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Recommendations to assure the quality, safety and efficacy of Enterovirus 71 vaccines (inactivated)

Proposed new recommendations

(Draft-version public consultation)

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed WHO Recommendations to assure the quality, safety and efficacy of Enterovirus 71 vaccines (inactivated) to a broad audience and to improve transparency of the consultation process.

These Recommendations were developed based on the outcomes and consensus of the WHO working group meeting convened in 2018 with participants from national regulatory authorities, national control laboratories, vaccine manufacturers and academia researchers.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Written comments proposing modifications to this text MUST be received **by 28 May 2020** in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Health Products Policy and Standards. Comments may also be submitted electronically to the Responsible Officer: Dr Dianliang Lei at email: leid@who.int.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide, second edition" (KMS/WHP/13.1).

Contents

Introduction

Enterovirus (EV)71 was first isolated from the faeces of a female suffering from encephalitis in 1969 in California (*1*) although a retrospective study in The Netherlands suggests that it could have emerged as early as 1963 (*2*), consistent with reports of possible epidemics due to EV71 in the late twentieth century across the world (*3).* EV71 has since been associated with a range of diseases, including Hand Foot and Mouth Disease (HFMD) throughout the world and with epidemics in Asia, Europe and America. The virus causes a range of effects from asymptomatic infection and mild HFMD, to neurological disease with severe central nerve system (CNS) complications and cardiopulmonary failure. In severe cases, mortality rates can be high, especially in children of 5 years age and younger. EV71 is considered to be the most severe neurotoxic enterovirus. Indeed, severe EV71 disease has become a major public health problem in China. In 2011, the WHO Western Pacific Regional Office issued a "*Guide to clinical Management and Public Health Response to Hand, Foot and Mouth Disease(HFMD)"* (*4*) to support the treatment, prevention and control of HFMD.

Several vaccines against EV71 virus are under development and three inactivated EV71 vaccines have already been licensed in China (*5-10*). The WHO Expert Committee on Biological Standardization (ECBS), at its 67th meeting in 2016, discussed the EV71 situation and considered it was of major regional significance (*11*). It noted that the joint effort of the National Institutes for Food and Drug Control (NIFDC) and the National Institute for Biological Standards and Control (NIBSC) had resulted in the development of the first International Standard for anti-EV71 serum (human) and recommended that consideration should also be given to the development of a written standard for EV71 vaccines. In addition, the 1st International standard for EV71 inactivated vaccine has been recently established by the WHO ECBS in October 2019 following a collaborative study led by NIBSC and NIFDC (*12, 13*). National standards for antigen content and neutralizing antibody responses for evaluating EV71 vaccines are also available in China (*14*) and have supported the development and clinical assessment of EV71 vaccines in China. In 2018, the ECBS endorsed a further proposal to develop WHO international standards for enterovirus RNA for NAT-based assays for EV71 (*11, 15, 16*).

Following requests from regulators and other stakeholders for WHO to develop Recommendations to assure the quality, safety and efficacy of EV71 vaccines, a series of meetings was convened by WHO to review the current status of development and licensure of such vaccines *(17).* These meetings were attended by experts from around the world involved in the research, manufacture, regulatory assessment and approval, control-testing and release of EV71 vaccines. Participants were drawn from academia, national regulatory authorities (NRAs), national control laboratories (NCLs) and industry. The following Recommendations are the first WHO recommendations for the production, quality control and evaluation of inactivated EV71 vaccines. They are based on the first three licensed EV71 vaccines and other vaccine candidates under development (*5-8,18-22*) and on the experience gained from other inactivated viral vaccines, such as inactivated polio vaccine and hepatitis A vaccines (*23, 24*).

Scope

These WHO Recommendations provide guidance to NRAs and manufacturers on the manufacturing processes, quality control and nonclinical and clinical evaluations, needed to assure the quality, safety and efficacy of inactivated EV71 vaccines.

They apply to EV71 vaccines prepared by the inactivation of whole EV71 virus for prophylactic use, grown in mammalian cells in culture, and using formaldehyde or other chemical inactivation procedures.

The document does not cover recombinant and other forms of subunit vaccines, vectored vaccines, virus-like particles (VLPs) or EV71-CA16 vaccines, which are at an early stage of development. However, some aspects outlined in this document may be relevant and may be taken into consideration during vaccine development.

The Recommendations should be read in conjunction with current WHO guidelines on nonclinical (*25*) and clinical evaluation of vaccines *(26*), GMP for biologicals (*27*), characterization of cell banks *(28*), nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (*29*), and lot release (*30*).

Terminology

The definitions given below apply to the terms as used in these WHO Recommendations. They may have different meanings in other contexts.

Adjuvant: a vaccine adjuvant is a substance, or a combination of substances, that is used in conjunction with a vaccine antigen to enhance (for example, increase, accelerate, prolong and/or possibly target) the specific immune response to the vaccine antigen and the clinical effectiveness of the vaccine.

Adventitious agents: contaminating microorganisms of the cell culture, or source materials used in its culture, that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced into the manufacturing process.

Cell-culture infectious dose 50% ($CCID_{50}$): the quantity of a virus suspension that will infect 50% of cell cultures.

Cell bank: a cell bank is a collection of appropriate containers whose contents are of uniform composition stored under defined conditions. Each container represents an aliquot of a single pool of cells.

The individual containers (for example, ampoules or vials) should be representative of the pool of cells from which they are taken and should be frozen on the same day by following the same procedure and by using the same equipment and reagents.

Cell seed: a quantity of well-characterized cells derived from a single tissue or cell of human or animal origin and stored frozen in liquid nitrogen in aliquots of uniform composition, one or more of which may be used for the production of a **master cell bank**.

EV71 antigen: the virus specific antigen found in infected cell cultures or purified from such cultures. It can be assayed by methods such as ELISA using EV71 specific antibodies. The antigen may consist of empty or full particles or both. The full and empty particles differ in their antigenic reactivity and both may be present in the final vaccine.

Final bulk: the finished vaccine present in the container from which the final containers are filled. The final bulk may be prepared from one or more vaccine bulks.

Final lot: a collection of sealed final containers of finished vaccine that is homogeneous with respect to the risk of contamination during the filling process. All of the final containers must therefore have been filled from a single vessel of **final bulk** in one working session.

Immunogenicity：The capacity of a vaccine to induce antibody-mediated and/or cell mediated immunity and/or immunological memory.

Inactivated purified pool: a filtered and purified pool of virus harvests in which the virus has been inactivated through the use of a validated method either before or after purification

Master cell bank (MCB): a quantity of well-characterized cells of human or animal origin derived from a **cell seed** at a specific population doubling level or passage level, dispensed into multiple containers, cryopreserved and stored frozen under defined conditions, such as the vapour or liquid phase of liquid nitrogen in aliquots of uniform composition. The MCB is prepared from a single homogeneously mixed pool of cells and is used to derive all **working cell banks**. The testing performed on a replacement MCB (derived from the same clone or from an existing master or working cell bank) is the same as for the initial MCB, unless a justified exception is made.

Potency：Quantitative measure of the specific ability or capacity of the product to achieve a defined biological effect.

Production cell culture: a collection of cell cultures derived from one or more ampoules of the WCB used for the production of EV71 vaccine.

Purified virus pool: a concentrated and purified pool of a number of single virus harvests processed at the same time.

Single harvest: a quantity of virus suspension harvested from cell cultures derived from the same **working cell bank** and prepared from a single production run.

Virus master seed lot: a quantity of virus suspension that has been processed at the same time to ensure a uniform composition, and passaged for a specific number of times that

does not exceed the maximum approved by the NRA. It is characterized to the extent necessary to support development of the **virus working seed lot**.

Virus working seed lot: a quantity of virus of uniform composition derived from the **virus master seed lot** used at a passage level approved by the NRA for the manufacturing of vaccine.

Working cell bank (WCB): a quantity of cells of uniform composition derived from one or more ampoules of the MCB at a finite passage level, stored frozen at -70 °C or below in aliquots, one or more of which would be used for vaccine production. All containers are treated identically and once removed from storage are not returned to the stock.

General considerations

Hand Foot and Mouth disease (HFMD) was first reported in New Zealand in 1957 and occurs mostly in young children with a peak incidence at about two years of age. The common mild disease involves lesions on the mucosal surfaces of the mouth and spots on the palms of the hands and soles of the feet which resolve in a few days; this is not life threatening. However, a more severe, potentially fatal form of the disease was reported in 1969 (*1*) and is now recognised to encompass meningitis/encephalitis, autonomic nervous system deregulation, cardiovascular collapse and pulmonary oedema. The mortality rate is of the order of one per 1-10 thousand cases.

The frequency of reported cases of the disease is geographically highly variable with most cases occurring in East Asian countries particularly China, but including Vietnam, Thailand, Singapore, Malaysia and Korea. Few cases of severe disease are normally reported in Europe or the USA and reports of mild HFMD are also less common, although under reporting is very likely. Typically, the total number of cases in Europe and USA is of the order of a few hundred per year whereas in 2008 in China alone there were 488,955 cases and 128 deaths (*4, 31, 32*). HFMD is now a reportable disease in China in contrast to Europe and the USA, and from 2013 to 2018 there have been 300,000 to 400,000 cases in May/June every year with a few deaths (*4, 31, 32*). The reason for the differences in disease burden in different geographic areas is not yet clear.

