INTRODUCTION

Mumps is an acute disease of children and young adults, caused by a paramyxovirus of which there is only a single serotype. Mumps virus infection produces no symptoms in about one-third of infected persons. In those with a clinical response, glandular and nerve tissue is most often affected. The most common signs are fever and swelling of the parotid glands. Other complications that may appear simultaneously with these signs, or in any sequence, are epididymoorchitis, 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.
Mumps is generally considered to be less contagious than measles or chickenpox; for this reason some people reach adulthood without developing immunity. Outbreaks have occurred frequently among young adult males in closed communities. Potential target populations for immunization include children and susceptible adults.

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.

Several strains of attenuated mumps virus have been developed for use in vaccines. The first vaccine strain to be developed, and that most often used, is the Jeryl Lynn strain, which is grown in chick embryo cell cultures. It was licensed in the United States of America in 1967 and by 1985 had been given to nearly 50 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. When mumps vaccine is combined with other vaccines, it should be shown that the vaccine viruses do not interfere with each other’s actions.

Vaccines based on the Leningrad-3 strain of attenuated mumps virus have been in use since 1974 in the USSR and subsequently in other countries. They are produced in cell cultures of Japanese quail embryo in the USSR and in chick embryo cell cultures in Yugoslavia. Approximately 20 million doses of vaccine based on this strain have so far been used, as monovalent vaccine or in combination with measles and rubella vaccines.

The Urabe strain of attenuated live mumps vaccine was first licensed in 1979 in Japan and thereafter in Belgium and France. It is produced either in the amnion of embryonated hen’s eggs or in chick embryo cell cultures. By 1985, about five million persons 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.

Two additional strains of attenuated mumps vaccine have been licensed in Japan. These are the Hoshino and Torii strains, both of which are grown in chick embryo cell culture. These strains have been less extensively used than the Jeryl Lynn, Urabe, and Leningrad-3 strains.
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, whilst 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.

GENERAL CONSIDERATIONS

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. The World Health Organization will provide such a reference preparation as soon as possible. A reference preparation of antibody to mumps virus is also required.

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 included in definitive national requirements. The parts of each section that are 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 an appendix.

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

The terms “national control authority” and “national control laboratory” as used in these requirements always refer to the country in which the final vaccine is manufactured.
PART A. MANUFACTURING REQUIREMENTS

1. Definitions

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.

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 vaccine or in combination with live measles and live rubella vaccines.

1.3 International reference materials

Since no international standards or reference reagents for live mumps vaccine have yet been established, no requirements for titre based on such standards or reagents can be formulated. National control authorities should therefore provide a reference preparation of live mumps virus for validating tests to determine virus concentration (see Part A, sections 3.3.2, 3.4.1, and 5.2).

1.4 Terminology

*Primary virus seed lot*: a quantity of virus suspension that has been processed as a single lot and has a uniform composition. It is used for the preparation of secondary seed lots.

*Secondary virus seed lot*: a quantity of virus suspension that has been processed as a single lot, has a uniform composition, and is only one passage from a primary seed lot produced on the same substrate. Material is drawn from secondary seed lots for inoculating cell cultures or eggs for the production of vaccine.

*Cell substrate lot*: a number of cell cultures derived from the same pool of cells and processed and prepared together, or a number of avian embryos from an approved source.
Single harvest: a virus suspension harvested in one continuous operation from one cell culture lot, or from one batch of embryonated eggs, all the cultures or eggs having been inoculated at the same time with the same inoculum.

Virus pool: a pool of single harvests before clarification.

Final bulk suspension: a quantity of vaccine after completion of the preparations for filling, and present in the container from which the final containers are filled.

Final lot: a collection of sealed final containers that are filled from the same final bulk and are homogeneous with respect to the risk of contamination during filling and freeze-drying. A final lot, therefore, consists of finished material distributed into containers in one working session and dried as a single lot.

2. General manufacturing requirements

The general requirements for manufacturing establishments contained in the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories, Annex 1 (1) shall apply to establishments manufacturing live mumps vaccine, with the addition of the following requirements.

