World Health Organization

POST-ECBS version
ENGLISH ONLY

Recommendations to assure the quality, safety and efficacy of rotavirus vaccines

Replacement of Annex 3 of WHO Technical Report Series, No. 941

Adopted on the recommendation of the eightieth meeting of the World Health Organization Expert Committee on Biological Standardization, 7–11 October 2024. This is the final edited version which will be published in the WHO Technical Report Series.

© World Health Organization 2024

All rights reserved.

The designations employed and the presentation of the material in this document do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this document. However, the material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use. The named authors [or editors as appropriate] alone are responsible for the views expressed herein.

Annex 2

Recommendations to assure the quality, safety and efficacy of rotavirus vaccines

Replacement of Annex 3 of WHO Technical Report Series, No. 941

Recommendations published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of rotavirus vaccines. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these WHO Recommendations are made only on condition that such modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the recommendations set out below.

Abbreviations

$CCID_{50}$	cell culture infective dose 50%
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FFU	focus-forming unit(s)
GMO	genetically modified organism
HTS	high-throughput sequencing
IgA	immunoglobulin A
IgG	immunoglobulin G
MCB	master cell bank
NAT	nucleic acid amplification technique
NCL	national control laboratory
NRA	national regulatory authority
PCV	porcine circovirus
PFU	plaque-forming unit(s)
PRNT	plaque reduction neutralization test
qPCR	quantitative polymerase chain reaction
rcDNA	residual cellular DNA
RNA	ribonucleic acid
ROA	route(s) of administration
RVGE	rotavirus gastroenteritis
SNA	serum neutralizing antibody
TSE	transmissible spongiform encephalopathy
VMS	virus master seed
VWS	virus working seed
WCB	working cell bank

Introduction

The WHO Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines (oral) were adopted on the recommendation of the WHO Expert Committee on Biological Standardization and published in 2007 *(1)*. Developments under way at that time and since have included the licensure of the first two live attenuated rotavirus vaccines in Europe, the United States of America (USA) and many other countries, with subsequent prequalification by WHO. A further two nationally licensed live attenuated rotavirus vaccines developed in India were prequalified by WHO in 2018. In addition, at least two other live rotavirus vaccines (one in China and one in Viet Nam) have been licensed and widely used in the country of manufacture but not yet prequalified by WHO *(2)*. Other candidate rotavirus vaccines are also in development, including non-replicating rotavirus vaccines *(3, 4)*. Furthermore, since the publication of the above Guidelines, WHO has developed or revised a number of its overarching general guidance documents on various aspects of vaccine manufacture and evaluation.[1]

In 2009, the WHO Strategic Advisory Group of Experts (SAGE) on Immunization recommended the universal rotavirus vaccination of infants. The WHO position paper on rotavirus vaccines was updated in 2021 *(2)* and continued to recommend the inclusion of rotavirus vaccination in all national immunization programmes.

In light of ongoing experience of the use of rotavirus vaccines and advances in the relevant fields, it was proposed that the 2007 WHO Guidelines be revised and updated. WHO convened a virtual informal consultation on 15–17 November 2022 attended by experts and representatives from academia, national regulatory authorities (NRAs), national control laboratories (NCLs), industry, and international health organizations and institutions from around the world to discuss and reach consensus on the issues to be addressed during the revision process *(5)*. WHO then set up a drafting group comprising regulatory experts from several countries to revise and update the following sections:

- General considerations and other sections to reflect developments and advancements in the relevant fields;
- Terminology;
- Part A to reflect up-to-date practices for the production and control of live attenuated rotavirus vaccines (oral);
- Part B to provide guidance on the pharmacological evaluation of candidate rotavirus vaccines built on different platforms, as well as to elaborate the regulatory considerations for toxicological testing, including the risk of intussusception;
- Part C to provide guidance on the design of clinical trials, including in the context of currently available licensed rotavirus vaccines and for different types of rotavirus vaccines;
- Part D and its associated appendices; and
- References.

Additional changes were also made to bring the document into line with other WHO Recommendations, Guidelines and guidance documents published since 2007.

[1] See: Vaccine standardization – General topics and regulatory guidance [webpage]. Geneva: World Health Organization (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/vaccine-standardization/).

Purpose and scope

These WHO Recommendations (formerly Guidelines) provide guidance to NRAs, NCLs and vaccine manufacturers on the quality, nonclinical and clinical evaluations needed to assure the quality, safety and efficacy of rotavirus vaccines.

The scope of the present document mainly encompasses live attenuated rotavirus vaccines for prophylactic use as this is the class of rotavirus vaccine currently licensed, and the updated recommendations provided should be taken into account during the development, manufacture and evaluation of all such vaccines. However, significant efforts are also under way to develop non-replicating rotavirus vaccines – though to date no such vaccines have been licensed. Therefore, while the manufacturing and quality control guidance provided in Part A is focussed on live attenuated rotavirus vaccines (oral), Part B (on nonclinical evaluation) and Part C (clinical evaluation) provide general guidance applicable to all types of candidate rotavirus vaccines, including live attenuated and non-replicating rotavirus vaccines. It is envisaged that as the development and use of non-replicating rotavirus vaccines advance, specific guidance on this class of rotavirus vaccine will be provided.

There are also a number of WHO Recommendations, Guidelines and guidance documents addressing various aspects of the development, manufacturing and evaluation of other types of vaccine that may be relevant to non-replicating rotavirus vaccines, including:

- Recommendations on inactivated vaccines *(6–8)*;
- Recommendations and Guidelines on protein antigens produced by recombinant technology *(9–12)*;
- Recommendations on virus-like particle vaccines *(13)*;
- Guidelines on plasmid DNA vaccines *(14)*;
- Regulatory considerations for messenger RNA vaccines *(15)*; and
- Guidelines on vectored vaccines *(16)*.

The principles outlined in these documents should be considered when applicable.

The current document should also be read in conjunction with WHO guidance on the nonclinical and clinical evaluation of vaccines *(17, 18)*, good manufacturing practices for biological products *(19)*, good manufacturing practices for sterile pharmaceutical products *(20)*, characterization of cell banks *(21)* and lot release *(22)*, as well as relevant WHO guidance on building an effective national pharmacovigilance system *(23)*.

Terminology

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

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

Adventitious agent: a contaminating microorganism of the cell substrates or source materials used in their culture, which may include bacteria, fungi, mycoplasmas, mycobacteria, rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents and endogenous/exogenous viruses that have been unintentionally introduced into the manufacturing process of a biological product.

Candidate vaccine: an investigational vaccine that is at the research and clinical development stage, and that has not yet been granted marketing authorization (also known as licensure) by a regulatory agency.

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

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

Cell seed: a quantity of well-characterized cells stored frozen under defined conditions (such as the vapour or liquid phase of liquid nitrogen) in aliquots of uniform composition, one or more of which may be used for the production of a **master cell bank**.

Cytopathic effect: a degenerative change in the appearance of cells, especially in tissue culture, when exposed to viruses, toxic agents or non-viral pathogens.

Drug product: a pharmaceutical product type in a defined and sealed container-closure system that contains a **drug substance** typically formulated with excipients and prepared in the final dosage form and packaged for use. The collection of all vials of the drug product resulting from one working session constitutes the **final lot**.

Drug substance: the active pharmaceutical ingredient and associated molecules.

Final bulk: a formulated vaccine preparation from which the final containers are filled. The final bulk may be prepared from one or more clarified monovalent virus pools formulated to contain all excipients and homogenous with respect to composition. The final bulk may contain one or more virus serotypes.

Final lot: a collection of sealed final containers of finished vaccine (drug product) that is homogeneous with respect to the risk of contamination during filling and freeze-drying. A final lot must therefore have been filled from a single vessel of **final bulk** in one working session, and if freeze-dried, processed under standardized conditions in the same chamber in one working session.

Focus-forming unit (FFU): the smallest quantity of a virus suspension that will infect host cells and cause a single visible focus of infection in a cell monolayer, as identified using rotavirus-specific antiserum.

Genetically modified organism (GMO): an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

Inoculum: stored virus intermediate culture, prepared from the **virus working seed lot** and used for inoculation of several successive lots of **production cell culture** to manufacture the desired drug substance lots of viral vaccines.

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

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

Plaque-forming unit (PFU): the smallest quantity of a virus suspension that will lyse host cells and cause a single visible focus of infection in a cell monolayer.

Production cell culture: a cell culture derived from one or more containers (for example, ampoules or vials) of the **working cell bank** or primary tissue used for the production of vaccines.

Single harvest: a quantity of virus suspension of one virus serotype derived from a batch of production cells inoculated with the same seed lot and processed together in a single production run.

Unit of infectivity (UI): relative viral infectivity of a sample inoculated in susceptible cell monolayers measured by quantitative polymerase chain reaction (qPCR) against a defined reference standard preparation.

Virus master seed lot: a quantity of virus suspension that has been processed at the same time in a single production run to assure a uniform composition, and passaged for a specific number of times that does not exceed the maximum approved by the NRA. It is characterized to the extent necessary to support development of the **virus working seed lot**.

Virus working seed lot: a quantity of virus suspension of uniform composition derived from the **virus master seed lot** by passaging (for a specific number of times that does not exceed the maximum approved by the NRA) and fully characterized. The virus working seed lot is used for vaccine production.

Working cell bank (WCB): a quantity of cells of uniform composition derived from one or more containers (for example, ampoules or vials) of the MCB at a specific population doubling level or passage level, dispensed in aliquots into individual containers, cryopreserved and stored frozen under defined conditions (such as the vapour or liquid phase of liquid nitrogen) in aliquots of uniform composition. The WCB is prepared from a single homogeneously mixed pool of cells. One or more of the WCB containers is used for each production culture. All containers are treated identically and once removed from storage are not returned to the stock.

General considerations

Infection and disease

Rotaviruses are a leading cause of severe, dehydrating gastroenteritis in children under the age of 5 years worldwide *(2, 24)*. The incubation period for rotavirus infection is short and estimated to be less than 48 hours, with the first infection having the greatest impact. Rotavirus disease in children presenting to emergency rooms and those requiring hospitalization is often characterized by watery diarrhoea, vomiting and fever that can result in electrolyte imbalance, shock and, in some cases, death *(25)*. The virus may be present at 10^{11} virus particles per gram of stool, with the infectious dose estimated to be only 100 virus particles. The disease is therefore highly infectious and chiefly transmitted by the faecal-oral route. Universal infection, usually in infancy, is found in all countries irrespective of economic status. However, the consequences of infection depend on economic circumstances and are most serious in low-income countries lacking access to health care facilities.

Rotavirus disease is the main cause of infant deaths from diarrhoeal disease globally *(2, 24)* with approximately half the global total of deaths occurring in Africa and South-East Asia. Rotavirus can also cause infections in older children and adults *(25)*. No specific antiviral therapy is currently available against rotaviruses and the only clinically effective intervention once severe symptoms develop is rehydration therapy.

According to a WHO position paper *(2)*, the number of deaths in children under 5 years of age attributable to rotavirus infection was estimated to be more than 500 000 in 2000 worldwide. This position paper also noted that between 2013 and 2017 the estimated annual number of deaths due to rotavirus reported in published studies was between 122 000 and 215 000, representing a decrease of 59–77% since 2000. A more recent review of cases in low- and middle-income countries reported through the Global Pediatric Diarrhea Surveillance network in 2022 *(24)* concluded that in 2018 the number of deaths among children under 5 years of age

was approximately 200 000, representing a reduction of about 40% compared to numbers before vaccines became available in 2006. Although the impact of vaccine usage has been substantial, rotavirus remains the main cause of mortality due to infant diarrhoea worldwide *(24)*. The further development of effective rotavirus vaccines therefore remains a high priority.

The virus

Rotavirus is a non-enveloped, double-stranded RNA virus belonging to the family Reoviridae *(26)*. It has a triple-shelled virion containing a genome of eleven segments. These segments encode for six viral structural proteins (VP1–VP4, VP6 and VP7) and six non-structural proteins (NSP1–NSP6). Each genome segment (with the exception of gene 11 encoding for NSP5 and NSP6) codes for a single viral protein. The VP4 (P) and VP7 (G) proteins found on the surface of the virion are the targets of neutralizing antibodies and are of the greatest current interest with respect to vaccine development. The inner protein VP6 has also been considered and is the target of most enzyme-linked immunosorbent assay (ELISA)-based antibody assays.

The G and P proteins are classified on the basis of their antigenic and molecular properties. Overall, at least 36 G types and 51 P types have been recognized, of which six G types (G1, G2, G3, G4, G9 and G12) and three P types (P4, P6 and P8) are those most commonly found in human infections. The distribution of genotypes varies from region to region and to some extent over time *(25, 27)*.

Live attenuated rotavirus vaccines

Live attenuated rotavirus vaccines have been developed using a range of strategies. The strains from which they have been derived include human isolates with minimal manipulation or animal viruses (bovine, ovine or other) (the Jennerian approach). Some vaccines have been monovalent (containing for example only the G1, G9 or G10 type) while others have been multivalent (containing for example the G1, G2, G3, G4 and G9 types). One strategy has been to exploit the segmented nature of the rotavirus genome to generate reassortants expressing the desired G type on a common core genotype. Monovalent and multivalent vaccines covering a range of serotypes have been successfully used in clinical trials and in vaccination programmes. To date, each vaccine used has been unique, with the strains used differing in their biological properties (such as growth characteristics in production and in recipients) so that the dosage required is specific to the vaccine in question.