The causative agents of HFMD are picornaviruses, most often of human enterovirus species A. The picornaviruses are a family of small non-enveloped viruses with a single strand positive sense RNA genome of about 7,500 nucleotides. The enteroviruses are one of the 35 taxa recognised by International Committee on Taxonomy of Viruses(ICTV) as of October 2019 and are sub-classified into numerous species including the four human enteroviruses A, B, C and D (*33*, https://talk.ictvonline.org/). The archetypal human picornavirus is poliovirus belonging to species C but the commonest cause of HFMD are the species A enteroviruses, chiefly the Coxsackie A viruses and EV71. The frequency of enterovirus species found varies from year to year when clinical isolates or environmental samples such as sewage are examined.

In recent years Coxsackie viruses such as A16, A10 and A6 have caused most HFMD in Asia. Other viruses of the human enterovirus picornavirus family are also implicated and as the different serotypes are antigenically distinct, development of a vaccine based on a single serotype to protect against all mild HFMD cases is unlikely with existing technologies. There is interest in combination vaccines containing several serotypes. However, while mild HFMD is caused by many strains of enterovirus, the great majority of severe disease in recent years has been caused by EV 71 which accounts for 70% of severe HFMD cases and 90% of HFMD-related deaths in China (*32).* Therefore, it has been the focus of vaccine development.

EV71 isolates can be clustered according to their genomic sequence into at least eight groups (A-H) (*33, 34*) but belong to one serotype. Genogroups B and C have been of greatest interest because of their frequency of isolation and implication in disease in Eastern Asia and they can each be subclassified into five sub-genogroups (C1-C5 and B1-B5). C4 is by far the major genogroup circulating in China while B4, B5 and C5 are found in other Asian countries. In contrast strains of genogroups C1 and C2 are predominantly found in Europe where severe disease is uncommon. It is possible that this has some effect on the disease burden with C4 being particularly virulent, but an outbreak of HFMD with severe disease caused by a C1 genogroup occurred in Spain in 2016 (*35, 36*).

A valid animal model would be useful in vaccine development to measure protective efficacy and potency as well as to resolve issues related to virulence. As yet the available models are imperfect. Neonatal mice are susceptible to EV71 by intracerebral inoculation and neonatal but not adult rhesus monkeys develop symptoms of HFMD on infection. Adult or infant mice are not susceptible to infection. Infant rhesus monkeys have been demonstrated to develop HFMD symptoms with inoculation of the virus therefore it could be used as a model of protection (*37, 38*). Picornaviruses are believed to use specific receptors to infect human cells. Human P-selecting glycoprotein ligand -1 (PSGL-1) is expressed in leucocytes and involved in their binding to endothelial cells in the early stages of inflammation and has been identified as a receptor for EV71. However, the disease produced by clinical EV71 strains in transgenic mice carrying PSGL-1 was not enhanced compared to non-transgenic strains. Human scavenger receptor class B, member 2 (SCARB2) has also been identified as a receptor for EV71. Transgenic mice carrying the SCARB gene are more susceptible to infection and disease than non-transgenic controls but the effect is not dramatic: two-week old transgenic mice develop mild symptoms and then recover (*39, 40).*

Three vaccines against EV71 have been licensed in China, all using C4 genogroup strains. Candidate vaccines containing B4 and B5 genogroups are in development elsewhere but have not yet reached licensure. In addition, development of vaccines against Coxsackie A16, A6 and A10 is being considered with a view to developing combination/multivalent vaccines. The efficacy of the three licensed EV71 vaccines after two doses ranges from 90.0% and 97.4% after one-year surveillance (*5-7*) and 95.1% after two years follow up (*8*).

The platforms that have produced licensed products against EV71 have involved growth of live virus in mammalian cells, which is then inactivated by validated techniques, in much the same way as is used for inactivated polio vaccine or hepatitis A vaccines. Tissue culture grown virus harvests include two types of particle forms, one containing the RNA genome and one empty. For polio and for EV71, the two particle forms have different antigenic and immunogenic properties; polio vaccine are purified so that they contain little if any empty

virus particles but the EV71 vaccines contain both types, which may complicate potency assays. The atomic structures of both full and empty particles of EV71 and polio have been solved by X-ray crystallography and cryo EM.

Other technologies for vaccine development including expression of viral proteins that then form virus like particles (VLPs) are progressing, but this document is concerned with the classical inactivated whole virus vaccine approaches.

Specific issues include:

1. The degree to which a vaccine based on one genogroup will protect against the others is not established. There is good cross neutralisation between genogroups, including sera induced by vaccination (*41*, *42*); it is not established that this translates into good cross protection in humans. A recent collaborative study indicates that assays of antigen content work acceptably on all genogroups tested. However clinical cross protection has not been demonstrated. Thus, the C4 genogroup vaccines may or may not protect against other genogroups.
2. There is a lack of a convenient and convincing animal model, the model most accurately reflects human disease at present being infant rhesus monkeys. This makes study of protective efficacy and immunogenic potency difficult other than by clinical trial. Neonatal mice are susceptible to disease and transgenic mice carrying the SCARB or the PSGL-1 genes which encode EV71 receptors have been developed and can prove useful without fully imitating human pathogenesis.
3. Virological issues include the existence of full and empty capsids in the licensed products. The different particles differ in their antigenic and immunogenic properties which complicates potency assays. It is not clear whether current national and international vaccine reference standards and antigen potency assays are suitable to specifically detect one or the other particle forms (*43*).

International reference materials

Subsequent sections of this document refer to WHO reference materials that may be used in laboratory or clinical evaluations. Key standards used in the control of EV71 vaccines include the following:

- An International Standard for Anti EV71 Serum Human is available for the standardization of diagnostic tests for use in seroprevalence studies and for assessing immunity. This standard was established by the WHO Expert Committee on Biological Standardization (ECBS) in 2015as the 1st International Standard for Anti EV71 Serum Human (code 14/140) and was assigned a unitage of 1000IU/ampoule *(44, 45)*. It is recommended the contents of each vial be reconstituted in 0.5ml distilled water. The preparation is held and distributed by the National Institute for Biological Standards and Control (NIBSC), Potters Bar, the United Kingdom and National Institutes of Food and Drug Control, Beijing, People's Republic of China (NIFDC).

- A WHO international reference reagent for anti-EV71 low human serum is also available. This reference reagent is for use in standardization of virus neutralization assays. This reference reagent was established by the WHO Expert Committee on Biological Standardization (ECBS) in 2015 as Anti-EV71 serum LOW (WHO International Reference Reagent) and was assigned a unitage of 300 IU/ampoule. It is recommended the contents of each vial be reconstituted in 0.5ml distilled water). WHO/BS/2015.2267) (*44, 45*). The preparation is held and distributed by the National Institute for Biological Standards and Control (NIBSC), Potters Bar, the United Kingdom and National Institutes of Food and Drug Control, Beijing, People's Republic of China (NIFDC).

- In 2019, the First WHO International Standard for inactivated EV71 vaccine (18/116) was established by the WHO ECBS. The assigned potency for this IS is 3,625 EV71 International Units (IU) of EV71 Antigen per ampoule. In addition, WHO Reference Reagents for genogroups C4 and B4 EV71 inactivated vaccine (18/120 and 18/156, respectively) with assigned potencies of 300 and 250 EV71 IU of EV71 Antigen per ampoule have been established, respectively. The preparation is held and distributed by the National Institute for Biological Standards and Control (NIBSC), Potters Bar, the United Kingdom (*12*,*13*).

  The International Standard for inactivated EV71 vaccine is intended for use in *in vitro* assays to measure the antigen content of vaccine products. It is a lyophilised commercial vaccine stored at -20^{o}C to be reconstituted in 250 µl of sterile distilled water before use. This material is for use in calibrating secondary reference preparations of EV71 vaccine, which are then used in potency tests to calculate antigen content. However, it is known that full and empty capsids, known to be present in EV71 vaccine preparations from all manufacturers, differ in their antigenicity and immunogenicity. The proportion of empty/full virus particles in the International standard is not known nor. whether this matters for the overall assessment of vaccines and whether the current IS is suitable to accurately measure antigen content across manufacturers. International standards and reference reagents for the control of in vivo potency assays are under investigation (*13*).

- Product-specific national standards for EV71 neutralizing antibody and EV71 antigen were established by the National Institutes of Food and Drug Control (NIFDC), Beijing, China, People's Republic of China (*14*). They were established following collaborative studies conducted by the National Institutes of Food and Drug Control (NIFDC) and the three main vaccine manufacturers in China and contributed to ensure the accuracy, comparability and repeatability of anti-EV71 neutralizing antibody and EV71 antigen detection assays and hence EV71 vaccine standardization. In addition, and the National Institutes of Food and Drug Control (NIFDC) also developed a new national reference for *in vivo* vaccine potency. These preparations are held and distributed by the National Institutes for Food and Drug Control (NIFDC), Beijing, People's Republic of China.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be enterovirus 71 vaccine (inactivated). The proper name should be equivalent to 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 recommendations formulated below.

