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

Proposed revision of Annex 3 of WHO Technical Report Series No. 941

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this draft is to provide information about the proposed document- *Recommendations to assure the quality, safety and efficacy of live attenuated rotavirus vaccines- Proposed revision of Annex 3 of WHO Technical Report Series No. 941,* to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. **Written comments proposing modifications to this text MUST be received by 29 February 2024 in the Comment Form available separately** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Health Products Policy and Standards. Comments may also be submitted electronically to the Responsible Officer: **Dr Tiequn Zhou at email: zhout@who.int**.

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

Recommendations published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance 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 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 guidance set out below.

Contents

Abbreviations

$CCID_{50}$	cell culture infectious dose 50%
ECBS	WHO Expert Committee on Biological Standardization
ELISA	enzyme-linked immunosorbent assay
FFU	focus forming unit
GCP	good clinical practice
GLP	good laboratory practice
GMO	genetically modified organism
GMP	good manufacturing practice(s)
HTS	high-throughput sequencing
IgA	immunoglobulin A
IgG	immunoglobulin G
LMIC	low- and middle-income countries
MCB	master cell bank
NAT	nucleic acid amplification technique
NCL	national control laboratory
NRA	national regulatory authority
PCR	polymerase chain reaction
PFU	plaque forming units
PRNT	plaque reduction neutralization test
RT-PCR	reverse transcription polymerase chain reaction
rcDNA	residual cellular DNA
RNA	ribonucleic acid
RVGE	rotavirus gastroenteritis
SAGE	WHO Strategic Advisory Group of Experts on Immunization
TSE	transmissible spongiform encephalopathy
VMS	virus master seed
VWS	virus working seed
WCB	working cell bank

Introduction

The World Health Organization (WHO) Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines (oral) were first adopted by the WHO Expert Committee on Biological Standardization in 2005 and published in Technical Report Series (TRS) No. 941 in 2007 (1). Developments since 2005 include the licensure of the first two live attenuated rotavirus vaccines in Europe, the United States of America, 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. At least two other live rotavirus vaccines, one in China and one in Vietnam, have been licensed and widely used in the country of manufacture but not yet prequalified by WHO (2). Other candidate rotavirus vaccines are in development including non-replicating rotavirus vaccines (3,4) but they are not licensed yet. Since 2005 WHO has published new or revised overarching general guidance documents in its Technical Report Series on various aspects of vaccines (available on WHO website: *https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/vaccine-standardization/*).

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

In light of the developing experience with the available rotavirus vaccines and advances in the relevant fields, it was proposed that the WHO Guidelines for live attenuated oral rotavirus vaccines (1) should be updated. WHO convened a virtual informal consultation meeting during 15 - 17 November 2022 which was attended by experts and representatives from academia, national regulatory authorities (NRAs), national control laboratories (NCLs), industry and other international health organizations and institutions from countries around the world to discuss and reach consensus on the issues for the TRS revision (5). WHO set up a drafting group composed of regulatory experts from several countries to prepare the draft revision of the 2005 Guidelines.

These updated recommendations (formally guidelines) should be taken into account in the development and manufacture of current and future rotavirus vaccines. In view of the interest in developing non-replicating rotavirus vaccines some relevant issues were also included in this document in particular in Part B and Part C, although the scope of the revision mainly concerns about live attenuated rotavirus vaccines.

The major issues addressed in this revision include updates of:

- General considerations and other sections to reflect the developments and advancements in relevant fields;
- Terminology;
- Part A, to reflect up-to-date practice of the production and control of live attenuated rotavirus vaccines;

- Part B, to provide guidance for pharmacological evaluation of new candidate rotavirus vaccines built on different platforms, as well as to elaborate regulatory considerations for toxicological testing including the risk of intussusception;
- Part C, to provide guidance on the design of future trials, including in the context of available licensed rotavirus vaccines, and for different types of vaccines;
- Part D and appendices; and
- References.

Additional changes have also been made to bring the document into line with other WHO Recommendations, Guidelines and guidance documents published since the 2005 adoption of the WHO Guidelines on rotavirus vaccines.