Although rotavirus is found globally, there are regional inequalities in the morbidity and mortality it causes *(24)*. However, the efficacy and effectiveness of the different vaccines are very similar in similar settings. In regions with low infant mortality and generally high or intermediate income, efficacy is of the order of 80–90%, while the same vaccine used in regions with high infant mortality has an efficacy of around 50–60% *(28–34)*. The reasons for the lower vaccine efficacy in low-income countries are complex and not fully understood *(35)*. Where monovalent vaccines have been used in programmes, there has been no evidence of a wild-type virus of a different serotype replacing the serotype found in each vaccine, implying that protection is not specific to a particular serotype. Rotavirus vaccination has led to substantial reductions in diarrhoeal deaths and hospitalizations *(24, 36)*.

There is currently no animal model that will reflect rotavirus virulence in children, and so comparisons of the attenuated phenotypes are possible only in clinical studies at present. The virological properties of current live attenuated rotavirus vaccines are highly varied, including with regard to the number and serotype(s) of the strains they contain, and their in vivo and in vitro growth properties. There are therefore major quality aspects that are specific to a particular vaccine. Although many of the points of possible concern considered in these

WHO Recommendations are generally applicable to all live attenuated rotavirus vaccines, it should be noted that each candidate vaccine is the result of a unique approach to the development of an attenuated product, and must be evaluated individually. This raises significant product-specific issues. The widely disparate nature of currently licensed and candidate rotavirus vaccines makes this a larger issue for rotavirus vaccines than for other live attenuated vaccines.

There is also no validated mechanistic correlate of protection for an individual vaccine. However, overall secretory immunoglobulin A (IgA) antibody and serum neutralizing antibody levels relate to protection after wild-type rotavirus infection and are considered a non-mechanistic indication of protection *(37–39)*. The higher the antibody level, the more likely it is that the individual is protected but a robust protective threshold has not yet been demonstrated *(38)*.

Special considerations

The development of any new rotavirus vaccine should take into account the experience of using the RotaShield vaccine, which was introduced in the USA in August 1998 and withdrawn less than 1 year later. An epidemiological relationship was established between vaccination with RotaShield and intussusception – a condition in which the gut invaginates and which can prove fatal unless treated. Early estimates indicated 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 cases were associated with the first dose, and occurred in children 4 months of age or older. This did not comply with the manufacturer's recommendation that the first dose be given at 2 months of age, and changed the early estimates of attributable risk of intussusception in the target population to less than one case per 10 000 children immunized *(40)*. The frequency of intussusception related to rotavirus vaccine administration has been the subject of many subsequent studies *(41)* which have suggested that it is influenced by the vaccine used, the age of the recipient and the epidemiological circumstances. The detailed pathogenic mechanisms for intussusception are unclear but are very likely to be complex.

Rotavirus is an acid-labile virus with a half-life of less than 12 minutes at pH 2.0. If rotavirus vaccines are intended to be administered to infants by the oral route, the vaccine virus might be inactivated by gastric acid prior to reaching the site of infection in the upper gastrointestinal tract. To prevent vaccine virus inactivation by gastric acid, antacids or buffers are usually administered before or in combination with the oral rotavirus vaccination. The need for, and 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.

Many rotavirus vaccines are produced in Vero cells. In 1986, a WHO study group *(42)* concluded that the risks posed by residual cellular DNA (rcDNA) in vaccines produced in continuous cell lines should be considered to be negligible for preparations given orally. This conclusion was based on the finding that polyoma virus DNA was not infectious when administered orally *(43)*. For such products, the principal requirement is the elimination of potentially contaminating viruses. Additional studies demonstrated that the uptake of DNA introduced orally was significantly lower than that of DNA introduced intramuscularly *(44)*. Nevertheless, the specifics of the manufacturing process and the formulation of a given product should be considered by the NRA *(21)* and, where possible, data should be generated on the levels of rcDNA in oral live attenuated rotavirus vaccines produced in Vero cells or any other cell line.

Cell banks should be characterized and shown to be free from adventitious agents *(21)*. In 2010, one rotavirus vaccine was shown to be contaminated with porcine circovirus (PCV)

which had infected the master and working cell banks. The original source of infection was most probably the porcine-derived trypsin used for the culture of the Vero cells during preparation of the banks *(45–48)*. Traces of PCV nucleic acid have also been found in other rotavirus vaccines as a contaminant from the trypsin used in production rather than viral infection of the cell production system *(46)*. The need to test for human, simian, bovine or porcine adventitious agents should be based on a risk assessment of potential contamination of the cell substrates used to propagate the virus, as well as the risk of inadvertent introduction of adventitious agents through the use of raw materials (for example, animal-derived culture medium components). If necessary, viruses such as bovine polyomavirus, porcine parvovirus and PCV may be screened for by using specific assays, including molecular assays such as nucleic acid amplification technique (NAT)-based assays and high-throughput sequencing (HTS).

International reference materials

A standardized vaccine reference material would be useful in the context of defining vaccine dose but given the range of live rotavirus vaccine types, their degree of attenuation and growth properties in culture, any such material would likely be specific to a particular vaccine. It is therefore not feasible to develop such international reference materials to standardize the virus content of different rotavirus vaccines. Common reference materials might nonetheless be useful in developing and comparing infectivity assays but the utility of a reference material in harmonizing the assay results obtained with different vaccine products is likely to be limited.

Similarly, although antibody reference materials are useful in harmonizing antibody assays, rotavirus immunoassays differ greatly from each other in terms of the source of the antigen (virus strain) and in the format and nature of the assay – for example, with regard to the cell used for neutralization assays or the design of ELISA binding assays in terms of the precise antigen against which it is directed.

As a result, it would be difficult at present to design universal international reference materials for rotavirus vaccines or serological assays. However, reference materials could be useful in establishing and validating immunoassays and comparing immune responses to different rotavirus vaccines.

Part A. Manufacturing recommendations for live attenuated rotavirus vaccines (oral)

A.1 Definitions

A.1.1 International name and proper name

The international name of the vaccine should be “live attenuated rotavirus vaccine (oral)” with additions to indicate the virus serotype(s) in the vaccine. The proper name should be the equivalent of the international name in the language of the country in which the vaccine is licensed.

The use of the international name should be limited to vaccines that meet all of the applicable specifications given below.

A.1.2 Descriptive definition

A live attenuated rotavirus vaccine (oral) is a preparation containing one or more live attenuated rotavirus strains (which could be of different serotypes) that have been grown through a seed lot system, prepared in a suitable approved cell substrate, and formulated in a

form suitable for oral administration. The preparation should satisfy all of the recommendations set out below, as applicable.

A.2 General manufacturing recommendations

The general manufacturing guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles *(49)*, WHO good manufacturing practices for biological products *(19)* and WHO good manufacturing practices for sterile pharmaceutical products *(20)* should be applied to the design, establishment, operation, control and maintenance of manufacturing facilities for live attenuated rotavirus vaccines. Production steps and quality control operations involving manipulations of live viruses should be conducted at a biosafety level that accords with the principles set out in the WHO *Laboratory biosafety manual (50)* and appropriate containment measures applied. The basis for this is a microbiological risk assessment which results in the classification of activities into different biosafety levels. The respective classification levels should be approved by the relevant authority of the country or region in which the manufacturing facility is located. As live attenuated rotavirus vaccines will be given to large numbers of healthy infants, the biological risk should be extremely low. Vaccine production must be appropriately contained to prevent contamination of the product by the environment and workers.

If vaccine virus strains have been derived by recombinant DNA technology and are regarded as genetically modified organisms (GMOs), national and/or regional regulations should be followed.

In general, the use of separate areas or a campaigned programme for the manufacturing of different virus serotypes is required. However, if the manufacturer can demonstrate and validate effective decontamination between production runs, then the use of the same production area to produce a different virus serotype may be justifiable. 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 are provided. More guidance on campaign production and containment can be found in the WHO good manufacturing practices for biological products *(19)*.

The use of appropriately validated in vitro methods for quality control is recommended, as it substantially reduces assay variability and enhances the predictability of the release of vaccine lots with acceptable quality. In all cases where in vivo assays are performed during vaccine development and production, the assay selection should be scientifically justified and the use of animals should adhere to the highest ethical standards.

A.3 Control of source materials

A.3.1 Cell lines

A.3.1.1 Master cell bank (MCB) and working cell bank (WCB)

The use of a cell line for the manufacture of rotavirus vaccines should be based on the cell bank system. The cell seed and cell banks should conform to WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks *(21)* and should be approved by the NRA. The maximum number of passages (or population doublings) allowed between the cell seed, the MCB, the WCB and the production passage level should be established by the manufacturer and approved by the NRA. Additional tests may include, but are not limited to, propagation of

the MCB or WCB cells to or beyond the maximum in vitro age for production, and examination for the presence of retrovirus and tumorigenicity in an animal test system *(21)*.

Cell banks should be assessed to confirm the absence of adventitious agents from the species of origin or that might inadvertently be introduced during their production.

The WHO Vero reference cell bank 10-87 is considered suitable for use as a cell substrate for generating an MCB *(21)* and is available to manufacturers upon application to WHO.[2]

The MCB is made in sufficient quantities and stored in a secure environment, and is used as the source material for producing the WCB. In normal practice, the MCB is expanded by serial subculture up to a passage number (or population doubling level, as appropriate) selected by the manufacturer and approved by the NRA, at which point the cells are combined to give a single pool, which is then distributed into containers and preserved cryogenically to form the WCB.

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

A.3.1.2 Identity test

Identity tests on the MCB and WCB should be performed in accordance with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks *(21)*.

The cell banks should be identified using tests such as biochemical tests, immunological tests, cytogenetic marker tests, and DNA fingerprinting or sequencing *(21)*. The tests used should be approved by the NRA.

A.3.2 Cell culture medium

Serum used for the propagation of cells should be tested to demonstrate freedom from bacterial, fungal and mycoplasmal contamination using appropriate tests – as specified in Part A, sections 5.2 *(51)* and 5.3 *(52)* of the WHO General requirements for the sterility of biological substances – as well as freedom from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks *(21)*.

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

The source(s) of animal components used in the cell culture medium should be approved by the NRA. Components derived from TSE-relevant animal species should comply with the *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (53)*.

Human serum should not be used. If human serum albumin derived from human plasma is used at any stage of product manufacture, the NRA should be consulted regarding the requirements, as these may differ from country to country. At a minimum, it should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives *(54)*. In addition, human albumin, as with all materials of

[2] All requests for the WHO Vero reference cell bank 10-87 should be sent to: Team Lead, Norms and Standards for Biologicals, Technical Specifications and Standards, Department of Health Product Policy and Standards, Access to Medicines and Health Products Division, World Health Organization, Geneva, Switzerland.

animal origin, should comply with the *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (53)*.

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

Bovine or porcine trypsin used for preparing cell cultures (or used to prepare culture medium components or activate rotavirus for infection) should be tested and found to be free of cultivatable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate. The methods used to ensure this should be approved by the NRA. The source(s) of trypsin of bovine origin, if used, should be approved by the NRA and should comply with the *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (53)*.

Recombinant trypsin is available and should be considered – however, it should not be assumed to be free of the risk of contamination and should be subject to the usual considerations for any reagent of biological origin *(21)*.

Penicillin and other beta-lactams should not be used at any stage of manufacture because they are highly sensitizing substances in humans. Other antibiotics may be used during early stages of production. In this case, the use of antibiotics should be well justified, and they should be cleared from the manufacturing process at the stage specified in the marketing authorization. Clearance should be demonstrated and validated through a residual removal study (or studies) and acceptable levels should be approved by the NRA.

Nontoxic pH indicators may be added – for example, phenol red at a concentration of 0.002%.

In all cases, only substances that have been approved by the NRA may be added.

A.3.3 Virus strains and seed lot system

A.3.3.1 Virus strains

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, or in other cases by multiple passages of human rotavirus in cell culture. The seed lot viruses should comply with the specifications outlined in the following sections. Development of the rotavirus strain(s) to be used for vaccine production may involve passage in continuous, diploid and/or primary cell lines.

- 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 (if applicable), whether the strains were cloned (for example, by plaque purification) prior to generation of the master seed lots, genome sequence information and the passage level at which attenuation for humans (if applicable) was demonstrated by clinical trials.
- The immunogenicity of each of the vaccine virus strains should be established in a dose–response study 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. 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, during stability monitoring and at expiry date. See Part B and Part C below.
- Live attenuated rotavirus strains may be derived by recombinant DNA. The entire nucleotide sequence of any complementary DNA clone used to generate vaccine

virus stocks should be determined prior to any nonclinical study or clinical trial. The cell substrate used for transfection to generate the virus should be appropriate for human vaccine production and should be approved by the NRA. In some countries, viruses derived by recombinant DNA technology are considered to be a GMO and should comply with the relevant regulations of the producing and recipient countries.

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

The genetic stability of the vaccine seed to a passage level comparable to final vaccine bulk, and preferably beyond the anticipated maximum passage level, should be demonstrated.

A.3.3.2 Virus seed lot system

Vaccine production should be based on the virus master seed (VMS) lot and virus working seed (VWS) lot system. Seed lots should be prepared in the same type of cells using similar conditions for virus growth as those used for production of the final vaccine.

The VWS should have a defined relationship to the VMS with respect to passage level and method of preparation such that the VWS retains the in vitro phenotypes and the genetic character of the VMS. Once the passage level of the VWS with respect to the VMS is established it should not be changed without approval from the NRA.

The maximum passage level of the VMS and VWS should be approved by the NRA. The inoculum for infecting cells used in the production of vaccine should be from a VWS with as few as possible intervening passages in order to ensure that the characteristics of the vaccine remain consistent with the lots shown to be satisfactory with respect to safety and efficacy in clinical trials.