A.1.2 Descriptive definition

Enterovirus 71 vaccine (inactivated) consists of a sterile preparation of EV71 grown in cell cultures, concentrated, purified and inactivated. It may be lyophilised. The antigen may be formulated for delivery with a suitable adjuvant. The preparation should satisfy all the recommendations formulated below.

A.2 General manufacturing recommendations

The general manufacturing requirements contained in the WHO Good manufacturing practices for pharmaceutical products: main principles (*46*) and Good manufacturing practices for biological products (*27)* should apply to the design, establishment, operation, control and maintenance of manufacturing facilities for EV71 vaccine.

Staff involved in the production and quality control of inactivated EV71 vaccine should be shown to be immune to EV71.

A.3 Control of source materials

A.3.1 Virus strains and seed lot system

A.3.1.1 Virus strains

Strains of EV71 used in the production of EV71 vaccine should be identified by historical records, which should include information on the strains' origin and subsequent manipulation or passage (for example, the genogroup and sub-genogroup of EV71). The strain identity should be determined by infectivity tests and immunological method, full or partial genomic sequencing and immunological methods.

Only virus strains that are approved by the NRA and that yield a vaccine complying with the recommendations set out in these WHO Recommendations should be used.

A.3.1.2 Virus seed lot system

Vaccine production should be based on the virus seed lot system. Unless otherwise justified and authorized, the virus in the final vaccine should not have undergone more passages from the virus master seed lot than were used to prepare a vaccine shown to be satisfactory with respect to safety and efficacy.

Virus master and working seed lots should be stored in dedicated temperature-monitored freezers at a temperature that ensures stability on storage (for example, ≤ -60 ºC).

A.3.1.3 Tests on virus master and working seed lots

Each virus master and working seed lot used for the production of vaccine batches should be subjected to the tests listed in this section and tests applicable to single harvests listed in sections A.4.3.1–A.4.3.3 below.

Each virus master and working seed lot should have been derived from materials that comply with the recommendations made in sections A.3.2 and A.3.3 and should be approved by the NRA.

A.3.1.3.1 Tests for adventitious viruses

A.3.1.3.1.1 Tests for adventitious viruses in cell cultures

The virus master and working seed lot used for the production of vaccine batches should be free from adventitious viruses in cell culture assays.

A sample of at least 20 ml of each virus master and working seed lot should be tested for the presence of adventitious agents. The sample should be neutralized by a high-titred antiserum against EV71.

If polyclonal antisera are used, the immunizing antigen used for the preparation of the antiserum should not be the same as the production seed.

The immunizing antigen should be shown to be free from adventitious agents and should be grown in cell cultures free from adventitious microbial agents that might elicit antibodies that could inhibit the growth of any adventitious agents present in the single harvest.

The sample should be tested in susceptible cells such as Vero, RD and human diploid cells. The tissue cultures should be incubated at 37 °C and observed for two weeks. At the end of this observation period, at least one subculture of supernatant fluid should be made in the same tissue culture system. The sample should be inoculated in such a way that the dilution of the supernatant fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm^2 per ml of supernatant fluid. At least one culture vessel of the cell cultures should remain uninoculated and should serve as a control. The cells inoculated with the supernatant fluid and the uninoculated control cultures should be incubated at 37 °C and observed at appropriate intervals for an additional two weeks.

The virus master and working seed lots pass the test if there is no evidence of the presence of adventitious agents. For the test to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the observation period.

New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: (a) degenerate nucleic acid amplification technique (NAT) for whole virus families, with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (b) NAT with random primers followed by analysis of the amplicons on large oligonucleotide micro-arrays of conserved viral sequencing or digital subtraction of expressed sequences; and (c) high-throughput sequencing. These methods might be used in the future to supplement existing

methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and with the approval of the NRA *(28)*.

The theoretical risk of the presence of potential human, simian, bovine or porcine adventitious agents in the seed lots, which may be derived from the use of bovine serum or porcine trypsin, should be assessed. If necessary, viruses such as bovine polyomavirus, porcine parvovirus or porcine circovirus (PCV) may be screened by using specific assays, such as molecular NAT-based assays *(28)*.

A.3.2 Cell lines

The general production precautions, as formulated in Good manufacturing practices for biological products *(27)*, should apply to the manufacture of EV71 vaccine, with the additional requirement that, during production, only one type of cell should be introduced or handled in the production area at any one time. Vaccines may be produced in a human diploid cell line or in a continuous cell line.

A.3.2.1 Master cell bank (MCB) and working cell bank (WCB)

The use of a cell line for the manufacture of EV71 vaccine should be based on the cell bank system. The cell seed and cell banks should conform to WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological products and for the characterization of cell banks *(28)*. The MCB should be approved by the NRA. The maximum number of passages (or population doublings) by which the WCB is derived from the MCB and the maximum number of passages of the production cultures should be established by the manufacturer and approved by the NRA.

The WHO Vero reference cell bank 10-87 is considered suitable for use as a cell seed for generating an MCB *(47)* and is available to manufacturers on application to the Group Lead, Norms and Standards for Biologicals, Technologies Standards and Norms, Department of Health Products Policy and Standards (HPS), Access to Medicines and Health Products (MHP) Division, World Health Organization, Geneva, Switzerland.

A.3.2.2 Identity test

Identity tests on the MCB and WCB are performed in accordance with WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological products and for the characterization of cell banks *(28)* and should be approved by the NRA.

The WCB should be identified by means of tests such as biochemical tests (for example, isoenzyme analysis), immunological tests, cytogenetic marker tests and DNA fingerprinting or sequencing. The tests should be approved by the NRA.

A.3.3 Cell culture medium

Where serum is used for the propagation of cells it should be tested to demonstrate freedom from bacterial, fungal and mycoplasmal contamination – as specified in Part A, sections 5.2 *(48)* and 5.3 *(49)* of the WHO General requirements for the sterility of biological substances – and freedom from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of WHO Recommendations for the evaluation of animal cell cultures as

substrates for the manufacture of biological medicinal products and for the characterization of cell banks *(28)*.

Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera if approved by the NRA. As an additional monitor of quality, sera may be examined for freedom from bacteriophage and endotoxin. Gamma irradiation may be used to inactivate potential contaminant viruses, while recognizing that some viruses are relatively resistant to gamma irradiation.

The source(s) of animal components used in the culture medium should be approved by the NRA. The components should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (50)*. The serum protein concentration should be reduced by rinsing the cell cultures with serum-free medium and/or purification of the virus harvests.

In some countries, control tests are carried out to detect the residual animal serum content in the final vaccine (see section A.6.6).

Human serum should not be used. If human serum albumin is used at any stage of product manufacture, the NRA should be consulted regarding the requirements, as these may differ from country to country. As a minimum, it should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives *(51)*. In addition, human albumin and materials of animal origin should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (50)*.

Manufacturers are encouraged to explore the possibilities of using serum-free media for the production of EV71 vaccine.

Penicillin and other beta-lactams should not be used at any stage of manufacture because they are highly sensitizing substances. Other antibiotics may be used during early stages of production. In this case, the use of antibiotics should be well justified, and they should be cleared from the manufacturing process at the stage specified in the marketing authorization. Acceptable residual levels should be approved by the NRA.

Bovine or porcine trypsin used for preparing cell cultures should be tested and found to be free of cultivatable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate *(28)*. The methods used to ensure this should be approved by the NRA.

In some countries, irradiation is used to inactivate potential contaminant viruses. If irradiation is used, it is important to ensure that a reproducible dose is delivered to all batches and to the component units of each batch. The irradiation dose must be low enough for the biological properties of the reagents to be retained but also high enough to reduce virological risk. Therefore, irradiation cannot be considered a sterilizing process *(52)*.

Recombinant trypsin is available and should be considered; however, it should not be assumed to be free from risk of contamination and should be subject to the usual considerations for any reagent of biological origin *(52)*.

The source(s) of trypsin of bovine origin, if used, should be approved by the NRA and should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (50)*.

A.4 Control of vaccine production

A.4.1 Control cell cultures

A fraction of the production cell culture equivalent to at least 5% of the total or 500 ml of cell suspension, or 100 million cells, at the concentration and cell passage level employed for seeding vaccine production cultures, should be used to prepare control cultures.

If bioreactor technology is used, the NRA should determine the size and treatment of the cell sample to be examined.

A.4.1.1 Tests of control cell cultures

The treatment of the cells set aside as control material should be similar to that of the production cell cultures, but they should remain uninoculated for use as control cultures for the detection of any adventitious agents.

These control cell cultures should be incubated under conditions as similar as possible to the inoculated cultures for at least two weeks, and should be tested for the presence of adventitious agents as described below. For the test to be valid, not more than 20% of the control cell cultures should have been discarded for nonspecific accidental reasons.

At the end of the observation period, the control cell cultures should be examined for evidence of degeneration caused by an adventitious agent. If this examination, or any of the tests specified in this section, shows evidence of the presence of any adventitious agent in the control culture, the EV71 grown in the corresponding inoculated cultures should not be used for vaccine production.

If not tested immediately, samples should be stored at -60 °C or below.

A.4.1.2 Tests for haemadsorbing viruses

At the end of the observation period, at least 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter cells have been stored, the duration of storage should not have exceeded seven days and the storage temperature should have been in the range of 2–8 °C. In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

Some NRAs require, as an additional test for haemadsorbing viruses, that other types of red blood cells, including cells from humans (blood group IV O), monkeys and chickens (or other avian species), should be used in addition to guinea-pig cells.