Purpose and scope

These WHO Recommendations provide guidance to NRAs/NCLs and vaccine manufacturers on the quality, nonclinical and clinical evaluation 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. There is a great deal of scientific effort aimed at developing non-replicating rotavirus vaccines but no such vaccine is licensed at the time of writing. While the manufacturing and quality control guidance provided in Part A is focussed on live attenuated rotavirus vaccines, the nonclinical section (Part B) and clinical section (Part C) provide general guidance on all types of candidate rotavirus vaccines including live attenuated and non-replicating rotavirus vaccines. It is perceived that when more experience becomes available in future on non-replicating rotavirus vaccines development, specific guidance on that class of rotavirus vaccines will be provided.

There are also many WHO guidance documents dealing with various other platforms that may be relevant to the development of non-replicating rotavirus vaccines including:

- inactivated vaccines (6-8)
- protein antigens produced by recombinant technology (9-12)
- virus-like particle vaccines (13)
- DNA vaccines (14)
- messenger RNA vaccines (15)
- vectored vaccines (16)

The principles in these general guidance documents should be considered when applicable.

This document should be read in conjunction with current WHO guidance documents on the nonclinical (17) and clinical (18) evaluation of vaccines, good manufacturing practices for biological products (19), good manufacturing practices for sterile pharmaceutical products (20), characterization of cell banks (21), lot release (22), as well as relevant WHO guidance on 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 vaccine adjuvant is a substance, or combination of substances, that is used in conjunction with a vaccine antigen to enhance (for example, increase, accelerate, prolong and/or possibly target) the specific immune response to the vaccine antigen and the clinical effectiveness of the vaccine.

Adventitious agents: contaminating microorganisms of the cell 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 or 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, such as in 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 infections.

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 a common 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 cell monolayers that is 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 working seed lot and used for inoculation of several successive lots of production cell cultures to manufacture the desired drug substance lots of virus 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 units (PFU): the smallest quantity of a virus suspension that will lyse host cells and cause a single visible focus of infection in cell monolayer.

Production cell culture: a cell culture derived from one or more ampoules of the working cell bank or primary tissue used for the production of vaccines.

Single harvest: a quantity or virus suspension of one virus type 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 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 of uniform composition derived from the virus master seed lot by a limited number of passages and fully characterized. The virus working seed lot is used for production of vaccine.

Working cell bank (WCB): a quantity of cells of uniform composition derived from one or more ampoules of the MCB at a finite passage level, dispensed in aliquots into individual containers, cryopreserved and stored frozen under defined conditions (such as in 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). No specific antiviral therapy is currently available against rotaviruses and the only clinically effective intervention once severe symptoms develop is rehydration therapy. The first infection with rotavirus has the greatest impact. The incubation period for rotavirus infection is short and is estimated to be less than 48 hours. 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). Virus may be present at 10^{11} virus particles per gram of stool and the infectious dose is estimated to be 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. The consequences of infection depend on the economic circumstances and are most serious in low-income countries without access to health care systems. Rotavirus disease is the main cause of infant deaths from diarrhoeal disease globally (24) and deaths are most common in Africa and Southeast Asia, which account for approximately half the global total. Effective vaccines are therefore a high priority.

A review of cases reported through the Global Pediatric Diarrhea Surveillance network concluded that in 2018 the number of deaths was approximately 200,000, representing a reduction of about 40% compared to numbers before vaccination. However, rotavirus remains the main cause of mortality due to infant diarrhoea (24).

The virus

Rotavirus is a non-enveloped, double-stranded RNA (dsRNA) virus, belonging to the family Reoviridae, with a triple shelled virion containing a genome of eleven segments. These segments encode for six viral structural proteins (VP1 to VP4, VP6, and VP7) and six non-structural proteins (NSP1 to NSP6). Each genome segment, with the exception of gene 11 (encoding 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 ELISA-based antibody assays.

The G and P proteins are classified on the basis of their antigenic and molecular properties. Overall, 36 G types and 51 P types have been recognised, of which 6 G types (G1, G2, G3, G4, G9, and G12) and 3 P types (P4, P6 and P8) are the commonest in human infections. The distribution of types varies from region to region and to some extent over time (25).