(a) Visitors not directly concerned with the production processes shall not be permitted to enter areas used for processing live mumps vaccine; other visitors having business in such areas shall be admitted only under supervision.

(b) Continuous cell lines shall not be introduced into areas used for the production of live mumps vaccine.

(c) Production and control shall be organized in two separate units of the manufacturing establishment that have independent responsibilities.

(d) Personnel involved in the production of the vaccine shall be shown to be immune to mumps.

Particular attention is drawn to the recommendations contained in Part A, section 1 of the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories, Annex 1, p. 13 (1) regarding the training and experience of persons in charge of production and
testing and of those assigned to various positions of responsibility in the manufacturing establishment.

3. Production control

Production shall be controlled according to the scheme recommended in 1980 by a group of WHO consultants (see Annex 3 of 2).

3.1 Control of source materials

3.1.1 Virus strain

The strain of mumps virus used in the production of live mumps vaccine shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. 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 3.1.3) and to yield live mumps vaccine of adequate immunogenicity and safety in human beings. The vaccine strain shall be approved by the national control authority.

3.1.2 Substrate for virus propagation

Mumps virus used in the production of vaccine shall be propagated only in substrates approved by the national control authority. If avian embryos or avian embryo cell cultures are used for the preparation of the vaccine, the eggs shall come from a closed, monitored healthy flock that is free from specific pathogens. Monitoring of chickens and Japanese quail, or their embryos, shall include tests for Mycobacterium avium, fowl or quail 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.
3.1.3 **Virus seed lot system**

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.

Each seed lot shall be identified as mumps virus by appropriate serological methods (see Part A, section 5.1).

Each primary and secondary seed lot shall be shown by appropriate tests to be free from all demonstrable adventitious microbial agents, including avian leukosis virus, and shall satisfy the requirements of Part A, sections 3.3.1 to 3.3.6.

In one country the tests for adventitious agents are not required for secondary seed lots, but they are mandatory for final vaccines derived from a specified seed lot, with the exception of the test in guinea-pigs.

Each seed lot shall be shown by neurovirulence tests in monkeys to cause no unexpected histopathological changes in the central nervous system. To avoid the unnecessary use of monkeys, virus seed lots should be prepared in large quantities.

Some national control authorities require manufacturers to perform the neurovirulence test on each of five consecutive lots of vaccine derived from each virus seed lot.

In some countries the test is performed as follows. Immediately before the test, each monkey should be shown to be serologically negative for mumps. At least 10 monkeys should be used for each test. The material under test should be given to each monkey by the inoculation of 0.5 ml into the thalamic region of each hemisphere. The total amount of mumps virus inoculated into each monkey should not be less than the amount contained in the recommended single human dose of vaccine. The monkeys should be observed for 17 to 21 days for symptoms of paralysis and other evidence of neuropathological changes. Animals that die within 48 hours of injection may be replaced. The test is invalid and should be repeated if more than 20% of the monkeys die from nonspecific causes. At the end of the observation period, each monkey should be anaesthetized and killed, and at autopsy histopathological examinations should be made of appropriate areas of the brain for evidence of pathological changes. It is recommended that the areas of the central nervous system examined include periventricular areas of the brain and the choroid plexus, since these structures are...
frequently affected under experimental conditions with neurotropic variants of mumps virus. At least four control animals shall be inoculated with diluent and similarly examined. Macaca and Cercopithecus monkeys are suitable for testing neurovirulence.

The seed preparation passes the neurovirulence test if there is no evidence of unexpected clinical or histopathological changes in the central nervous system that are attributable to the inoculated virus or to contaminating adventitious microbiological agents.

Histologically mild lesions of ependymal cells and inflammatory reactions in the choroid plexus of the ventricles, which are caused by some mumps vaccine strains, may be acceptable.

If freeze-dried, the seed lot shall be stored at or below −20 °C. If not freeze-dried, the seed lot shall be stored at or below −60 °C. The virus in the final vaccine should be at the minimum passage level beyond that used in the preparation of a vaccine that has been shown to be immunogenic and safe in clinical studies. The passage level must be acceptable to the national control authority.

