Annex 2

Requirements for hepatitis A vaccine (inactivated)
(Requirements for Biological Substances No. 49)

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Appendix 1
Example, for guidance, of a summary protocol for the production and testing of hepatitis A vaccine (inactivated)  

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Introduction

Hepatitis A virus (HAV) infection is a significant cause of morbidity and attendant economic loss in many parts of the world. The virus is present worldwide and rates of infection are inversely associated with levels of environmental sanitation and personal hygiene. Improvements in sanitation and hygiene can reduce the transmission of HAV. However, in less developed countries, an improvement in sanitary conditions may result in an increase in the burden of clinical disease as peak rates of infection shift from early childhood, when infection is largely asymptomatic, to older age groups in which it is more often symptomatic. Passive immunoprophylaxis using immune globulin can prevent disease in individuals who are exposed to the virus; however, the protective effect is temporary and immune globulin is not suitable for the control of HAV in large populations. Within the past few years, several vaccines have been developed that provide active immunity and potentially long-lasting protection against HAV. The development of these vaccines represents a major advance in the ability to control HAV infection and reduce the burden of disease.

General considerations

Hepatitis A is caused by HAV, a non-enveloped virus with positive-sense single-stranded RNA, belonging to the genus Hepatovirus (Heparnaviridae) of the Picornaviridae family. All human isolates of HAV, while having up to 20-25% variation in nucleic acid sequence, belong to a single serotype. Epidemiological evidence in humans and in vitro and in vivo studies support the concept that antibodies to HAV induced by any isolate of the virus will protect against all viral strains.

The isolation and propagation of HAV in cell culture were critical steps in the successful development of hepatitis A vaccines. Several HAV isolates have been propagated in cell culture and have been used to make both inactivated and attenuated vaccines. These Requirements relate only to the production of hepatitis A vaccine (inactivated) in human diploid cells or continuous cell lines. If any other production process is used, different requirements may apply.

Inactivated hepatitis A vaccines are prepared by the purification of virus propagated in cell culture followed by formalin inactivation, an approach similar to that used for inactivated poliovirus vaccines. Inactivation is one of the critical production steps, and the inactivation process should be carefully monitored. The vaccines produced to date have been shown to be highly immunogenic when two different adjuvants (aluminium and liposomal formulation) were used. Two large studies have demonstrated the efficacy of the vaccines obtained from two manufacturers.
Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements so that, if a national control authority so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, an example of a summary protocol for recording the results of tests is provided as Appendix 1.

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

**Part A. Manufacturing requirements**

A.1 **Definitions**

A.1.1 **International name and proper name**

The international name shall be “Vaccinum hepatitidis A inactivatum”. The proper name shall be the equivalent of the international name in the language of the country of origin.

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

A.1.2 **Descriptive definition**

“Vaccinum hepatitidis A inactivatum” shall consist of hepatitis A virus grown in cell cultures and inactivated. The preparation shall satisfy all the requirements formulated below.

A.1.3 **International reference materials**

An International Reference Preparation of Hepatitis A Immunoglobulin is available from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands. Samples are distributed free of charge, on request, to national control laboratories.

A.1.4 **Terminology**

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

*Master cell bank:* A quantity of adequately characterized cells stored at −60 °C or below in aliquots of uniform composition, one or more of which may be used for the production of a manufacturer’s working cell bank.
Manufacturer’s working cell bank (MWCB): A quantity of cells of uniform composition derived from one or more ampoules of the master cell bank, which may be used for the production of cell cultures. A master cell bank is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to give a single pool and preserved cryogenically in aliquots to form the MWCB. One or more of the ampoules from such a pool may be used for the production cell culture.

Production cell culture: A cell culture derived from one or more ampoules of the MWCB and used for the production of HAV.

Adventitious agents: Contaminating microorganisms of the cell substrate or materials used in its culture, including bacteria, fungi, mycoplasmas and endogenous and exogenous viruses.

Virus master seed lot: A quantity of virus that has been prepared as a single lot and has a uniform composition. It is used for the preparation of working seed lots.

Virus working seed lot: A quantity of virus of uniform composition derived by passage from the master seed lot, by a method and at a passage level approved by the national control authority.