A reading should be taken after incubation at 2–8 °C for 30 minutes, and again after a further incubation for 30 minutes at 20–25 °C.

If a test with monkey red blood cells is performed, readings should also be taken after a final incubation for 30 minutes at 34–37 °C.

In some countries the sensitivity of each new batch of red blood cells is demonstrated by titration against a haemagglutinin antigen before use in the test for haemadsorbing viruses.

A.4.1.3 Tests for other adventitious agents in cell supernatant fluid

At the end of the observation period, a sample of the pooled supernatant fluid from each group of control cultures should be tested for adventitious agents. For this purpose, 10 ml of each pool should be tested in the same cells, but not the same batch of cells, as those used for the production of vaccine.

A second indicator cell line should be used to test an additional 10 ml sample of each pool. When a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line. When a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line *(28)*.

The pooled fluid should be inoculated into culture vessels of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of pooled fluid. At least one culture vessel of each kind of cell culture should remain uninoculated and should serve as a control.

The inoculated cultures should be incubated at a temperature of 35–37 °C and observed at appropriate intervals for a period of at least 14 days.

Some NRAs require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least an additional 14 days. Furthermore, some NRAs require that these cells should be tested for the presence of haemadsorbing viruses.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvests produced from the batch of cells from which the control cells were taken should be discarded.

Some selected viruses may be screened using specific validated assays which are approved by the NRA, such as molecular NAT-based assays *(28)*.

If these tests are not performed immediately, the samples should be kept at a temperature of -60 °C or below.

A.4.1.4 Identity tests

At the production level, the control cells should be identified by means of tests approved by the NRA.

Suitable methods include, but are not limited to, biochemical tests (for example, isoenzyme analyses), immunological tests, cytogenetic tests (for example, for chromosomal markers), morphological identification and tests for genetic markers (for example, DNA fingerprinting or sequencing).

A.4.2 Cell cultures for vaccine production

A.4.2.1 Observation of cultures for adventitious agents

On the day of inoculation with the virus working seed lot, each cell culture or a sample from each culture vessel should be examined visually for degeneration caused by infective agents.

If this examination shows evidence of the presence in a cell culture of any adventitious agent, the culture should not be used for vaccine production.

If animal serum is used for cell cultures before the inoculation of virus, the medium should be removed and replaced with serum-free maintenance medium after the cells have been washed with serum-free medium, if appropriate.

A.4.3 Control of single harvests

After inoculation of the production cells with virus, the culture conditions of inoculated and control cell cultures should be standardized and kept within limits agreed with the NRA.

Samples required for the testing of single harvests should be taken immediately on harvesting.

Samples may be taken after storage and filtration with the agreement of the NRA.

A.4.3.1 Identity test

The strain identity of the single harvest should be determined by infectivity tests. The test for antigen content described in section A.4.4.2.4 can be used to identify the single harvest.

A.4.3.2 Sterility test for bacteria, fungi and mycoplasmas

A volume of at least 10 ml of each single harvest should be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests, as specified in Part A, sections 5.2 *(48)* and 5.3 *(49)* of the WHO General requirements for the sterility of biological substances, or by a method approved by the NRA.

NAT alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and the agreement of the NRA *(52)*.

In some countries this test is performed on the purified virus harvest instead of on the single harvest.

A.4.3.3 Virus titration

The virus concentration of each single harvest should be determined by titration of infectious virus using tissue culture methods to monitor production consistency and as a starting point for monitoring the inactivation curve. This titration should be carried out in not more than 10-fold dilution steps using 10 cultures per dilution, or by any other arrangement yielding equal precision.

The use of RD, human diploid or Vero cells in microtitre plates is suitable for this purpose *(52)*. The same cells should be used for virus titrations throughout the production process.

Information on virus titre will help in selecting single harvests that can be expected to meet potency requirements after inactivation.

The virus titration may be carried out on the pooled harvest after demonstration of consistency of production at the stage of the single harvest.

A.4.4 Control of virus pools

Several single harvests may be mixed to prepare a pool of virus before inactivation. The order in which purification, filtration and inactivation of virus pools is conducted should be carefully

established by the manufacturer to ensure consistent full virus inactivation and absence of residual infectivity. Based on experience with production of poliovirus inactivated vaccines, the WHO Recommendations to assure the quality, safety and efficacy if poliomyelitis vaccines (inactivated) (*23*) recommends purification, filtration and inactivation steps in this order.

> Requirement for filtration before and during inactivation was introduced in the IPV production process following the Cutter incident during which a number of paralytic polio cases occurred in children following vaccination with a defective IPV (*23*). The vaccine used was later found to contain aggregates which led to incomplete virus inactivation likely due to formaldehyde not accessing some virus particles inside the aggregates.

Any deviation from the production sequence shown to be acceptable for IPV for a vaccine against a virus similar to poliovirus such as EV71 should be fully and carefully validated and justified in terms of yielding a product of equivalent safety.

Inactivation of virus may be performed before or after purification according to current approved procedures of production of licensed vaccines. The method of purification and inactivation as well as the agent used for inactivation should be appropriately validated and should be approved by the NRA.

If inactivation of the virus pool is conducted after purification, go to section A.4.4.1. If inactivation of the virus pool is conducted before purification go to section A.4.4.2.

A.4.4.1 Purification of virus pools

Each pool of virus should be purified before inactivation. Removal of host cell protein should be assessed during process validation *(28)*.

> An acceptable method is to clarify the virus suspension by filtration, to concentrate the virus by ultrafiltration and, thereafter, to collect the virus peak after passing it through a gel-filtration column. Further purification is achieved by passing the virus through an ion-exchange column. Other purification procedures resulting in acceptable release criteria may be used – for example, passing the preparation through an immobilized DNA-ase column.

A.4.4.2 Tests on virus pools (purified or not) before inactivation

A.4.4.2.1 Virus titration

The virus concentration of each virus pool should be determined by titration of infectious virus using tissue culture validated methods. This titration should be carried out in not more than 10-fold dilution steps using 10 cultures per dilution, or by any other arrangement yielding equal precision.

> The use of RD, human diploid or Vero cells in microtitre plates is suitable for this purpose (52). The same cells should be used for virus titrations throughout the production process.

> Information on virus titre will help in selecting pools that can be expected to meet potency requirements following inactivation.

A.4.4.2.2 Virus antigen content

The antigen content of each virus pool should be determined by use of a validated immunochemical method and should be calculated by using a reference vaccine calibrated against a suitable standard preferably the WHO International Standard (see section International reference materials) and expressed in antigen unit.

A.4.4.2.3 Specific activity

The ratio of virus concentration or the antigen content to the total protein content (specific activity) of the virus pool before inactivation should be within the limits of material shown to be safe and effective in clinical trials and approved by the NRA. This would allow a consistent ratio of the chemical agent to the viral protein and a consistent inactivation process.

A. 4.4.3 Filtration before inactivation

In order to avoid interference with the inactivation process, virus aggregation should be prevented or aggregates should be removed immediately before and during the inactivation process. For this reason, each virus pool should be filtered before inactivation.

Satisfactory results have been reported with several filter types but a final filtration using a 0.22 µm filter should be used.

Inactivation should be initiated as soon as possible and, in any case, not later than 72 hours after filtration.

It is preferable to start inactivation within 24 hours of filtration. 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 increase on standing after filtration, efforts should be made to keep within this time limit.

A sample of the filtered virus pool should be retained and its virus titre determined as described in section A.4.4.2.1.

The main purpose of determining the titre of filtered virus pools destined for inactivation is to provide the starting titre to monitor the kinetics of inactivation.

A.4.5 Control of inactivated pools

A.4.5.1 Inactivation procedure

The virus in the filtered pools should be inactivated by a validated method approved by the NRA. Prior to inactivation, the concentration of the filtered pool, based on viral titre, virus antigen and protein content, should be adjusted to the acceptable range established during the process validation.

Most manufacturers currently use formaldehyde as the method for inactivation but at least one manufacturer is using other inactivating agents such as beta-propiolactone.

The method of inactivation should have been shown to consistently inactivate EV71 virus without destroying the antigenic and immunogenic activity. Inactivation of the virus pool may take place before or after purification depending on the approved production process. The progress of inactivation should be monitored by suitably spaced determinations of virus titres. The inactivation period should usually exceed the time taken to reduce the titre of live virus to undetectable amounts by a factor of at least 2 and agreed by the NRA.

A second filtration should be made during the process of inactivation. This step is made after the virus titre has fallen below detectable levels but before the first sample for the safety test is taken.

The kinetics of viral inactivation should be established by each manufacturer and approved by the NRA. During these validation studies, an inactivation curve should be established with at least 4 time-points showing the decrease in live virus concentration with

time. The consistency of the inactivation process should be monitored; the virus titre and antigen content of each pool before, during and at the end of inactivation should be determined.

A record of consistency (effective inactivation and kinetic of inactivation) should be established by the production of at least five consecutive lots and, if broken, a root-cause analysis should be performed and a further five consecutive filtered purified virus pools should be prepared and shown to be satisfactory for establishing this record.

A.4.5.2 Purification of inactivated virus pool

If inactivation is conducted using a non-purified virus pool, inactivated pool should be purified as described in section 4.4.1.