Live attenuated rotavirus vaccines

Live attenuated rotavirus vaccines have been developed using a range of individual 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 including for example only the G1, G9 or G10 serotypes, while others have been multivalent, including G1, G2, G3, G4 and G9 or other serotypes. 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 of a range of types have

been successfully used in clinical trials and in vaccination programmes. The vaccine strains differ 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. In summary each vaccine is unique in its properties.

While 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 that may be 50-60% (26-31). The low vaccine efficacy in low income countries is a complex issue, which is not fully understood (32). Where monovalent vaccines have been used in programmes there has been no evidence of wild type strains replacing the serotypes found in the vaccine implying that protection is not specific for a particular serotype. Rotavirus vaccination has led to substantial reductions in diarrhoeal deaths and hospitalizations (24, 33).

There is currently no animal model that will reflect rotavirus virulence in children so that comparisons of the attenuated phenotypes are possible only in clinical studies at present. The virological properties of the available live attenuated rotavirus vaccines are highly varied including the number and types of 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 this document are generally applicable to all live attenuated rotavirus vaccines , it must be remembered that each candidate is the result of a unique approach in development of an attenuated product and candidates must be examined individually. This raises significant product-specific issues. The widely disparate nature of the licensed and candidate rotavirus vaccines makes this a larger issue for rotavirus vaccines than other live attenuated vaccines.

There is no validated mechanistic correlate of protection for an individual vaccine. However overall secretory IgA antibody and serum neutralizing antibody levels relate to protection after wild type rotavirus infection and are considered a non-mechanistic indication of protection (34-36). 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 (35).

Special considerations

Development of new rotavirus vaccines should take into account experience with one vaccine (RotaShield) which was introduced in the United States of America in August 1998 and was withdrawn less than one year later. An epidemiological relationship was established between vaccination and intussusception, a condition where the gut invaginates and which can prove fatal unless treated. Early estimates suggested a risk of one case per 2500 children immunized. Re-analysis of the case–control study that examined intussusception and RotaShield revealed that the majority of the cases of intussusception 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 should 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 10000 children

immunized (37). The detailed pathogenic mechanisms for intussusception are unclear but are very likely to be complex.

Rotavirus is an acid labile virus which has 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 virus would be inactivated by stomach gastric acid prior to reaching the site of infection in the upper gastrointestinal tract. To prevent inactivation of the virus by gastric acid, antacids or buffers are usually administered before or in combination with the oral rotavirus vaccination. The 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 (38) 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 (39). 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 (40). Nevertheless, the specifics of the manufacturing process and the formulation of a given product should be considered by NRAs (21) and, where possible, data should be accumulated on the levels of rcDNA in oral live attenuated rotavirus vaccines produced in Vero cells or any other cell line.

Cell banks should be characterised and shown to be free of 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 (41-44). 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 (42). 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 adventitious agents that may be inadvertently introduced through the use of raw materials, e.g. animal-derived culture medium components. If necessary, viruses such as bovine polyomavirus, porcine parvovirus or PCV may be screened for using specific assays, such as molecular assays based on nucleic acid amplification techniques (NAT).

International reference materials

A standardised reference preparation of vaccine would be useful in the context of defining the dose of vaccine but in view of the range of live rotavirus vaccine types, their virulence and their growth properties in culture, any reference is likely to be specific for a particular vaccine. It is therefore not feasible to develop such international reference materials to standardize virus content between vaccine types. Common materials might nonetheless be useful in developing and comparing infectivity assays

Similarly, antibody references are useful in controlling the differences between assays due to variation in their execution. Rotavirus immune assays differ a great deal from each other in the source of the antigen and the basis of the assay including the strain of virus used as well as the format and nature of the assay, such as the cell used where the assay is for neutralization or the design of the ELISA assay and the precise antigen to which it is directed. Universal reagents for rotavirus vaccines or serological assays are difficult to design at this stage.

Nevertheless, reference materials could be helpful in establishing and validating immune assays and comparing responses to different vaccine types.

Part A. Recommendations on the manufacturing and control of live attenuated rotavirus vaccines

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) of 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 satisfy the specifications formulated below.

A.1.2 Descriptive definition

A live attenuated rotavirus vaccine (oral) is a sterile preparation containing one or more live attenuated rotavirus strains, which could be of different serotypes and have been grown through a seed lot system, prepared in a suitable approved cell substrate, formulated in a form suitable for oral administration and satisfying all of the recommendations set out in this document, as applicable.

