogen was produced</th>
<th>Human cells</th>
<th>Nature of cells</th>
<th>Date of inoculation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simian cells</td>
<td>Nature of cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Not mandatory if already performed on the primary virus seed
D.8 Additional tests

1. If chicken fibroblasts are used as substrate (A.4.4.3.5)
   Tests in embryonated eggs inoculated by allantoic route
   No. and age of embryonated eggs inoculated

   Results

   Tests in embryonated eggs inoculated by yolk sac route
   No. and age of embryonated eggs inoculated

   Results

2. If human diploid cells (HDC) are used (A.4.4.3.4)
   Reference of batch no. of HDC
   Date of inoculation
   Results

E. Certification

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 seed:

I certify that the working virus seed lot of measles vaccine No. .................... meets the requirements in Part A, sections 2 to 4.4.4.2, of the WHO Requirements for Biological Substances No. 12 (Requirements for Measles Vaccine (Live)) (Revised 1987).

Name (typed) ........................................
Signature ........................................
Date ........................................
II. PROTOCOL FOR FINAL BULK SUSPENSION

A. Summary Information

Name and address of manufacturer

Virus strain

Reference no. of secondary virus seed

Date of completion of final bulk suspension

B. Control of Cell Cultures

Give all relevant information, following as a guide section B of the Protocol for Working Virus Seed Lot. If human diploid cells were used as substrate, do not repeat information on the production of the manufacturer's working cell bank (MWCB) (Part C of the Requirements) unless a different MWCB has been used.

C. Single Harvests

Give all relevant information as indicated in section C of the Protocol for Working Virus Seed Lot.

D. Virus Pool

Reference no. of virus pool

Give all relevant information as indicated in section D of the Protocol for Working Virus Seed Lot, except that the information on tests for neurovirulence and on tests on small laboratory animals need not be repeated.

E. Clarified Virus Pool

Date and result of test for clarification (A.4.4.4.1)

Date, cell substrate used for the assay and result of the test for virus content (A.4.4.4.2)

Reference preparation used in the assay

F. Final Bulk Suspension

Nature of stabilizer and final concentration

Reference of final bulk suspension

Total volume of final bulk suspension

Result of test for virus content (optional)
Tests for residual heterologous serum proteins

Date

Method

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

G. Certification

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:

I certify that final bulk suspension lot No. ...................... of measles vaccine meets the requirements in Part A, section 4.4.5, of the WHO Requirements for Biological Substances No. 12 (Requirements for Measles Vaccine (Live)) (Revised 1987).

Name (typed)

Signature

Date
III. PROTOCOL FOR FINAL LOT

A. Summary Information

Name and address of manufacturer

Proprietary name of vaccine
Reference no. of freeze-drying lot
Expiry date
No. of containers in the lot
No. of doses per container

B. Production Details

Lot no. of final bulk suspension
Date of submission of bulk protocol
Date of filling of final lot

Tests of final product¹

1. Identity test (A.6.1)
   Date
   Method used
   Results

2. Tests for bacterial and mycotic sterility (A.6.2)
   Date of inoculation
   Media used
   Observation period
   Results

3. Tests for virus concentration (A.6.3)
   Date of inoculation
   Type of cell cultures
   Reference preparation used

<table>
<thead>
<tr>
<th>Vaccine containers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not kept at 37°C</td>
</tr>
<tr>
<td>Kept 7 days at 37°C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of containers tested</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus concentration found in each container (in human doses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

¹ Give details of all tests and retests
Mean of virus concentration
Lower 95% fiducial limit

<table>
<thead>
<tr>
<th>4. General safety tests (A.6.4)</th>
<th>Not kept at 37°C</th>
<th>Kept 7 days at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date of inoculation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. of mice given injections</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Volume and route of injection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observation period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Results (give all relevant details in case of deaths)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. of guinea-pigs given injections</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Volume and route of injection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observation period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Results (give all relevant details in case of deaths)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5. Residual moisture (A.6.5)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Method used</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Size of sample</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Moisture content (%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6. Inspection of final containers (A.6.6)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date and result</strong></td>
<td></td>
</tr>
</tbody>
</table>

**C. Certification**

Name (typed) and signature of head of laboratory ____________________________

Date ____________________________

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

I certify that lot No. ................. of measles vaccine meets the requirements in Part A of the WHO Requirements for Biological Substances No. 12 (Requirements for Measles Vaccine (Live)) (Revised 1987).

Name (typed) ____________________________

Signature ____________________________

Date ____________________________
Appendix 2

MODEL CERTIFICATE FOR THE RELEASE OF MEASLES VACCINES BY NATIONAL CONTROL AUTHORITIES

(to be completed by the national control authority of the country where vaccines have been manufactured, and to be provided by the vaccine manufacturer to importers)

The following final lots of measles vaccine produced by .................. 1 in ........................ 2, the numbers of which appear on the labels of the final containers, meet all national requirements 3 and Part A 4 of WHO Requirements for Biological Substances No. 12 (Requirements for Measles Vaccine (Live)) (Revised 1987), and WHO Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (Revised 1965).

<table>
<thead>
<tr>
<th>Final Lot No.</th>
<th>Expiry Date</th>
<th>Final Lot No.</th>
<th>Expiry Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

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

The Director of the National Control Laboratory (or Authority as appropriate) 5

Name (typed)..................................................................................................................
Signature............................................................................................................................
Date....................................................................................................................................

---

1 Name of manufacturer.
2 Country.
3 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.
4 With the exception of provisions on distribution and shipping, which the national control authority may not be in a position to assess.
5 Or his or her representative.