Virus seed lots should be stored as recommended in the WHO good manufacturing practices for biological products *(19)* in dedicated temperature-monitored freezers (for example, at or below −60 °C) to ensure stability on storage, and the storage arrangements should ensure the appropriate security of the virus seed lots.

A.3.3.3 Tests on virus master and working seed lots

A.3.3.3.1 Identity

Each seed lot should be identified by virus type using an immunological assay and/or molecular methods (such as HTS) approved by the NRA.

A.3.3.3.2 Genotype/phenotype characterization

The genotypic stability of the virus seed on passage should be assessed. Although phenotypic stability may provide additional information, markers for attenuation are still in development and are probably specific to the particular vaccine being considered. The choice of tests is therefore the responsibility of the manufacturer, but could include phenotypic properties such as growth characteristics in culture or the use of HTS to identify the variability of nucleotide polymorphisms between batches. Acceptable limits for variation should be defined by the manufacturer and agreed by the NRA.

A.3.3.3.3 Tests for bacteria, fungi, mycoplasmas and mycobacteria

Each virus seed lot should be tested for bacterial, fungal, mycoplasmal and mycobacterial contamination using appropriate tests, as specified in Part A, sections 5.2 *(51)* and 5.3 *(52)* of

the WHO General requirements for the sterility of biological substances, and/or by methods approved by the NRA.

Molecular assays (for example, NAT-based assays alone or in combination with cell culture) may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and with the agreement of the NRA *(21)*.

A.3.3.3.4 Tests for adventitious agents

Each virus seed lot should be tested in cell cultures for adventitious agents relevant to the origin and the passage history of the seed virus.

When antisera are used to neutralize rotavirus, the antisera should be shown to be free from antibodies that may neutralize specific adventitious viruses being tested for. Suitable indicator cells should be selected to enable the detection of such viruses. The choice of indicator cells should be guided by the species and legacy of the production cell substrate, taking into consideration the types of viruses to which the cell substrate could potentially have been exposed. Infection with such viruses should then be tested for, using a suitable assay method. For test details, refer to section B.11 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks *(21)*.

Each virus master or working seed lot should also be tested in animals if the risk assessment indicates that such testing would provide a risk mitigation, taking into account the overall testing package. The animals used might include guinea-pigs and suckling mice as appropriate, while embryonated chicken eggs are also an option. For test details, refer to section B.11 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks *(21)*.

Validated in vitro assay alternatives are recommended whenever available.

New molecular methods with broad detection capabilities are available for the detection of adventitious agents. These methods include: (a) degenerate nucleic acid testing for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (b) NAT-based assay with random primers followed by analysis of the amplicons on large oligonucleotide microarrays of conserved viral sequencing or digital subtraction of expressed sequences; and (c) HTS. These methods may be used to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and with the approval of the NRA.

A.3.3.3.5 Virus concentration

Each seed lot should be assayed for infectivity using a sensitive assay in a cell culture system.

A plaque-forming assay or immunofocus assay may be used in MA-104, Vero or other sensitive cells to determine virus concentration. Such assays are 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 plaque-forming units per mL (PFU/mL) or focus-forming units per mL (FFU/mL).

A cell culture infective dose assay may also be used to determine virus concentration. Results should be recorded as cell culture infective dose 50% per mL ($CCID_{50}$/mL).

Alternatively, qPCR 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 per mL (UI/mL).

The detailed procedures for carrying out the tests and interpreting the results should be approved by the NRA.

Because of the diversity of rotavirus vaccines produced by different manufacturers (for example, in terms of strain composition, biological properties and formulation) it is unlikely that international reference materials will be suitable for the standardization of assays across all rotavirus vaccine products. Manufacturers should therefore establish a product-specific reference preparation. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters, and the reference vaccine replaced when necessary. A procedure for replacing reference vaccines should be in place and agreed with the NRA *(55)*.

A.4 Control of vaccine production

A.4.1 Control cell cultures

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

If bioreactor technology is used, the size and treatment of the cell sample to be examined should be well documented and approved by the NRA.

A.4.1.1 Tests of control cell cultures

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

The control cell cultures should be incubated under conditions as similar as possible to the inoculated cultures for at least 2 weeks and should be tested for the presence of adventitious agents as described below. For the test to be valid, not more than 20% of the control cell cultures should have been discarded for any reason by the end of the test period.

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

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

A.4.1.2 Tests for haemadsorbing viruses

At the end of the observation period, at least 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter cells have been stored, the duration of storage should not have exceeded 7 days and the storage temperature should have been in the range 2–8 °C. In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

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

Read half of the cultures after incubation at 2–8 °C for 30 minutes, and the other half after incubation at 20–25 °C for 30 minutes.

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

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

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

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

A second indicator cell line should be used to test an additional 10 mL sample of each pool. When a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line. When a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line *(21)*.

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

The inoculated cultures should be incubated at the same temperature (± 1 °C) as that used for the production of the rotavirus vaccine, and should be examined at intervals for cytopathic effects over a period of at least 14 days.

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

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

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

Some selected viruses may be screened for using specific validated assays approved by the NRA – such as assays based on molecular techniques (for example, NAT-based assays or HTS) *(21)*.

If these tests are not performed immediately, the samples should be stored at or below −60 °C.

A.4.1.4 Identity test

At the production level, the control cells should be identified by means of tests approved by the NRA. Suitable methods include, but are not limited to, biochemical tests (for example, isoenzyme analyses), immunological tests, cytogenetic marker tests (for example, for chromosomal markers) and tests for genetic markers (for example, DNA fingerprinting or sequencing).

A.4.2 Cell cultures for vaccine production

A.4.2.1 Observation of cultures for adventitious agents

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

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

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

A volume of at least 20 mL of the pooled supernatant fluids from the production cell culture should be tested for bacterial, fungal, mycoplasmal and mycobacterial contamination using appropriate tests, as specified in Part A, sections 5.2 *(51)* and 5.3 *(52)* of the WHO General requirements for the sterility of biological substances, and/or by methods approved by the NRA.

Molecular assays (for example, NAT-based assays alone or in combination with cell culture) may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and with the agreement of the NRA *(21)*.

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 or an inoculum at a defined multiplicity of infection. The number of passages from working seed to inoculum should be defined by the manufacturer during product development and approved by the NRA. After viral adsorption, cell cultures are fed with maintenance medium and incubated within a defined temperature range and for a defined period, usually established based upon the degree of cytopathic effect.

The permitted ranges of multiplicity of infection, temperature, pH and time period of incubation will depend on the vaccine strain and production. Defined ranges should be established by the manufacturer and be approved in the marketing authorization by the NRA.

A.4.3.2 Monovalent virus pools

A virus single harvest is harvested within a defined time period post inoculation established during process development. 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 containers of the WCB and the same virus working seed lot recovered at the same time. 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. In cases where an antibiotic is added during vaccine production, the upper limit for residual antibiotic content in the final product should be approved by the NRA (see section A.6.9 below).

Samples of monovalent virus pools should be taken for testing and if not tested immediately should be stored at or below –60 °C. Any alternative storage temperature should be justified based on stability data and approved by the NRA.

A.4.3.3 Tests on single harvest or monovalent virus pools

Tests may be done on single harvests or on virus pools.

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 described in section A.4.3.3.4 below are not performed immediately, the samples taken for these tests should be stored at or below –60 °C and subjected to no more than one freeze–thaw cycle. Any alternative storage temperature should be justified based on stability data and approved by the NRA.

A.4.3.3.2 Identity

Each single harvest or virus pool should be identified as the appropriate rotavirus serotype by immunological assay and/or molecular assay such as reverse transcription PCR (RT-PCR) or by DNA sequencing (such as Sanger or HTS). All tests used should be validated by the manufacturer and approved by the NRA.

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

Each single harvest or virus pool should be tested for bacterial, fungal, mycoplasmal and mycobacterial contamination using appropriate tests, as specified in Part A, sections 5.2 *(51)* and 5.3 *(52)* of the WHO General requirements for the sterility of biological substances, and/or by methods approved by the NRA.

Molecular assays (for example, NAT-based assays alone or in combination with cell culture) may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and with the agreement of the NRA *(21)*.

A.4.3.3.4 Tests for adventitious agents

For the purposes of the requirements set out in this section, the volume of each single harvest or virus pool sample 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. Neutralization of the rotavirus is necessary for many tests as 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 the rotavirus is not possible, the test sample may be passaged in trypsin-free media prior to initiating the assay to reduce the ability of the 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 by using a molecular method with broad detection capabilities (such as HTS or microarray).

A.4.3.3.5 Virus concentration

Each virus pool should be assayed for infectivity using a sensitive assay in a cell culture system to monitor the consistency of production. See section A.3.3.3.5 above.

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 are not intended for batch release. Tests should be conducted 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. Examples of evidence to support the consistent quality of the virus produced may include in vitro growth characteristics, thermal stability profile, ratio of infectious (triple shelled) to non-infectious (double shelled) particles, sensitivity to neutralization by polyclonal serum and/or monoclonal antibodies, 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 residual host cell protein, rcDNA, endotoxin, bovine serum, trypsin and antibiotics. The reduction of these during processing can be monitored to assess consistency of the manufacturing process. The reduction level should be approved by the NRA.

Once the consistency of the production process has been shown to reduce the impurities to acceptable levels, and the drug substance consistently meets the acceptance criteria, these tests for impurities may be omitted from routine lot release testing with the agreement of the NRA.

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

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 below in this section. If not tested immediately, the samples should be stored at or below –60 °C. Any alternative storage temperature should be justified based on stability data and approved by the NRA.

A.4.3.4.2 Tests for bacterial and fungal contamination

The clarified virus pool should be tested for bacterial and fungal sterility as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances *(51)*, or by methods approved by the NRA.

Alternatively, in agreement with the NRA, a bioburden test with a low bioburden limit that has been established based on batch data and process validation (for example, not more than 10 CFU/100 mL) may be acceptable. In this case, a sterile filtration step must be performed prior to or during preparation of the final bulk.

A.4.3.4.3 Virus concentration

Each clarified virus pool should be assayed for infectivity using a sensitive assay in a cell culture system to monitor the consistency of production. See section A.3.3.3.5 above.

A.4.3.4.4 Tests for residual cellular DNA

If continuous cell lines are used for production, the virus pool should be tested for rcDNA and the purification procedure should have been shown to consistently reduce its level *(21)*. Consideration should also be given to determining the size of rcDNA as part of the validation process *(21)*. An upper limit should be established by the manufacturer and approved by the NRA.

These tests may be omitted from routine lot release testing, with the agreement of the NRA, if the manufacturing process is validated as consistently achieving the specification.

A.4.4 Final bulk

Final bulk must be sterile and prepared from one or more serotypes each derived from one or more monovalent virus pools that pass the tests specified in sections A.4.1–A.4.3 above.

If a monovalent virus pool has been manufactured aseptically and shown to be sterile when tested, no further sterile filtration step is required. However, if the monovalent virus pool has been controlled for bioburden (see section A.4.3.4.2 above), then sterile filtration must be

incorporated in preparing the final bulk. In either case, filling should be carried out under aseptic conditions.

All aseptic processes (including sterile filtration steps) should be conducted in accordance with the principles and guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles *(49)*, WHO good manufacturing practices for biological products *(19)* and WHO good manufacturing practices for sterile pharmaceutical products *(20)*.

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 substance (such as a diluent or stabilizer) that is added to the product should have been shown, to the satisfaction of the NRA, to not impair the safety and efficacy of the vaccine in the concentration used.

A.4.4.1 Tests on the final bulk

A.4.4.1.1 Tests for residual materials

The manufacturer should demonstrate by testing each final bulk or by validating the manufacturing process that any residual materials used in the manufacturing process (such as animal serum, trypsin, antibiotics and DNases), as well as any rcDNA, are consistently reduced to a level acceptable to the NRA.

These tests may be omitted for routine lot release upon demonstration that the purification process consistently eliminates the residual components from the final bulks to the satisfaction of the NRA, and after validation.

A.4.4.1.2 Bacterial and fungal sterility

Each final bulk suspension should be tested for bacterial and fungal sterility as specified in the WHO General requirements for the sterility of biological substances *(51)* or by an alternative method approved by the NRA.

A.4.4.2 Storage

Prior to filling, if the final bulk suspension needs to be stored, it should be stored under conditions shown by the manufacturer to allow the final bulk to retain the desired biological activity.

A.5 Filling and containers

The relevant requirements concerning filling and containers given in WHO good manufacturing practices for pharmaceutical products: main principles *(49)* and WHO Good manufacturing practices for biological products *(19)* 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 the vaccine and its diluent. To this end, a container closure integrity test and assessment of extractables and/or leachables for the final container closure system are generally required for the qualification of containers, and may be needed as part of stability assessments. Assessment of extractables and/or leachables might also be required for container systems used for long-term storage of bulks and formulated bulks.

When a freeze-drying process is used for vaccine production, its validation should be submitted to the NRA for approval. If multi-dose vaccine containers are used, their use should

be compliant with the WHO Policy Statement: multi-dose vial policy (MDVP) *(56)*. The multi-dose container should prevent microbial contamination of the contents after opening. The extractable volume of multi-dose vials should be validated and the results of in-use stability studies provided to the NRA.

The manufacturer should also provide the NRA with adequate data demonstrating the stability of the product under appropriate conditions of storage and shipping.