Single virus harvest: A virus suspension harvested from production cell cultures that were processed together. Multiple harvests from the same production cell culture may be pooled and considered a single virus harvest.

Purified pool: A purified single virus harvest or a pool of single virus harvests purified at the same time.

Final bulk: The preparation present in the container from which the final containers are filled. The final bulk is prepared from one or more purified pool(s) or part of a pool after inactivation.

Final lot: A collection of sealed final containers of vaccine that are homogeneous with respect to the risk of contamination during the preparation of finished vaccine, including the filling process and, if applicable, the freeze-drying. The containers of a final lot must therefore have been filled, or the final lot prepared in one working session.

A.2 General manufacturing requirements

The general manufacturing requirements contained in Good Manufacturing Practices for Pharmaceutical (1) and Biological (2) Products shall apply to hepatitis A vaccine with the following addition: all staff involved in the production of hepatitis A vaccine should be shown to be immune to hepatitis A.
A.3 Control of source materials

A.3.1 Cell cultures for virus propagation

A.3.1.1 Cell banks used for providing production cell cultures
If human diploid cells are used for the propagation of HAV, they shall meet the Requirements for Human Diploid Cells used for the Production of Varicella Vaccine (Live) (3) and shall be approved by, and registered with, the national control authority.

If continuous cell lines are used for the propagation of HAV, they shall be approved by, and registered with, the national control authority and meet the Requirements for Continuous Cell Lines used for Biologicals Production (4).

The cells in any cell bank shall have been characterized with respect to their genealogy, growth characteristics, and viability during storage and shall have been shown to be free from detectable adventitious agents.

A.3.1.2 Cell culture medium
Serum used for the propagation of cells for hepatitis A vaccine production shall be tested to demonstrate freedom from bacteria, fungi and mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (5) and to demonstrate freedom from viruses. Serum of bovine origin must come from countries or herds certified to be free of bovine spongiform encephalopathy.

Some countries require that bovine serum should come from herds that have not been given feed derived from ruminant protein.

Suitable tests for detecting viruses in calf or newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (6). In some countries sera are also examined for freedom from certain phages.

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

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

A.3.1.3 Trypsin used for preparing cell cultures
Trypsin used for preparing cell cultures shall be bacteriologically sterile and free from mycoplasmas and viruses, especially porcine parvoviruses. If bovine trypsin is used, it must come from countries or herds certified to be free of bovine spongiform encephalopathy. The methods used to ensure this shall be approved by the national control authority.

A.3.2 Virus seed

A.3.2.1 Strain of virus
Strains of HAV used in the production of vaccine shall be approved by the national control authority. Each strain shall be identified by means of
historical records that include information on the origin of the virus. Any strain that will yield a vaccine meeting the requirements set forth in the present document, and that has been shown by clinical trials to produce a safe and effective vaccine, may be used.

A.3.2.2 Virus seed lot system
The production of vaccine shall be based on the virus seed lot system. The virus working seed lot used for the production of vaccine batches shall be prepared from a master seed lot by a method approved by the national control authority. All virus seed lots shall be stored at a temperature of −60 °C or below.

A.3.2.3 Tests on virus seed lots
The seed lot used for the production of vaccine shall be free from detectable adventitious agents and shall be produced in conditions that satisfy the requirements of sections A.4.1 and A.4.2.1.

Identity and infectivity. Each virus seed lot shall be identified as HAV by appropriate serological methods.

Appropriate serological methods include enzyme immunoassay or hepatitis A neutralization assay, using a reference serum or a monoclonal antibody known to neutralize HAV.

The infectivity of each seed lot shall be established in the cell culture system selected for production.

Freedom from bacteria, fungi and mycoplasmas. Each virus seed lot shall be shown to be free from bacterial, mycotic and mycoplasmal contamination by appropriate tests as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (5).

Tests for adventitious viruses. Each virus seed lot shall be tested in cell cultures for adventitious viruses. Neutralization of HAV may not be necessary for many tests because the virus is generally not cytopathogenic and has a limited host range.

A volume of each seed lot of at least 10 ml shall be tested for adventitious viruses by inoculation into simian cells. Similar volumes shall likewise be tested in human cell cultures and also in cell cultures of the same type but not the same batch as that used in the preparation of the virus seed. Uninoculated control cell cultures shall be included in the tests. All cell cultures shall be incubated at 35–37 °C and observed for at least 14 days.