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

3.2 Production precautions

The general production precautions formulated in Annex 1 of the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories (1) shall apply to the manufacture of live mumps vaccine, with the addition of the following.

3.2.1 Control cell cultures

From the cells used in the preparation of the cell cultures for growing attenuated mumps virus, an amount of processed cell suspension equivalent to that used to prepare 500 ml of cell culture 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 beyond the time of inoculation of the production vessels with mumps virus. After 14 days, fluids collected from the control
cultures and cell monolayers from some of the control vessels shall be tested for the presence of adventitious agents as described below.

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 cell culture lot, the control fluid taken at each harvest is frozen and stored at or below -60°C until the last virus harvesting from that tissue culture lot is completed. The control fluids are then pooled in proportion to their amounts and submitted to the required tests.

The results of the tests on control cultures shall be considered satisfactory only if there is no evidence of adventitious agents and if at least 80% of the control vessels are available for testing at the end of the observation period.

Test for haemadsorbing or haemagglutinating viruses. The cell monolayers from one-quarter of the control vessels shall be tested at the end of the observation period for the presence of haemadsorbing viruses by the addition of guinea-pig erythrocytes that have not been stored for more than 7 days.

Test in cell cultures for adventitious agents. Samples of at least 5 ml of the fluids from each of the control cell cultures shall be inoculated into cell cultures of human and simian origin and into the cell substrate used for virus production, and shall be tested for adventitious agents by the method prescribed in Part A, section 3.3.4.

Test for avian leukosis virus. A sample of fluids pooled from the control cultures shall be tested for avian leukosis virus by a method approved by the national control authority. Suitable methods include the test for resistance-inducing factor, the complement fixation test, and the test for reverse transcriptase.

3.2.2 Control embryonated eggs

Of each batch of eggs used for producing the vaccine, 2% (or 20, whichever is the largest 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 harvesting the virus, amniotic fluids shall be taken from the control eggs and examined individually for haemagglutinating agents (see Annex 3, Part A, section 3.2.1, p. 179 of 4). In addition, a pool of amniotic fluid shall be tested for adventitious agents, including avian leukosis virus, by the methods specified in section 3.2.1.
3.2.3 Cell substrate for vaccine production

The cell cultures for vaccine production shall be grown and maintained under aseptic conditions. The maintenance medium shall contain no added proteins, except human albumin if required. Any human albumin added shall meet the Requirements for biological substances no. 27. Requirements for the collection, processing, and quality control of human blood and blood products (5).

If animal serum is used in the growth medium for the cell cultures, the cells shall be washed free from serum before the virus suspension is harvested, so that the final vaccine shall not contain more than 1 μl of animal serum per litre. Human serum shall not be used.

Penicillin or other β-lactams shall not be used at any stage of manufacture.

Minimal concentrations of other suitable antibiotics, such as neomycin, may be used where approved by the national control authority.

3.3 Harvesting and testing the virus

Virus fluid shall be harvested by a method approved by the national control authority. Each single harvest or pool of harvests shall be tested as described in the following sections.

Samples of single harvests or harvest pools shall be taken for testing at the time of harvesting and if not tested immediately shall be kept at a temperature below −60 °C.

For those tests that require prior neutralization of mumps 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 cultures from a species different to that used for the production of vaccine. Such cell cultures shall be free from extraneous microbial agents that might elicit antibodies inhibitory to the growth of extraneous agents present in the mumps virus pool.

3.3.1 Sterility tests

Each single harvest or pool of harvests shall be tested for bacterial and mycotic sterility according to Part A, sections 5.2 and 5.3 of the Requirements for biological substances no. 6. General requirements for the sterility of biological substances (6). Tests for mycoplasmas shall be performed by a method approved by the national control authority.
Tests for mycoplasmas should be done with both solid and liquid media that have been shown to be capable of supporting the growth of mycoplasmas. Certain nutritionally fastidious mycoplasmas are best detected by DNA fluorescent staining on the surface of cultured indicator cells.

