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Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines (oral)

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This document provides guidance to national regulatory authorities and vaccine manufacturers on the production, quality control and evaluation of safety and efficacy of live attenuated rotavirus vaccines (oral) by outlining, in detail, international regulatory expectations for product characterization. It should be read in conjunction with the WHO guidelines on nonclinical evaluation of vaccines (*1*), and the WHO guidelines on clinical evaluation of vaccines: regulatory expectations (*2*), in order to understand the whole process of vaccine evaluation. Advice that is specific to the nonclinical or clinical evaluation of live attenuated rotavirus vaccines is provided to supplement these two generic documents.

The following text is written in the form of guidelines instead of recommendations. Guidelines allow greater flexibility than recommendations with respect to expected future developments in the field.

Introduction

The high incidence of rotavirus disease around the world has prompted international agencies including the World Health Organization (WHO), the Global Alliance for Vaccines and Immunization (GAVI), Children's Vaccine Program and the Rotavirus Vaccine Programme at the Program for Appropriate Technology in Health (PATH) to identify rotavirus vaccine development as one of their highest priorities. In response to this interest in the development of candidate rotavirus vaccines, WHO held an informal consultation on the Quality, Safety and Efficacy Specifications for Live Attenuated Rotavirus Vaccines in Mexico City, from 8–9 February 2005 (*3*). The experts present considered it timely for WHO to develop production and quality control guidelines and technical specifications for these vaccines. Draft guidelines on production and quality control specifications for live attenuated rotavirus vaccine (oral) were developed by a small drafting group established by WHO and were comprehensively reviewed by a WHO Informal Consultation on the proposed WHO Guidelines to Assure the Quality, Safety and Efficacy of Live Attenuated Rotavirus Vaccines held in Geneva, from 29–30 August 2005. After taking into account the comments made during the consultation, the revised document was submitted to and approved by the WHO Expert Committee on Biological Standardization at its 56th meeting.

The scope of this document covers live attenuated rotavirus vaccines (oral), and vaccine candidates, that are intended for international use. The aim of vaccination against rotavirus infection is to induce immunity against relevant rotavirus serotypes in one series of inoculations for the prevention of rotavirus gastroenteritis. The prevalence of multiple and different rotavirus serotypes throughout the world complicates vaccine development strategies. According to the data available in 2005, the majority of rotavirus disease

in humans is caused by five distinct serotypes of rotavirus, designated G1, G2, G3, G4 and G9 on the basis of the molecular composition of one outer coat viral protein (VP7). In the last decade, intense surveillance of rotavirus and improved laboratory assay methods have resulted in the detection of additional G serotypes that play a role in human disease. Although serotypes G1–G4 still predominate worldwide, serotypes G5, G8 and G9 have been isolated in Latin America, Africa and India respectively, and are beginning to cause a higher percentage of cases of the disease. The relative benefits of monovalent, multivalent or regional specific vaccines will remain unclear until efficacy data demonstrating heterotypic protection against relevant rotavirus serotypes become available.

The scope of this document is restricted to live vaccines, but the properties of the possible candidates are varied, particularly with respect to their degree of attenuation. This affects the extent to which they grow in culture and in the human gut and applies to strains currently being investigated as well as those studied previously. There will therefore be major quality issues specific to a particular vaccine, such as the assay of vaccine potency, the stability of the virus in production, the yield of virus and the extent of contamination by cell-derived materials. Clinical issues which will vary between candidates include the dose required to obtain immunity in recipients, the possibility of transmission to contacts of vaccinees and the genetic stability of the virus on replication in the gut of recipients. Although many of the points of possible concern considered in this document are generally applicable to rotavirus vaccines, it must be remembered that each candidate must be examined individually and that this may raise significant product-specific issues.

Several oral rotavirus vaccines are currently approved or in various stages of vaccine development. Each vaccine is the result of a unique approach in developing an attenuated phenotype and to the prevention of rotavirus disease. As more vaccines become readily available around the world, data will be provided to help in making decisions on the compositions of future vaccines. Examples of vaccines approved or in development include multivalent human–bovine and human–rhesus reassortant vaccines and monovalent vaccines containing a single attenuated human rotavirus strain; natural human–bovine reassortants or animal rotavirus strains. Vaccines for which controlled efficacy studies have been completed have demonstrated high levels of efficacy (> 80%) against severe rotavirus gastroenteritis and have also shown a range of heterotypic protection against multiple serotypes. The impact of introduction of the vaccine on the epidemiology of natural rotavirus infection is as yet unknown.

All of the vaccine candidates are claimed to be attenuated; however, the basis for the attenuation phenotype of the current vaccine candidates is

unknown. Typically the method of attenuation is to exploit the host range restriction properties of animal rotaviruses (Jennerian) by laboratory or natural (neonatal rotavirus strains) genetic reassortment with human rotavirus with the desired serotypes or insertion of multigenic mutations by serial passage in cell culture. Because laboratory markers of attenuation are not well defined and animal models demonstrating attenuation are not readily available, the claim of attenuation is based on clinical experience in human subjects. How the product will actually behave in clinical use is therefore based on clinical trials and consistency of production rather than on any specific laboratory tests. Potential laboratory markers of product consistency include information on the genetic sequence of the virus seed to show that a new seed material is similar to the previous seed and that each can be distinguished from the parent virus. Multiple passages of virus seed materials under defined cell culture conditions as well as examination of vaccine virus isolated from stool samples of vaccinees may be helpful to generate data on the conditions favouring genetic stability of the virus. Studies on consistency of production would need to take into account the variability inherent in replication of RNA virus and assess the presence of minority populations, as revealed for example by the occurrence of mixed base signals in sequencing studies. If minority populations are detected, it will be necessary to assess their biological importance, for example, by careful comparison of the level of heterogeneity between the master or working seed and higher passage levels e.g. clinical trial material.

The development of any new rotavirus vaccines must take into account the events which led to the withdrawal of one vaccine (RotaShield) from the marketplace. RotaShield was introduced in the United States in August 1998 and was withdrawn less than 1 year later when an epidemiological relationship was established between RotaShield vaccination and intussusception (IS). Early estimates suggested a risk of one case per 2500 children immunized. Re-analysis of the case–control study that examined intussusception and RotaShield revealed that the majority of the cases of IS associated with the first dose occurred in children 4 months of age or older. This did not comply with the manufacturer's recommendation that the first dose should be given at 2 months of age and thus changed the early estimates of attributable risk of IS in the target population (*4*). The detailed pathogenic mechanisms for IS are unclear, but are very likely to be complex.

General considerations

Considerable experience in the manufacturing, testing and clinical evaluation of rotavirus vaccines has been gained from over 20 years of their development. Candidate vaccines have been and are being studied in a number of countries with diverse economic conditions and geographical

boundaries. The regulatory approvals of two rotavirus vaccines and the submission for approval of another provide a solid foundation for the preparation of these guidelines and for the continuing development of new rotavirus vaccines; they also support the global introduction of rotavirus vaccines. These experiences have enabled standards to be set for vaccine efficacy, identified safety concerns, highlighted areas of scientific weakness, and led to the introduction of new manufacturing technologies and testing strategies. Issues relevant to the development of rotavirus vaccines are discussed below.

Rotavirus is an acid-labile virus which is rapidly inactivated at an acid pH and has a half-life of less than 12 minutes at pH 2.0. Because rotavirus vaccines are intended to be administered to infants by the oral route, the virus would be inactivated by stomach gastric acid prior to reaching the site of infection in the upper gastrointestinal tract. To prevent inactivation of the virus by gastric acid, antacids or buffers are usually administered before or in combination with the oral rotavirus vaccination. The composition of the antacid and the mode of administration (in combination with vaccine or administered separately) will depend upon the biological characteristics of the vaccine virus.

The processing of live rotavirus vaccines, like that of many other live viral vaccines, involves cell disruption and, if any, incomplete purification of the virus. In-process steps for the inactivation of adventitious agents are not included for live viral vaccines, as these steps may compromise the live nature of the vaccine itself. As a result, validation of clearance of any adventitious agents may not be possible. For these reasons a comprehensive set of tests for adventitious agents and qualification of the vaccine source materials is essential as part of the vaccine safety control. As with any viral vaccines, production of rotavirus vaccines also involve cells, virus seeds and biological reagents (such as growth supplements, serum, trypsin and any virus stabilizers used in the final product). Hence, each of these vaccine source components must be shown to be free from adventitious agents. The full passage history of the seed materials used for vaccine development should identify all substrates through which they have been passed to aid the development of appropriate programmes of testing for adventitious agents.Viral seed lots should be assessed for absence of adventitious agents from all species that they may have been exposed to from isolation, through passage, and during production, including those that may be present in the raw materials used at each of these stages. The testing required will depend on available documentation on cell substrate donor, virus seed passage and derivation history and the seed virus may require purification (e.g. molecular cloning and plaque purification), if passage and derivation history is uncertain, or if it is contaminated with a known agent.

A risk assessment for transmissible spongiform encephalopathies would need to be included for the seed materials. The revised WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (*5*) provide guidance on risk assessments for master and working seeds and should be consulted.

Many candidate vaccines are being developed in Vero cells. WHO has set a limit of 10 ng of DNA per human dose for parenterally administered vaccines based upon a WHO risk assessment (*6*) that established a 2×10^8 safety factor. For orally delivered vaccines, the acceptable limit of residual cellular DNA has not been previously established. However, recent studies in rats, which compared the uptake efficiency of Vero cell DNA following administration by the oral and intramuscular routes indicated that Vero cell DNA given orally could be found in rat tissues at a concentration of 10 000-fold less than when equivalent amounts of Vero DNA were given parenterally (*7*). Similarly, experiments in mice using polyoma DNA also showed a difference in uptake between the oral and parenteral routes (*8*). These preliminary data and the lack of evidence that cellular DNA is tumorigenic suggest that a DNA level of 100 μg/dose in an orally administered vaccine is equivalent to 10 ng in parenterally administered vaccines. In addition to the quantity of DNA, the size distribution of DNA should be evaluated during assessments of manufacturing consistency and process validation. It may be desirable for manufacturers to include steps in the process to reduce the level of high-molecular-weight cellular DNA. The national regulatory authority may require the further purification of virus harvests derived from continuous cell lines to remove cellular DNA, and/or the use of DNase treatment to reduce the size of DNA fragments. If the virus harvests are derived from diploid cell cultures, further purification is not required.