The cells shall be observed microscopically for cytopathic changes. At the end of the observation period, the cells or fluids shall be tested for haemadsorbing viruses and other adventitious agents as specified in sections A.4.1.1 and A.4.1.2. For a test to be valid, no more than 20% of the culture vessels should have been discarded for non-specific reasons by the end of the test period. For the seed virus to be satisfactory, no cytopathic changes or adventitious agents shall be detected.
If primary monkey kidney cultures were used in the adaptation of the virus strain to cell culture, tests for simian viruses should be done.

Specific tests may be required for simian immunodeficiency virus (SIV), simian virus 40 (SV40), Marburg virus and B virus. In some countries virus seed lots are also tested in animals.

Tests for SIV may include reverse transcriptase assay, infectivity in a sensitive cell line, hybridization tests with appropriate recombinant DNA probes or the polymerase chain reaction (PCR), inoculation of a sensitive host monkey, and determination of infection by seroconversion or molecular diagnostic tests.

A.4 Control of vaccine production

A.4.1 Control cell cultures

An amount of the production cell suspension equivalent to at least 5% of the total volume but not more than 1000 ml shall be used to prepare control cultures of uninfected cells.

These cells should be maintained under similar conditions of time, temperature and media as the infected cells. The control cells shall be maintained until the time of harvest of the production cells. At the time of the HAV harvest (usually 2–4 weeks after inoculation), the control cells shall be examined for degeneration caused by adventitious agents.

In addition, at the end of the observation period, fluids collected from the control culture shall be pooled and tested for the presence of adventitious agents as described below. Samples that are not tested immediately shall be stored at –60 °C or below.

If multiple virus harvests are made from the same production cell culture lot, the control fluid taken at the time of each harvest shall be frozen and stored at or below –60 °C until the last virus harvesting is completed. The control fluids shall then be pooled in proportion to their amounts and submitted to the required tests.

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

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

In some countries, the national control authority may permit reduced testing for adventitious agents where the manufacturer has demonstrated that:

(a) all raw materials of animal origin used in the cell culture process, the MWCB and the virus seed lots are free from detectable adventitious agents;
(b) at the end of the cell culture, the cell culture system is free from bacterial, mycotic and mycoplasmal contamination as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (5);
(c) the manufacturing process is consistent and validated to remove/inactivate a panel of representative adventitious agents.

A.4.1.1 Test for haemadsorbing adventitious viruses
At the end of the observation period, 25% of the control cells shall be tested for the presence of haemadsorbing viruses, using guinea-pig red cells. If the red cells have been stored, the duration of storage shall not have exceeded 7 days, and the temperature of storage shall have been in the range of 2–8 °C.

Some national control authorities require that the cell cultures described in section A.4.1.2 should be tested for the presence of haemadsorbing viruses at the end of the incubation period(s). If this is the case, the test for haemadsorbing viruses described here may be deleted.

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

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

A.4.1.2 Tests for non-haemadsorbing adventitious agents
At 14 days after the day of inoculation of the production cultures or at the time of final virus harvest, 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 cell cultures, but not the same batch, as that used for virus growth. Additional 10 ml samples of each pool shall be tested in both human and simian cells.

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 culture shall remain uninoculated as a control.

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

The cell culture safety tests are satisfactory if no cytopathic changes attributable to adventitious agents in the test sample are detected.
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, no more than 20% of the control culture vessels should have been discarded for non-specific reasons by the end of the test period.

A.4.1.3 Identity test
The cells used in hepatitis A vaccine production should be documented to be derived from the characterized MWCB.

An identity test shall be performed on the control cell cultures by a method approved by the national control authority, as specified in Appendix 1, section 2.2.2 of the Requirements for Human Diploid Cells used for the Production of Varicella Vaccine (Live) (3) or section 3.4 of the Requirements for Continuous Cell Lines used for Biologicals Production (4).

Methods used for identity testing include biochemical (e.g. isoenzyme analysis), immunological (e.g. histocompatibility antigen assays), and cytogenic marker tests or, for diploid cells, karyotyping of at least one metaphase spread of chromosomes.