3.3.2 Virus titration

The live virus content of each single harvest or pool of harvests may be determined by titration in cell culture against a reference preparation of live mumps virus (see Part A, section 1.3).

3.3.3 Tests for mycobacteria

Regardless of which substrate is used for producing the virus vaccine, each single harvest or pool of harvests shall be tested for the presence of *Mycobacterium tuberculosis* and *Mycobacterium bovis*. If the vaccine is produced in fibroblasts of avian origin, it shall also be tested for the presence of *Mycobacterium avium*. Tests for mycobacteria shall be done after centrifugation of 20 ml of the virus pool or single harvest.

3.3.4 Tests in cell cultures

A volume of each virus pool equivalent to at least 500 human doses of vaccine shall be tested for adventitious agents by inoculation into cell cultures of human and simian origin. The human cells may include those from continuous or diploid cell lines. The simian cells shall be derived from monkey kidneys. The tissue cultures shall be observed for at least 14 days.

Suitable cell lines for these tests are Vero, LLCMK₂, HeLa, and MRC5.

In one country, the virus pool is tested for the absence of adventitious agents in cell cultures derived from the same species (but not from the same group of embryos) as that used for vaccine production.

The virus pool passes the tests if at least 80% of the cell cultures remain viable and none of the tissue cultures show evidence of the presence of adventitious agents attributable to the virus pool.

3.3.5 Tests in embryonated hen’s eggs

A volume of the neutralized virus pool, equivalent to at least 100 human doses of vaccine, shall be tested in a group of embryonated
hen's eggs aged between 9 and 12 days, by inoculation of 0.5 ml per egg by the allantoic and/or amniotic route.

The virus pool passes the test if, after 7 days, at least 80% of the inoculated eggs still survive, and the allantoic/amniotic fluids fail to show the presence of any haemagglutinating agent. In addition, none of the embryos or chorioallantoic membranes shall show gross pathological changes.

In some countries, it has been found that the inoculation of the yolk sac of 6-day-old to 7-day-old embryos, with incubation for 9 days, is a sensitive method for isolating nutritionally fastidious bacteria, which may then be detected by microscopic inspection of suitably stained smears.

3.3.6 Tests in small laboratory animals

Since a group of WHO consultants considered that tests for extraneous agents in small laboratory animals do not add to the safety of vaccines (see Annex 3 of 2), national control authorities may permit manufacturers to omit such tests on the virus harvest. However, these tests remain mandatory for seed lots.

Tests 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 pool. 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 examined after death 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 five additional mice, which shall be observed for 21 days.

The virus pool 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 agents attributable to the virus pool.

In certain countries the national control authority requires only a 60% survival of the original animals.

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 pool. 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 examined after death 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 five additional suckling mice, which shall be observed daily for 14 days.

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

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

Tests in guinea-pigs. The virus pool is tested for adventitious agents by the intraperitoneal inoculation of 5.0 ml of the virus pool into each of at least five 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 examined macroscopically after death, and their tissues shall be examined both microscopically and in tissue culture for evidence of infection. Animals that survive the observation period without signs of illness shall be killed and examined in a similar manner.

The virus pool passes the test if at least 80% of the guinea-pigs survive the observation period and if none of the animals shows evidence of infection with any adventitious agents attributable to the virus pool.

3.4 Clarification, and control of clarified virus pool

After successful completion of appropriate testing, the virus pool shall be clarified by a method that will ensure removal of all intact cells and cell debris. Microscopic observation of a smear of a concentrated sample of the virus pool is a suitable method of checking the efficiency of the clarification process.

3.4.1 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 Part A, section 1.3).
3.5 Control of final bulk suspension

The final bulk suspension shall be prepared from one or more clarified virus pools obtained from substrates that satisfy the requirements of Part A, section 2; the virus pools shall pass the tests of Part A, sections 3.3.1 to 3.3.5, and 3.4.

Only stabilizers, diluents, or 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.