The concentration of virus can be determined by infectivity titrations, e.g. plaque-forming or focus-forming assays. However, confirming the quantity of each rotavirus serotype in the final mixture of a multivalent, as opposed to a single component, vaccine is challenging. Conventional infectivity titrations require an antibody specific to each of the vaccine components. Molecular methods of measuring virus concentration that do not require the use of specific antibody, such as quantitative real-time polymerase chain reaction (PCR), have been developed and are being evaluated. One method uses Vero cell monolayers which are seeded in 96-well plates and inoculated with dilutions of a multivalent reference standard and samples. Viral replication is allowed to proceed for 23–24 hours and the cells are then lysed by addition of Triton and one freeze–thaw cycle. Cell lysates are assayed by real-time quantitative reverse transcriptase-PCR (QPCR) and rotavirus nucleic acid is quantified (*9*). Individual QPCR reactions are performed employing primer/probe sets specific to each of the reassortant

strains. Virus concentration is determined by parallel line analysis between the reference standard and each sample. International titration standards do not yet exist and WHO is considering developing such reagents and their subsequent characterization by international collaborative study. It should be borne in mind however that the differences in the properties of the candidate vaccines in terms of their attenuation may mean that such reference material will be product-specific. Manufacturers should set aside a preparation for use as an in-house standard.

The real-time stability of each serotype in the final vaccine formulation should be measured to verify that the infectivity titre is at or above that level of virus required for vaccine efficacy at the end of the product shelf-life. The thermal stability of each individual serotype should be determined in an appropriate stability study programme. It is advisable to assign a shelf-life to the intermediates that are intended to be stored on the basis of stability studies. WHO is developing further guidance on this issue. Based on the results of the stability testing programme, an accelerated stability test should be done on each new batch of vaccine.

If the final product is mixed with vaccine diluent prior to use, the stability of this combination should also be determined.

The consistency of a manufacturing process for live virus vaccine can best be determined by monitoring the content and quality of virus produced throughout the process, as well as the level of impurities following processing and the stability of the final product. Manufacturers should select relevant parameters based upon the specific production process used and develop methods to establish a programme to assure the consistency of production process. For example, the amount of virus produced by a standardized production process and the amount lost during processing should be consistent; the quality of the virus produced can be assessed by its thermal stability profile; the stability of the genomic sequence through multiple cell culture passages may be evaluated; process impurities and residuals such as residual host cell protein, residual cellular DNA, endotoxin, bovine serum, trypsin and antibiotics can be measured and their reduction during processing can be monitored. If the consistency of production has been validated, the monitoring programme could be conducted based on a reduced frequency of testing, but not on a batch-to-batch basis, with the agreement of the national regulatory authority.

Special considerations

Use of primary cells for the production of rotavirus vaccine

The majority of live attenuated rotavirus vaccines that have been developed or are currently under development are produced in continuous cell lines

or diploid cells which are based on the cell bank system, so the main focus of these guidelines is on vaccines produced under such conditions. But the vaccine may also be produced in primary cells, in which case, the manufacturers should consult additional relevant guidelines or documents to ensure the safety of the final product. However, as suitable alternative cell substrates become available, primary cell cultures are less likely to be used in the future for many reasons (*6*).The following text provides brief guidance on issues that need to be considered if primary cells are used for rotavirus vaccine production, but there are some specific issues relating to the production of rotavirus vaccines in primary cell substrates which are not covered in these guidelines.

If vaccine is produced in primary cells obtained directly from trypsinized tissue of normal healthy animals, such animals should be of a species approved by the national regulatory authority and not have been previously employed for experimental purposes. However, primary cell cultures prepared from wild animals show a high frequency of viral contamination. The number of viruses isolated and the frequency of isolation depend on many factors, including methods of isolation, test cell systems used, number of passages, duration of incubation and co-cultivation, and are directly proportional to the duration of the incubation period of the cultures. The frequency of cell culture contamination can be reduced by careful screening of the source animals to be used in production for the absence of antibodies to the relevant viruses. The use of animals bred in a carefully controlled colony, especially those which are specific-pathogen free, is strongly recommended. In addition, the use of secondary or tertiary cells on which testing for adventitious agents could be performed will reduce the frequency of contamination of the production cell culture. Production of uninfected control cultures and the extensive testing required for relevant adventitious agents becomes more challenging when producing live rotavirus vaccines in primary cells.

A live attenuated rotavirus vaccine prepared from primary calf kidney cells has been approved for use in one country. There are no existing WHO guidelines for the production of vaccines on primary calf kidney cells. The WHO Requirements for the use of animal cells as in vitro substrates for the production of biologicals (*6*) provide general recommendations. The WHO Recommendations for the production and control of poliomyelitis vaccine (Oral) (*10*), Guidelines for the production and control of Japanese encephalitis vaccine (live) for human use (*11*) and Guidelines for the production of candidate live attenuated dengue virus vaccines (*12*) provide guidance on the use of primary cell substrates of monkey, hamster and dog kidney, respectively, and illustrate the principles that should be followed for vaccines produced in primary cell substrates from other species.

Part A. Guidelines on manufacturing

A.1 Definitions

A.1.1 *International name and proper name*

The international name should be Live attenuated rotavirus vaccine (LARV) (oral) with additions to indicate the virus serotype(s) of the vaccine. 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 specifications formulated below.

A.1.2 *Descriptive definition*

Live attenuated rotavirus vaccine (oral) is a sterile vaccine preparation containing one or more virus serotypes, which have been grown through a seed lot system, prepared in a suitable approved cell substrate, formulated in a form suitable for oral administration and satisfying all the recommendations formulated in this document.

At least five types of live attenuated rotavirus vaccine have been developed and/or are under development:

— a candidate rhesus rotavirus strain live attenuated vaccine;
— a candidate human-rhesus rotavirus strain live attenuated vaccine;
— a human rotavirus strain live attenuated vaccine;
— a candidate human–bovine reassortant rotavirus strain live attenuated vaccine;
— a lamb rotavirus strain live attenuated vaccine.

The preparation should satisfy all of the specifications formulated in these guidelines.

Live attenuated rotavirus vaccine with an appropriate stabilizer may be freeze-dried or liquid.

A.1.3 *International reference preparations*

No international reference preparations are available at the time of preparing this document.

A.1.4 *Terminology*

The definitions given below apply to this document only.

Candidate vaccine. A vaccine under development which is used in human clinical trials to assess its safety and efficacy.

Virus master seed lot. A quantity of virus of uniform composition derived from an original isolate, processed at one time and passaged for a documented number of times.

Virus working seed lot. A quantity of virus of uniform composition derived from the master seed lot by a limited number of passages by a method approved by the national regulatory authority and fully characterized. The virus working seed lot is used for production of vaccine.

Cell seed. A quantity of well-characterized cells, derived from a single tissue or cell of human, animal or other origin, 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.

Cell bank. A collection of ampoules containing material of uniform composition stored under defined conditions, each ampoule containing an aliquot of a single pool of cells.

Master cell bank (MCB). A quantity of fully characterized cells of human, animal or other origin stored frozen in liquid nitrogen in aliquots of uniform composition, derived from the cell seed. The master cell bank is itself an aliquot of a single pool of cells generally prepared from a selected cell clone under defined conditions, dispensed into multiple containers and stored under defined conditions. The master cell bank is used to derive all working cell banks. The testing performed on a replacement master cell bank (derived from the same cell clone, or from an existing master or working cell bank) is the same as for the initial master cell bank, unless a justified exception is made.

Working cell bank (WCB). A quantity of cells of uniform composition derived from one or more ampoules of the master cell bank at a finite passage level, dispensed in aliquots into individual containers appropriately stored, usually stored frozen in liquid nitrogen, one or more of which would be used for production of cell culture. All containers are treated identically and once removed from storage, are not returned to the stock.

Production cell culture. A cell culture derived from one or more ampoules of the WCB or primary tissue used for the production of vaccines.

Adventitious agents. Contaminating microorganisms of the virus seed or cell substrate or materials used in its culture, which may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses.

Single harvest. A virus suspension of one virus type harvested from cell cultures prepared from a single production run.

Monovalent virus pool. A homogenous pool of a number of single harvests of the same virus serotype, collected into a single vessel before clarification.

Final bulk. The finished biological preparation present in the container from which the final containers are filled. The final bulk may be prepared from one or more clarified monovalent virus pools and may contain one or more virus serotypes.

Filling lot (final lot). A collection of sealed final containers of vaccine that are homogeneous with respect to the risk of contamination during the filling process or freeze-drying. A filling lot must therefore have been filled or prepared in one working session.

Focus forming unit (FFU). Viral infectivity identified on cell monolayers using rotavirus-specific antiserum.

Plaque forming unit (PFU). The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

Cell culture infective dose 50% ($CCID_{50}$). The quantity of a virus suspension that will infect 50% of cell cultures.

Unit of infectivity (UI). Relative viral infectivity of a sample inoculated in 96-well Vero cell monolayers measured by QPCR against a defined reference standard preparation.

A.2 General manufacturing recommendations

The general manufacturing requirements contained in *Good manufacturing practices for biological products* (*13*) should apply to establishments manufacturing oral rotavirus vaccine, with the addition of the following:

Production steps and quality control operations involving manipulations of live rotavirus should be conducted under Biosafety Level 2 (*14*).

Separate areas or a campaigned programme for the manufacturing of different virus serotypes are required.

In production areas used for bulk formulation and filling, multiple serotypes may be present at the same time and these production areas may be campaigned with other vaccines provided sufficient cleaning validation and product changeover data is provided.

A.3 Control of source materials

A.3.1 *Cell cultures for virus production*

A.3.1.1 *Master cell bank and working cell bank*

The use of a continuous cell line such as Vero cells (low passage, non-tumorigenic phenotype) or diploid cells such as fetal rhesus lung cells, FrHL-2 (well-defined non-tumorigenic phenotype) for the manufacture of rotavirus vaccines should be based on the cell bank system. The cell substrates and cell banks should conform with the WHO Requirements for use of animal cells as in vitro substrates for the production of biologicals (*6*), as appropriate to continuous cells and diploid cells, and should be approved by the national regulatory authority. The maximum number of passages (or population doublings) allowable between the cell seed and the WCB and the

production cells should be approved by the national regulatory authority. Additionally, for Vero cells, the MCB or WCB cells should be propagated to or beyond the maximum production level and be examined for the presence of retroviruses and tumorigenicity in an animal test system (*6*).

WHO has established a cell bank of Vero cells characterized in accordance with the requirements in the forty-seventh report of the WHO Expert Committee on Biological Standardization (*6*), which is available as a well-characterized source material (*15*) to manufacturers for preparation of their own MCB and WCB on application to the Coordinator, Quality Assurance and Safety of Biologicals, WHO, Geneva, Switzerland.