A.4.2 Control of single virus harvests

A.4.2.1 Sterility tests
A sample of each single virus harvest or virus culture supernatant shall be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests as specified in Part A, sections 5.2 and 5.3, of the Revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (5). Any single harvest in which contamination is detected shall be discarded.

A.4.2.2 Virus content
A sample removed from each virus harvest shall be tested for antigen content by an immunological assay and/or for virus content by an infectivity assay to confirm consistency of production.

A.4.3 Preparation and control of purified pool

A.4.3.1 Pooling of single harvests
Only virus harvests meeting the requirements for sterility and virus content of sections A.4.2.1 and A.4.2.2 shall be pooled.

A.4.3.2 Purification procedures
Each pool of virus shall be purified before inactivation. The methods used to purify HAV from virus harvests shall be approved by the national control authority.
Each manufacturer shall demonstrate, by testing each lot, or by validation of the purification process, that residual contaminants are consistently reduced to a level acceptable to the national control authority.

The protein content per human dose and the minimum purity shall be approved by the national control authority.

Animal serum albumin shall be reduced to less than 50 ng per human dose.

The upper limit of cellular DNA for vaccine produced in continuous cell lines shall be 100 pg per dose.

Consistency of purification within the limits approved by the national control authority shall be demonstrated. For each lot there shall be a residual marker substance whose clearance is monitored.

Serum albumin is an appropriate candidate for a marker, though other components may be more appropriate for specific manufacturing processes.

**A.4.3.3 Inactivation of virus**

The virus in the purified pools shall be inactivated by a method that has been validated. The kinetics of inactivation shall be suitably monitored and demonstrated by the manufacturer to be consistently effective. Virus aggregates, if present, shall be removed before inactivation. The method shall be approved by the national control authority.

Chemical or physical steps, such as filtration, may be used to remove aggregates.

The importance of filtration or clarification of the crude virus suspensions as a means of improving the consistency of the inactivation process has been clearly established. Satisfactory results have been reported with several types of filter but a 0.2 μm filter should be used for the final filtration.

If the crude virus suspension is filtered, it is preferable to start inactivation within 24 hours. Since the purpose of the filtration step is to remove particulate matter and other interfering substances that may diminish the effectiveness of the inactivation process, and since aggregates tend to form if the filtrate is left to stand after filtration, efforts should be made to keep within this time limit.

Formaldehyde may be used as an inactivating agent in the production of vaccines. It has been recommended that tests for free formaldehyde should be performed at intervals and the concentration maintained at the desired level by intermittent readjustments. Some manufacturers use a combination of initial formaldehyde treatment with some other method of inactivation.

If the virus pool is filtered before inactivation is initiated, a second filtration shall be carried out during the process of inactivation.

This is done after the infective virus titre has fallen below detectable levels but before the first sample for the safety test is taken.

**Test for effective inactivation.** Each undiluted bulk suspension shall be tested for effective inactivation of HAV by a method approved by the national control authority. Two samples shall be tested, one of which
shall be taken at the end of the inactivation period and the other not later than three-quarters of the way through this period. The total sample size for these two samples shall be 5% of the batch or not more than 1500 adult doses. After removal or neutralization of the inactivating agent, the samples shall be tested by inoculation into cell cultures for the absence of infective HAV. The test samples shall be inoculated into sensitive cell cultures preferably of the same type as that from which the vaccine was prepared. The inoculated cells shall be incubated for a validated period of time at the optimum growth temperature of the particular strain of HAV used in the vaccine. Since HAV is relatively difficult to detect in cell culture when present in small amounts, any residual virus shall be amplified by a blind passage. A portion of the entire cell lysate from the first passage shall be used as the inoculum for the second passage in similar cultures, which shall be incubated for a period equal to that used for the first passage. Absence of HAV replication shall be monitored by an assay approved by the national control authority.

The approved assay could be an immunofluorescence assay, radioimmunoassay, enzyme-linked immunosorbent assay, direct radioimmunoassay or possibly an assay for HAV-specific RNA replication.

Suitable infectious HAV inocula shall be used concurrently as positive controls to demonstrate cellular susceptibility and absence of interference.