A.4.5.3 Tests on purified inactivated pools (bulk)

A.4.5.3.1 Test for effective inactivation

After removal or neutralization of the inactivating agent (where applicable), the absence of residual live EV71 virus should be verified by inoculating a quantity of the inactivated virus pool equivalent to 5 per cent of the batch or, not less than 1,500 doses of vaccine into sensitive cell cultures of the same type as those used for vaccine production.

> If the sample is taken before the final filtration process, the sample should be filtered before the cell culture assays are conducted using a 0.22 µm filter as described in section A.4.4.2.4

The virus sample should be incubated for a total of no less than 21 days making no fewer than 2 cell passages during that period. The dilution of the sample in the nutrient fluid should not exceed 1 in 4 and the area of the cell sheet should be at least 3 cm^2 per ml of sample. One or more culture vessels of each batch of cultures should be set aside to serve as uninoculated control culture vessels with the same medium.

> If formaldehyde has been used as the inactivating agent, samples of vaccine for tissue culture tests are generally neutralized at the time of sampling by the addition of bisulfite. Usually, the samples are subsequently dialysed.

> It is possible to conduct tissue culture tests on non-dialysed material. However, this is often found to be toxic to cells, even with a dilution of 1 in 4. If in such tests nonspecific degeneration of cells occurs, or if the sensitivity of the tissue culture system is reduced, the test should be repeated on dialysed material. The virus antigen content after dialysis should be determined to ascertain whether the viral antigen was lost during the dialysis process.

If infectious virus is detected, the pool should not be used for further processing. The isolation of live virus from an inactivated pool should be regarded as a break in the manufacturing consistency record and a production process review and revalidation should be undertaken.

It is important to demonstrate that each test retains sensitivity to detect partially inactivated EV71. At the end of the observation period, the cell culture used for the detection of residual live virus should be challenged with a validated amount of live EV71 virus of the same strain as that of the inactivated virus pool. The details of the challenge procedure should be approved by the NRA. The ability to detect infectious virus should be checked concurrently for each test by including a positive control at the beginning of each test. Positive control flasks should be inoculated with a low quantity of virus close to the detection

limit of the method. Alternatively, if no positive control is used, a challenge test should be performed as described above.

The problem of detecting residual active virus in an inactivated vaccine is not the same as that of measuring infective virus in untreated suspensions. Other similar viruses that have been exposed to the action of formaldehyde without becoming inactivated have been shown to require a much longer period to produce cytopathic changes than untreated virus. For this reason, it is desirable that tissue cultures in tests for the presence of residual active virus are observed for as long a time as is technically possible. A satisfactory tissue culture system for this purpose depends, therefore, not only on the sensitivity of the cells used for the preparation of the cultures but also on the nutrient fluid.

The serum added to the nutrient fluid should be tested for inhibitors to EV71 at serum concentrations up to 50%. Only serum free from inhibitors should be used.

Maintenance of the cultures in good condition may require frequent changes of culture medium. However, it should be borne in mind that early changes of fluid may result in unadsorbed virus being removed and the validity of the test would thus be impaired. Therefore, the fluid should be changed no earlier than 5–7 days after inoculation.

A.4.5.3.2 Sterility test for bacteria and fungi

Each inactivated pool should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances *(48)*, or by methods approved by the NRA.

A.4.5.3.3 Antigen content

The EV71 antigen content of each inactivated purified virus pool should be determined by use of a validated immunochemical method and should be calculated by use of a reference vaccine calibrated against the WHO International Standard (see section International Standards). The results obtained should be within the required limits established by the NRA.

A.4.5.3.4 Residual inactivating agent

The content of free residual formaldehyde or any other chemical used for inactivating the virus, should be determined by a method approved by the NRA. The limits should be approved by the NRA.

A.4.5.3.5 Residual cellular DNA

If continuous cell lines are used for production, the purification shall have been shown to reduce consistently the level of host-cell DNA (*28*). The content and size of host cell DNA should not exceed the maximum levels agreed with the NRA, taking into consideration issues such as those discussed in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*28*). Human diploid cell lines have been used successfully for many years for the production of viral vaccines, and the residual cellular DNA deriving from these cells has not been (and is not) considered to pose any significant risk (*28*).

This test can be performed on the purified virus pool (see section A.4.4.2) and may be omitted from routine testing, with the agreement of the NRA, if the manufacturing process is validated to achieve this specification (28).

If assessed, the size distribution of the DNA may be considered as a characterization test, taking into account the amount of DNA detectable using appropriate methods, as approved by the NRA (*28*).

A.4.4.3.6 Residual Chemicals

If chemical substances are used during the purification process, tests for these substances should be carried out. The concentration should not exceed the limits approved for the particular product.

A.4.6 Control of final bulk

Preservatives, excipients or other substances that might be added to form the final bulk should have been shown, to the satisfaction of the NRA, to have no deleterious effect on the immunizing potency and the safety profile of the EV71 antigens. Preservative efficacy should be demonstrated during product development using a method approved by the NRA.

The operations necessary for preparing the final bulk from the purified inactivated pool should be conducted in such a manner as to avoid contamination of the product. In preparing the final vaccine bulk, any substances such as diluents, stabilizers or adjuvants that are added to the product should have been shown, to the satisfaction of the NRA, not to impair the safety and efficacy of the vaccine in the concentration used. Until the final bulk is filled into containers, the final vaccine bulk suspension should be stored under conditions shown by the manufacturer to retain the desired biological activity.

A.4.6.1 Sterility test for bacteria and fungi

The final bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances *(48)*, or by methods approved by the NRA.

A.4.6.2 Potency tests

Each final bulk should be tested in an *in vivo* assay for immunogenicity by tests approved by the NRA. Product-specific reference preparations may be used in these tests.

The EV71 antigen content of each final bulk should be determined using a validated immunochemical method and calculated using a reference vaccine calibrated against the WHO International Standard (see section International reference materials). The in vitro assay that has been found most suitable for measuring the antigen content is the EV71 antigen ELISA. The results obtained should be within the required limits established by the NRA.

When consistency of production has been established on a suitable number of consecutive final bulks, the *in vivo* assay may be omitted with the agreement of the NRA. This can occur once it has been demonstrated that the acceptance criteria for the EV71 antigen determination are such that the in vitro test yields a comparable result to the in vivo assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary by heat treatment or other means of diminishing the immunogenic activity.

If an adjuvant is used in the final bulk, a desorption or treatment step may be necessary before performing the EV71 antigen ELISA.

If the final bulk is formulated with other antigens into a combination vaccine, the suitability of performing the EV71 antigen ELISA on the final bulk will have to be determined. If the EV71 ELISA is not suitable for a particular combination, an in vivo assay should be used.

The potency of the final bulk for each virus type should be approved by the NRA.

A.4.6.3 Preservative content

If preservative is added, its content in the final bulk should be determined by a method approved by the NRA. The preservative used and content permitted should be approved by the NRA. The preservative should not adversely affect the quality of the antigen.

A.4.6.4 Adjuvant (if applicable)

Each final vaccine bulk should be assayed for adjuvant content. This test may be omitted if it is performed on the final lot. Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose.

A.5 Filling and containers

The requirements concerning filling and containers given in Good manufacturing practices for biological products *(27)* should apply to vaccine filled in the final form. Single- and multiple-dose containers may be used.

The requirements concerning filling and containers given in WHO good manufacturing practices for pharmaceutical products: main principles *(46)* and WHO good manufacturing practices for biological products *(27)* should apply to vaccine filled in the final form.

Care should be taken to ensure that the materials of which the container and, if applicable, the transference devices and closure are made do not adversely affect the quality of the vaccine.

Manufacturers should provide the NRA with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

A.6 Control tests on the final lot

Samples should be taken from each final lot for the tests described in the following sections. The following tests should be performed on each final lot of vaccine (that is, in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers from each final lot by means of validated methods approved by the NRA. All tests and specifications, including methods used and permitted concentrations, should be approved by the NRA, unless otherwise specified.

A.6.1 Inspection of final containers

Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities should be discarded and recorded for each relevant abnormality. A limit

should be established for the percentage of rejection to trigger investigation of the cause, potentially resulting in batch failure.

A.6.1.1 Appearance

The appearance of the vaccine should be described with respect to its form and colour.

A.6.2 Identity test

An identity test should be performed on at least one labelled container from each final lot by an appropriate method. The potency test described in section A.6.4 may serve as the identity test.

A.6.3 Sterility test for bacteria and fungi

Each final lot should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances *(48)*, or by methods approved by the NRA.

A.6.4 Potency test

The EV71 antigen content of each final lot should be determined using a validated immunochemical method (see sections A.4.6.2) if such a test has not been performed on the final bulk and calculated using a reference vaccine calibrated against a reference preparation or the WHO International Standard (see section International reference materials).

> In some countries, this test is omitted provided that the determination of the EV71 antigen content has been carried out with satisfactory results on the final bulk product and provided that a validation has been performed to demonstrate that there is no loss of potency between the final bulk product and the final lot, subject to approval by the NRA.

If the use of an adjuvant in the final bulk interferes with the assay, a desorption or treatment step may be necessary. If treatment/desorption is not possible, the interference of the adjuvant should be documented and an in vivo assay should be performed (see section A.4.7.2 and Appendix 2).