A.2 General manufacturing recommendations

The general manufacturing recommendations contained in WHO good manufacturing practices for pharmaceutical products: main principles (45) and WHO good manufacturing practices for biological products (19), and WHO good manufacturing practices for sterile pharmaceutical products (20) should apply 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 according to the principles of the latest WHO Laboratory biosafety manual (46) and should follow the containment criteria. The basis for this is a microbiological risk assessment which results in the classification of activities into different biosafety levels. The respective classification level should be approved by the relevant authority of the country/region in which the manufacturing facility is located. Live attenuated rotavirus vaccines will be given to large numbers of healthy infants so the biological risk should be extremely low. However, production must still be appropriately contained, in this case to prevent contamination of the product by the environment and workers rather than vice versa.

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

Whenever in vivo tests are performed during vaccine development or manufacturing, it is desirable for ethical reasons to apply the 3Rs principles (Replacement, Reduction, Refinement) to minimize the use of animals where scientifically appropriate (47).

In general, separate areas or a campaigned programme for the manufacturing of different virus serotypes are required. However, if the manufacturer can demonstrate and validate effective containment and decontamination of the live microorganisms and viruses; then the use of multi-product facilities 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 is provided. More guidance on campaign production and containment can be found in the WHO good manufacturing practices for biological products (19).

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 be inadvertently 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 on application to the 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.

The master cell bank, which is made in sufficient quantities and stored in a secure environment is used as the source material to make manufacturer's working cell banks. In normal practice a master cell bank is expanded by serial subculture up to a passage number (or population doubling,

as appropriate) selected by the manufacturer and approved by the NRA, at which point the cells are combined to give a single pool distributed into ampoules and preserved cryogenically to form the WCB.

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

A.3.1.2 Identity test

Identity tests on the MCB and WCB should be performed in accordance with *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.3.1.3 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 (48) and 5.3 (49) 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 bank (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 current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (50).

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 (51). In addition, human albumin, as with all materials of animal origin, should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (50).

Manufacturers are encouraged to explore the possibilities of using serum-free media for the production of 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 current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (50).

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, e.g. phenol red at a concentration of 0.002%.

Only substances that have been approved by the NRA may be added.

A.3.2 Virus strains and seed lot system

A.3.2.1 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 with the desired serotypes or in other cases by multiple passages of human rotavirus in cell culture. The seed lot viruses should comply with the specifications of this section. Development of the rotavirus strains to be used for vaccines 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, potential method of attenuation, whether the strains have been 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, based upon the quantity of infectious virus of each serotype present in the vaccine that induces seroconversion when susceptible individuals are immunized with the vaccine, should be established in a dose–

response study. Any potential interference or potentiation between the serotypes in an infectivity assay should be evaluated prior to establishing this value. The immunizing dose established in this way serves as a basis for establishing parameters for potency at the time of release, stability and expiry date. See Part B and Part C.

- Live-attenuated rotavirus strains may be derived by recombinant DNA. The entire nucleotide sequence of any complementary DNA (cDNA) 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 a GMO and should comply with the regulations of the producing and recipient countries regarding GMOs**.**

Only virus strains that are approved by the NRA and that yield a vaccine complying with the recommendations set out in these WHO Guidelines 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.3.2.2 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 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 arrangement should ensure appropriate security of the virus seed lots.

A.3.2.3 Tests on virus master and working seed lots

A.3.2.3.1 Identity

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

A.3.2.3.2 Genotype/phenotype characterization

The genotypic stability of the virus seed on passage should be assessed. Phenotypic stability may provide additional information, however markers for attenuation are still in development and are probably specific to the particular vaccine 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.3.2.3.3 Tests for bacteria, fungi and mycoplasmas

Each virus seed lot should be tested for bacterial, fungal, and mycoplasmal contamination using appropriate tests, as specified in Part A, sections 5.2 (48) and 5.3 (49) of the WHO General requirements for the sterility of biological substances, or by methods approved by the NRA.

Nucleic acid amplification techniques , either alone or in combination with cell culture and with an appropriate detection method, may be used for mycoplasma detection after suitable validation and agreement with the NRA.