The master cell bank, which is made in sufficient quantities and stored in a secure environment is used as the source material to make manufacturer's working cell banks. In normal practice a master cell bank is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national regulatory authority, at which point the cells are combined to give a single pool distributed into ampoules and preserved cryogenically to form the WCB.

The manufacturer's WCB is used for the preparation of production cell culture, and thus for production of vaccine batches.

A.3.1.1.1 Cell culture medium

Serum used for the propagation of cells should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, according to the requirements given in Part A, sections 5.2 and 5.3 of the *General requirements for the sterility of biological substances* (*16*), as amended in 1995 and from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of the *WHO Recommendations for production and control of poliomyelitis vaccine* (Oral) (*10*).

Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera.

As an additional indicator of quality, sera may be examined for freedom from phage and endotoxin. Irradiation may be used to inactivate potential contaminant viruses.

The acceptability of the source(s) of any components of bovine, porcine, ovine or cervine origin used should be approved by the national regulatory authority. These components should comply with current WHO guidelines in relation to animal transmissible spongiform encephalopathies (*5*).

If trypsin is used for preparing cell cultures and aiding in virus infection, it should be tested and found free of cultivable bacteria, fungi, mycoplasmas and infectious viruses, especially bovine or porcine parvoviruses, as appropriate.

The methods used to ensure this should be approved by the national regulatory authority. The trypsin should be gamma irradiated if possible.

Human serum should not be used. If human albumin is used, it should meet the revised *Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives* (Requirements for Biological Substances No. 27) (*17*), as well as current guidelines in relation to human transmissible encephalopathies (*5*).

Penicillin and other beta-lactams should not be used at any stage of the manufacture. Other antibiotics may be used at any stage in the manufacture provided that the quantity present in the final product is acceptable to the national regulatory authority.

Nontoxic pH indicators may be added, e.g. phenol red at a concentration of 0.002%. Only substances that have been approved by the national regulatory authority may be added.

A.3.1.1.2 **Tests on master and working cell banks**

Tests on the MCB and WCB are performed in accordance with WHO *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (*6*, *15*). It is important to show that the cell banks are free of adventitious agents relevant to the species used in its derivation. Cell banks should be assessed for absence of adventitious agents that may have been present during production, including those that may be present in the source materials used at each of these stages.

Full characterization may be performed on either the MCB or on the WCB (*6*, *15*).

A.3.2 *Virus seeds*

A.3.2.1 *Virus strains*

Virus strains of rotavirus used for master and working seed lots to produce vaccines have in some cases been derived by genetic reassortment of animal rotavirus with human rotavirus with the desired serotypes or in other cases by multiple passages of human rotavirus in cell culture. The seed lot viruses should comply with the specifications of this section. Viruses may be passed in continuous, diploid, and/or primary cell lines. The candidate vaccine strains should be approved by the national regulatory authority.

- The strains of rotavirus used in the production of candidate rotavirus vaccines should be identified by historical records, which will include information on the origin of each strain, method of attenuation, whether the strains have been biologically cloned prior to generation of the master seed lots, genetic sequence information and the passage level at which attenuation for humans was demonstrated by clinical trials.

- The vaccine production strain(s) should have been shown by appropriate laboratory tests (see A.3.2.3) and/or clinical studies to yield vaccines that are safe and efficacious in humans. Only strains approved by the national regulatory authority should be used.
- The immunogenicity of the virus strains, based upon the quantity of infectious virus of each serotype present in the vaccine that induces seroconversion when susceptible individuals are immunized with the vaccine, should be established in a dose–response study. Any potential interference or potentiation between the serotypes in an infectivity assay should be evaluated prior to establishing this value. The immunizing dose established in this way serves as a basis for establishing parameters for potency at the time of release, stability and expiry date.

A.3.2.2 *Virus seed lot system*

The production of vaccine should be based on the virus master seed lot and virus working seed lot system. Seed lots should be prepared in the same type of cells and by the same production process as those used for production of final vaccine. Virus seed lots should be stored in a dedicated temperature-monitored refrigerator at a temperature that ensures stability and the storage arrangement should ensure appropriate security of the virus seed lots.

Seed lots of rotavirus used in the production of live attenuated rotavirus vaccine (oral) should be identified by historical records, which should include information on their origin. Only virus seed lots that are approved by the national regulatory authority should be used (see General considerations). The virus master seed lot is generally produced from a biological clone of the attenuated parent virus (animal/human reassortant or cell-culture passaged human virus) and is made in sufficient quantities and stored in a secure environment and is used as the source material to make the manufacturer's virus working seed lots. Either the virus master seed lots or the virus working seed lots should be fully characterized and be tested extensively for adventitious agents, and approved by the national regulatory authority.

The virus master seed lot also serves as a benchmark against which to compare virus produced by subsequent passage in cell culture or shed virus following vaccination.

The manufacturer's virus working seed lot is used for the production of vaccine batches and is prepared from the master virus seed lot. It is recommended that a large lot of virus working seed be set aside as the basic material that the manufacturer should use for the preparation of each batch of vaccine. The virus working seed lot should be prepared by defined number of passages from the virus master seed lot by a method and a passage level from the original virus seed that is established through clinical and

vaccine development studies. Once the passage level of the working seed lot is established, it may not be changed without approval from the national regulatory authority.

A.3.2.3 *Tests on virus master and working seed lots*

A.3.2.3.1 **Identity**

Each seed lot should be identified by virus type by an immunological assay or by sequencing. A molecular identity test, electropherotyping, is one recognized method for identifying rotaviruses. Comparing human and animal rotaviruses and differentiating between subgroups can be achieved through electropherotyping. Electropherotyping can be used as a means for identifying gross alterations in genomic differences between the parent wild type viruses and/or animal donor virus (if employed) and the vaccine strains. The identity of the vaccine virus is confirmed by comparing the electrophoretic profile of the vaccine virus to the RNA electrophoretic profile of a known human rotavirus serotype. Other molecular identity tests may include RNA/RNA hybridization and enzyme restriction maps or genetic sequences of PCR-amplified VP7 gene segments. The tests should be approved by the national regulatory authority.

A.3.2.3.2 **Genotype/phenotype characterization**

Genotype and phenotype stability of the seed lots upon passage should be measured using relevant assays to ensure uniformity of vaccine lots. Genetic characterization of the viruses has played a major role in the development of the vaccines already licensed or currently nearing licensure. It helps to assess the genetic and phenotypic stability of the seed lots on passage, and molecular methods have been used intensively. Other approaches including in vitro phenotypic markers might be considered.

Genetic characterization of the seed lot viruses has included determination of the nucleotide sequence of the complete viral genome, analysis of the molecular basis of the attenuated phenotype and determination of the genetic stability of the virus seed lots by comparison of the nucleotide sequence of the viral genome at different passage levels. It should be noted that full-length sequencing may not identify minority populations of variants that may be present in vaccines. In some studies the entire genomic sequence of all 11 gene segments of the virus master seed lot has been sequenced and compared with the sequence of the virus working seed lot, vaccine production lots and vaccine virus shed in stools of vaccinees. If the attenuation loci are known, sequencing around these sites can be performed instead of on the entire genome. Given the frequency of errors of the RNA replicating polymerase, base changes are likely to be found. The potential impact of any changes observed (silent or in protein-coding regions) should be evaluated.

Specific assays for markers of attenuation are still in development and the manufacturer should make an effort to investigate appropriate assays that are relevant to their vaccine.

A.3.2.3.3 Tests for bacteria, fungi and mycoplasmas

Each virus seed lot should be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests as specified in Part A, sections 5.2 and 5.3, of the *General requirements for the sterility of biological substances*, as amended in 1995 (*16*).

A.3.2.3.4 Tests for adventitious viruses

Each virus seed lot should be tested in cell cultures for adventitious viruses appropriate to the origin and the passage history of the seed virus (*18*). Neutralization of rotavirus is necessary for many tests because the virus is cytopathogenic. Antisera used for this purpose should be shown to be free from antibodies that may neutralize specific adventitious viruses being tested for. If neutralization of rotavirus is not possible, the test sample may be passaged in trypsin-free media prior to initiating the assay, to reduce the ability of rotavirus to infect the indicator cell substrates. The cells inoculated should be observed microscopically for cytopathic changes. At the end of the observation period, the cells should be tested for haemadsorbing viruses.

Each master or working seed lot should also be tested in animals that may include guinea-pigs, mice and embryonated chicken eggs, as appropriate. For test details refer to the WHO *Requirements for measles vaccines* (Live) (*19*), and the *European Pharmacopoeia*, 2002 (*18*)

Additional testing for specific adventitious viruses may be performed, for example, using PCR amplification techniques.

A.3.2.3.5 Virus concentration

Each seed lot should be assayed for infectivity in a sensitive assay in a cell culture system. An immunofocus or plaque forming assay may be used in MA-104, Vero or other sensitive cells to determine virus concentration. The assay is based on the visualization of infected areas (plaques or focus of infection) of a cell monolayer directly or by probing with rotavirus-specific antibodies. Results should be recorded as focus-forming units (FFU/ml) or plaque-forming units (PFU/ml).

A cell culture infectious dose assay may also be used to determine virus concentration. Results should be recorded as $CCID_{50}$/ml.

Alternatively, quantitative PCR detection of virus replication in a cell culture system may be used to provide an appropriate measure of infectivity. Results should be recorded as units of infectivity (UI/ml).

The detailed procedures for carrying out the tests and for interpreting the results should be those approved by the national regulatory authority.

A.4 Control of vaccine production

A.4.1 *Control of cell cultures*

From the cell suspension used to prepare cell cultures for growing attenuated rotavirus, an amount of processed cell suspension equivalent to at least 5% or 500 ml of cell suspension, whichever is greater, should be used to prepare control cultures of uninfected cells. If fermenter technology is used, the size and treatment of the cell sample to be examined should be approved by the national regulatory authority.

These control cultures should be observed microscopically for changes attributable to the presence of adventitious agents for at least 14 days after the day of inoculation of the production cultures or at the time of final virus harvest if this is later. The control cultures should be incubated under essentially similar conditions to those used for the production cultures with agreement of the national regulatory authority. At the end of the observation period, fluids collected from the control culture from each single virus harvest should be tested for the presence of adventitious agents as described below (A.4.1.2). Samples that are not tested immediately should be stored at –60 ºC or below.

If any test shows evidence of the presence of adventitious agents in control cultures, the harvest of virus from these cultures should not be used for vaccine production.

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

A.4.1.1 *Tests for haemadsorbing viruses*

At the end of the observation period, cells comprising no less than 25% of the control cells should be tested for the presence of haemadsorbing viruses, using guinea-pig red blood cells. If the red blood cells have been stored, the duration of storage should not have exceeded 7 days, and the temperature of storage should have been in the range of 2–8 ºC.