Some strains of cell-culture-adapted HAV are essentially non-pathogenic in humans at a dose many log_{10} infectious units (powers of 10) greater than any that could conceivably be present as residual infectious virus after inactivation.

A record of consistency shall be established by the production of five consecutive lots. If any bulk suspension fails the test for effective inactivation, a further five consecutive lots shall be prepared and shown to be satisfactory for re-establishing production.

Antigen content of bulk suspension. The antigen content of the vaccine shall be assayed prior to the addition of adjuvant (see section A.4.2.2). The assay for antigen content shall be approved by the national control authority.

A.4.4 Preparation and control of final bulk

A.4.4.1 Preservatives
Preservatives that might be added to the vaccine shall have been shown to have no deleterious effect on the immunizing potency of the product. The preservatives used and their concentrations shall be approved by the national control authority.

A.4.4.2 Addition of adjuvant
Where the final bulk contains an adjuvant, the adjuvant and the concentration used shall be approved by the national control authority.

Where aluminium salts are used, the concentration of aluminium should not exceed 1.25 mg per single human dose.
A.4.4.3 Test for completeness of adsorption to adjuvant
Tests shall be carried out to confirm that hepatitis A antigen is adsorbed to the adjuvant. The tests and limits shall be approved by the national control authority.

A.4.4.4 Sterility tests
Each final bulk shall be tested for bacterial and mycotic sterility and for mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (5), or by a method approved by the national control authority.

A.4.4.5 Tests for chemicals used in production
The concentration of any organic solvent and inactivating agent remaining in the final vaccine shall be determined by methods approved by the national control authority. These concentrations shall not exceed upper limits specified by the national control authority.

A.4.4.6 Potency tests
A potency test on the final bulk may be performed. The required potency and the assay method shall be approved by the national control authority.

A.5 Filling and containers
The requirements concerning filling and containers in Good Manufacturing Practices for Biological Products (2, section 4) shall apply.

A.6 Control tests on final lot
The national control authority may permit tests for endotoxin, protein content, preservatives and adjuvants to be performed on the final bulk instead of on the final lot.

A.6.1 Identity test
An identity test based on immunological reactivity shall be performed on the vaccine in the final labelled container.

The potency test may serve as an identity test.

A.6.2 Potency test
A potency test on the vaccine in the final container shall be performed if it has not been performed on the final bulk. The required potency of the vaccine and the assay method shall be based on evidence submitted to prove efficacy in clinical trials and shall be approved by the national control authority.

Potency assays for hepatitis A vaccines have not been worked out in detail. Manufacturers should devise a validated assay for their product.
A.6.3 Sterility test
Each final lot shall be tested for sterility as specified in Part A, sections 5.1 and 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (5).

A.6.4 General safety tests
Each final lot shall be tested for the absence of abnormal toxicity in mice and guinea-pigs by parenteral injection. The test procedures shall be approved by the national control authority.

A.6.5 Test for endotoxin
Each final lot shall be tested for endotoxin. The test and limits shall be approved by the national control authority.

A.6.6 Test for preservative
Each final lot shall be tested for the presence of preservative, if used. The test used and the permitted concentration shall be approved by the national control authority.

A.6.7 Protein content
Limits shall be defined for the protein content of the final product.

A.6.8 Adjuvants
Each final lot shall be assayed for the content of adjuvant. The method used and the permitted concentration shall be approved by the national control authority.

The formulation shall be such that a homogeneous suspension is administered to the patient.

A.6.9 Residual moisture
If the vaccine is freeze-dried, the residual moisture shall be determined in the final product by a method and with an upper limit approved by the national control authority.

A.6.10 Inspection of final containers
Every container in each final lot shall be inspected visually and those showing abnormalities shall be discarded.

A.7 Records
The requirements in Good Manufacturing Practices for Biological Products (2, section 8) shall apply.
A.8 **Samples**

The requirements in Good Manufacturing Practices for Biological Products (2, section 9) shall apply.

A.9 **Labelling**

The requirements in Good Manufacturing Practices for Biological Products (2, section 7) shall apply, with the addition of the following:

The leaflet accompanying the package shall state:

- the nature of the cell culture used;
- the virus strain used for the production of the vaccine;
- the method used for inactivating the virus;
- the nature and amount of adjuvant and preservative present.