These general requirements apply to the final containers (final lot) not product-administration devices. There are multiple options for administration devices (for example, syringes, squeezable tubes and droppers) for rotavirus vaccines, all of which should comply with the relevant requirements. Any information related to vaccine-administration devices should be included in the product packaging label and be considered on a case-by-case basis by the NRA.

A.6 Control tests on the final lot

Samples should be taken from each final lot for the tests described in the following sections. The tests should be performed on each final lot of vaccine (that is, in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers from each final lot by means of validated methods approved by the NRA. The specifications should be defined on the basis of the results of tests on lots that have been shown to be safe and effective in clinical studies. All tests and specifications should be approved by the NRA.

Both freeze-dried vaccine and its diluent, if applicable, should be tested and should fulfil the requirements set out in the following sections.

A.6.1 Inspection of final containers

Each container in each final lot should be inspected visually and/or in an automated manner, and those showing abnormalities (for example, improper sealing or unexpected presence of particles) should be discarded and recorded for each abnormality. A maximum limit should be established for the percentage of containers that can be rejected before triggering investigation of the cause, potentially resulting in lot failure.

A.6.2 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 for the appearance test.

A.6.3 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 NRA, such as immunoassays in cell culture or molecular methods suitable for detecting the presence of a specific rotavirus serotype included in the vaccine.

A.6.4 Bacterial and fungal sterility

Liquid or reconstituted vaccine should be tested for bacterial and fungal sterility as specified in the WHO General requirements for the sterility of biological substances *(51)* or by an alternative method approved by the NRA.

A.6.5 pH

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

A.6.6 Residual moisture (if applicable)

The residual moisture in a representative sample of each freeze-dried lot should be determined by a method approved by the NRA. The upper limit for moisture content should be approved by the NRA based on the results of stability testing.

A.6.7 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. See section A.3.3.3.5 above.

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 approved by the NRA.

The NRA should approve the reference preparation(s) of live attenuated rotavirus vaccine used in tests to determine the concentration of each serotype in the vaccine.

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 not suitable for use in the selected assay. If a different diluent is used for this test, data to allow for comparison of the results obtained using each of the two diluents (approved and alternative) should be submitted for the approval of the NRA.

Virus concentration limits should be established by the manufacturer taking into account the vaccine dose shown to be safe and effective in clinical trials, and should be agreed with the NRA.

A.6.8 Thermal stability

Thermal stability should be considered as a vaccine characteristic that provides an indicator of production and shelf-life consistency of the finished product. The thermal stability test is not designed to provide a predictive value of real-time stability but rather to evaluate whether the product complies with a defined stability specification. Additional guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines *(57)*.

A representative number of the final containers should be exposed to an elevated temperature for a defined time, 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 specified amount during the period of exposure. Estimation of the virus titre in 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 NRA. 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.6.9 Residual antibiotics (if applicable)

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

A.6.10 Stabilizer (if applicable)

If a stabilizer is added during vaccine production, its concentration in the vaccine should be determined and should be within limits approved by the NRA. This test may be omitted for routine lot release on final product if stabilizer content is determined at the final bulk stage and once consistency of production has been established by the manufacturer, with the agreement of the NRA.

A.6.11 Diluents (if applicable)

The requirements set out in the WHO good manufacturing practices for pharmaceutical products: main principles *(49)* 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 concentration of key components should be performed.

A.6.12 Extractable volume (if applicable)

It should be demonstrated that the nominal volume shown on the label can consistently be extracted from the containers.

A.7 Records

The guidance provided in WHO good manufacturing practices for biological products *(19)* should be followed.

A.8 Retained samples

The guidance provided in WHO good manufacturing practices for biological products *(19)* should be followed.

A.9 Labelling

The guidance provided in WHO good manufacturing practices for biological products *(19)* should be followed.

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

- the designation of the strain(s) of rotavirus contained in the vaccine, and whether the vaccine strains were derived by molecular methods;
- the minimum amount of virus of each serotype contained per human dose;
- the cell substrate used for the preparation of the vaccine;
- a statement that the vaccine should be administered orally;

- the nature and amount of any antibiotics present in the vaccine;
- the number of doses, if the product is issued in a multi-dose container;
- the volume of each dose;
- a statement regarding the concomitant administration of oral rotavirus vaccine with other oral vaccines and non-orally administered vaccines;
- a statement concerning vaccine administration to HIV-positive or other immunocompromised individuals;
- if applicable, 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;
- if applicable, a statement that after the vaccine has been reconstituted, it should be used without delay, or if not used immediately, stored under defined conditions and in the dark up to the maximum period defined by the manufacturer's stability studies;
- a statement concerning storage conditions (temperature), expiry date, volume and, if applicable, instructions for reconstitution; and
- if applicable, a statement on 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 final vaccine producer and the source of the bulk material if the producer of the final vaccine did not prepare it.

Unused vaccine should be disposed of as specified in the WHO good manufacturing practices for biological products *(19)* and WHO *Laboratory biosafety manual (50)*.

A.10 Distribution and transport

The guidance provided in WHO good manufacturing practices for biological products *(19)* and WHO good manufacturing practices for pharmaceutical products: main principles *(49)* should be followed. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products *(58)*.

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

A.11 Stability testing, storage and expiry date

A.11.1 Stability testing

Adequate stability studies form an essential part of vaccine development. These studies should follow the general principles outlined in the WHO Guidelines on stability evaluation of vaccines *(57)* and WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions *(59)*. Stability testing should be performed at different stages of production when intermediate product is stored, namely on single harvests, monovalent bulk, final bulk and final lot. Stability-indicating parameters should be defined appropriately according to the stage of production. The shelf-life of the final product and the hold time of each process intermediate (such as single harvests, monovalent bulk and final bulk) should be established based on the results of real-time, real-condition stability studies, and freeze and thaw studies (if applicable), and should be approved by the NRA.

The stability of the vaccine in its final container, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA on at least three consecutive lots of final product.

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

The formulation of the vaccine should be shown to minimize potency loss throughout its shelf-life. Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile *(57)*.

The final stability testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection of stability data and criteria for the rejection of vaccine(s). Data should be provided to the NRA in accordance with local regulatory requirements.

Any extension of the shelf-life should be based on real-time real-condition stability data and be approved by the NRA.

A.11.2 Storage conditions

Before being released by the manufacturing facility or before being distributed from a storage site, all vaccines in final containers should be stored at a temperature shown by the manufacturer to be compatible with minimal titre loss. The maximum duration of storage should be fixed with the approval of the NRA 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 be maintained until the end of the shelf-life.

A.11.3 Expiry date

The expiry date should be based on the shelf-life as supported by the stability studies and approved by the NRA.

The start of the dating period should be specified (for example, based on the date of filling or the date of the first valid potency test on the final lot) and should be approved by the NRA.

Part B. Nonclinical evaluation of rotavirus vaccines

This section addresses the pharmacological and toxicological assessment of candidate rotavirus vaccines. Currently, all licensed rotavirus vaccines are live attenuated vaccines. However, although no non-replicating rotavirus vaccine is licensed at the time of writing there is considerable interest in their development. Therefore, the following sections are intended to provide guidance on the nonclinical evaluation of both live attenuated and non-replicating candidate rotavirus vaccines.

The guidance provided should be read in conjunction with the principles outlined in the WHO guidelines on nonclinical evaluation of vaccines *(17)* and WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines *(60)*, if applicable. In addition, WHO guidance on the evaluation of DNA and messenger RNA vaccines *(14, 15)* and regional documents on live recombinant viral-vectored vaccines – see for example *(61)* – may also be informative, if applicable.

B.1 Primary pharmacodynamics

To date, there is no well-established immune correlate of protection against rotavirus disease *(34–36, 59–62)*. Although protection in animals against challenge with human rotavirus would provide an alternative measure of protection, this is not generally required as small animals

such as mice or rabbits are not susceptible to infection with human rotavirus strains *(63–65)* – though they can be used for studies of immune responses to vaccine strains and are used in vaccine development. Although gnotobiotic piglets are well known to be susceptible to human rotavirus infections, and able to develop diarrhea upon challenge with them *(66–69)*, the use of such large animals is limited for practical reasons, including high cost, limited accessibility, and the need for specialized equipment, facilities and staff. Therefore, no recommendation on the use of animal challenge-protection studies is made at this point. Further research is encouraged to develop a suitable animal model that can be economic, tractable and commonly used in a laboratory setting.

Primary pharmacodynamics (immunogenicity) studies should be carried out in relevant species (for example, mice, rats, guinea-pigs, gnotobiotic piglets or rabbits) prior to commencing human trials. In these studies, the method of vaccine delivery, including the route of administration (ROA), should correspond to that intended for use in the clinical trials. Depending on the vaccine characteristics, its putative mechanism(s) of action and ROA, the immunological parameters to be measured may include the humoral, cellular and functional immune responses to each rotavirus antigen included in the vaccine, as appropriate (for example, IgG and IgA antibodies, B cells or T cells, both in the circulation and in faecal specimens). Given the importance of the heterotypic immunity observed for live oral rotavirus vaccines, it is recommended that studies that evaluate immune function include an evaluation of immune responses to diverse human rotavirus serotypes. It is essential that the suitability of the analytical methods used for these studies is demonstrated for the intended purpose.

Studies that evaluate the immunogenicity of a rotavirus vaccine should include the dose-range testing of vaccine antigen(s). Ideally, the readouts should be assessed after each dose of vaccine if more than one dose is proposed for the vaccination schedule. This information is useful for the selection of vaccine dose and dosing regimen.

When a candidate rotavirus vaccine (such as an inactivated rotavirus vaccine) is formulated with an adjuvant, it is important to evaluate the use of vaccine formulations both with and without the adjuvant(s) to justify the inclusion of the adjuvant(s) in the vaccine formulation *(60)*. For a new combination vaccine designed to contain the rotavirus antigen(s) and antigens derived from other infectious disease pathogens, immune interference is a pertinent issue and should be adequately evaluated in animals.

B.2 Pharmacokinetics

The need for pharmacokinetic studies should be considered on a case-by-case basis depending on the route of administration and vaccine type. Although pharmacokinetic studies are not normally needed, evaluating the biodistribution of vaccine components following administration can provide important information during the evaluation of vaccines containing novel vectors and/or adjuvants, based on new formulations or technologies, or involving a new route of administration *(17, 60, 61)*.

B.3 Toxicology studies

The toxicology testing of a candidate rotavirus vaccine should be undertaken in compliance with the recommendations provided in the WHO guidelines on nonclinical evaluation of vaccines *(17)* and the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines *(60)*, as applicable. In addition, the assessment of local tolerance, single-dose toxic effects and safety pharmacology end-points, where appropriate *(60)*, should be incorporated into the design of a repeated-dose toxicity study, in accordance with the principles of regulatory acceptance of 3Rs (Replace, Reduce, Refine) testing approaches *(70)*.

The pivotal toxicity studies should be good laboratory practice (GLP) compliant and conducted in a relevant animal species that demonstrates an immune response to all important components of the vaccine. The route and dosing regimen should mimic the intended clinical use. In addition, the test vaccine used in these studies should be representative of clinical trial material in terms of its quality attributes, including impurity profile.

The use of live oral rotavirus vaccines has been associated with a low risk of intussusception in vaccinated infants. Currently, the pathogenic mechanisms for such rare events are unknown and there is no suitable animal model available to evaluate such a risk. Therefore, the pre-licensure nonclinical evaluation of intussusception risk is not deemed necessary, either for live oral rotavirus vaccines or non-replicating rotavirus vaccines, but post-marketing surveillance of intussusception risk should be carried out. As rotavirus is not neurotropic, a neurovirulence test is not required if the live oral rotavirus vaccine candidates have never been passaged in tissues of the central nervous system. Furthermore, the examination of reproductive and developmental toxicity is not relevant to rotavirus vaccines as the vaccination of humans with such vaccines occurs during infancy.

Genotoxicity studies are normally not needed. However, a standard battery of genotoxicity studies is generally recommended for most novel adjuvants that are (or contain) new chemical entities *(60)*.

B.4 Environmental risk assessment

Administering a live oral rotavirus vaccine or replicating rotavirus vaccine based on a GMO may lead to rotavirus infections in unvaccinated humans and/or animals if the vaccine virus is shed from vaccinated individuals. For such investigational products, an environmental risk assessment may be required as part of the preclinical evaluation. An investigation into the possible shedding of the vaccine virus following administration is considered relevant. In addition, information on the likelihood of recombination (reassortment) of excreted vaccine virus with wild-type rotaviruses may be required, and suitable nonclinical tests may be designed to provide data for this purpose.

Part C. Clinical evaluation of rotavirus vaccines

C.1 Introduction

Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products *(71)*. General guidance on vaccine clinical development programmes is provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(18)*.

The following sections address only issues for clinical development programmes that are specific to, or of special concern for, vaccines intended to prevent rotavirus gastroenteritis (RVGE) due to one or more rotavirus serotypes. Although the guidance provided below is applicable to candidate rotavirus vaccines generally, there are a number of specific considerations with regard to the ROA (that is, oral or parenteral) and the vaccine construct (that is, live attenuated, live reassortant or non-replicating vaccines). It is assumed throughout that candidate rotavirus vaccines will be intended for the prevention of RVGE in infancy.

C.2 Safety and immunogenicity studies

As part of the initial studies to investigate the safety and immunogenicity of the candidate vaccine, and regardless of the ROA, sera obtained from vaccinees may be assayed to determine:

- serum neutralizing antibody (SNA) titres using a plaque reduction neutralization test (PRNT) that uses a defined percentage reduction end-point with results reported as PRNT titres or SNA titres; and
- rotavirus-specific serum IgG and IgA.