In some countries, the national regulatory authority requires that additional tests for haemadsorbing viruses be performed using other species of red blood cells including those from humans (blood group O), monkeys and chickens (or other avian species). In all tests readings should be taken after incubation for 30 minutes at 0–4 ºC, and again after a further incubation for 30 minutes at 20–25 ºC. A further reading for the test with monkey red blood cells should be taken after an additional incubation for 30 minutes at 34–37 ºC.

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.

A.4.1.2 *Tests for other adventitious agents*

At the end of the observation period, a sample of the pooled 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 virus, and additional 10-ml samples of each pool should be tested in human cells and at least one other sensitive cell system.

Each sample should be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cells should be at least 3 cm^2 per ml of pooled fluid. At least one bottle of each kind of cell cultures should remain uninoculated to serve as a control.

The inoculated cultures and control cultures should be incubated at a temperature of 35–37 ºC and should be observed for cytopathic effects for a period of at least 14 days.

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.

Some national regulatory authorities require that, at the end of this observation period, a subculture is made by inoculating the fluid onto fresh cells in the same culture system and observed for at least 7 days. Furthermore, some national regulatory authorities require that these cells should be tested for the presence of haemadsorbing viruses.

A.4.1.3 *Identity test*

At the production level, the cells should be identified by means of tests approved by the national regulatory authority. Suitable methods are, but are not limited to, biochemical tests (e.g. isoenzyme analyses), immunological tests (e.g. HLA assays), cytogenetic tests (e.g. for chromosomal markers), and tests for genetic markers (e.g. DNA fingerprinting).

A.4.2 ***Cell cultures for vaccine production***

A.4.2.1 *Tests for adventitious agents*

On the day of inoculation with the virus working seed lot, each cell culture and/or cell culture control should be examined for degeneration caused by infectious agents. If such examination shows evidence of the presence in a cell culture of any adventitious agents, the culture should not be used for vaccine production. Prior to infection, samples of each cell culture are removed for sterility and mycoplasma testing.

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

A.4.2.2 *Tests for bacteria, fungi and mycoplasmas*

A volume of at least 20 ml of the pooled supernatant fluids from the production cell culture should be tested for bacterial, fungal and mycoplasmal sterility by appropriate tests as specified in Part A, sections 5.2 and 5.3, of the *General requirements for the sterility of biological substances, as amended in 1995* (*16*).

A.4.3 *Control of single harvests and monovalent virus pools*

A.4.3.1 *Virus inoculation*

Cell cultures are inoculated with rotavirus working seed at a defined multiplicity of infection. After viral adsorption, cell cultures are fed with maintenance medium and incubated within a defined temperature range and for a defined period of time, usually established based upon the degree of cytopathic effect.

The range of multiplicity of infection, temperature, pH and time period of incubation will depend on the vaccine strain and production, a defined range should be established by the manufacturer and be approved in the marketing authorization by the national regulatory authority.

A.4.3.2 *Monovalent virus pools*

A virus single harvest is harvested within a defined time period post-inoculation. A monovalent virus pool may be the result of one or more single harvests (from multiple tissue culture flasks, cell factories or bioreactors) in which all harvests were derived from one or a small number of ampoules of the WCB and the same virus working seed lot recovered at the same time. If multiple single harvests are taken, each single harvest should be sampled for testing, stabilized and stored under suitable conditions until pooling. No antibiotics should be added at the time of harvesting or at any later stage of manufacture. Samples of monovalent virus pools should be taken for testing and stored at a temperature of –60 ºC or below.

A.4.3.3 *Tests on single harvest or monovalent virus pools*

Tests may be done on single harvest or on virus pool. If the tests are done on the virus pool, the protocol should be approved by the national regulatory authority.

A.4.3.3.1 **Sampling**

Samples required for the testing of virus harvests should be taken immediately on harvesting prior to further processing. If the tests for adventitious agents

as described in Part A, section A.4.3.3.4, are not performed immediately, the samples taken for these tests should be kept at a temperature of –60 ºC or below and subjected to no more than one freeze–thaw cycle.

A.4.3.3.2 **Identity**

Each single harvest or virus pool should be identified as the appropriate rotavirus serotype by immunological assay or by a molecular-based assay, e.g. electropherotyping, RNA/RNA hybridization or PCR. The tests should be approved by the national regulatory authority.

A.4.3.3.3 **Tests for bacteria, fungi and mycoplasmas**

Each single harvest or virus pool should be shown to be free from bacterial, fungal and mycoplasmal contamination by appropriate tests as specified in Part A, sections 5.2 and 5.3, of the *General requirements for the sterility of biological substances,* as amended in 1995 (*16*).

A.4.3.3.4 **Tests for adventitious agents**

For the purposes of the recommendations set out in this section of Part A, the volume of each single harvest or virus pool taken for neutralization and testing should be at least 10 ml and should be such that a total of at least 50 ml or the equivalent of 500 doses of final vaccine, whichever is the greater, has been withheld from the corresponding final bulk.

Each virus pool should be tested in cell cultures for adventitious viruses appropriate to the passage history of the seed virus (*18*). Neutralization of rotavirus is necessary for many tests because the virus is cytopathogenic. Antisera used for this purpose should be shown to be free from antibodies that may neutralize the adventitious viruses being tested for. If neutralization of rotavirus is not possible, the test sample may be passaged in trypsin-free media prior to initiating the assay to reduce the ability of rotavirus to infect the indicator cell substrates. The cells inoculated should be observed microscopically for cytopathic changes. At the end of the observation period, the cells should be tested for haemadsorbing viruses.

Additional testing for specific adventitious viruses may be performed, for example using PCR amplification techniques.

A.4.3.3.5 **Virus concentration**

Each virus pool should be assayed for infectivity using a sensitive assay in cell cultures to monitor the consistency of production.

An immunofocus or plaque assay may be used in MA-104, Vero or other sensitive cells to determine virus concentration. The assay is based on the visualization of infected areas of a cell monolayer directly or by probing

with rotavirus serotype-specific monoclonal antibodies. Results should be recorded as FFU/ml or PFU/ml.

Alternatively, quantitative PCR detection of virus replication in a cell culture system may be used to provide an appropriate measure of infectivity. Results should be recorded as units of infectivity (UI/ml).

A cell culture infectious dose assay may also be used to determine virus concentration. Results should be recorded as $CCID_{50}$/ml.

The detailed procedures for carrying out the tests and for interpreting the results should be those approved by the national regulatory authority.

A.4.3.3.6 **Tests for consistency of virus characteristics**

Tests for consistency of virus characteristics are performed during vaccine development, and process validation and not intended for batch release. Examples of studies that might be considered to characterize rotavirus are given here. Tests should be sought to compare the rotavirus in the harvest pool with the master seed virus, or suitable comparator, to ensure that the vaccine virus has not undergone critical changes during its multiplication in the production culture system. Phenotypic or genotypic characteristics (genomic sequence analysis) may be suitable. Examples of evidence to support the consistent quality of the virus produced may include in vitro growth characteristics, thermal stability profile, the ratio of infectious (triple-shelled) to non-infectious (double-shelled) particles produced and the stability of the genomic sequence through multiple cell culture passages.

Other aspects of process consistency may also be monitored and validated, such as process impurities and residuals as residual host cell protein, residual cellular DNA, endotoxin, bovine serum, trypsin and antibiotics. Their reduction during processing can be monitored to assess consistency of the manufacturing process. The reduction level should be accepted by the national regulatory authority.

A.4.3.3.7 **Storage**

Virus pools should be stored at a temperature that will ensure stability until formulation.

A.4.3.4 *Control of clarified monovalent virus pool (bulk)*

The monovalent virus pool may be clarified or filtered to remove cell debris and stored at a temperature that ensures stability before being used to prepare the final bulk.

A.4.3.4.1 **Sampling**

Samples of the clarified virus pool should be taken immediately after clarification and prior to further processing to ensure that no cells or cell

debris is left. Samples should also be tested as described in this section. If not tested immediately, the samples should be kept at a temperature below –50 °C until testing is done.

A.4.3.4.2 **Sterility**

The clarified virus pool should be tested for bacterial and fungal sterility according to the requirements in Part A, sections 5.2 of the *General requirements for the sterility of biological substances* (*16*), by acceptable methods approved by the national regulatory authority.

A.4.3.4.3 **Virus concentration**

Each clarified virus pool should be assayed for infectivity in a sensitive assay in cell cultures to monitor the consistency of production.

An immunofocus or plaque assay may be used in MA-104, Vero or other sensitive cells to determine virus concentration. The assay is based on the visualization of infected areas of a cell monolayer directly or by probing with rotavirus serotype-specific monoclonal antibodies. Results should be recorded as FFU/ml or PFU/ml.

Alternatively, quantitative PCR detection of virus replication in a cell culture system may be used to provide an appropriate measure of infectivity. Results should be recorded as units of infectivity (UI/ml).

A cell culture infectious dose assay may also be used to determine virus concentration. Results should be recorded as $CCID_{50}$/ml.

The detailed procedures for carrying out the tests and for interpreting the results should be those approved by the national regulatory authority.

A.4.3.4.4 **Tests for residual cellular DNA**

For viruses grown in continuous cells, the virus pool should be tested for residual cellular DNA. The removal process, at production scale, should be shown to consistently reduce the level of cellular DNA. The limit should be established and approved by the national regulatory authority. Based on relevant studies in animals, WHO established the acceptable limit of not more than 100 µg of cellular DNA per human dose, which is likely to provide an adequate margin of safety for orally-administered vaccines. Consideration should also be given to determining the size of residual cellular DNA as part of the validation process.

These tests may be conducted on the final product, in which case, the product should also conform to the specifications described here.

These tests may be omitted from routine testing, with the agreement of the national regulatory authority, if the manufacturing process is validated as consistently achieving the specification.

A.4.4 *Final bulk*

The final bulk should be aseptically prepared from one or more serotypes each derived from one or more virus pools obtained from substrates of which control cultures pass the tests specified in Section A.4.1. The virus concentration in the final formulation should be sufficient to ensure the dose which is consistent with that shown to be safe and effective in human clinical trials. The virus pools and final bulk should pass the tests specified in Sections A.4.3.3 and A.4.4.1.

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

In preparing the final bulk, any substances such as diluents or stabilizers that are added to the product should have been shown to the satisfaction of the national regulatory authority not to impair the safety and efficacy of the vaccine at the concentration used.

A.4.4.1 *Tests on the final bulk*

A.4.4.1.1 **Sterility**

Each final bulk suspension should be tested for bacterial and fungal sterility according to Part A, sections 5.2 of the *General requirements for the sterility of biological substances* (*16*), or by a method approved by the national regulatory authority.