The potency of the vaccines should be approved by the NRA.

A.6.5 Protein content

EV71 vaccine (inactivated) should not less than the amount per human dose which demonstrated efficacious in clinical trial. This test may be omitted for routine lot release once consistency of production has been established to the satisfaction of the NRA.

If animal serum is used for the growth of cell cultures, the serum protein concentration (bovine serum albumin) in the final lot should be no more than 50 ng per human dose. The test for bovine serum albumin may be omitted if performed on the final bulk, subject to approval by the NRA.

A.6.6 Preservative content

Where appropriate, the preservative content of each final lot should be determined by a method approved by the NRA. The method used and content permitted should be approved by the NRA. This test may be omitted if conducted on the final bulk.

A.6.7 Endotoxin content

The endotoxin content of each final lot should be determined by a method approved by the NRA. Levels should be consistent with levels found to be acceptable in vaccine lots used in pre-licensure clinical trials and approved by the NRA.

A.6.8 Test for residual formaldehyde

The content of free residual formaldehyde in each final lot should be determined by a method approved by the NRA. The limit should be approved by the NRA. This test may be omitted if performed on the final bulk.

A.6.9 Test for pH

The pH of each final lot should be determined and should be within limits approved by the NRA.

A.6.10 Adjuvant and degree of adsorption (if applicable)

If an adjuvant is used in the formulation, each final lot should be assayed for adjuvant content. Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose. This test may be omitted on the final lot if performed on the final bulk.

The degree of adsorption of the antigen to the aluminium compounds (aluminium hydroxide or hydrated aluminium phosphate) in each final lot should be assessed. This test may be omitted for routine lot release upon demonstration of product consistency, subject to the agreement of the NRA.

A.6.11 Residual antibiotics (if applicable)

If any antibiotics are added during vaccine production, the residual antibiotic content should be determined and should be within limits approved by the NRA. This test may be omitted for routine lot release once consistency of production has been established to the satisfaction of the NRA.

Due to aluminium adsorption has an impact on the test, the antibiotics content test may be done in the bulk.

A.6.12 Extractable volume

For vaccines filled into single-dose containers, the extractable content should be checked and shown to be not less than the intended dose.

For vaccines filled into multi-dose containers, the extractable content should be checked and should be shown to be sufficient for the intended number of doses.

A.7 Records

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles *(46)* and Good manufacturing practices for biological products *(27)* should apply.

A.8 Retained samples

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles *(46)* and Good manufacturing practices for biological products *(27)* should apply.

A.9 Labelling

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles *(46)* and Good manufacturing practices for biological products *(27)* should apply, and additionally the label on the container or package should include the following information:

- the designation(s) of the strain(s) of EV71 contained in the vaccine
- the cell substrate used for the preparation of vaccine
- the antigen content
- the method and inactivating agent used to inactivate the virus
- the nature and amount of any stabilizer and preservative present in the vaccine
- the nature and amount of adjuvant, if applicable.

It is desirable for the label to carry the names both of the producer and of the source of the bulk material if the producer of the final vaccine did not prepare it. The nature and amount of antibiotics present in the vaccine, if any, may be included.

A.10 Distribution and shipping

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles *(46)* and Good manufacturing practices for biological products *(27)* should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products *(53)*.

A.11 Stability, storage and expiry date

A.11.1 Stability testing

Adequate stability studies form an essential part of vaccine development. Current guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines *(54)*. Stability testing should be performed at different stages of production when intermediate product is stored, namely on single harvests, inactivated purified pool, final bulk and final lot. Stability-indicating parameters should be defined or selected appropriately

according to the stage of production. During vaccine production a shelf-life should be assigned to all in-process materials – particularly intermediates such as single harvests, inactivated purified virus pool, bulk and final bulk.

The stability of the vaccine in its final containers, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA. As a guide, containers from at least three consecutive final lots, and derived from different pools may be tested.

Accelerated stability tests may be undertaken to provide additional information on the overall characteristics of a vaccine and may also aid in assessing comparability should the manufacturer decide to change aspects of manufacturing.

The formulation of the vaccine should be stable throughout its shelf-life. Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile *(54)*. Data should be provided to the NRA in accordance with local regulatory requirements.

The final stability testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection and sharing of stability data, and criteria for rejecting vaccine(s).

A.11.2 Storage conditions

EV71 vaccine (inactivated) should be stored at all times at a temperature between 2 °C and 8 °C.

If a vaccine has been shown to be stable at temperature ranges higher than the approved 2–8 °C range, it may be stored under extended controlled temperature conditions for a defined period, subject to the agreement of the NRA *(55)*.

A.11.3 Expiry date

The expiry date should be based on the shelf-life, and should be supported by stability studies and approved by the NRA. The expiry date should be based on the date of blending of the final bulk, the date of filling or the date of the first valid potency test on the final lot.

> Where an in vivo potency test is used, the date of the potency test is the date on which the test animals were inoculated with the final bulk.

Part B Nonclinical Evaluation of EV71 vaccines

Nonclinical evaluation of a new EV71 vaccine should follow the principles outlined in the WHO Guidelines on nonclinical evaluation of vaccines (*25*) which provide details on the design, conduct, analysis and evaluation of nonclinical studies. Further guidance on the general principles for the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines can be

found in separate WHO guidelines (*29*). This section provides specific guidance to address the important issues that are related to the nonclinical development of a new inactivated whole EV71 virus vaccine.

B.1 Product characterization and process development

The vaccine lots used in nonclinical studies should be adequately characterized as described in Part A, taking into consideration the stage of the product development. Both the antigen(s) of the vaccine and the end-product need to be clearly defined, and the manufacturing process be carefully monitored for all crucial steps so as to ratify the consistency of production. It is essential that sufficient data are generated to testify the full inactivation of vaccine virus with the absence of virulent virus in the end-product. Furthermore, sufficient stability data of the vaccine are necessary to support its suitability for use in the nonclinical studies.

It is crucially important that vaccine production processes are appropriately standardized and controlled to ensure consistency in manufacturing. The extent of product characterization may vary according to the stage of development. To support the validity of studies, nonclinical studies should be done on the vaccine lots that are adequately representative of the concurrent clinical lots, in terms of physicochemical data, stability, qualitative and quantitative impurity profiles, and formulation.

B.2 Primary pharmacodynamics studies

B.2.1 Evaluation of immunogenicity in animal models

Unless otherwise justified, the immunogenicity of the vaccine needs to be characterized for any new EV71 vaccine in suitable animal models (e.g., mice, rats, rabbits), before proceeding into human trials. These proof-of-concept nonclinical studies should reflect the clinically proposed use of the vaccine, including the administration route, and should include an evaluation of serum neutralizing antibody response, and dose-range testing of the antigen. The immune response to the candidate vaccine should ideally be assessed after each dose of vaccine, and whenever possible, comparing with a licensed EV71 vaccine as an active control. Data on cross-neutralizing antibodies should be obtained from nonclinical immunogenicity studies using a range of heterologous viruses of different sub-genogroups. These data may guide selection of the doses, dosing regimen and administration route to be evaluated in clinical trials.

When a candidate EV71 vaccine is formulated with a new adjuvant, a rationale for selection of the adjuvant should be provided and the benefit for its inclusion in vaccine formulation be demonstrated by the immunogenicity data.

The immunogenicity studies in animals may additionally be considered, when appropriate, as part of comparability exercise to demonstrate the reproducibility of the manufacturing process, when major changes have been introduced during the different stages

of process development or during the validation phase of a new candidate EV71 manufacturing process.

B.2.2 Challenge-protection studies

Existing evidence suggests that serological immune responses play an essential role in mediating protection by the formalin-inactivated whole EV71 virus vaccines.Animal studies,conducted in mice and nonhuman primate, have demonstrated that vaccination with inactivated EV71 vaccines induces protective immunity against EV71 and that protection in challenged animals is primarily mediated by neutralizing antibodies (*38, 41, 56*). Importantly, human efficacy trials conducted with several formalin-inactivated EV71 vaccines show strong correlation between vaccine-induced serum neutralizing antibodies and the protection against EV71-associated diseases (*5-7*). Based on these observations, it is considered that, for a candidate EV71 vaccine as such similarly manufactured, no further challenge-protection studies in animal models need to be performed.

However, protection studies may be usefulfor a candidate EV71 vaccine that is based on a novel production process or intended with novel mechanisms of action.

Since evidence for the cross-protection against EV71 disease has thus far been limited from epidemiological studies and clinical trials, in general, claims of cross-protection should be supported by appropriate animal data. Specifically, challenge studies should be conducted in appropriate animal models to evaluate the potential for protection against heterologous viruses of different genogroups, as it could indicate the breadth of protection.

B.3 Nonclinical safety studies

For a new EV71 vaccine based on inactivated whole EV71 virus, a repeat-dose toxicity study in a relevant animal species is generally needed to assess the potential local and systemic toxicity and any undesirable effects. Omission of stand-alone local tolerance and single-dose toxicity studies is possible, if the assessment of acute toxic effects and local tolerance has been incorporated into the repeat-dose toxicity study.

If the candidate vaccine contains a novel adjuvant, principles given in the WHO Guidelines on nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (29) should be consulted, for example, consideration should be given to assessing the toxicity of the adjuvant alone.