For live and live-vectored candidate vaccines intended for oral administration, the sponsor should document faecal shedding of the vaccine strain post-administration. The duration of shedding should be determined and the potential risk of transmission of the vaccine strain to close contacts of vaccinees should be assessed during the clinical development programme (see section C.6 below). Furthermore, the sponsor should develop a method to differentiate the vaccine strain(s) from wild-type strains in faeces to enable confirmation of RVGE episodes with onset while vaccine strains may still be present in faecal samples.

C.3 Dose and regimen

There is no established immune correlate of protection for the prevention of RVGE. The preliminary selection of dose and regimen may be based on safety and immunogenicity studies, including studies conducted in the target population. The serological data should be sufficient to determine if the immune response reaches a plateau, such that there is no appreciable increment in functional and/or total binding antibody above a certain dose level, and whether sequential doses administered at timed intervals achieve potentially important increments in immune responses.

C.4 Vaccine efficacy against RVGE

In the absence of an established immune correlate of protection for the prevention of RVGE, there is only a limited rationale for immunobridging a candidate vaccine to a licensed oral rotavirus vaccine based on immunogenicity. Thus, a clinical demonstration of efficacy against RVGE is recommended.

Due to the widespread recommendations for the use of licensed vaccines to prevent RVGE in infancy, and due to the observed efficacy and effectiveness of these vaccines, it is not expected that placebo-controlled clinical efficacy studies will be feasible.

In principle, it could be acceptable that a candidate rotavirus vaccine against RVGE in infants demonstrates protective efficacy that is non-inferior to that of a licensed rotavirus vaccine for which efficacy was established in a placebo-controlled study. However, this approach would require that the same primary end-point is applicable to both the candidate and reference (licensed) vaccines, and that a robust and well-justified non-inferiority margin can be determined. There are several potential issues to be considered, both for study design and for determining an appropriate non-inferiority margin. Such issues include, but are not limited to, those set out in the remainder of this section.

The primary analyses of efficacy of the first oral rotavirus vaccines to be licensed were based on protection against RVGE due to the rotavirus serotype(s) included in each of the vaccines. A new candidate vaccine is unlikely to have the same content as a licensed vaccine and will likely be developed to cover as many of the currently circulating rotavirus serotypes as possible. A study that aims to demonstrate non-inferiority for efficacy against RVGE due to rotavirus serotype(s) for which the efficacy of the licensed vaccine is not known or is estimated to be suboptimal is not an appropriate basis for licensure.

Secondary analyses in the efficacy studies for the first licensed vaccines examined the prevention of RVGE due to any rotavirus serotype, as well as efficacy against specific rotavirus serotypes included in the vaccine and serotypes not included in the vaccine. However, these analyses are not sufficient to underpin the selection of a valid non-inferiority margin that could be applied to a study comparing the efficacy of a candidate and a reference vaccine against RVGE due to any rotavirus serotype and/or against selected rotavirus serotypes.

The placebo-controlled efficacy studies conducted for the first licensed vaccines enrolled infants resident in selected regions. Where efficacy by geographical location was explored within any one study, there was some variability in vaccine efficacy by region. Furthermore, cross-study comparisons of the pre-licensure studies conducted outside of Africa and the subsequent placebo-controlled studies conducted in various parts of Africa also suggested that there could be considerable differences in vaccine efficacy in different populations. Such differences likely reflect the effects on risk for, and severity of, RVGE associated with several host factors (for example, general health and level of nutrition) and with concomitant infections (for example, helminthic infections). Therefore, it is not possible to select a valid non-inferiority margin for a comparative efficacy study performed in a population different to that enrolled in the placebo-controlled study originally conducted for the reference vaccine.

Additionally, changes may occur in a number of background factors over time – for example, the factors that led to observed geographical variations in vaccine efficacy in the previously conducted placebo-controlled studies with licensed vaccines are unlikely to apply to a similar extent to a population enrolled into a prospective comparative efficacy study at a later time, even in the same geographical location(s). This compounds the many difficulties in identifying a relevant and robust non-inferiority margin.

Due to these and other issues, it is recommended that the primary objective of comparative vaccine efficacy studies is to demonstrate superiority in the prevention of RVGE for a candidate vaccine (regardless of construct and ROA) compared to a licensed vaccine for which absolute vaccine efficacy against RVGE has been documented. In this approach, all infants randomized to the control group receive a licensed vaccine that is currently standard of care. Since study success is based on demonstrating the superiority of the candidate vaccine in preventing RVGE, it does not matter if the efficacy of the licensed vaccine is not known or is estimated to be suboptimal against certain rotavirus serotypes and/or in certain populations.

The primary end-point for such a study will depend on the composition of the candidate vaccine and what is expected from it in terms of rotavirus serotype-specific protection against RVGE. Thus, if the vaccine is designed to provide protection against specific rotavirus serotypes, the primary end-point could be RVGE due to these rotavirus serotypes, with a secondary analysis based on all RVGE. However, if it is anticipated that the candidate vaccine will confer protection against a very broad range of rotavirus serotypes, the primary end-point could be RVGE due to any rotavirus serotype, with secondary analyses of efficacy against specific rotavirus serotypes.

The protocol must include a primary case definition for laboratory-confirmed RVGE and the severity of RVGE should be assessed using an appropriate grading scale. It is acceptable that the primary case definition will include a minimum time to symptom onset since the last rotavirus vaccine dose was administered. This should be justified based on what is known about the immune response kinetics of the candidate and reference vaccines. Sensitivity analyses should count all cases from the time of the first dose and from the time of sequential doses, assuming that a multi-dose regimen is required. Secondary analyses could examine vaccine efficacy against mild/moderate versus severe RVGE. For the primary analysis, the number of cases meeting the primary case definition accrued during the first rotavirus season (if the disease is mainly seasonal) could be compared and/or an alternative

duration of follow-up could be defined. Beyond the primary analysis it is appropriate to continue documenting RVGE cases for at least 1 year from administration of the last dose of vaccine.

Hospitalization is not appropriate for defining a case and/or its severity because the reasons for admission are not solely influenced by severity of RVGE, and policies differ by country/region. However, hospitalization and/or other forms of contact with health-care professionals could be designated as secondary or exploratory end-points.

If the candidate and licensed vaccines are administered by different routes, a double dummy approach is recommended so that a double-blind study design is possible.

If no preliminary efficacy study was conducted on the candidate vaccine (that is, the sponsor initiated the pivotal efficacy study having selected a dose solely from safety and immunogenicity data), it is recommended that the protocol includes a planned futility analysis.

Finally, it is recognized that there may be individual NRAs who consider that a non-inferiority study that compares the efficacy of a candidate vaccine with a vaccine that was licensed in their jurisdiction based on an estimate of absolute vaccine efficacy could suffice to support national approval. In such cases, it is recommended that the rationale for the agreed non-inferiority margin applied to the primary analysis is clearly explained. Moreover, further considerations for efficacy study design will apply in future if new rotavirus vaccines are approved based on superior efficacy, which leads to replacement of the vaccines currently available and in routine use.

C.5 Concomitant administration with routine childhood vaccines

Rotavirus vaccines have been incorporated into routine childhood immunization programmes based on experience with co-administration during the pre-licensure efficacy studies and on pre-licensure and post-licensure serological data supporting lack of negative immune interference.

Depending on where the candidate vaccine is to be licensed and expected to be used, sponsors should consider generating data to support co-administration with widely used routine infant vaccines. Such data could be obtained in specific co-administration studies and/or by including subsets to evaluate co-administration in pivotal efficacy studies.

C.6 Vaccine safety

Due to experience with an initial reassortant rotavirus vaccine, the live oral rotavirus vaccines that were developed subsequently underwent pre-licensure assessments of the risk for vaccine-attributable intussusception. These assessments provided estimates of the relative and absolute risks compared to placebo. Post-marketing safety surveillance followed, suggesting that the risk of vaccine-associated intussusception is far outweighed by the benefit of vaccination in terms of prevention of RVGE in infants.

It is reasonable to expect that the risk of vaccine-associated intussusception will differ by vaccine construct and content. Sponsors should identify cases of intussusception as adverse events of special interest in clinical studies of candidate vaccines and should consider the need for and value of post-authorization safety studies to examine the risk in addition to routine safety surveillance.

In the case of live rotavirus candidate vaccines, the clinical programme should include an assessment of the risk of transmission of the vaccine virus (or viruses), the duration of any such risk after sequential doses and any possible consequences there may be for close contacts of vaccinated infants (see section C.2 above). If the vaccine is likely to be used in regions where there are substantial numbers of HIV-infected infants, sponsors should consider conducting

studies to assess safety, immunogenicity and risk of transmission in this specific sub-population.

The specific guidance provided by WHO on the post-marketing surveillance of rotavirus vaccine safety *(72)* should be followed.

Part D. Recommendations for NRAs

D.1 General recommendations

The guidance for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products *(73)* and WHO Guidelines for independent lot release of vaccines by regulatory authorities *(22)* should be followed. These guidelines specify that no new biological product should be released until consistency of lot manufacturing and product quality have been established and demonstrated by the manufacturer.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety or efficacy of rotavirus vaccines, should be discussed with and approved by the NRA.

For control purposes, the NRA may obtain the product-specific or working reference(s) from the manufacturer to be used for lot release until an international or national standard reference material is established.

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

D.2 Official release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations *(22)*.

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

A lot release certificate signed by the appropriate NRA/NCL official should then be provided if requested by the manufacturing establishment, and should certify that the lot of vaccine meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot, including the basis of the release decision (by summary protocol review and/or independent laboratory testing). The purpose of this official national lot release certificate is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccine.

A model NRA/NCL Lot Release Certificate for live attenuated rotavirus vaccine (oral) is provided in Appendix 2.

Authors and acknowledgements

The first draft of these WHO Recommendations was prepared by a WHO drafting group comprising: Dr G.I. Parra, Center for Biologics Evaluation and Research, US Food and Drug Administration, USA; Dr M. Powell, Health Products Regulatory Authority, Ireland; Dr Y. Sun, Paul-Ehrlich Institute, Germany; Dr M. Xu, National Institutes for Food and Drug

Control, China; and Dr T.Q. Zhou, World Health Organization, Switzerland, with direct inputs from Dr P. Minor, consultant, St Albans, United Kingdom; and Dr D. Feigelstock, Center for Biologics Evaluation and Research, US Food and Drug Administration, USA, based on discussions held during a WHO informal consultation on the revision of guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines, held virtually on 15–17 November 2022 and attended by: Dr N. Aldosri and Dr M. Alnufeay, Saudi Food and Drug Authority, Saudi Arabia; Dr M. Blahoianu, Biologic and Radiopharmaceutical Drugs Directorate, Health Canada, Canada; Dr K. Brusselmans, Sciensano, Belgium; Dr M. Bruysters, National Institute for Public Health and the Environment, Netherlands (Kingdom of the); Dr G. Ciréfice, European Directorate for the Quality of Medicines & Healthcare, France; Dr S. Cryz, PATH, USA; Dr V. Dirix, Federal Agency for Medicines and Health Products, Belgium; Miss D. Doraswami, South African Health Products Regulatory Authority, South Africa; Dr Đ.T. Dương, Hanoi Medical University, Viet Nam; Dr S. Fakhrzadeh, Food and Drug Administration, Iran (Islamic Republic of); Dr D. Feigelstock, Center for Biologics Evaluation and Research, US Food and Drug Administration, USA; Dr E. Fitriyani, Indonesian Food and Drug Authority, Indonesia; Professor M.A. Franco Cortés, Institute of Human Genetics, Colombia; Dr Y. Fujii, National Institute of Infectious Diseases, Japan; Dr M. Groome, National Institute for Communicable Diseases, South Africa; Dr M. Iturriza-Gomara, PATH, Switzerland; Mr P. Jagtap, Ministry of Health and Family Welfare, India; Mrs T. Jivapaisarnpong, King Mongkut's University of Technology Thonburi, Thailand; Dr J.A. Dahlan, Directorate of Drug Registration, Indonesia; Dr C. Kirkwood, Bill & Melinda Gates Foundation, USA; Miss K. Kullabutr, Ministry of Public Health, Thailand; Dr R. Levis, Center for Biologics Evaluation and Research, US Food and Drug Administration, USA; Dr A. Lommel, Paul-Ehrlich-Institut, Germany; Dr P. Manavalan, Ministry of Health and Family Welfare, India; Dr E. Mee, Medicines and Healthcare products Regulatory Agency, United Kingdom; Dr P. Minor, consultant, United Kingdom; Dr J. Mitchell, Medicines and Healthcare products Regulatory Agency, United Kingdom; Dr K. Murakami, National Institute of Infectious Diseases, Japan; Dr E. Nkansah, Food and Drugs Authority, Ghana; Dr N. Rose, Medicines and Healthcare products Regulatory Agency, United Kingdom; Mr M. Salahuddin, Directorate General of Drug Administration, Bangladesh; Dr K. Smits, Federal Agency for Medicines and Health Products, Belgium; Dr J. Song, Ministry of Food and Drug Safety, Republic of Korea; Dr J. Southern, South African Health Products Regulatory Authority, South Africa; Dr D. Steele, Bill & Melinda Gates Foundation, USA; Dr M.F. R S Thees, Brazilian Health Regulatory Agency, Brazil; Dr N.T. Lý, National Institute for Control of Vaccine and Biologicals, Viet Nam; Dr X. Yin, National Medical Products Administration, China. *Representatives of the Developing Countries Vaccine Manufacturers Network (DCVMN)*: Dr S. Desai and Dr J. Zade, Serum Institute of India, India; Dr R.C. Guimarães and Dr L.A.D. Santos Lima, Biomanguinhos, Brazil; Dr N.D. Hien and Dr N.T. Huong, Polyvac, Viet Nam; Mr S. Prasad and Dr C. Roy, Bharat Biotech International Limited, India; and Mr. Z. Wei, Lanzhou Institute of Biological Products Co. Ltd, China. *Representatives of the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA)*: Dr B. Baras and Dr L. Moerman, GSK, Belgium; Dr N. Kanibir, MSD, Switzerland; and Dr C. Hoath and Dr Y. Tu, MSD, USA. *WHO Secretariat*: Dr C. Ondari, Dr I. Knezevic and Dr T.Q. Zhou, Health Products Policy and Standards Department, World Health Organization, Switzerland; Dr M. Alali, Regulation and Prequalification Department, World Health Organization, Switzerland; and Dr B. Giersing, Immunization Vaccines and Biologicals Department, World Health Organization, Switzerland.