In certain countries, the national control authority may approve the addition of certain antibiotics, for example neomycin at a concentration of no more than 50 µg/ml.

The final bulk suspension should be prepared under aseptic conditions, and should be stored, until it is distributed into containers and lyophilized, in conditions shown by the manufacturer to retain the activity of the vaccine.

3.5.1 Sterility tests

Each final bulk suspension shall be tested for bacterial and mycotic sterility according to Part A, sections 5.2 and 5.3 of the Requirements for biological substances no. 6. General requirements for the sterility of biological substances (6), or by a method approved by the national control authority.

4. Filling and containers

The requirements for filling and containers given in Annex 1, Part A, section 4 of the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories (1) shall apply.

Single-dose containers are recommended, except for mass immunization campaigns.
5. Control tests on final product

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

5.1 Identity test

An identity test for mumps virus shall be performed by appropriate methods on material from two or more individual labelled containers from each final lot.

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

5.2 Virus concentration

The live virus concentration of each freeze-dried final lot shall be determined by titration in a suitable cell culture system against a reference preparation of live mumps virus (see Part A, section 1.3). The minimum acceptable virus titre per human dose shall be approved by the national control authority.

It is desirable to titrate individually the contents of five or more containers.

5.3 General safety test

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.

5.4 Inspection of final containers

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

5.5 Sterility tests

Final containers shall be tested for bacterial and mycotic sterility by a method approved by the national control authority.

Many countries have standard regulations for sterility testing of the final product. Where these are not available, WHO requirements should be followed (6).
5.6 Residual moisture

The residual moisture in a representative sample of each freeze-dried lot may be determined by a method approved by the national control authority. The upper limit of the moisture content may be specified by the national control authority. Generally moisture levels of less than 2% are considered satisfactory.

5.7 Test for thermostability

Samples of each final freeze-dried vaccine shall be incubated in the dry state at 37 °C for 7 days. At the end of the incubation period, heated samples shall be assayed in parallel with unheated ones and with the reference preparation; the heated vaccine should meet the minimum requirement for potency established by the national control authority. The loss in titre shall be no more than one log_{10} unit.

6. Records

The requirements given in Part A, section 6, of the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories (I) shall apply.

7. Samples

The requirements given in Part A, section 7, of the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories (I) shall apply.

8. Labelling

The requirements given in Part A, section 8, of the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories (I) 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 statement that the vaccine meets Part A of the WHO Requirements for mumps vaccine (live);
— the nature of the preparation, i.e., the designation of the strain of mumps virus contained in the vaccine and the origin of the substrate used to prepare vaccine;
— 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 to be added to reconstitute the vaccine, and specifying that such diluent should be supplied by the manufacturer;
— the statement that after the vaccine is reconstituted it should be used without delay.

9. Distribution and shipping

The requirements given in Part A, section 9, of the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories (1) shall apply. Shipment should be at a temperature of 8 °C or below.

10. Storage and expiry date

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as required in Part A, section 10 of the revised Requirements for biological substances no. 1. General requirements for manufacturing establishments and control laboratories (1), shall be based on experimental evidence and shall be submitted for approval to the national control authority.

10.1 Storage conditions

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

The expiry date shall be fixed with the approval of the national control authority and shall relate to the date of the last satisfactory test to determine virus concentration, this being the date on which the test system was inoculated.

In some countries, manufacturers and national control authorities have observed that mumps vaccines continuously stored in the lyophilized state at or below $-20\,^\circ\text{C}$ do not lose potency over a period of several years. In such cases, the national control authority may allow the dating period to start when the vaccine is taken out of the frozen state, provided that a satisfactory potency test has been carried out within the preceding 12 months.

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 (1) shall apply.

The national control authority shall provide a reference preparation of live mumps virus (see Part A, section 1.3) for tests to determine virus concentration (see Part A, sections 3.3.2, 3.4.1, and 5.2), and shall specify the virus content required to achieve adequate immunization of human beings with the recommended human dose.