A.4.4.2 *Storage*

Until the bulk is filled into containers, the final bulk suspension should be stored under conditions shown by the manufacturer to allow it retain the desired biological activity.

A.5 Filling and containers

The requirements concerning filling and containers given in *Good manufacturing practices for biological products* (*13*) 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, transference devices and closure are made do not adversely affect the quality of vaccine and its diluent.

When a freeze-drying process is used for vaccine production, its validation should be submitted to the national regulatory authority for approval.

The manufacturers should provide the national regulatory authority with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

A.6 Control tests on final lot

Samples should be taken from each final lot for the following tests. Both freeze-dried vaccine and its diluent, if applicable, should be tested and should fulfil the requirements discussed in this section.

A.6.1 *Vaccine*

A.6.1.1 *Inspection of final containers*

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

A.6.1.1.1 *Appearance*

The appearance of the freeze-dried or liquid vaccine should be described with respect to its form and colour. In the case of freeze-dried vaccines, a visual inspection should be performed of the freeze-dried vaccine, its diluent and the reconstituted vaccine. If reconstitution with the product diluent does not allow for the detection of particulates, an alternative diluent may be used.

A.6.1.2 *Identity*

The virus in one or more individually labelled final containers should be identified as rotavirus and, for multivalent vaccine formulations each serotype should be identified, by appropriate methods approved by the national regulatory authority. Methods such as plaque neutralization, and immunofocus assays in cell culture are suitable to identify the presence of rotavirus using rotavirus-specific polyclonal antiserum. PCR may also be appropriate, in this case the virus titration by quantitative PCR may serve as the identity test.

A.6.1.3 *Sterility*

Liquid or reconstituted vaccine should be tested for bacterial and fungal sterility according to the requirements in Part A, section 5.2 of the *General requirements for the sterility of biological substances* (*16*), or by the methods approved by the national regulatory authority.

A.6.1.4 *pH*

The pH of the final lot should be tested in a pool of final containers and an appropriate limit set to guarantee virus stability. In case of freeze-dried vaccines, pH should be measured after reconstitution of the vaccine with the diluent.

A.6.1.5 *Residual moisture*

The residual moisture in a representative sample of each freeze-dried lot should be determined by a method approved by the national regulatory authority and an appropriate limit set to ensure vaccine stability.

A.6.1.6 *Virus concentration*

The virus concentration in each of at least three final containers of the rotavirus vaccine final lot should be assayed individually for infectivity in a sensitive assay system in which interference or potentiation between the serotypes present in the vaccine does not occur.

An immunofocus or plaque assay may be used in MA-104, Vero or other sensitive cells to determine virus concentration. The assay is based on the visualization of infected areas of a cell monolayer directly or by probing with rotavirus serotype-specific monoclonal antibodies. Results should be recorded as FFU/ml or PFU/ml.

If immunological based assays are used, the specificity and lack of cross-reactivity of the antiserum must be verified. Alternatively, quantitative PCR detection of virus replication in a cell culture system may be used to provide an appropriate measure of infectivity. Results should be recorded as units of infectivity (UI/ml).

A cell culture infectious dose assay may also be used to determine virus concentration. Results should be recorded as $CCID_{50}$/ml.

The titre of each individual serotype should be determined and should fall within the specifications for potency. The assay method should include suitable qualified reference reagents for each serotype in the vaccine. The detailed procedures for carrying out the tests and for interpreting the results should be those approved by the national regulatory authority.

The national regulatory authority should approve a reference preparation of live attenuated rotavirus vaccine for use in tests to determine virus concentration.

Freeze-dried vaccine should be reconstituted with its diluent to determine virus concentration. A validated alternative diluent may be needed if the approved diluent is toxic to the cell cultures used in the assay. If a different diluent is used for this test, data to allow a comparison between the results with both diluents should be submitted for the approval of the national regulatory authority.

Internal consistency limits should be established by the manufacturer taking into account the vaccine dose shown to be safe and effective in human clinical trials. Specifications for virus concentration should essentially specify the minimum titre guaranteed to be contained in one human dose and this should be agreed with the national regulatory authority.

A.6.1.7 *Accelerated stability tests*

A representative number of the final containers should be exposed to an elevated temperature for a defined time period, using conditions based on

the manufacturer's experience. The geometric mean of infectious virus titre of the containers that have been exposed should not have been decreased by more than a specific amount during the period of exposure. Estimation of the virus titre of non-exposed and exposed vials should be made in parallel and results expressed in terms of PFU, FFU, $CCID_{50}$ or UI per human dose. The maximum allowable loss of titre during the accelerated stability test should be confirmed on the basis of the manufacturer's experience and approved by the national regulatory authority. For a multivalent vaccine, if there is no significant difference in the virus loss between serotypes, the loss may be based upon total virus concentration.

A.6.2 *Diluents*

The requirements given in *Good manufacturing practices for pharmaceutical products* (*20*) should apply for the manufacturing and control of diluents used to reconstitute live attenuated rotavirus vaccines and, if required, the antacid buffer used. An expiry date should be established for the diluent based upon stability data. If an antacid is to be used, the stability of the rotavirus in the presence of the antacid should be confirmed. For lot release of the diluent, tests for identity, appearance, pH, volume, sterility, and the content of key components should be done.

A.7 Records

The requirements given in *Good manufacturing practices for biological products* (*13*) should apply.

A.8 Samples

The requirements given in *Good manufacturing practices for biological products* (*13*) should apply.

A.9 Labelling

The requirements given in *Good manufacturing practices for biological products* (*13*) should apply, with the addition of the following.

The label on the carton enclosing one or more final containers, or the leaflet accompanying the container, should include the following information:

— a statement that the candidate vaccine fulfils Part A of these Guidelines;
— a statement of the nature of the preparation, specifying the designation of the strain(s) of rotavirus contained in the vaccine, the minimum amount of virus contained per human dose, the origin of the substrates used in the preparation of the vaccine and whether the vaccine strains were derived by molecular methods;

- the fact that the vaccine is not to be injected;
- a statement of the nature and quantity, or upper limit, of any antibiotic present in the vaccine;
- an indication that contact with disinfectants is to be avoided;
- a statement regarding the concomitant administration of rotavirus vaccine with other oral vaccines such as oral poliovirus vaccine and with other non-orally administered vaccines;
- a statement concerning administration to HIV-positive or other immunocompromised individuals;
- a statement indicating the volume and nature of the diluent to be added to reconstitute the vaccine, and specifying that the diluent to be used is that supplied by the manufacturer;
- a statement that after the vaccine is reconstituted, it should be used without delay, or if not used immediately, stored under defined conditions and in the dark for a maximum period defined by stability studies;
- a statement concerning storage conditions (temperature), expiry date, volume and instructions for reconstitution. Only one storage temperature and expiry date should be stated on the label and leaflet; and
- a statement indicating whether an antacid is to be given prior to or in combination with the vaccine at the time of vaccination.

It is desirable for the label or the leaflet to carry the names of both the producer and the source of the bulk material if the producer of the final vaccine did not prepare it.

A.10 Distribution and shipping

The requirements given in *Good manufacturing practices for biological products* (*13*) should apply.

Vaccine shipments should be maintained at the approved temperatures and parcels should contain cold-chain monitors.

For some products, freezing of the diluent should be avoided.

A.11 Storage and expiry date

The statements concerning storage temperature and expiry date of the vaccine and its diluent, if applicable, that appear on the label and in the leaflet, as recommended in *Good manufacturing practices for biological products* (*13*), should be based on experimental evidence and should be submitted for approval to the national regulatory authority.

A.11.1 *Storage conditions*

Before being distributed by the manufacturing establishment or before being issued from a storage site, the vaccine should be stored at a temperature

shown by the manufacturer to be compatible with a minimal titre loss. The maximum duration of storage should be fixed with the approval of the national regulatory authority and should be such as to ensure that all quality specifications for final product including the minimum titre specified on the label of the container (or package) will still be maintained until the end of the shelf-life.

A.11.2 *Expiry date*

The expiry date should be defined on the basis of shelf-life and supported by the stability studies with the approval of the national regulatory authority and should relate to the date of the last satisfactory determination of virus concentration, performed in accordance with Part A, section A.4.3.3.5, i.e. the date on which the test system was inoculated.

The expiry dates for the vaccine and the diluent may be different.

Part B. Nonclinical evaluation of live attenuated rotavirus vaccines

Nonclinical evaluation of rotavirus vaccines should be based on existing WHO guidelines (*1*); however, the following rotavirus-specific issues should be considered.

In animal studies of rotavirus vaccines, oral dosing with antacid which corresponds to that intended for use in clinical trials is necessary; for example, in mice or in rats (using a strain of rat susceptible to human rotavirus infection) with the full human dose of vaccine. As rotavirus is not neurotropic, and neural tissue passage has not been used in the derivation of any of the vaccine strains, a neurovirulence test for each batch is not justified, nor is there a need to test the master or working seed lots. As with all live attenuated vaccines, special attention should be paid to evaluating the stability of attenuation phenotype by appropriate in vitro and in vivo assays. Nonclinical experimental studies to predict risks from excreted vaccine virus are not expected to be informative.

On the basis of a review of the literature, manufacturers should evaluate any risks to humans or animals if the vaccine virus is subsequently shown to be shed from vaccinees, taking into account the likelihood of excreted vaccine virus reassorting with wild-type viruses. Finally, the pathogenic mechanisms of intussusception associated with oral rotavirus vaccination are currently unknown and no suitable animal model is readily available to evaluate the risk of intussusception. Nevertheless, manufacturers should keep abreast of the evolving scientific knowledge and plan their strategy for preclinical vaccine evaluation accordingly.

Part C. Clinical evaluation of live attenuated rotavirus vaccines

The WHO guidelines on regulatory expectations for clinical evaluation are appropriate for development of rotavirus vaccines (*2*). Consideration should be given to the following rotavirus-specific issues.

C.1 Immune responses to rotavirus

Live attenuated rotavirus vaccines have been developed on the basis of evidence that natural rotavirus infections elicit protective immune responses, particularly against future severe rotavirus disease. For a period after rotavirus vaccination, much of the serum IgA is rotavirus-specific, so that rotavirus serum IgA levels act as a measure of seroconversion. Rotavirus serum IgA antibody responses have been used as measures of vaccine immunogenicity of all the candidate live attenuated rotavirus vaccines evaluated so far. Thus rotavirus serum IgA antibody responses should also be recorded for other vaccine candidates.