For the candidate EV71 vaccine that is manufactured using novel cell substrate, attempts should be made to explore the biomarkers indicative of the potential of allergic reactions during the nonclinical safety study, e.g. by measure of Type 2 CD4 T cell responses.

Part C. Clinical evaluation of inactivated EV71 vaccines

C.1 Introduction

Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products *(56) and the WHO Guidelines on* clinical evaluation of vaccines: regulatory expectations *(26).* This section focuses only on issues relevant or specific to the clinical development of inactivated EV71 vaccines.

To date, no efficacy data on cross-protection are available from completed clinical trials with inactivated EV71 vaccines due to the distinct regional circulation of specific sub-genogroups. In addition, no internationally recognised immune correlate of protection or surrogate marker of protection is established. Although immunogenicity results from clinical trials suggest that a neutralizing antibody titer of 1:16 to 1:32 might be related to protection, further analysis by applying a scaled logit model indicate that significantly higher levels of neutralizing antibodies might be needed to achieve protection (*5-7, 42, 58*).

If cross-protection against heterologous EV71 viruses is to be claimed, then appropriate non-clinical and/or clinical studies should be conducted to evaluate the potential for such cross-protection as addressed in B.2.1. In addition, data demonstrating the ability of antibodies of vaccinated individuals to neutralize *in vitro* various sub-genogroups of EV71 viruses including recently circulating isolates are expected to be provided. Continuous evaluation of protective vaccine efficacy post licensure is encouraged due to the evolution of new EV71 strains or a rapid change of sub-genogroups in different countries and regions, which may result in outbreaks.

C.2 Assays

General guidance on the use and validation of assays for the evaluation of immune responses is provided in section 5.3.3 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(26).*

This section provides some guidance on assays of relevance to the investigation of immune responses to human EV71 vaccines in clinical trials and to confirm vaccine efficacy in pivotal studies

- serological assays for establishing the baseline serostatus of trial subjects and evaluating the humoral immune response to vaccination (see also section C.3); and
- detection assays for laboratory confirmation of hand, foot and mouth disease and herpangina caused by EV71 infection in vaccine efficacy trials (see also section C.4).

C.2.1 Serological assays

C.2.1.1 Functional antibody

Direct measurement of anti-EV71 neutralizing antibody is well established. Neutralizing antibody has been estimated using methods such as plaque reduction neutralization assays employing either isolated virus strains or pseudoviruses (*5-7, 58, 59*). Sponsors are encouraged to develop high-throughput assays for anti-EV17 neutralizing antibody. The assay should be standardized by use of the International Standard for Anti EV71 Serum Human and the WHO

international reference reagent for anti-EV71 low human serum (see Section international reference materials). In addition, a reference neutralising antibody panel for the evaluation of neutralizing antibody responses was established in China (*14*).

C.2.2 Virus detection assays

Since HFMD and herpangina can be caused by a group of human enteroviruses including EV71 coxsackie and echoviruses, appropriate RNA or virus detection assays are required to confirm the presence of EV71 in throat and vesicle swaps and/or stool samples of cases of HFMD and herpangina (see section C.4). International guidance on enterovirus diagnostics and characterization should be considered (*4, 60*).

As for other enterovirus infections confirmed diagnoses based on cell culture, virus isolation and identification of enteroviruses is still a standard method for laboratory diagnosis. It is recommended to use established cell lines such as RD (human rhabdomyosarcoma) or Vero cells for virus isolation. RD cells are available from the Global Polio Laboratory Network and the quality control of the cells is routinely carried out according to the Polio Laboratory Manual (60).

Various quantitative PCR assays are commercially available. Although several different EV71 specific PCR systems have been described, the ability of assays to reliably detect EV71 RNA from specific sub-genogroup should be taken into account when selecting the method to be used in trials. In general, it is recommended to determine the sub-genogroup based upon VP1 gene sequences.

Sponsors should provide full details of the methodology applied and appropriate controls should be used.

In addition, EV71 infection can be confirmed by use of anti-EV71 IgM assays.

C.3 Immunogenicity

C.3.1 Formulation, dose and regimen

C.3.1.1 Primary series

EV71 vaccines will be used mainly or exclusively in regions with relatively high rates of clinically apparent infections. However, pre-vaccination testing for EV71 serostatus will not be feasible in routine use. In naturally primed individuals the first dose of EV71 vaccine may elicit large increments in antibody due to an anamnestic response. In contrast, multiple doses of the same vaccine may be required to achieve similar antibody levels in EV71-naive subjects. Consequently, it is important that the primary series should be selected on the basis of the immune responses observed in subjects who are seronegative prior vaccination.

In the absence of an internationally established immune correlate of protection (ICP) for EV71, according to the WHO definition of ICP (26) the selection of the vaccine dose and regimen may be based on reaching an antibody plateau response unless this is precluded by

concerns over reactogenicity. It is desirable that immunogenicity studies should explore the minimum number of doses and the shortest dose interval(s) required to achieve a plateau immune response.

C.3.1.2 Cross-protection

The ability of a candidate EV71 vaccine to protect against a range of wild-type strains covering the main EV71 genogroups may vary according to the vaccine strain used. For example lower cross-neutralization against an atypic C2-like strain was observed in naturally infected EV71 patients (*61*) and in sera clinical trials using B4 based vaccine strains (*62*).

In clinical trials in which vaccine-elicited antibody is determined against the antigen in the vaccine, it is recommended that neutralizing activity is also measured using antigens derived from a range of circulating wild-type EV71 strains from different (sub)genogroups. If marked differences are observed in antibody when measured using vaccine versus non-vaccine strains and/or by EV sub-genogroup, it would be of particular interest to assess whether a similar effect is observed for functional antibody levels in naturally infected individuals.

C.4 Efficacy

C.4.1 Requirement for a demonstration of vaccine efficacy

It is currently recommended that the protective efficacy of a candidate vaccine against clinically apparent HFMD and herpangina caused by EV71 infection should be evaluated in a pre-licensure vaccine efficacy trial. The following considerations apply:

- At the time of preparing these WHO Recommendations there are three vaccines against human EV71 that are licensed in one country (See General considerations) *(4, 17, 60)*.
- These licensed vaccines are not yet widely used internationally. As a result, the use of a control group that does not receive vaccination against EV71 is possible.
- In jurisdictions in which a licensed vaccine is available, it is possible that individual NRAs may consider that licensure can be based on a trial that evaluates the efficacy of the candidate vaccine relative to that of the licensed vaccine in a population similar to that in which the efficacy of the licensed vaccine was established.
- The lack of an established immune correlate of protection against EV71 does not rule out immunobridging a candidate vaccine to a licensed vaccine that has been shown to be efficacious. However, this approach is possible only if both vaccines contain the same antigen(s) so that immune responses can be compared directly. In addition, the demonstration of efficacy of all three licensed vaccines was confined to EV71 sub-genogroup C4 and it is not known whether the protective efficacy may vary between genogroups circulating in different regions.

Taking these considerations into account, the focus of this section is on clinical development programmes that include vaccine efficacy trials in which the control group does

not receive vaccination against EV71. Most of the recommendations are also applicable to trials in which the control group receives a licensed vaccine against EV71. Clinical programmes leading to licensure based on immunobridging are not addressed in this guidance. The general principles to consider are discussed in sections 5.6.2 and 6.3.3 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(26)*.

C.4.2 Considerations for efficacy trial design

C.4.2.1 Primary objective

The primary objective will be to demonstrate that the candidate vaccine protects against clinically apparent (that is, symptomatic) HFMD and herpangina caused by EV71 infection regardless of the genogroup (see section C.4.2.4).

- It is not required for efficacy to be shown against asymptomatic EV71 infection. Asymptomatic infection is of no clinical significance.
- It is not required for vaccine efficacy trials to be powered to demonstrate genogroup-specific efficacy (see section C.4.2.2).

C.4.2.2 Trial sites

Efficacy trials will be conducted in endemic areas in which the estimated attack rate for HFMD and herpangina due to EV71 infection is sufficient to complete enrolment into an adequately powered vaccine efficacy trial within a reasonable time frame. Sites may be chosen on the basis of available public health disease-surveillance data and/or pre-trial evaluations of epidemiology conducted by the sponsor. In three prior efficacy trials *(14, 59, 60)*, EV71 genogroups that caused clinically apparent HFMD and herpangina related to EV71 infections were limited to strains of C4 sub-genogroup circulating at the trial sites in the years in which they were conducted. Sponsors are encouraged to consider selecting sites in a range of geographical areas in which strains of different genogroups are circulating and/or to conduct separate vaccine efficacy trials in regions with different genogroup distributions.

C.4.2.3 Subject selection criteria

Because of the age incidence and severity of EV71 infections it is likely that vaccine efficacy trials will target infants and children. An upper age limit may be set depending on the age-specific attack rates.

C.4.2.4 Primary end-point

In accordance with the recommended primary objective, the primary end-point should be clinically apparent HFMD or herpangina that is confirmed to be due to EV71 infection. Sponsors could consider appointing an independent data-adjudication committee to review the data and determine which subjects meet the case definition to be counted in the primary analysis.