A.3.2.3.4 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 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 this test provides a risk mitigation taking into account the overall testing package. The animals used might include guinea-pigs and suckling mice as appropriate; 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).

For ethical reasons it is desirable to apply the 3Rs principles (Replacement, Reduction, Refinement) to minimize the use of animals where scientifically appropriate (47).

New molecular methods with broad detection capabilities are available for the detection of adventitious agents. These methods include: (a) degenerate nuclei acid testing for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (b) NAT with random primers followed by analysis of the amplicons on large oligonucleotide 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.3.2.3.5 Virus concentration

Each seed lot should be assayed for infectivity in 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. The assay is based on the visualization of infected areas (plaques or focus of infection) of a cell monolayer directly or by probing with rotavirus-specific antibodies. Results should be recorded as plaque-forming units (PFU/mL) or focus forming units (FFU/mL).

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

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

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

Because of the diversity of rotavirus vaccines produced by different manufacturers such as in the composition, strains, biological properties and formulation, it is unlikely that International Standards will be suitable for the standardization of assays of vaccines from all manufacturers. 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 should be replaced when necessary. A procedure for replacing reference vaccines should be in place with the agreement of the NRA (52).

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 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 −60 °C or below.

A.4.1.2 Tests for haemadsorbing viruses

At the end of the observation period, at least 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter cells have been stored, the duration of storage should not have exceeded 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.

A reading should be taken after incubation at 2–8 °C for 30 minutes, and again after further 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 for 30 minutes at 34–37 °C.

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 +/- 1º C as that of 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 or HTS) (21).

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

A.4.1.4 Identity 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 (e.g., isoenzyme analyses), immunological tests, cytogenetic marker tests (e.g. for chromosomal markers), and tests for genetic markers (e.g. 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 infection, samples of each cell culture are removed for sterility and mycoplasma testing.

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

A.4.2.2 Tests for bacteria, fungi and mycoplasmas

A volume of at least 20 mL of the pooled supernatant fluids from the production cell culture should be tested for bacterial, fungal, mycoplasmal and mycobacterial sterility using appropriate tests, as specified in Part A, sections 5.2 (48) and 5.3 (49) of the WHO General requirements for the sterility of biological substances, or by methods approved by the NRA.

NATs, either alone or in combination with cell culture and with an appropriate detection method, may be used for compendial mycoplasma detection after 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 range of multiplicity of infection, temperature, pH and time period of incubation will depend on the vaccine strain and production. A defined range should be established by the manufacturer and be approved in the marketing authorization by the 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 ampoules 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.

Samples of monovalent virus pools should be taken for testing and if not tested immediately should be stored at a temperature of –60 °C or below. 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. If the tests are done on the virus pool, the protocol should be approved by the NRA.

A.4.3.3.1 Sampling

Samples required for the testing of virus harvests should be taken immediately on harvesting prior to further processing. If the tests for adventitious agents as described in Part A, section A.4.3.3. 4, are not performed immediately, the samples taken for these tests should be kept at a temperature of –60 ºC or below and subjected to no more than one freeze–thaw cycle. 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 by a molecular based assay, e.g. reverse transcription PCR (RT-PCR), DNA sequencing (such as Sanger or HTS). The tests should be validated by the manufacturer and approved by the NRA.

A.4.3.3.3 Sterility tests for bacteria, fungi and mycoplasmas

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 (48) and 5.3 (49) of the WHO General requirements for the sterility of biological substances, 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 rotavirus is necessary for many tests because the virus is cytopathogenic. Antisera used for this purpose should be shown to be free from antibodies that may neutralize the adventitious viruses being tested for. If neutralization of rotavirus is not possible the test sample may be passaged in trypsin-free media prior to initiating the assay, to reduce the ability of rotavirus to infect the indicator cell substrates. The cells inoculated should be

observed microscopically for cytopathic changes. At the end of the observation period, the cells should be tested for haemadsorbing viruses.

Additional testing for specific adventitious viruses may be performed, for example any of the new molecular methods with broad detection capabilities (such as HTS, microarrays).

A.4.3.3.5 Virus concentration

Each virus pool should be assayed for infectivity using a sensitive assay in cell cultures to monitor the consistency of production. See A.3.2.3.5.