Nevertheless it should be noted that rotavirus serum IgA is not an immune correlate of protection for rotavirus vaccines. A role for neutralizing antibody in protective efficacy has been suggested, but the correlation between neutralizing antibody titres and clinical efficacy has not been demonstrated. These data have been recently reviewed for the different live attenuated rotavirus vaccine candidates (*21*).

C.2 Special considerations

There are additional specific issues that may affect the safety and efficacy of oral rotavirus vaccines. Factors which are considered important for evaluation pre-licensure include the following:

C.2.1 *Vaccine virus shedding and transmission*

Manufacturers should undertake studies to determine the amounts of the vaccine virus (if applicable, by serotype) shed by vaccinees and the duration of shedding. They should assess the transmissibility of vaccine strains to unvaccinated people during clinical studies of safety and efficacy. If vaccine virus is shed in sufficient quantities to make transmission feasible and/or if transmission from vaccines is demonstrated, then studies of the likelihood of reversion to wild type and the likelihood of the vaccine virus reassorting with wild-type rotaviruses are indicated.

C.2.2 *Dose regimen*

Clinical trials should be designed to determine the number of doses to be administered to elicit a measurable immune response and clinical efficacy.

Based on experience to date, at least two or three doses of live attenuated oral rotavirus vaccines are necessary to overcome interfering factors which may include maternal antibodies or concomitant administration of oral poliovirus vaccine (OPV).

C.2.3 *Concomitant administration with routine childhood vaccines*

As it is intended that live attenuated rotavirus vaccines would be incorporated into routine childhood immunization programmes, it is important to generate information on immune responses to co-administration with the routine childhood vaccines at the target ages of administration. In particular, co-administration with OPV should be studied, as both are live attenuated oral vaccines.

C.2.4 *Vaccine safety*

After licensure of a rhesus reassortant rotavirus vaccine, postmarketing surveillance in one country revealed an association with intussusception, a rare serious adverse event following administration of the vaccine to infants. This occurred in infants given the vaccine at an older age than that recommended by the manufacturer. In light of this experience, rotavirus vaccines must be assessed for any vaccine-attributable intussusception within 30 days after each dose of vaccine. However, even very large pre-licensure studies (for example in 70 000 infants) cannot rule out an association between vaccine and intussusception. They can only provide an estimate of the relative and absolute risk compared to placebo together with 95% confidence intervals that give an idea of the degree of risk that cannot be excluded. Such data are generated in clinical trials in selected populations and in which the doses of the regimen under study are controlled, so that the data may not predict risk in the post-licensure period. If post-licensure studies, currently in progress, reveal an age-related risk of vaccine-attributable intussusception, specifications may need to be considered for the age at first dose of vaccine (*22*).

In planning the size of future clinical safety trials for a new rotavirus vaccine, assumptions need to be made about the likely background incidence of intussusception. However, it may not be feasible to obtain accurate data on naturally occurring intussusception in all countries in which a study is to be performed. The calculation should state the degree of risk of vaccine-associated intussusception that the study should be able to assess. With appropriate justifications, sample sizes of < 70 000 for future pre-licensure clinical trials of new rotavirus vaccines may be acceptable.

C.2.5 *Definition of clinical end-point in trials of protective efficacy*

The results from clinical trials to date, show that rotavirus vaccines are more efficacious against severe illness than against mild or moderate disease. Standard definitions of a diarrhoea episode and the severity of illness

(recommended as the primary end-point of efficacy studies) are critical. Several methods for assessing the "severity" of rotavirus infection are available, for example, based on the scale described by Ruuska et al (*23*) and Clark et al (*24*). Hospitalization may not be appropriate as the only end-point because this is a context-sensitive situation which may not always reflect the severity of illness. However, hospitalization combined with grading of severity on an appropriate scale should give comparable results between study sites.

Usually the primary analysis is focused on prevention of acute rotavirus gastroenteritis due to the serotype(s) in the vaccine. It is acceptable that such primary analysis be based on those cases of acute rotavirus gastroenteritis that occur from 14 days onwards after administration of the last dose of the tested regimen. Consideration should also be given to sensitivity analysis to investigate efficacy against all cases of acute rotavirus gastroenteritis that occur from the time of the first dose onwards.

C.2.6 *Other factors*

Several other factors need to be considered when assessing new rotavirus vaccines, although some of these data may be generated post-licensure. Such data include those on use in special populations and in diverse geographical regions (developed or developing country settings), and interference by other health factors.

C.2.6.1 *Vaccine safety in immunocompromised infants*

No data on immunocompromised children are currently available. As with other live vaccines, live attenuated rotavirus vaccines should be shown to be safe in HIV-infected infants prior to general implementation in countries with high prevalence of HIV. Natural rotavirus infection has not been shown to be more serious or to be associated with greater safety risks in HIV-infected infants than in non-HIV-infected infants, although a slightly longer period of shedding of rotavirus was observed in one study.

C.2.6.2 *Seasonality of rotavirus infection*

Depending on where the pre-licensure efficacy data were generated (i.e. in areas with seasonal or year-round rotavirus infection), it may be appropriate to generate further data in alternative geographical areas. For example, in those settings with a marked seasonal pattern of rotavirus infection, the efficacy of rotavirus vaccine may be higher when it is administered shortly before the peak period for rotavirus circulation, possibly due to a booster effect of natural infection during the season. For this reason, it may be useful to assess efficacy in sequential seasons.

C.2.6.3 *Diverse geographical and population settings*

The efficacy and/or effectiveness of rotavirus vaccine needs to be studied in diverse geographical regions with different populations and different

socioeconomic conditions. Generally, data from clinical trials conducted in one part of the world would not necessarily be predictive of vaccine efficacy in other parts of the world (*3*). For example, clinical trials have shown variable levels of rotavirus-specific serum IgA with the same vaccine in populations in developed and less developed or developing countries. Furthermore, the assessment of protection provided by each serotype in the vaccine and/or against serotypes not in the vaccine (i.e. cross-protection) is inherently limited by the serotypes circulating in the countries where the study/studies was/were done during the observation period. Studies may lack the power to demonstrate efficacy by specific serotype. In these circumstances, data must be assessed on a case-by-case basis when considering any claims for vaccine serotype-specific and heterotypic protection.

Many factors are likely to be responsible for observed differences in immunogenicity and protective efficacy of the rotavirus vaccines in diverse geographical settings. In designing rotavirus vaccine trials (pre- or post-licensure), consideration should be given to controlling for the possible effects of factors such as the predominant circulating rotavirus serotypes, other vaccinations, malnutrition, vitamin and mineral deficiencies, concurrent enteric infections, malaria, parasitic infections, HIV, hepatitis, immunodeficiences, maternally transmitted antibodies and breastfeeding.

C.2.6.4 *Interchangeability*

At present there are no data on safety, immunogenicity or efficacy when more than one vaccine type is used in one infant in the priming series. Although there may be difficulties in interpreting immunogenicity data in terms of predicting efficacy, and of complexities that would arise in studies of safety and efficacy when more than one vaccine type is given (e.g. a monovalent human rotavirus strain live attenuated vaccine followed by a multivalent human-bovine reassortant rotavirus strain live attenuated vaccine), such data would be welcome.

C.3 Postmarketing studies and surveillance

Large-scale postmarketing surveillance studies of safety and effectiveness are indicated once vaccines have been approved. In the case of rotavirus, knowledge of distribution of strains, establishment of country-specific laboratory expertise and education of physicians and policy-makers will be required to conduct these studies effectively. The aims of postmarketing surveillance of rotavirus vaccines may include evaluation of vaccine effectiveness in different populations and in settings in which more varied rotavirus serotypes predominate; the impact of emerging strains of rotavirus; monitoring the risk of vaccine-associated intussusception and of other potential adverse events; and evaluation of compliance with the recommended vaccination schedule. A standardized protocol for postmarketing surveillance is under development.

Part D. Guidelines for national regulatory authorities

D.1 General

The general recommendations for control laboratories given in the *Guidelines for national authorities on quality assurance for biological products* (*25*), which specify that no new biological substance should be released until consistency of manufacturing and quality as demonstrated by a consistent release of batches has been established, should apply.

The detailed production and control procedures and any significant changes made to them should be discussed with and approved by the national regulatory authority. The national regulatory authority should obtain the working reference from the manufacturers to establish a national working reference preparation for use until an international reference reagent becomes available.

D.2 Release and certification

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

A statement signed by the appropriate official of the national control laboratory should be provided if requested by a manufacturing establishment and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these Guidelines. The certificate should also state the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, the date of the last satisfactory determination of virus concentration as well as the expiry date assigned on the basis of shelf-life should be stated. A copy of the official national release document should be attached. The certificate should be based on the model given in Appendix 2.

The purpose of the certificate is to facilitate the exchange of live attenuated rotavirus vaccines between countries.

Authors

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Appendix 1

Summary protocol of manufacturing and control of live attenuated rotavirus vaccines (oral)

The following protocol is intended for guidance, and indicates the information that should be provided as a minimum by the manufacturer to the national regulatory authority. The protocol must be accompanied by a lot release certificate from the licensing authority which may or may not be the country of manufacturing origin. Information and tests may be added or deleted as required by the national regulatory authority of the importing country, if applicable.

It is thus 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 guidelines for a particular product should be given in the protocol submitted.

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

Summary information on the finished product (final lot)

International name: Live attenuated rotavirus vaccine (oral)
Commercial name: ______________________________
Product licence (marketing authorization) number ______________
Country: ______________________________
Name and address of manufacturer: ______________________
Name and address of licence holder if different: ______________
____

Final packing lot number: ______________________
Type of container: ______________________
Number of containers in this packing lot: ______________
Final container lot number: ______________________
Number of filled containers in this final lot: ______________
Date of manufacture (filling or lyophilizing, if applicable): ________
Date on which last determination of virus concentration was started: ______________________

Shelf-life approved (months): ___________________________
Expiry date: ___________________________
Storage conditions: ___________________________
Volume of single dose: ___________________________
Volume of vaccine per container: ___________________________
Number of doses per container: ___________________________
Virus concentration per human dose:
Serotype: ___________________________
Serotype: ___________________________
Serotype: ___________________________
Serotype: ___________________________

Nature of any antibiotics present in vaccine and amount
per human dose: ___________________________
Production cell substrate: ___________________________
Bulk No. of monovalent virus pools blended in multivalent
vaccine (if applicable): ___________________________
Diluent or antacid (if applicable):___________________________
Lot number: ___________________________
Date of manufacture: ___________________________
Expiry date: ___________________________

A genealogy of the lot numbers of all vaccine components used in the formulation of the final product, diluent and antacid will be informative. An example of a genealogy is given in Appendix 2.