2. Release and certification

A vaccine shall be released only if it fulfils Part A of these requirements. A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether the lot of vaccine in question meets all national requirements, as well as Part A of these requirements. The certificate shall state the date of the last
satisfactory determination of virus concentration, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached. The purpose of the certificate is to facilitate the exchange of live mumps vaccine between countries.

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Dr P. Parkman, Office of Biologics Research and Review, Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD, USA
Dr Pranter, Behringwerke AG, Marburg, Federal Republic of Germany
Dr G.V. Quinnan, Director, Division of Virology, Office of Biologics Research and Review, Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD, USA
Dr J. J. Walsh, Director, Quality Assurance of Animal Services, Commonwealth Serum Laboratories, Parkville, Victoria, Australia

REFERENCES

Appendix

SUMMARY PROTOCOL FOR PRODUCTION AND TESTING OF MUMPS VACCINE (LIVE)

Based on Requirements for biological substances no. 38.
Requirements for mumps vaccine (live)

Identification of final lot

Name and address of manufacturer 

No. of final lot 

Date of initiation of last test for determining virus concentration 

Expiry date 

Proprietary name of vaccine 

No. of ampoules or vials in the final lot 

No. of doses in each ampoule or vial 

3.1 Production control

3.1 Control of source materials

Seed virus strain

Substrate for virus propagation

For closed colonies of birds, duration of monitoring for specific pathogens

Virus seed lot system

Reference no. of seed lot

Date(s) of satisfactory test(s) for freedom from extraneous agents

Neurovirulence test

Result of blood serum test of monkeys before inoculation

1 Numbers refer to the corresponding numbered sections in the text of the Requirements.
<table>
<thead>
<tr>
<th>Date of inoculation of seed lot</th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. and species of monkeys inoculated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quantity inoculated into each test monkey¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of monkeys surviving observation period without specific symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results of histopathological examination (specify any abnormal findings)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2 Production precautions

*Control cell cultures or embryonated eggs*

Substrate used for vaccine production

Quantity of cell suspension or no. of eggs used for control

Period of observation of uninoculated controls

Test for haemadsorbing or haemagglutinating viruses

Test method

Results

Cell culture tests

Sample size

Cell cultures inoculated

Results

Test for avian leukosis virus

Test method

Results

Other tests if done (embryonated eggs)

Type of test(s)

Results

*Cell substrate for vaccine production*

Antibiotics added (if used)

Concentration

¹ In terms of TCID₅₀, the quantity of virus suspension that will infect 50% of inoculated cell cultures.
### 3.3 Harvesting and testing the virus

*Single harvests used in virus pool*

<table>
<thead>
<tr>
<th>No. of passages from the primary seed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference no. of harvests</td>
<td></td>
</tr>
<tr>
<td>Virus concentration (optional)</td>
<td></td>
</tr>
<tr>
<td>Sterility test</td>
<td></td>
</tr>
<tr>
<td>Dates</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma test</td>
<td></td>
</tr>
<tr>
<td>Dates</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
</tbody>
</table>

**Tests on virus pool for absence of contamination**

If any test had to be repeated or any abnormal result was observed, this must be specified. If necessary a separate sheet of paper can be used.

| Virus concentration (optional) |                          |
| Sterility test                |                          |
| Date                          |                          |
| Media used                    |                          |
| Results                       |                          |
| Mycoplasma test               |                          |
| Date                          |                          |
| Media used                    |                          |
| Results                       |                          |
| Test for *Mycobacterium tuberculosis* |                      |
| Date                          |                          |
| Media used                    |                          |
| Results                       |                          |
| Tests in cell culture         |                          |
| Volume tested                 |                          |
| Cell cultures used            |                          |
| Observation period            |                          |
| Results                       |                          |
| Tests in embryonated hen's eggs |                      |
| Volume tested                 |                          |
| No. of eggs used              |                          |
| Route(s) of inoculation       |                          |
| Results                       |                          |
| Test in adult mice*           |                          |
| No. and weight of mice        |                          |