The first draft was then circulated among the above informal consultation meeting participants for review during the period 24 April to 25 May 2023. Comments were received from: Dr M. Alali, World Health Organization, Switzerland; Dr B. Baras, GSK, Belgium; Dr

K. Brusselmans, Sciensano, Belgium; Dr S. Fakhrzadeh, Food and Drug Administration, Iran (Islamic Republic of); Professor M.A. Franco Cortés, Institute of Human Genetics, Colombia; Dr V. Dirix, Federal Agency for Medicines and Health Products, Belgium; Dr C. Kirkwood, Bill & Melinda Gates Foundation, USA; Dr K. Murakami, National Institute of Infectious Diseases, Japan; Dr G.I. Parra, Center for Biologics Evaluation and Research, US Food and Drug Administration, USA; Dr N. Rose, Medicines and Healthcare products Regulatory Agency, United Kingdom; Dr C. Roy, Bharat Biotech International Limited, India; Dr K. Smits, Federal Agency for Medicines and Health Products, Belgium; Dr J. Southern, South African Health Products Regulatory Authority, South Africa; Dr M. Xu, National Institutes for Food and Drug Control, China; Dr X. Yin, National Medical Products Administration, China; and Dr J. Millogo (coordinated comments from MSD, USA).

The second draft document was prepared by the WHO drafting group and Dr P. Minor, taking into account all comments received. The document was then posted on the WHO Biologicals website during July and September 2023 for a first round of public consultation. Comments were received from: Dr S.B. Amor, National Control Laboratory, Tunisia; Dr J.A. Dahlan, Directorate of Standardization of Drug, Narcotics, Psychotropics, Precursors and Addictive Substances, Indonesia; Dr D. Feigelstock, Center for Biologics Evaluation and Research, US Food and Drug Administration, USA; Dr A.C. Geyer, Prequalification Unit, World Health Organization, Switzerland; S. Laghnimi-Hahn (*on behalf of IFPMA, Vaccines Policy*); Dr T. Lu, Therapeutic Goods Administration, Australia; Dr J. Millogo, Merck Vaccines, USA; Dr K. Mohan, Bharat Biotech International Limited, India; Dr S. Silveira, Brazilian Health Regulatory Agency, Brazil; Dr J. Southern, South African Health Products Regulatory Authority, South Africa; Dr S. Wendel, Hospital Sírio-Libanês, Brazil; Dr T. Wu, Health Canada, Canada; and Dr M. Yang, Ministry of Food and Drug Safety, Republic of Korea.

Following completion of the first round of public consultation, the third draft document was prepared by the WHO drafting group and Dr P. Minor, with inputs received from Dr T. Wu, Health Canada, Canada, and taking into account the comments received. The resulting document was then was posted on the WHO Biologicals website during January and February 2024 for a second round of public consultation. Comments were received from: Dr B. Baras, GSK, Belgium; Dr U. Desselberger, University of Cambridge, United Kingdom; Dr S. Fakhrzadeh, Food and Drug Administration, Iran (Islamic Republic of); Dr T. Guo, China Quality Associations for Pharmaceuticals, China; S. Laghnimi-Hahn, IFPMA, Switzerland (*on behalf of IFPMA*); Dr J. Millogo, Merck Vaccines, USA; Dr K. Mohan, Bharat Biotech International Limited, India; Dr D. Robinson and Dr M.M. Lumpkin, Bill & Melinda Gates Foundation, USA; Dr J. Southern, South African Health Products Regulatory Authority, South Africa; Dr W. Tangkeangsirisin, Ministry of Public Health, Thailand; Mrs Y. Wang, Wuhan Institute of Biological Products Co. Ltd, China; Dr S. Wendel, Hospital Sirio-Libanês, Brazil; Mr Z. Wei, Lanzhou Institute of Biological Products Co. Ltd, China; and Dr J.K. Zade, Serum Institute of India, India.

The key issues raised during the second round of public consultation were then presented to the WHO Expert Committee on Biological Standardization in March 2024 and the amended text consulted on with rotavirus vaccine manufacturers. Feedback was received from: Dr B. Baras, GSK, Belgium; Dr J. Millogo, Merck Vaccines, USA; Dr K. Mohan, Bharat Biotech International Limited, India; Mrs Y. Wang, Wuhan Institute of Biological Products Co. Ltd, China; Mr Z. Wei, Lanzhou Institute of Biological Products Co. Ltd, China; and Dr J.K. Zade, Serum Institute of India, India. The resulting document (WHO/BS/2024.2474) was then prepared by the WHO drafting group and Dr P. Minor, with inputs received from Dr T. Wu, Health Canada, Canada, and taking into account the comments received during the above consultation.

Editorial review of document WHO/BS/2024.2474 was then completed by Dr T. Waddell, United Kingdom in accordance with WHO requirements for all documents appearing in the WHO Technical Report Series.

The document WHO/BS/2024.2474 was posted on the WHO Biologicals website for a third round of public consultation during July and September 2024. Written comments were received from: Dr P. Stickings, Medicines and Healthcare products Regulatory Agency, United Kingdom; Dr A. Ramkishan, Central Drugs Standard Control Organization, India; Dr S. Wendel, Hospital Sírio-Libanês, Brazil; Dr S. Silveira, Agência Nacional de Vigilância Sanitária-Anvisa, Brazil; Dr I. Feavers, consultant, Nacton, United Kingdom; Professor M.A. Franco Cortés, Instituto de Genética Humana, Colombia; Dr J.K. Zade, Serum Institute of India Pvt Ltd, India; Mr Z. Wei, Lanzhou Institute of Biological Products Co., Ltd, China; Dr Q. Meyer, South African National Control Laboratory for Biological Products, South Africa; Ms W. Wang, Wuhan Institute of Biological Products, China; Dr K. Mohan, Bharat Biotech International Limited, India; and Dr K. Djalilov and Mr. A. Zaynidinov, Ministry of Health of the Republic of Uzbekistan, Uzbekistan.

Further changes were made to document WHO/BS/2024.2474 by the WHO Expert Committee on Biological Standardization.

References

1. Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines (oral). In: WHO Expert Committee on Biological Standardization: fifty-sixth report. Geneva: World Health Organization; 2007: Annex 3 (WHO Technical Report Series, No. 941; https://iris.who.int/handle/10665/43594, accessed 18 October 2024).

2. Rotavirus vaccines: WHO position paper – July 2021. Weekly Epidemiological Record. 2021;96:301–20 (https://apps.who.int/iris/bitstream/handle/10665/342904/WER9628-eng-fre.pdf, accessed 18 October 2024).

3. Kirkwood CD, Ma L-F, Carey ME, Steele AD. The rotavirus vaccine development pipeline. Vaccine. 2019;37(50): 7328–35 (https://pmc.ncbi.nlm.nih.gov/articles/PMC6892263/, accessed 18 October 2024).

4. Cárcamo-Calvo R, Muñoz C, Buesa J, Rodríguez-Díaz J, Gozalbo-Rovira R. The rotavirus vaccine landscape, an update. Pathogens. 2021;10(5):520 (https://doi.org/10.3390/pathogens10050520, accessed 18 October 2024).

5. WHO informal consultation on revision of guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines. Executive summary. Virtual meeting, 15–17 November 2022 (https://www.who.int/publications/m/item/executive-summary--who-informal-consultation-on-revision-of-guidelines-to-assure-the-quality--safety-and-efficacy-of-live-attenuated-rotavirus-vaccines, accessed 18 October 2024).

6. Recommendations for inactivated rabies vaccine for human use produced in cell substrates and embryonated eggs. In: WHO Expert Committee on Biological Standardization: fifty-sixth report. Geneva: World Health Organization; 2007: Annex 2 (WHO Technical Report Series, No. 941; https://iris.who.int/handle/10665/43594, accessed 18 October 2024).

7. Recommendations to assure the quality, safety and efficacy of enterovirus 71 vaccines (inactivated). In: WHO Expert Committee on Biological Standardization: report of the seventy-second and seventy-third meetings. Geneva: World Health Organization; 2021: Annex 3 (WHO Technical Report Series, No. 1030; https://iris.who.int/handle/10665/341239, accessed 18 October 2024).

8. Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated). In: WHO Expert Committee on Biological Standardization: sixty-fifth report. Geneva: World Health Organization; 2015: Annex 3 (WHO Technical Report Series, No. 993; https://iris.who.int/handle/10665/173739, accessed 18 October 2024).

9. Recommendations to assure the quality, safety and efficacy of recombinant hepatitis B vaccines. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 4 (WHO Technical Report Series, No. 978; https://iris.who.int/handle/10665/89148, accessed 18 October 2024).

10. Recommendations to assure the quality, safety and efficacy of recombinant hepatitis E vaccines. In: WHO Expert Committee on Biological Standardization: sixty-ninth report. Geneva: World Health Organization; 2019: Annex 2 (WHO Technical Report Series, No. 1016; https://iris.who.int/handle/10665/325184, accessed 18 October 2024).

11. Guidelines on the quality, safety and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum*. In: WHO Expert Committee on Biological Standardization: sixty-third report. Geneva: World Health Organization; 2014: Annex 3 (WHO Technical Report Series, No. 980; https://iris.who.int/handle/10665/98870, accessed 18 October 2024).

12. Recommendations for the production and control of influenza vaccines (inactivated). In: WHO Expert Committee on Biological Standardization: fifty-fourth report. Geneva: World Health Organization; 2005: Annex 3 (WHO Technical Report Series, No. 927; https://iris.who.int/handle/10665/43094, accessed 18 October 2024).

13. Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 4 (WHO Technical Report Series, No. 999; https://iris.who.int/handle/10665/208900, accessed 18 October 2024).

14. Guidelines on the quality, safety and efficacy of plasmid DNA vaccines. In: WHO Expert Committee on Biological Standardization: seventy-first report. Geneva: World Health Organization; 2021: Annex 2 (WHO Technical Report Series, No. 1028; https://iris.who.int/handle/10665/340016, accessed 18 October 2024).

15. Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations. In: WHO Expert Committee on Biological Standardization: seventy-fourth report. Geneva: World Health Organization; 2022: Annex 3 (WHO Technical Report Series, No. 1039; https://iris.who.int/handle/10665/353069, accessed 18 October 2024).

16. Guidelines on the quality, safety and efficacy of Ebola vaccines. In: WHO Expert Committee on Biological Standardization: sixty-eighth report. Geneva: World Health Organization; 2018: Annex 2 (WHO Technical Report Series, No. 1011; https://iris.who.int/handle/10665/272807, accessed 18 October 2024).

17. WHO guidelines on nonclinical evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-fourth report. Geneva: World Health Organization; 2005: Annex 1 (WHO Technical Report Series, No. 927; https://iris.who.int/handle/10665/43094, accessed 18 October 2024).

18. Guidelines on clinical evaluation of vaccines: regulatory expectations. In: WHO Expert Committee on Biological Standardization: sixty-seventh report. Geneva: World Health Organization; 2017: Annex 9 (WHO Technical Report Series, No. 1004; https://iris.who.int/handle/10665/255657, accessed 18 October 2024).

19. WHO good manufacturing practices for biological products. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 2 (WHO Technical Report Series, No. 999; https://iris.who.int/handle/10665/208900, accessed 18 October 2024).

20. WHO good manufacturing practices for sterile pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: fifty-sixth report. Geneva: World Health Organization; 2022: Annex 2 (WHO Technical Report Series, No. 1044; https://iris.who.int/handle/10665/365397, accessed 18 October 2024).

21. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 3 (WHO Technical Report Series, No. 978; https://iris.who.int/handle/10665/89148, accessed 18 October 2024).

22. Guidelines for independent lot release of vaccines by regulatory authorities. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 2 (WHO Technical Report Series, No. 978; https://iris.who.int/handle/10665/89148, accessed 18 October 2024).

23. Global vaccine safety blueprint 2.0 (GVSB2.0) 2021–2023. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/9789240036963, accessed 18 October 2024).

24. Cohen AL, Platts-Mills JA, Nakamura T, Operario DJ, Antoni S, Mwenda JM et al. Aetiology and incidence of diarrhoea requiring hospitalisation in children under 5 years of age in 28 low-income and middle-income countries: findings from the Global Pediatric Diarrhea Surveillance network. BMJ Glob Health. 2022;7(9):e009548 (https://doi:10.1136/bmjgh-2022-009548, accessed 18 October 2024).

25. WHO immunological basis for immunization series. Module 21: rotavirus. Update. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/331323, accessed 18 October 2024).

26. Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M et al. Rotavirus infection. Nat Rev Dis Primers. 2017;3:17083 (https://doi.org/10.1038/nrdp.2017.83, accessed 18 October 2024).

27. Tatsi EB, Koukou DM, Dellis C, Dourdouna MM, Efthymiou V, Michos A et al. Epidemiological study of unusual rotavirus strains and molecular characterization of emerging P[14] strains isolated from children with acute gastroenteritis during a 15-year period. Arch Virol. 2023;168(5):149 (https://link.springer.com/article/10.1007/s00705-023-05769-8, accessed 18 October 2024).

28. Henschke N, Bergman H, Hungerford D, Cunliffe NA, Grais RF, Kang G et al. The efficacy and safety of rotavirus vaccines in countries in Africa and Asia with high child mortality. Vaccine. 2022;40(12):1707–11 (https://doi.org/10.1016/j.vaccine.2022.02.003, accessed 18 October 2024).

29. Cunliffe NA, Witte D, Ngwira BM, Todd S, Bostock NJ, Turner AM et al. Efficacy of human rotavirus vaccine against severe gastroenteritis in Malawian children in the first two years of life: a randomized, double-blind, placebo controlled trial. Vaccine. 2012;30(1):A36–A43 (https://doi.org/10.1016/j.vaccine.2011.09.120, accessed 18 October 2024).

30. Madhi SA, Kirsten M, Louw C, Bos P, Aspinall S, Bouckenooghe A et al. Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen in South African children over two consecutive rotavirus-seasons: a randomized, double-blind, placebo-controlled trial. Vaccine. 2012;30(1):A44–A51 (https://doi.org/10.1016/j.vaccine.2011.08.080, accessed 18 October 2024).

31. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. N Engl J Med. 2010;362(4):289–98 (https://www.nejm.org/doi/full/10.1056/NEJMoa0904797, accessed 18 October 2024).

32. Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. Lancet. 2010;376(9741):606–14 (abstract: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(10)60889-6/abstract, accessed 18 October 2024).

33. Zaman K, Dang DA, Victor JC, Shin S, Yunus M, Dallas MJ et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. Lancet. 2010;376(9741):615–23 (abstract: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(10)60755-6/abstract, accessed 18 October 2024).

34. Bergman H, Henschke N, Hungerford D, Pitan F, Ndwandwe D, Cunliffe N et al. Vaccines for preventing rotavirus diarrhoea: vaccines in use. Cochrane Database Syst

Rev. 2021;11(11):CD008521 (https://doi.org/10.1002/14651858.CD008521.pub6, accessed 18 October 2024).

35. Glass RI, Parashar U, Patel M, Gentsch J, Jiang B. Rotavirus vaccines: successes and challenges. J Infect. 2014;68(1):S9–S18 (abstract: https://www.journalofinfection.com/article/S0163-4453(13)00277-6/abstract, accessed 18 October 2024).

36. Burnett E, Parashar UD, Tate JE. Real-world effectiveness of rotavirus vaccines, 2006–19: a literature review and meta-analysis. Lancet Glob Health. 2020;8(9):e1195–e1202 (https://www.thelancet.com/journals/langlo/article/PIIS2214-109X(20)30262-X/fulltext, accessed 18 October 2024).

37. Angel J, Steele AD, Franco MA. Correlates of protection for rotavirus vaccines: possible alternative trial endpoints, opportunities, and challenges. Hum Vaccin Immunother. 2014;10(12):3659–71 (https://doi.org/10.4161/hv.34361, accessed 18 October 2024).

38. Liu GF, Hille D, Kaplan SS, Goveia MG. Postdose 3 G1 serum neutralizing antibody as correlate of protection for pentavalent rotavirus vaccine. Hum Vaccin Immunother. 2017;13(10):2357–63 (https://doi.org/10.1080/21645515.2017.1356522, accessed 18 October 2024).

39. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. J Infect Dis. 2013;208(2):284–94 (https://doi.org/10.1093/infdis/jit166, accessed 18 October 2024).

40. Simonsen L, Viboud C, Elixhauser A, Taylor RJ, Kapikian AZ. More on RotaShield and intussusception: the role of age at the time of vaccination. J Infect Dis. 2005;192(l):S36–S43 (https://doi.org/10.1086/431512, accessed 18 October 2024).

41. Burnett E, Riaz A, Anwari P, Myat TW, Chavers TP, Talat N et al. Intussusception risk following oral monovalent rotavirus vaccination in 3 Asian countries: a self-control case series evaluation. Vaccine. 2023;41(48):7220–5 (https://doi.org/10.1016/j.vaccine.2023.10.042, accessed 19 October 2024).

42. Acceptability of cell substrates for production of biologicals. Report of a WHO Study Group. Geneva: World Health Organization; 1987 (https://iris.who.int/handle/10665/38501, accessed 18 October 2024).

43. Israel MA, Chan HW, Hourihan SL, Rowe WP, Martin MA. Biological activity of polyoma viral DNA in mice and hamsters. J Virol. 1979;29(3):990–6 (https://doi.org/10.1128/jvi.29.3.990-996.1979, accessed 18 October 2024).

44. Lebron JA, Troilol PJ, Pacchione S, Griffiths TG, Harper LB, Mixson LA et al. Adaptation of the WHO guideline for residual DNA in parenteral vaccines produced on continuous cell lines to a limit for oral vaccines. Dev Biol (Basel). 2006;123:35–44 (abstract: https://pubmed.ncbi.nlm.nih.gov/16566435/, accessed 18 October 2024).

45. Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S et al. Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. J Virol. 2010;84(12):6033–40 (http://dx.doi.org/10.1128/JVI.02690-09, accessed 18 October 2024).

46. McClenahan SD, Krause PR, Uhlenhaut C. Molecular and infectivity studies of porcine circovirus in vaccines. Vaccine. 2011;29(29–30):4745–53 (abstract: https://pubmed.ncbi.nlm.nih.gov/21569811/, accessed 18 October 2024).

47. Dubin G, Toussaint JF, Cassart JP, Howe B, Boyce D, Friedland L et al. Investigation of a regulatory agency enquiry into potential porcine circovirus type 1 contamination of the human rotavirus vaccine, Rotarix: approach and outcome. Hum Vaccin Immunother. 2013;9(11):2398–408 (https://doi.org/10.4161/hv.25973, accessed 18 October 2024).

48. Salamanca de la Cueva I, Pahud B, Huang LM, Leonardi M, Garcia-Sicilia J, Céspedes J et al. Immunogenicity and safety of porcine circovirus-free human rotavirus vaccine in healthy infants: a phase III, randomized trial. J Infect Dis. 2020;225(12):2106–15 (https://doi.org/10.1093/infdis/jiaa210, accessed 18 October 2024).

49. WHO good manufacturing practices for pharmaceutical products: main principles. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-eighth report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 986; https://iris.who.int/handle/10665/112733, accessed 18 October 2024).

50. Laboratory biosafety manual. Fourth edition. Geneva: World Health Organization; 2020 (https://iris.who.int/handle/10665/337956, accessed 18 October 2024).

51. General requirements for the sterility of biological substances (Requirements for Biological Substances No. 6) (Revised 1973). In: WHO Expert Committee on Biological Standardization: twenty-fifth report. Geneva: World Health Organization; 1973: Annex 4 (WHO Technical Report Series, No. 530; https://iris.who.int/handle/10665/41053, accessed 18 October 2024).

52. General requirements for the sterility of biological substances (Requirements for Biological Substances No. 6, revised 1973, amendment 1995). In: WHO Expert Committee on Biological Standardization: forty-sixth report. Geneva: World Health Organization; 1998: Annex 3 (WHO Technical Report Series, No. 872; https://iris.who.int/handle/10665/42058, accessed 18 October 2024).

53. WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products. Geneva: World Health Organization; 2003 (https://iris.who.int/handle/10665/68932, accessed 18 October 2024).

54. Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (revised 1992). In: WHO Expert Committee on Biological Standardization: forty-third report. Geneva: World Health Organization; 1994: Annex 2 (WHO Technical Report Series, No. 840; https://iris.who.int/handle/10665/39048, accessed 18 October 2024).

55. WHO manual for the establishment of national and other secondary standards for vaccines. Geneva: World Health Organization; 2011 (Document WHO/IVB/11.03; https://iris.who.int/handle/10665/70669, accessed 18 October 2024).

56. WHO Policy Statement: multi-dose vial policy (MDVP). Revision 2014. Handling of multi-dose vaccine vials after opening. Geneva: World Health Organization; 2014 (Document WHO/IVB/14.07; https://iris.who.int/handle/10665/135972, accessed 18 October 2024).

57. Guidelines on stability evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-seventh report. Geneva: World Health Organization; 2011: Annex 3 (WHO Technical Report Series, No. 962; https://iris.who.int/handle/10665/44678, accessed 18 October 2024).

58. Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-fifth report. Geneva: World Health Organization; 2011: Annex 9 (WHO Technical Report Series, No. 961; https://iris.who.int/handle/10665/44079, accessed 18 October 2023).

59. Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 5 (WHO Technical Report Series, No. 999; https://iris.who.int/handle/10665/208900, accessed 18 October 2024).

60. Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines. In: WHO Expert Committee on Biological Standardization: sixty-fourth report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 987; https://iris.who.int/handle/10665/129494, accessed 18 October 2024).

61. Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines. London: European Medicines Agency; 2010 (Document EMA/CHMP/VWP/141697/2009; https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-and-clinical-aspects-live-recombinant-viral-vectored-vaccines_en.pdf, accessed 18 October 2024).

62. Angel J, Franco MA, Greenberg HB. Rotavirus vaccines: recent developments and future considerations. Nat Rev Microbiol. 2007;5:529–39 (https://www.nature.com/articles/nrmicro1692, accessed 19 October 2024).

63. Ciarlet M, Conner ME. Evaluation of rotavirus vaccines in small animal models. Methods Mol Med. 2000;34:147–87 (abstract: https://pubmed.ncbi.nlm.nih.gov/21318860/, accessed 19 October 2024).

64. Fix A, Kirkwood CD, Steele D, Flores J. Next-generation rotavirus vaccine developers meeting: summary of a meeting sponsored by PATH and the bill & melinda gates

foundation (19–20 June 2019, Geneva). Vaccine. 2020;38(52):8247–54 (abstract: https://doi.org/10.1016/j.vaccine.2020.11.034, accessed 19 October 2024).

65. Desselberger U, Huppertz H-I. Immune responses to rotavirus infection and vaccination and associated correlates of protection. J Infect Dis. 2011;203(2):188–95 (https://pmc.ncbi.nlm.nih.gov/articles/PMC3071058/, accessed 19 October 2024).

66. Wang Y, Vlasova A, Velasquez DE, Saif LJ, Kandasamy S, Kochba E et al. Skin vaccination against rotavirus using microneedles: proof of concept in gnotobiotic piglets. PLoS ONE. 2016;11(11):e0166038 (https://doi.org/10.1371/journal.pone.0166038, accessed 19 October 2024).

67. Ramesh A, Mao J, Lei S, Twitchell E, Shiraz A, Jiang X et al. Parenterally administered P24-VP8* nanoparticle vaccine conferred strong protection against rotavirus diarrhea and virus shedding in gnotobiotic pigs. Vaccines (Basel). 2019;7(4):177 (https://doi.org/10.3390/vaccines7040177, accessed 19 October 2024).

68. Kumar D, Shepherd FK, Springer NL, Mwangi W, Marthaler DG. Rotavirus infection in swine: genotypic diversity, immune responses, and role of gut microbiome in rotavirus immunity. Pathogens. 2022;11(1)):1078 (https://doi.org/10.3390/pathogens11101078, accessed 19 October 2024).

69. Saif LJ, Ward LA, Yuan L, Rosen BI, To TL. The gnotobiotic piglet as a model for studies of disease pathogenesis and immunity to human rotaviruses. Arch Virol Suppl. 1996;12:153–61 (https://doi.org/10.1007/978-3-7091-6553-9_17, accessed 19 October 2024).

70. Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches. London: European Medicines Agency; 2016 (Document EMA/CHMP/CVMP/JEG-3Rs/450091/2012; https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-principles-regulatory-acceptance-3rs-replacement-reduction-refinement-testing-approaches_en.pdf, accessed 18 October 2024).

71. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. In: WHO Expert Committee on the Use of Essential Drugs: sixth report. Geneva: World Health Organization; 1995: Annex 3 (WHO Technical Report Series, No. 850; https://iris.who.int/handle/10665/37340, accessed 18 October 2024).

72. Post-marketing surveillance of rotavirus vaccine safety. Geneva: World Health Organization; 2009 (Document WHO/IVB/09.01; https://iris.who.int/handle/10665/70017, accessed 18 October 2024).

73. Guidelines for national authorities on quality assurance for biological products. In: WHO Expert Committee on Biological Standardization: forty-second report. Geneva: World Health Organization; 1992: Annex 2 (WHO Technical Report Series, No. 822; https://iris.who.int/handle/10665/39431, accessed 18 October 2024).

Appendix 1

Model summary protocol for the manufacturing and control of live attenuated rotavirus vaccines (oral)

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by the manufacturer to the NRA or NCL.

Information and tests may be added or omitted as necessary with the approval of the NRA or NCL. In cases where the testing method is different from the one listed in this model protocol, it should be approved by the NRA. For example, if molecular methods (such as NAT-based assays and HTS) are used for the testing of adventitious agents or mycoplasmas, their key parameters and information should be identified and provided, covering, as a minimum, the testing method, date of testing, specification and result.