The following sections are intended for the reporting of the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production; thus if any test has to be repeated, this must be indicated. Any abnormal results should be recorded on a separate sheet. If any cell lot, virus harvest or other intermediates intended for production of the current lot was rejected, this should also be recorded either in the following sections or on a separate sheet.

A.3 Control of source materials

The results of tests on the same master or manufacturing working cell bank and the same virus master and manufacturing working seed lots are submitted to and approved by the national regulatory authority during the procedure for granting the licence or its variations and need not be re-submitted at the time of lot release.

A.3.1 *Cell cultures for virus production*

Name and identification of cell substrate: ___________________________
Origin and short history: ___________________________
Authority that approved cell bank: ___________________________

Master cell bank (MCB)

Lot number: ____________________

Date MCB was established: ____________________

Date of approval by the national regulatory authority: ____________________

Total number of ampoules stored: ____________________

Passage/population doubling level of MCB: ____________________

Maximum passage/population doubling level approved for MCB: ____________________

Storage conditions: ____________________

Percentage of total MCB ampoules tested: ____________________

Identity test: ____________________

Date of test ____________________

Method used ____________________

Results ____________________

Results of tests for adventitious agents: ____________________

Results of tests for tumorigenicity (if applicable): ____________________

Tests for retroviruses (if applicable)

Date of test ____________________

Method used ____________________

Results ____________________

Manufacturer's working cell bank (MWCB)

Lot number: ____________________

Date MWCB was established: ____________________

Total number of ampoules stored: ____________________

Passage/population doubling level of MWCB:____________________

Maximum passage/population doubling level approved for MWCB: ____________________

Storage conditions ____________________

Percentage of total MWCB ampoules tested: ____________________

Identity test:

Date of test ____________________

Method used ____________________

Results ____________________

Results of tests for adventitious agents: ____________________

Results of tests for tumorigenicity (if applicable): ____________________

Tests for retroviruses (if applicable):

Date of test ____________________

Method used ____________________

Results ____________________

Cell culture medium

Serum used in cell culture medium

Animal origin of serum: ____________________

Batch number: ______________________________
Vendor: ______________________________
Country of origin: ______________________________
Certificate of freedom from TSE (yes/no): ______________________________
Tests performed on serum: ______________________________
    Methods used ______________________________
    Results ______________________________

Trypsin used for preparation of cell cultures

Animal origin of trypsin: ______________________________
Batch number ______________________________
Vendor: ______________________________
Country of origin: ______________________________
Certificate of freedom from TSE (yes/no): ______________________________
Tests performed on trypsin: ______________________________
    Date of tests ______________________________
    Methods used ______________________________
    Results ______________________________

A.3.2 *Virus seeds*

Virus strain(s) and serotype(s): ______________________________
Substrate used for preparing seed lots: ______________________________
Origin and short history: ______________________________
Authority that approved virus strain(s): ______________________________
Date approved: ______________________________

Virus master seed lot (VMS)

Lot number: ______________________________
Date VMS was established: ______________________________
Date approved by the National Regulatory Authority: ______________________________
Total quantity of VMS stored: ______________________________
Storage conditions: ______________________________
Passage level of VMS: ______________________________
Maximum passage level approved for VMS: ______________________________

Manufacturer's virus working seed lot (MVWS)

Lot number: ______________________________
Date MVWS was established: ______________________________
Total quantity of MVWS stored: ______________________________
Storage conditions: ______________________________
Passage level of MVWS: ______________________________
Maximum passage level approved for MVWS: ______________________________

Tests on virus master and working seed lots

Identity test:

Date of test ______________________________

Method used ______________________________

Results ______________________________

Genotype/phenotype characterization:

Date of test ______________________________

Method used ______________________________

Results ______________________________

Tests for bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Incubation	Media used	Inoculum	Date test on	Date test off	Results
20–25 °C	________	________	________	________	_____
30–36 °C	________	________	________	________	_____
Negative control	________	________	________	________	_____

Tests for mycoplasmas

Standard culture method

	Inoculum	Date test began	Date test ended	Incubation conditions	Results
Solid media	______	________	________	________	_____
Liquid media	______	________	________	________	_____
Negative control	______	________	________	________	_____
Positive control cultures	______	________	________	________	_____

Indicator cell-culture method

Cell substrate used ______________________________

Inoculum ______________________________

Date of test ______________________________

Passage number ______________________________

Negative control ______________________________

Positive controls ______________________________

Date of staining ______________________________

Results ______________________________

Tests for adventitious viruses

Volume of virus seed samples for neutralization and testing ________

Batch number(s) of antisera/antiserum used for neutralization of virus seeds ______________________________

Tests in tissue cultures

Type of simian cells______________________________

Quantity of neutralized sample inoculated ______________

Incubation conditions ______________________________

Date test began ______________________________________

Date test ended ______________________________________

Ratio of cultures viable at end of test ______________________

Results ____

Type of human cells ___________________________________

Quantity of neutralized sample inoculated __________________

Incubation conditions __________________________________

Date test began ______________________________________

Date test ended ______________________________________

Ratio of cultures viable at end of test ______________________

Results ____

Other cell types____

Quantity of neutralized sample inoculated __________________

Incubation conditions __________________________________

Date test began ______________________________________

Date test ended ______________________________________

Ratio of cultures viable at end of test ______________________

Results ____

Tests in animals

Test in adult mice

Weight and number of animals ____________________________

Routes and quantity of neutralized sample inoculated ___________

Date test began ______________________________________

Date test ended ______________________________________

Ratio of animals surviving the observation period _____________

Results ____

Test in suckling mice

Age and number of animals ______________________________

Routes and quantity of neutralized sample inoculated ___________

Date test began ______________________________________

Date test ended ______________________________________

Ratio of animals surviving the observation period _____________

Results ____

Test in guinea-pigs

Weight and number of animals ____________________________

Routes and quantity of neutralized sample inoculated ___________

Date test began ______________________________________

Date test ended ______________________________________

Ratio of animals surviving the observation period _____________

Results ____

Additional tests ______________________________
Date of tests ______________________________
Methods used ______________________________
Results ______________________________

Virus concentration

Date of test ______________________________
Method used ______________________________
Results ______________________________

A.4 Control of vaccine production

Production cells

Lot number: ______________________________
Date of thawing ampoule of MWCB: ______________________________
Passage/population doubling level at virus inoculation: ______________
Maximum passage/population doubling level approved for vaccine production: ______________________________
Nature and concentration of antibiotics used in production cell culture maintenance medium: ______________________________
Identification and source of starting materials used in preparing production cells including excipients and preservative (particularly any materials of human or animal origin): ______________

A.4.1 *Control of Cell Cultures*

(*Note*: If more than one virus single harvest is used to produce a monovalent virus pool, then data on each lot of control cells should be provided.)

Amount or ratio of control cultures to production cell cultures:

____

Incubation conditions: ______________________________
Period of observation of cultures: ______________________________
Date started ______________________________
Date ended ______________________________
Ratio of cultures discarded and reason: ______________________________
Results of observation: ______________________________
Date fluids collected: ______________________________
Date fluids pooled (if applicable): ______________________________

Tests for haemadsorbing viruses:

Quantity of cells tested ______________________________
Type of RBC used ______________________________
Storage time and temperature of RBC ______________________________
Incubation time and temperature of RBC ______________________________

Date test began ______________________________
Date test ended ______________________________
Results ______________________________

Additional tests if performed ______________________________

Tests for other adventitious agents:

Test in production cells

Date of sampling ______________________________
Quantity of sample inoculated ______________________________
Incubation conditions ______________________________
Date test began ______________________________
Date test ended ______________________________
Ratio of cultures viable at end of test ______________________________
Uninoculated cell control ______________________________
Results ______________________________

Test in human cells

Type of human cells ______________________________
Quantity of sample inoculated ______________________________
Incubation conditions ______________________________
Date test began ______________________________
Date test ended ______________________________
Ratio of cultures viable at end of test ______________________________
Uninoculated cell control ______________________________
Results ______________________________

Test in other cell system

Type of cells ______________________________
Quantity of sample inoculated ______________________________
Incubation conditions ______________________________
Date test began ______________________________
Date test ended ______________________________
Ratio of cultures viable at end of test ______________________________
Uninoculated cell control ______________________________
Results ______________________________

Identity test:

Date of test ______________________________
Method used ______________________________
Results ______________________________

A.4.2 *Cell cultures for vaccine production*

Tests for adventitious agents

Date of examination (inoculation) ______________________________
Results ______________________________

Tests for bacteria, fungi and mycoplasmas

Date and volume of sampling ___________________________

Volume of samples tested ______________________________

Tests for bacteria and fungi

Incubation	Media used	Inoculum	Date test on	Date test off	Results
20–25 °C	________	_______	___________	__________	____
30–36 °C	________	_______	___________	__________	____
Negative control	________	_______	___________	__________	____

Tests for mycoplasmas

Standard culture method

	Inoculum	Date test on	Date test off	Incubation conditions	Results
Solid media	______	__________	__________	_______________	_____
Liquid media	______	__________	__________	_______________	_____
Negative control	______	__________	__________	_______________	_____
Positive control cultures	______	__________	__________	_______________	_____

Indicator cell-culture method

Cell substrate used ____

Inoculum ____

Date of test ____

Passage number ____

Negative control ____

Positive controls ____

Date of staining ____

Results ____

A.4.3 *Control of single harvests*

For multivalent vaccine, the following information for each virus serotype should be submitted.

If more than one single harvest is used to prepare a monovalent virus pool, the following information for each single harvest should be submitted.

Virus serotype ____

Lot number of single harvest ___________________________

Date of virus inoculation ______________________________

Multiplicity of infection _______________________________

Incubation conditions _________________________________

Date of harvesting ____________________________________

Volume harvested _____________________________________

Date of sampling ______________________________________

Volume of sampling ____________________________________

Storage conditions and period __________________________

Monovalent virus pool (pre-clarification)

Lot number of virus pool ____
Date of pooling ____
Virus single harvests pooled

Lot number __________________ Volume pooled _________________

Volume of virus pool after pooling ________________________________
Date of sampling ____
Volume of sampling ______________________________________
Storage of samples (if applicable) _________________________________

Tests on single harvest or monovalent virus pools

(Tests may be done on individual single harvest or on the virus pools as approved by the national regulatory authority.)