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. Examples of studies that might be considered to characterize rotavirus are given here. Tests should be sought to compare the rotavirus in the harvest pool with the master seed virus, or suitable comparator, to ensure that the vaccine virus has not undergone critical changes during its multiplication in the production culture system. Phenotypic or genotypic characteristics (genomic sequence analysis) may be suitable. Examples of evidence to support the consistent quality of the virus produced may include in vitro growth characteristics, thermal stability profile, the ratio of infectious (triple shelled) to non-infectious (double shelled) particles produced, 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, residual cellular DNA, endotoxin, bovine serum, trypsin and antibiotics. Their reduction during processing can be monitored to assess consistency of the manufacturing process. The reduction level should be approved by the NRA.

Once consistency of production process has been established to reduce the impurities to acceptable levels and the drug substance meets the acceptance criteria consistently, these tests for impurities may be omitted from routine lot release after approval by 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 (bulk)

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

A.4.3.4.1 Sampling

Samples of the clarified virus pool should be taken immediately after clarification and prior to further processing to ensure that no cells or cell debris is left. Samples should also be tested as described in this section. If not tested immediately, the samples should be kept at a temperature

below –60 °C until testing is done. 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 (48), or by methods approved by the NRA. However, in agreement with the NRA, a bioburden test with a low bioburden limit (e.g., not more than 10 CFU/100 mL) may be acceptable, provided that a bacteria-retentive filtration step is performed prior to storage if applicable, and that adequate measures are in place to avoid contamination and growth of microorganisms during storage of the intermediate.

A.4.3.4.3 Virus concentration

Each clarified virus pool should be assayed for infectivity in a sensitive assay in cell cultures to monitor the consistency of production. See section A.3.2.3.5.

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 residual cellular DNA and the purification procedure should have been shown to consistently reduce the level of residual host cell DNA (21). Consideration should also be given to determining the size of residual cellular DNA as part of the validation process. The limit should be established by the manufacturer and approved by the NRA.

These tests may be omitted from routine 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 should be sterile and prepared from one or more serotypes each derived from one or more virus pools obtained from substrates of which control cultures pass the tests specified in Section A.4.1. The process used to prepare the final bulk should incorporate sterile filtration steps and aseptic process based on the principles and guidance contained in the current WHO good manufacturing practices for sterile pharmaceutical products (20). The virus concentration in the final formulation should be sufficient to ensure the dose which is consistent with that shown to be safe and effective in human clinical trials. The virus pools and final bulk should pass the tests specified in Sections A.4.3.3 and A.4.4.1.

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

In preparing the final bulk, any substance such as diluents or stabilizers that is added to the product should have been shown to the satisfaction of the NRA not to impair the safety and efficacy of the vaccine in the concentration used.

A.4.4.1 Tests on the final bulk

A.4.4.1.1 Test 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, DNases, and residual cellular DNA– are consistently reduced to a level acceptable to the NRA.

A.4.4.1.2 Bacterial and fungal sterility

Each final bulk suspension should be tested for bacterial and fungal sterility. Sterility testing should be carried out as specified in the WHO General requirements for the sterility of biological substances (48, 49) 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 (45) 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 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, it should be compliant with the WHO Policy Statement: multi-dose vial policy (53). The multi-dose container should prevent microbial contamination of the contents after opening. The extractable volume of multi-dose vials should be validated and in use stability studies should be provided.

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

This section provides general requirements for final containers (final lot), not product administration devices. There are multiple options for administration devices (e.g. syringes, squeezable tubes, droppers) for rotavirus vaccines which should comply with relevant

requirements. Any information related to vaccine administration devices should be included in the product packaging label and considered in a case-by-case by each 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 have acceptable performance 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 discussed in this section.

A.6.1 Vaccine

A.6.1.1 Inspection of final containers

Each container in each final lot should be inspected visually and/or in an automated manner, and those showing abnormalities (for example, improper sealing, clumping or the 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 batch failure.

A.6.1.1.1 Appearance

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

A.6.1.2 Identity

The virus in one or more individually labelled final containers should be identified as rotavirus and, for multivalent vaccine formulations each serotype should be identified by appropriate methods approved by the NRA, such as immunoassays in cell culture suitable to identify the presence of a specific rotavirus serotype included in the vaccine.