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

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet (package insert) that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a lot release certificate (see Appendix 2) from the NRA or from the NCL of the country in which the vaccine was produced and/or released, stating that the product meets national requirements as well as the recommendations in Part A of this document.

Summary information on the finished product (final lot)

International name: Live attenuated rotavirus vaccine (oral)
Trade name/ Commercial name: _____
Product licence (marketing authorization) number _____
Country: _____
Name and address of manufacturer: _____
_____
Name and address of licence holder, if different: _____
Final packaging lot number: _____
Type of container: _____
Number of containers in this packaging lot: _____
Final container lot number: _____
Number of filled containers in this final lot: _____
Bulk numbers of monovalent bulk suspensions blended in monovalent/multivalent vaccine: _____
Site of manufacture of each monovalent bulk: _____
Date of manufacture of each monovalent bulk: _____
Date of manufacture of final bulk (or blending, if applicable): _____
Date of manufacture of finished product (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: _____
Release 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.

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

Summary of source materials

The information requested below is to be presented on each submission. Full details on master and working seed lots should be provided upon first submission only and whenever a change has been introduced.

Control of source materials (section A.3)

Cell lines (section A.3.1)

Cell banks – every submission

Information on cell banking system

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

Master cell bank (MCB) and working cell bank (WCB) (section A.3.1.1) – every submission

Lot numbers: _____
Date of preparation: _____
Date the MCB and WCB were established: _____
Date of approval by NRA: _____
Total number of containers stored: _____
Passage/population doubling level of cell bank: _____
Maximum passage/population doubling level approved: _____
Storage conditions: _____
Method of preparation of cell bank in terms of freezes, and efforts made to ensure that an homogeneous population is dispersed into the containers: _____

Tests on MCB and WCB (section A.3.1.2) – first submission only

Percentage of total cell bank containers tested: _____

Identity test

Date of test: _____
Method used: _____
Results: _____
Biochemical data: _____
Immunological marker: _____
Cytogenetic marker: _____
DNA fingerprinting (or sequencing) data: _____
Results of other identity tests: _____

Tests for adventitious agents

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Date test started: _____
Date test ended: _____
Result: _____

Tests for bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Volume of medium per vial: _____
Observation period (specification) _____

Incubation	Media used	Inoculum	Date test started	Date test ended	Results

20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Test for mycoplasmas

Method used: _____
Volume tested: _____
Media used: _____
Temperature of incubation: _____
Observation period (specification): _____
Positive controls – list of species used and results: _____

	Date test started	Date test ended	Results
Subcultures at day 3	_____	_____	_____
Subcultures at day 7	_____	_____	_____
Subcultures at day 14	_____	_____	_____
Subcultures at day 21	_____	_____	_____

Indicator cell culture method (if applicable)

Cell substrate used: _____
Inoculum: _____
Date of test: _____
Passage number: _____
Negative control: _____
Positive control: _____
Date of staining: _____
Results: _____

Results of tests for tumorigenicity (if applicable)

_____

Test for retroviruses (if applicable)

Date of test: _____
Method used: _____
Results: _____

Cell culture medium (section A.3.2)

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

Date of tests: _____
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: _____

Virus seeds (section A.3.3) – every submission

Virus strains (section A.3.3.1)

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

Virus seed lot system (section A.3.3.2) – every submission

Source of VMS: _____
VMS and VWS lot number: _____
Name and address of manufacturer: _____
VWS passage level from VMS: _____
Date of inoculation: _____
Date of harvest: _____
Date of preparation: _____
Date approved by NRA: _____
Total quantity stored: _____
Storage conditions: _____
Passage level of VMS: _____
Maximum passage level authorized: _____

Tests on VMS and VWS (section A.3.3.3) – first submission only

Identity test

Date of test: _____
Method used: _____
Results: _____

Genotype/phenotype characterization

Date of test: _____

Method used: _____
Results: _____

HTS (for virus seed, if applicable)

Specification: _____
Date of test: _____
Result: _____

Tests for bacteria, fungi, mycoplasmas and mycobacteria

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Volume of medium per vial: _____
Observation period _____

Incubation	Media used	Inoculum	Date test started	Date test ended	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Test for mycoplasmas

Method used: _____
Volume tested: _____
Media used: _____
Temperature of incubation: _____
Observation period: _____
Positive controls – list of species used and results: _____

	Date test started	Date test ended	Results
Subcultures at day 3	_____	_____	_____
Subcultures at day 7	_____	_____	_____
Subcultures at day 14	_____	_____	_____
Subcultures at day 21	_____	_____	_____

Test for mycobacteria

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Indicator cell culture method (if applicable)

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

Tests for adventitious agents

Date(s) of satisfactory test(s) for freedom from adventitious agents: _____
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 started: _____
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 started: _____
Date test ended: _____
Ratio of cultures viable at end of test: _____
Results: _____

Other cell types: _____
Quantity of neutralized sample inoculated: _____
Incubation conditions: _____
Date test started: _____
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 started: _____
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 started: _____
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 started: _____
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: _____
Reference lot no.: _____
Results: _____

Control of vaccine production (section A.4)

Control of production cell cultures (section A.4.1)

Lot number of MCB: _____
Lot number of WCB: _____
Date of thawing ampoule of WCB: _____
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): _____

Control of cell cultures (section A.4.1)

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.

Tests of control cell culture (section A.4.1.1)

Amount or ratio of control cultures to production cell cultures: _____
Incubation conditions: _____
Period of observation of cultures: _____
Date started: _____
Date ended: _____
Ratio or proportion of cultures discarded and reason: _____
Results of observation: _____
Date fluids collected: _____
Date fluids pooled (if applicable): _____

Tests for haemadsorbing viruses (section A.4.1.2)

Quantity of cells tested: _____
Type of red blood cell used: _____
Storage time and temperature of red blood cell: _____
Incubation time and temperature of red blood cell: _____
Date test started: _____
Date test ended: _____
Results: _____
Additional tests if performed: _____

Tests for other adventitious agents in cell supernatant fluids (section A.4.1.3)

Test in production cells

Date of sampling: _____
Quantity of sample inoculated: _____
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 started: _____
Date test ended: _____

Ratio of cultures viable at end of test: _____
Uninoculated cell control: _____
Results: _____

Test in other cell system

Type of cell: _____
Quantity of sample inoculated: _____
Incubation conditions: _____
Date test started: _____
Date test ended: _____
Ratio of cultures viable at end of test: _____
Uninoculated cell control: _____
Results: _____

Identity test (section A.4.1.4)

Date of test: _____
Method used: _____
Results: _____

Cell cultures for vaccine production (section A.4.2)

Tests for adventitious agents (section A.4.2.1)

Date of examination (inoculation): _____
Results: _____

Tests for bacteria, fungi, mycoplasmas and mycobacteria (section A.4.2.2)

Date and volume of sampling: _____
Volume of samples tested: _____

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Volume of medium per vial: _____
Observation period (specification): _____

Incubation	Media used	Inoculum	Date test started	Date test ended	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Test for mycoplasmas

Method used: _____
Volume tested: _____
Media used: _____

Temperature of incubation: _____
Observation period (specification): _____
Positive controls (list of species used and results): _____

	Date test started	Date test ended	Results
Subcultures at day 3	_____	_____	_____
Subcultures at day 7	_____	_____	_____
Subcultures at day 14	_____	_____	_____
Subcultures at day 21	_____	_____	_____

Test for mycobacteria

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Indicator cell-culture method (if applicable)

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

Control of single harvests and monovalent virus pools (section A.4.3)

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

Note: 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) (section A.4.3.2)

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 conditions and period: _____

Tests on single harvest or monovalent virus pools (section A.4.3.3)

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

Identity (section A.4.3.3.2)

Date of test: _____

Method used: _____

Results: _____

Tests for bacteria, fungi, mycoplasmas and mycobacteria (section A.4.3.3.3)

Tests for bacteria and fungi

Method used: _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Volume of medium per vial: _____

Observation period (specification): _____

Incubation	Media used	Inoculum	Date test started	Date test ended	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Test for mycoplasmas

Method used: _____

Volume tested: _____

Media used: _____

Temperature of incubation: _____

Observation period (specification): _____

Positive controls (list of species used and results): _____

	Date test started	Date test ended	Results
Subcultures at day 3	_____	_____	_____
Subcultures at day 7	_____	_____	_____

Subcultures at day 14 _____ _____ _____

Subcultures at day 21 _____ _____ _____

Test for mycobacteria

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Indicator cell-culture method (if applicable)

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 antiserum/antisera used for neutralization _____

Tests in tissue cultures

Type of simian cells: _____
Quantity of neutralized sample inoculated: _____
Incubation conditions: _____
Date test started: _____
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 started: _____
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 started: _____
Date test ended: _____
Ratio of cultures viable at end of test: _____
Results: _____

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

Additional tests (if applicable):
Date of tests: _____
Methods used: _____
Results: _____

Virus concentration (section A.4.3.3.5)

Date of test: _____
Method used: _____
Reference lot no.: _____
Results: _____

Tests for consistency of virus characteristics (section A.4.3.3.6)

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

Item tested: _____
Date of test: _____
Methods used: _____
Results: _____

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

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 or bioburden:	_____	_____	_____	_____

Virus concentration:	_____	_____	_____	_____
Tests for residual cellular DNA:	_____	_____	_____	_____

Final bulk (section A.4.4)

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:	_____	_____	_____	_____
Tests for residual materials:	_____	_____	_____	_____
Storage conditions and period:	_____	_____	_____	_____
Approved storage period:	_____	_____	_____	_____

Filling and containers (section A.5)

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: _____

Control tests on the final lot (section A.6)

Appearance (section A.6.2)

Date of test: _____
Results: _____
Before reconstitution: _____

After reconstitution: _____
Diluent used: _____
Lot number of diluent used: _____

Identity (section A.6.3)

Date test started: _____
Date test ended: _____
Method used: _____
Results: _____
Lot number of reference reagents: _____

Bacterial and fungal sterility (section A.6.4)

Specification	Date test initiated	Method	Results
_____	_____	_____	_____

Diluent used: _____
Lot no. of diluent used: _____

pH (section A.6.5)

Date of test: _____
Method used: _____
Results: _____
Diluent used: _____
Lot number of diluent used: _____

Residual moisture (if applicable) (section A.6.6)

Date of test: _____
Method used: _____
Results: _____

Virus concentration (section A.6.7)

Date titration started: _____
Date titration 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: _____

Thermal stability tests (section A.6.8)

Duration of exposure: _____
Temperature of exposure: _____
Date titration began and ended: _____
Method used for titration: _____

Results

Exposed sample virus titre: _____
Non-exposed sample virus titre: _____
Titre reduction: _____

Lot number of reference virus: _____
Lot number of other reference reagents if used: _____
Diluent used: _____
Lot number of diluent used: _____

Residual antibiotics (if applicable) (section A.6.9)

Date of test: _____
Method used: _____
Results: _____

Stabilizer (if applicable) (section A.6.10)

Date of test: _____
Method used: _____
Results: _____

Diluents (if applicable) (section A.6.11)

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:

_____
_____
_____
_____

Antacid (if applicable) (section A.6.11)

Nature and volume: _____
Lot number: _____

Date of manufacture: _____
Storage conditions and period: _____
Expiry date: _____

Extractable volume (if applicable) (section A.6.12)

Extractable volume (mL): _____
The number of drops, using the approved dropper, in a minimum of five individual final containers: _____

Certification by the manufacturer

Certification by the person from the control laboratory of the manufacturing company taking over all 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 containers, meets all national requirements and/or satisfies Part A[3] of the WHO Recommendation to assure the quality, safety and efficacy of rotavirus vaccines.[4]

Signature: _____

Name (typed): _____

Date: _____

Certification by the NRA/NCL

If the vaccine is to be exported, attach the model NRA/NCL Lot Release Certificate for live attenuated rotavirus vaccine (oral) (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.

[3] With the exception of provisions on distribution and transport, which the NRA or NCL may not be in a position to assess.
[4] WHO Technical Report Series, No. XXXX, Annex 2.

Appendix 2

Model NRA/NCL Lot Release Certificate for the release of live attenuated rotavirus vaccines (oral)

This certificate is to be provided by the NRA or NCL of the country in which the vaccine has been manufactured, on request by the manufacturer.

Certificate no. _____

The following lot(s) of live attenuated rotavirus vaccine (oral) produced by _____[5]

in _____,[6] whose lot numbers appear on the labels of the final containers, meet all national requirements[7] and Part A[8] of the WHO Recommendations to assure the quality, safety and efficacy of rotavirus vaccines,[9] and comply with WHO good manufacturing practices for pharmaceutical products: main principles;[10] WHO good manufacturing practices for biological products;[11] and the WHO Guidelines for independent lot release of vaccines by regulatory authorities.[12]

The release decision is based on _____[13]

Final lot number _____

Number of human doses released in this final lot _____

Expiry date _____

The certificate may include the following information:

[5] Name of manufacturer.
[6] Country of origin.
[7] If any national requirements have not been met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the NRA or NCL.
[8] With the exception of provisions on distribution and shipping, which the NRA or NCL may not be in a position to assess.
[9] WHO Technical Report Series, No. XXXX, Annex 2.
[10] WHO Technical Report Series, No. 986, Annex 2.
[11] WHO Technical Report Series, No. 999, Annex 2.
[12] WHO Technical Report Series, No. 978, Annex 2.
[13] Evaluation of the product-specific summary protocol, independent laboratory testing and/or specific procedures laid down in a defined document, and so on as appropriate.

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

The Director of the NRA/NCL (or other appropriate authority)

Signature: _____

Name (typed):_____

Date: _____