Identity

Date of test ____
Method used ____
Results ____

Tests for bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Incubation	Media used	Inoculum	Date test began	Date test ended	Results
20–25 °C	_________	________	_____________	____________	_____
30–36 °C	_________	________	_____________	____________	_____
Negative control	_________	________	_____________	____________	_____

Tests for mycoplasmas

Standard culture method

	Inoculum	Date test began	Date test ended	Incubation conditions	Results
Solid media	_______	___________	___________	________________	______
Liquid media	_______	___________	___________	________________	______
Negative control	_______	___________	___________	________________	______
Positive control cultures	_______	___________	___________	________________	______

Indicator cell-culture method

Cell substrate used ____
Inoculum ____
Date of test ____
Passage number ____
Negative control ____
Positive controls ____
Date of staining ____
Results ____

Tests for adventitious agents

Volume of samples for neutralization and testing ____________________

Batch number(s) of antisera/antiserum used for neutralization __________

Tests in tissue cultures

Type of simian cells ______________________________

Quantity of neutralized sample inoculated ____________________

Incubation conditions ______________________________

Date test began ______________________________

Date test ended ______________________________

Ratio of cultures viable at end of test ____________________

Results ______________________________

Type of human cells ______________________________

Quantity of neutralized sample inoculated ____________________

Incubation conditions ______________________________

Date test began ______________________________

Date test ended ______________________________

Ratio of cultures viable at end of test ____________________

Results ______________________________

Type of other cells ______________________________

Quantity of neutralized sample inoculated ____________________

Incubation conditions ______________________________

Date test began ______________________________

Date test ended ______________________________

Ratio of cultures viable at end of test ____________________

Results ______________________________

Cell substrate	Specification	**Primary passage**			**Subculture passage**		
		Test initiation date	No. of flasks tested	Results	Test initiation date	No. of flasks tested	Results
	Cytopathic effect						
	Haemadsorption						
	Positive control virus						
	Negative control						

Virus concentration

Date of test ______________________________

Method used ______________________________

Results ______________________________

Tests for consistency of virus characteristics

(Tests are performed during vaccine development and process validation, may not be required for batch release.)

Item tested ____________________

Date of test ____________________

Methods used ____________________

Results ____________________

Storage conditions and period ____________________

Control of clarified monovalent virus pool

Lot number of monovalent virus pool ____________________

Date of clarification ____________________

Methods used for clarification ____________________

Volume of virus pool before clarification ____________________

Volume of virus pool after clarification ____________________

Date of sampling ____________________

Volume of sampling ____________________

Storage conditions of samples ____________________

	Specification	**Date test initiated**	**Method**	**Results**
Sterility	______	______	______	______
Virus concentration	______	______	______	______
Tests for residual	______	______	______	______
cellular DNA	______	______	______	______

A.4.4 *Final bulk*

Lot number ____________________

Date of formulation ____________________

Total volume of final bulk formulated ____________________

Monovalent virus pools used for formulation

Serotype	Lot number	Volume added	Virus concentration
______	______	______	______
______	______	______	______
______	______	______	______

	Name	Lot number	Volume added
Stabilizer if used	______	______	______
Diluent used	______	______	______

	Specification	**Date test initiated**	**Method**	**Results**
Sterility	______	______	______	______

Storage conditions and period ____________________

Approved storage
period ______________________________

A.5 Filling and containers

Lot number ______________________________
Date of filling ______________________________
Volume of final bulk filled ______________________________
Filling volume per container ______________________________
Number of containers filled (gross) ______________________________
Date of lyophilization (if applicable) ______________________________
Number of containers rejected during inspection ______________________________
Number of containers sampled ______________________________
Total number of containers (net) ______________________________
Maximum period of storage approved ______________________________
Storage temperature and period ______________________________

A.6 Control tests on final lot

A.6.1 *Vaccine*

Inspection of final containers

Appearance ______________________________
Date of test ______________________________
Results ______________________________
Before reconstitution ______________________________
After reconstitution ______________________________
Diluent used ______________________________
Lot number of diluent used ______________________________

Identity

Date test began and ended ______________________________
Method used ______________________________
Results ______________________________
Lot number of reference reagents ______________________________

	Specification	Date test initiated	Method	Results
Sterility	______	______	______	______

Diluent used ______________________________
Lot number of diluent used ______________________________

pH

Date of test ______________________________
Method used ______________________________
Results ______________________________

Diluent used ______________________________

Lot number of diluent used ______________________________

Residual moisture (if applicable)

Date of test ______________________________

Method used ______________________________

Results ______________________________

Virus concentration

Date titration began and ended ______________________________

Method used for titration ______________________________

Results

Serotype	Virus titre

Lot number of reference virus ______________________________

Lot number of other reference reagents if used ______________

Diluent used ______________________________

Lot number of diluent used ______________________________

Accelerated stability tests

Duration of exposure ______________________________

Temperature of exposure ______________________________

Date titration began and ended ______________________________

Method used for titration ______________________________

Results ______________________________

Total virus titre

Exposed sample ______________________________

Non-exposed sample ______________________________

Titre reduction ______________________________

Lot number of reference virus ______________________________

Lot number of other reference reagents if used ______________

Diluent used ______________________________

Lot number of diluent used ______________________________

A.6.2 ***Diluents***

Nature and volume ______________________________

Lot number ______________________________

Date of manufacture ______________________________

Storage conditions and period ______________________________

Expiry date ______________________________

	Specification	Date test initiated	Method	Results
Sterility	___________	___________	___________	___________
Identity	___________	___________	___________	___________
pH	___________	___________	___________	___________

Physical inspection ____

Content of key components:

____

____

____

____

Certification by the manufacturer

Name of head of production (typed) ____________________________

Certification by the person from the control laboratory of the manufacturing company taking over responsibility for the production and control of the vaccine:

I certify that lot no. ______________ of live attenuated rotavirus vaccine (oral), whose number appears on the label of the final container, meets all national requirements and satisfies Part A of the Requirements for Biological Substances No. __________ of live attenuated rotavirus vaccines (oral).

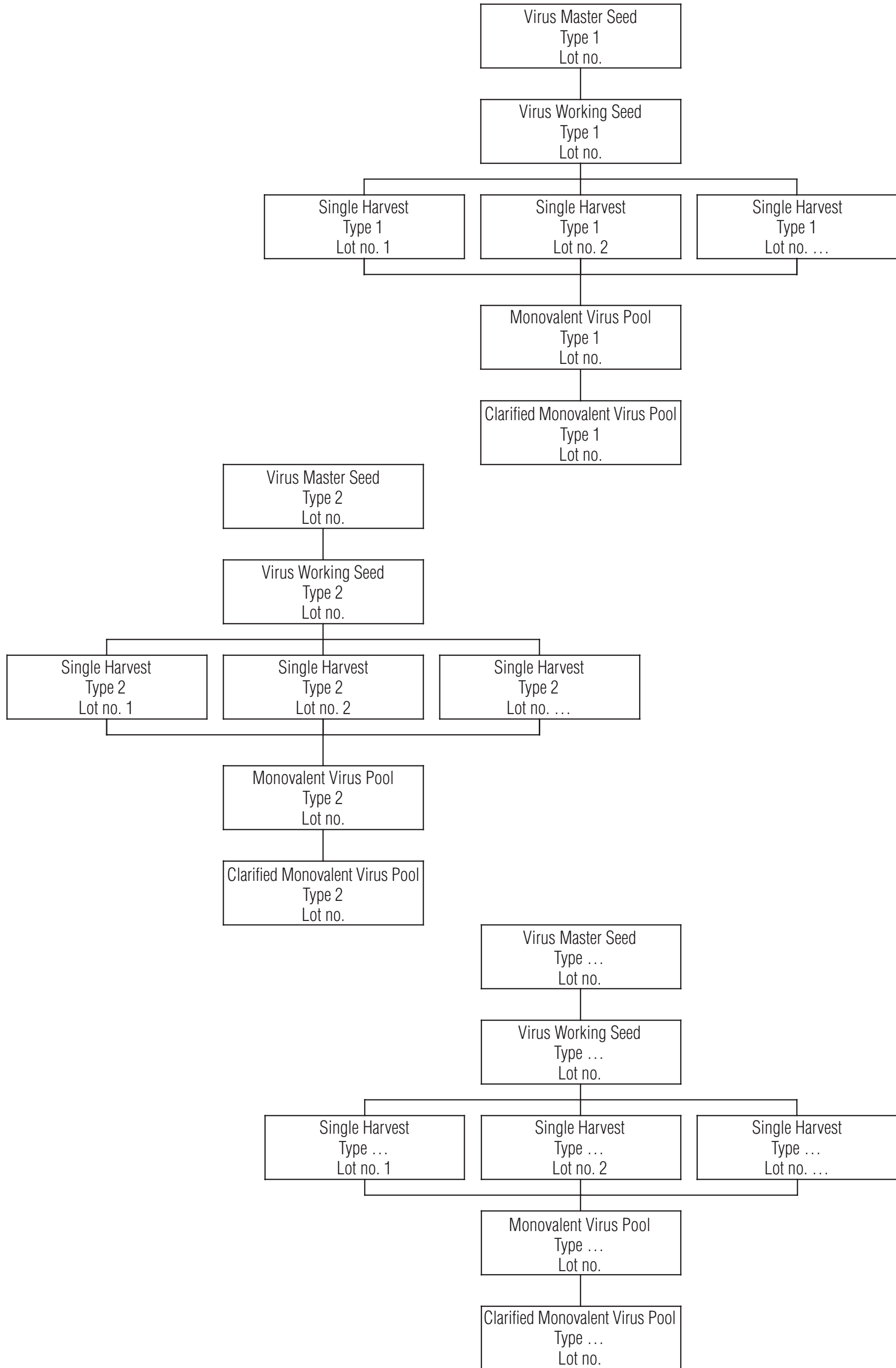
Signature: ____

Name (typed): ____

Date: ____

Appendix 2

Genealogy of vaccine production process



Genealogy of final bulk lot and final lot

Appendix 3

Model certificate for the release of live attenuated rotavirus vaccines

This certificate is to be provided by the national regulatory authority of the country where the vaccines have been manufactured, on request by the manufacturer

Certificate no. ________________

Lot release certificate

The following lot(s) of live attenuated rotavirus vaccine (oral) produced by ____________________________[1] in _______________[2] whose numbers appear on the labels of the final containers, meet all national requirements[3] and Parts A[4], B and C of Guidelines for Live Attenuated Rotavirus Vaccine (oral), (2007)[5] and comply with Good Manufacturing Practices for pharmaceutical products[6] and Good manufacturing practices for biological products.[7]

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

Final lot no.	Number of released human doses in this final lot	Expiry date

The director of the national regulatory authority
(or authority as appropriate):

Name (typed) ____

Signature ____

Date ____

[1] Name of manufacturer.

[2] Country of origin.

[3] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national regulatory authority.

[4] With the exception of provisions on distribution and shipping, which the national regulatory authority may not be in a position to assess.

[5] WHO Technical Report Series, No. 941, 2007, Annex 3.

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

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