C.4.2.4.1 Clinical Features for the Case definition

The clinical features that trigger subjects to present to study site staff or to a local designated health-care facility for laboratory investigations for acute HFMD or herpangina should be selected with the aim of capturing as many cases as possible while limiting unnecessary investigations. On this basis it is reasonable to define a possible case of HFMD or herpangina requiring laboratory investigation as an illness presenting with febrile illness accompanied by a papular or vesicular rash in the characteristic distribution on the oral mucosa, hands, feet, or buttocks. A severe case of HFMD should be defined as associated with neurologic, respiratory, or circulatory complications as published by WHO (*4*).

C.4.2.4.1 Laboratory confirmation of HFMD caused by EV71 infection

It is recommended that the laboratory confirmation of HFMD and herpangina cases should be conducted in a designated central laboratory. If more than one central laboratory is necessary for practical reasons, it is essential that the laboratories use identical methodologies, and consideration should be given to testing a randomly selected subset of samples at each laboratory to assess concordance.

The confirmation of EV71 as causative of the clinical picture should be based on any of the following:

- detection of EV71 RNA in vesicle / throat swabs or in stool.
- Virus isolation and analysis of VP1 sequence
- IgM against EV71 – which is often detectable at the time of onset of clinical symptoms but may peak after 1–2 weeks;

To avoid cases being missed, protocols should plan for appropriately timed repeat specimens to be collected from individuals with a first positive test for EV71 RNA (for example, at 3 to 7 days after the first sample).

Samples obtained at first presentation and repeat specimens should also be tested to detect infection with other enteroviruses such as coxsackie and echoviruses that can cause HFMD and regularly co-circulate in affected countries.

C.4.2.5 Primary, secondary and other analyses

In a vaccine efficacy trial, it may be permissible that the primary analysis includes only confirmed cases of HFMD and herpangina caused by EV71 as follows:

- in subjects who completed the vaccination series within predetermined visit windows, if more than one dose is required; and
- with symptom onset occurring more than a defined period after the only or final dose of the series that takes into account what is known about the timing of the post-dose anti-EV71 IgG peak.

This approach gives the most optimistic estimation of vaccine efficacy.

If the primary analysis is confined to cases counted as described above it is essential that predefined secondary analyses are carried out to estimate vaccine efficacy based on

confirmed cases of clinically apparent HFMD and herpangina caused by EV71 infection defined and counted as follows:

- all cases in subjects who received at least one assigned dose as randomized and regardless of adherence to study visit windows;
- cases that occurred at any time after the last dose received (that is, counted from the day of dosing) in those who completed the assigned number of doses;
- cases that occurred after each sequential dose, depending on the number of doses in the series and counted from the day of dosing.

Vaccine efficacy should be explored according to EV71 genogroup if this is feasible, depending on the numbers of cases that occur due to individual genogroups.

It is recommended that an additional analysis should explore any differences in clinical or laboratory features (including severity) between cases that occur in the candidate vaccine group and the control group (whether the control group receives placebo or a licensed vaccine against EV71). The analysis should take into account whether the severity observed in individual subjects could reflect coinfection with other enteroviruses.

C.4.2.6 Case ascertainment

It is recommended that an active case-ascertainment strategy is used throughout the time frame of a vaccine efficacy trial. This is essential at least up to the time of the primary analysis, which may be conducted after a specific number of total cases has been accumulated or after a predefined period in which a sufficient number of cases are expected to occur to estimate vaccine efficacy.

C.4.2.7 Duration of protection

While the primary analysis may lead to licensure, it is recommended that trials continue to use active case ascertainment to follow up subjects for several years to provide data on waning vaccine protection without unblinding of treatment assignment at the level of the individual. These data can be reported at some time after licensure of the vaccine and may point to the need for further doses to be administered at intervals to maintain protection or change to the vaccine strains used.

C.4.2.8 Vaccine effectiveness

The need for vaccine effectiveness studies should be established at the time of licensure.

If longer-term follow-up within a pre-licensure trial is not considered to be feasible, the duration of vaccine protection should be investigated within a vaccine effectiveness study and/or as part of routine disease surveillance conducted by public health authorities. Furthermore, the efficacy of the vaccine against individual sub-genogroups should be explored as part of a vaccine effectiveness study and/or during routine disease surveillance.

C.5 Safety

Evaluation of the safety of candidate EV71 vaccines should be undertaken in accordance with the recommendations made in section 7 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(26)*. If the primary series consists of several vaccine doses it is important to document whether reactogenicity increases with sequential doses. Additionally, the safety of post-primary doses should be evaluated. There may be special considerations for vaccine safety depending on the vaccine construct and the intended target population.

If a candidate vaccine is evaluated in a large pre-licensure trial, and if the safety profile documented during immunogenicity trials did not give rise to any major concerns, it may be acceptable for a full assessment of safety (that is, including detailed documentation of local and systemic reactogenicity, as well as all unsolicited adverse events) to be confined to a randomized subset of the total subjects. Serious adverse events should be documented in all subjects enrolled at all trial sites.

Part D. Recommendations for NRAs

D.1 General recommendations

The general recommendations for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products *(27)* and WHO Guidelines for independent lot release of vaccines by regulatory authorities *(30)* should apply. These recommendations specify that no new biological substance should be released until consistency of lot manufacturing and quality has been demonstrated.

The detailed production and control procedures – as well as any significant changes in them that may affect the quality, safety and efficacy of inactivated EV71 vaccines – should be discussed with and approved by the NRA *(63)*. For control purposes, the relevant international reference preparations currently in force should be obtained for the purpose of calibrating national, regional and working standards *(64)*. The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release.

Consistency of production has been recognized as an essential component in the quality assurance of EV71 vaccines. In particular, the NRA should carefully monitor production records and quality control test results for clinical lots, as well as for a series of consecutive lots of the vaccine.

D.2 Official release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations *(30)*.

A summary protocol for the manufacturing and control of EV71 vaccines, based on the model summary protocol provided in Appendix 1 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for the release of a vaccine for use.

A Lot Release Certificate signed by the appropriate NRA official should then be provided if requested by a manufacturing establishment and should certify whether or not the lot of vaccine in question meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot including the basis of the release decision (by summary protocol review or independent laboratory testing). The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided in Appendix 2.

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Appendix 1 Model Summary protocol for the manufacturing and control of Enterovirus 71 vaccines (inactivated)

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by the manufacturer to the NRA. Information and tests may be added or deleted/omitted as necessary with approval of the NRA.

It is possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO recommendations for a particular product should be provided in the protocol submitted.

The section concerning the final product must/should be accompanied by a sample of the label and a copy of the leaflet (package insert) that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must/should also be accompanied by a lot release certificate from the NRA of the country in which the vaccine was produced or released, stating that the product meets national requirements as well as the recommendations in Part A of this document.

Summary information on final lot

International name: _____

Trade name/commercial name: _____

Products license (marketing authorization) number: _____

Country: _____

Name and address of manufacturer: _____

Name and address of licence holder, if different: _____

Final packaging lot number: _____

Type of container: _____

Number of containers in this final lot: _____

Final container lot number: _____

Date of manufacture: _____

Nature of final product (adsorbed): _____

Preservative and nominal concentration: _____

Volume of each single human dose: _____

Number of doses per final container: _____

Summary of the composition (include a summary of the qualitative and quantitative composition of the vaccine per human dose, including any adjuvant used and other excipients):

_____

_____

Shelf-life approved (months): _____

Expiry date: _____

Storage condition: _____

The following sections are intended for recording the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. If any test has to be repeated, this must be indicated. Any abnormal result must be recorded on a separate sheet.

Detailed information on manufacture and control

Starting materials

Identity of seed lot strain used for vaccine production:

Reference number of seed lot: _____

Date(s) of reconstitution (or opening) of seed lot ampoule(s) _____

Identity of cell bank used for vaccine production:

Reference number of cell bank: _____

Date(s) of reconstitution (or opening) of cell bank ampoule(s) _____

Single harvests used for preparing the bulk

Name of the culture medium: _____

Date of inoculation: _____

Temperature of incubation: _____

Control of bacterial purity

Methods: _____

Result: _____

Date: _____

Date of harvest _____

Volume of harvest: _____

Yield (mg?/ml): _____

To be completed after finalization of the Recommendations.

Appendix 2 Model NRA Lot Release Certificate for Enterovirus 71 vaccines (inactivated)

Certificate No._____

The following lot(s) of recombinant hepatitis E vaccine produced by _____ _____ [1] in _____, [2] whose numbers appear on the labels of the final containers, meet all national requirements[3] and Part A[4] of the WHO Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated)[5] and comply with WHO good manufacturing practices for pharmaceutical products: main principles;[6] WHO good manufacturing practices for biological products;[7] and Guidelines for independent lot release of vaccines by regulatory authorities.[8]

The release decision is based on _____[9]

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container used;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and expiry date;
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other appropriate authority)
Name (typed) _____
Signature _____
Date _____

1 Name of manufacturer.
2 Country of origin.
3 If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA.
4 With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.
5 WHO Technical Report Series, No. 000, Annex 0.
6 WHO Technical Report Series, No. 986, Annex 2.
7 WHO Technical Report Series, No. 999, Annex 2.
8 WHO Technical Report Series, No. 978, Annex 2.

9 Evaluation of the summary protocol, independent laboratory testing and/or procedures specified in a defined document etc., as appropriate.[1]

[1]