A.10 **Distribution and shipping**

The requirements in Good Manufacturing Practices for Biological Products (2, section 8) shall apply.

A.11 **Storage and expiry date**

The requirements in Good Manufacturing Practices for Biological Products (2) shall apply.

A.11.1 **Storage conditions**

Hepatitis A vaccine (inactivated) shall be stored at all times at a temperature between 2 °C and 8 °C. Alternative storage temperatures shall be justified and approved by national control authorities. Aluminium-absorbed vaccines shall not be frozen.

A.11.2 **Stability of vaccine and expiry date**

The stability of the vaccine shall be established at the recommended storage temperature. The expiry date shall be fixed with the approval of the national control authority.

*Accelerated degradation studies at 37°C may provide useful additional information.*

**Part B. National control requirements**

B.1 **General**

The general requirements for control laboratories in the Guidelines for National Authorities on Quality Assurance for Biological Products (7) shall apply with the addition of the following:
The national control authority shall approve the strains to be used and the cell substrate, and specify the potency requirements, and shall be satisfied that the results of all tests, including those done on pools during the process of manufacture, are satisfactory and that consistency has been established.

B.2 Release and certification

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

At the request of the manufacturing establishment, the national control authority shall provide a certificate that states whether the vaccine meets all national requirements and/or Part A of the present Requirements. The certificate shall be based on the model given in Appendix 2.

The purpose of the certificate is to facilitate the exchange of hepatitis A vaccines among countries.

Authors

The first draft of these Requirements was prepared in February 1993 by Dr S. Feinestone, Division of Viral Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA, and by Dr V. Grachev, Scientist, Biologicals, and Dr D. Macrae, Chief, Biologicals, World Health Organization, Geneva, Switzerland. A revised draft was formulated at an informal consultation held in Geneva from 15 to 17 November 1993, attended by the following people:

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Dr D. Wood, Virologist, National Institute for Biological Standards and Control, Potters Bar, Herts., England (Rapporteur)
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Secretariat (WHO, Geneva, Switzerland)
Dr D. McGreal, Chief, Biologicals
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Dr M. Kane, Microbiology and Immunology Support Services

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References


Appendix 1

Example, for guidance, of a summary protocol for the production and testing of hepatitis A vaccine (inactivated)¹

Data on the control of source materials are required only on first submission of a product and whenever a change is made.

Identification of final lot

Name and address of manufacturer

Lot no. of vaccine

Date of manufacture of final lot

Expiry date

Total volume of final lot

Number of containers and doses

Control of source materials (A.3)

Cell cultures for virus propagation (A.3.1)

Cell banks used for providing production

Cell cultures (A.3.1.1)

Origin and short history of cell bank

Authority that approved cell seed

Characteristics of cell bank

Cell culture medium (A.3.1.2)

Origin of serum used for cell cultures

Results of tests performed on serum

Trypsin used for preparing cell cultures (A.3.1.3)

Results of tests performed on trypsin

Virus seed (A.3.2)

Strain of virus (A.3.2.1)

Short history

Authority that approved strain of HAV

Virus seed lot system (A.3.2.2)

Date of preparation of virus master seed lot

Date of preparation of virus working seed lot

Number of passages between master and working seed lots

Number of subcultures between working seed lot and production

Tests on virus seed lots (A.3.2.3)

Identification of the virus seed lot
  Method used
  Results

Infectivity of seed lot
  Method used
  Results

Freedom from bacteria, fungi and mycoplasmas

<table>
<thead>
<tr>
<th>bacteria</th>
<th>fungi</th>
<th>mycoplasmas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
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<tr>
<td>Media used</td>
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<tr>
<td>Observation period</td>
<td></td>
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<tr>
<td>Results</td>
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</table>

Tests in cell cultures for adventitious viruses
  Methods used
  Results

Control of vaccine production (A.4)

Control cell cultures (A.4.1)
  Observation period
  Percentage of culture vessels discarded for non-specific reasons
  Results

Tests for haemadsorbing adventitious viruses (A.4.1.1)
  Methods
  Results
Tests for non-haemadsorbing adventitious viruses (A.4.1.2)