* May be omitted if the cell cultures used for production were derived from monitored closed colonies of animals and if permission was given by the national control authority.
<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of inoculation</td>
<td></td>
</tr>
<tr>
<td>Quantity inoculated</td>
<td></td>
</tr>
<tr>
<td>Results (survival numbers, etc.)</td>
<td></td>
</tr>
</tbody>
</table>

**Test in suckling mice¹**

<table>
<thead>
<tr>
<th>No. and weight of mice</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td></td>
</tr>
<tr>
<td>Route of inoculation</td>
<td></td>
</tr>
<tr>
<td>Quantity inoculated</td>
<td></td>
</tr>
<tr>
<td>Results (survival numbers, etc.)</td>
<td></td>
</tr>
</tbody>
</table>

**Test in guinea-pigs¹**

<table>
<thead>
<tr>
<th>No. and weight of guinea-pigs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td></td>
</tr>
<tr>
<td>Route of inoculation</td>
<td></td>
</tr>
<tr>
<td>Quantity inoculated</td>
<td></td>
</tr>
<tr>
<td>Results (survival numbers, etc.)</td>
<td></td>
</tr>
</tbody>
</table>

**Additional tests (depending on cells used)**

<table>
<thead>
<tr>
<th>Test method used</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Results of test</td>
<td></td>
</tr>
</tbody>
</table>

### 3.4 Clarification, and control of clarified virus pool

<table>
<thead>
<tr>
<th>Reference no.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Result of test for intact cells</td>
<td></td>
</tr>
<tr>
<td>Virus titre²</td>
<td></td>
</tr>
</tbody>
</table>

### 3.5 Control of final bulk suspension

<table>
<thead>
<tr>
<th>Date of preparation</th>
<th></th>
</tr>
</thead>
</table>

**Sterility test**

<table>
<thead>
<tr>
<th>Date</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Media used</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td></td>
</tr>
</tbody>
</table>

¹ May be omitted if the cell cultures used for production were derived from monitored closed colonies of animals and if permission was given by the national control authority.

² In terms of TCID₅₀, the quantity of virus suspension that will infect 50% of inoculated cell cultures.
5. Control tests on final product

### 5.1 Identity test
- **Date**: 
- **Method used**: 
- **Result**: 

### 5.2 Virus concentration
- **Date**: 
- **Method**: 
- **Result**: 

### 5.3 General safety test

*Test in mice*
- **Date**: 
- **No. of mice**: 
- **Volume and route**: 
- **Observation period**: 
- **Results (give details of deaths)**: 

*Test in guinea-pigs*
- **Date**: 
- **No. of guinea-pigs**: 
- **Volume and route**: 
- **Observation period**: 
- **Results (give details of deaths)**: 

### 5.4 Inspection of final containers
- **Result**: 

### 5.5 Sterility tests
- **Date**: 
- **Media used**: 
- **Result**: 
- **Were repeat tests necessary?**  
  (if so, give details) 

### 5.6 Residual moisture
- **Size of sample**: 
- **Moisture content (%)**: 

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163
5.7 Test for thermostability

<table>
<thead>
<tr>
<th>Control (unheated) samples</th>
<th>Samples incubated at 37(^{\circ}) C for 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of containers tested</td>
<td></td>
</tr>
<tr>
<td>Virus concentration in each container</td>
<td></td>
</tr>
<tr>
<td>Mean virus titre per human dose</td>
<td></td>
</tr>
<tr>
<td>Mean loss in titre due to heat exposure (in log(_{10}) units)</td>
<td></td>
</tr>
</tbody>
</table>

Reference preparation

<table>
<thead>
<tr>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical titre</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Actual titre</th>
</tr>
</thead>
</table>

Internal certification

*Certification by person taking overall responsibility for production of the vaccine*

I certify that lot no. _______ of mumps vaccine (live) satisfies Part A of the WHO Requirements for mumps vaccine (live).

Signature __________________________

Name (typed) __________________________

Date __________________

The protocol must be accompanied by a sample of the label and a copy of the leaflet. If the vaccine is to be exported, the protocol must also be accompanied by a certificate of the national control authority stating that the given product meets national as well as WHO requirements.