Methods

Results

Identity test (A.4.1.3)

Methods

Results

Control of single virus harvests (A.4.2)

Sterility tests (A.4.2.1)

<table>
<thead>
<tr>
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<tr>
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Virus content (A.4.2.2)

Method

Results

Preparation and control of purified pool (A.4.3)

Pooling of single harvests (A.4.3.1)

Number of single harvests

Volume of bulk material

Purification procedures (A.4.3.2)

Protein nitrogen test

Method

Results

Animal serum test

Method

Results

Residual DNA test

Method

Results

55
*Inactivation of virus* (A.4.3.3)

Treatment before inactivation

Method of inactivation

Agent and concentration

Temperature

Date of start of inactivation

Second sterile filtration

(if applicable)

Date of completion of inactivation

Test for effective inactivation

Method

Results

Antigen content of bulk suspension

Method

Results

*Preparation and control of final bulk* (A.4.4)

Composition of final bulk

(after mixing of all ingredients)

and identification number

*Preservatives* (A.4.4.1)

Concentrations

*Addition of adjuvant* (A.4.4.2)

Volume of bulk

Nature and volume of adjuvant added

and final concentration

*Tests for completeness of adsorption to adjuvant* (A.4.4.3)

Method

Results

Date
**Sterility tests** (A.4.4.4)

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**Tests for chemicals used in production** (A.4.4.5)

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**Potency tests** (A.4.4.6)

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<td>Results</td>
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**Control tests on final lot** (A.6)

**Identity test** (A.6.1)

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**Potency test** (A.6.2)

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**Sterility test** (A.6.3)

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**General safety tests** (A.6.4)

**Test in mice**

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<td>Section</td>
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<tr>
<td>---------------------------------</td>
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<tr>
<td>No. of mice tested</td>
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<td>Volume and route of injection</td>
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<td>Observation period</td>
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<td>Results (give details of deaths)</td>
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<tr>
<td><strong>Test in guinea-pigs</strong></td>
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<tr>
<td>Date of inoculation</td>
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<tr>
<td>No. of guinea-pigs tested</td>
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<tr>
<td>Volume and route of injection</td>
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<tr>
<td>Results (give details of deaths)</td>
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<td><strong>Test for endotoxin</strong> (A.6.5)</td>
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<td><strong>Test for preservatives</strong> (A.6.6)</td>
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<td><strong>Protein content</strong> (A.6.7)</td>
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<td><strong>Adjuvants</strong> (A.6.8)</td>
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<td><strong>Residual moisture (if applicable)</strong> (A.6.9)</td>
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<td><strong>Inspection of final containers</strong> (A.6.10)</td>
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</table>
Submission addressed to national control authority for batch release

Name (typed) and signature of head of production laboratory

Date

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

I certify that lot no. _____ of hepatitis A vaccine (inactivated) satisfies national requirements and/or Part A of the Requirements for Biological Substances No. 49 (Requirements for Hepatitis A Vaccine (Inactivated)).

Signature

Name (typed)

Date
Appendix 2

Model certificate for the release of hepatitis A vaccine (inactivated) by national control authorities

The following lots of hepatitis A vaccine (inactivated) produced by ________ in ________, whose numbers appear on the labels of the final containers, meet all national requirements and Part A of Requirements for Biological Substances No. 49 (Requirements for Hepatitis A Vaccine (Inactivated)) and comply with Good Manufacturing Practices for Pharmaceutical and Biological Products.

Lot no. | Date of last potency test by manufacturer | Expiry date | Lot no. | Date of last potency test by manufacturer | Expiry date
---|---|---|---|---|---
_______ | _______ | _______ | _______ | _______ | _______
_______ | _______ | _______ | _______ | _______ | _______
_______ | _______ | _______ | _______ | _______ | _______

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

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

Name (typed) ________________________________________________

Signature ____________________________________________

Date ____________________________________________

---

1 To be completed by the national control authority of the country where the vaccine has been manufactured, and to be provided by the vaccine manufacturer to importers.
2 Name of manufacturer.
3 Country.
4 If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national control authority.
5 With the exception of the provisions on distribution and shipping, which the national control authority may not be in a position to assess.
9 Or his or her representative.