A.6.1.3 Bacterial and fungal sterility

Liquid or reconstituted vaccine should be tested for bacterial and fungal sterility. Sterility testing should be carried out as specified in the WHO General requirements for the sterility of biological substances (48, 49) or by an alternative method approved by the NRA.

A.6.1.4 pH

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

A.6.1.5 Residual moisture (if applicable)

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

A.6.1.6 Virus concentration

The virus concentration in each of at least three final containers of the rotavirus vaccine final lot should be assayed individually for infectivity in a sensitive assay system in which interference or potentiation between the serotypes present in the vaccine does not occur. See section A.3.2.3.5.

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 a reference preparation of live attenuated rotavirus vaccine for use in tests to determine virus concentration.

Freeze-dried vaccine should be reconstituted with its diluent to determine virus concentration. A validated alternative diluent may be needed if the approved diluent is not suitable for the execution of the assay. If a different diluent is used for this test, data to allow a comparison between the results with both diluents should be submitted for the approval of the NRA.

Virus concentration limits, both minimum and maximum, should be established by the manufacturer taking into account the vaccine dose shown to be safe and effective in human clinical trials, and be agreed with the NRA. Specifications for virus concentration should essentially specify the minimum and maximum titre guaranteed to be contained in one human dose and this should be agreed with the NRA.

A.6.1.7 Thermal stability

Thermal stability should be considered as a vaccine characteristic that provides an indicator of production and shelf-life consistency of 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 (54).

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 specific 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.6.1.8 Residual antibiotics (if applicable)

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

A.6.1.9 Stabilizer (if applicable)

If a stabilizer is added during vaccine production, the content of the stabilizer present in the vaccine should be determined and should be within limits approved by the NRA.

A.6.2 Diluents (if applicable)

The requirements given in Good manufacturing practices for pharmaceutical products: main principles (45) should apply for the manufacturing and control of diluents used to reconstitute live attenuated rotavirus vaccines and, if required, the antacid buffer used. An expiry date should be established for the diluent based upon stability data. If an antacid is to be used, the stability of the rotavirus in the presence of the antacid should be confirmed. For lot release of the diluent, tests for identity, appearance, pH, volume, sterility, and the content of key components should be done.

A.6.3 Extractable volume (if applicable)

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

A.7 Records

The requirements given in WHO good manufacturing practices for biological products (19) should apply.

A.8 Retained samples

The requirements given in WHO good manufacturing practices for biological products (19) should apply.

A.9 Labelling

The requirements given in WHO good manufacturing practices for biological products (19) should apply.

The label on the carton enclosing one or more final containers, or the leaflet accompanying the 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 type contained per human dose
- the cell substrate used for the preparation of the vaccine
- A statement that the vaccine should be administered orally
- a statement of the nature and amount of the antibiotics present in the vaccine, if any
- 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 rotavirus vaccine with other oral vaccines and non-orally administered vaccines
- a statement concerning 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 is reconstituted, it should be used without delay, or if not used immediately, stored under defined conditions and in the dark for a maximum period defined by stability studies
- a statement concerning storage conditions (temperature), expiry date, volume and instructions for reconstitution
- if applicable, a statement describing whether an antacid is to be given prior to or in combination with the vaccine at the time of vaccination.

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

Unused vaccine should be disposed of as specified in the WHO GMP guidelines and biosafety manual (19, 46).

A.10 Distribution and shipping

The requirements given in WHO good manufacturing practices for pharmaceutical products: main principles (45) and WHO good manufacturing practices for biological products (19) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (55).

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 (54) and WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (56). 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, 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 (54).

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 condition, real time stability data and approved by the NRA.

A.11.2 Storage conditions

Before being released by the manufacturing establishment 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 a 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 still 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.

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

Part B. Nonclinical evaluation of rotavirus vaccines

This section addresses the pharmacological and toxicological assessment of a new candidate rotavirus vaccine. Currently, all licensed rotavirus vaccines are live attenuated vaccines. No non-replicating rotavirus vaccine is licensed at the time of writing although there is a great deal of interest in their development. Therefore Part B of current document is intended to provide guidance on nonclinical evaluation of candidate live attenuated rotavirus vaccines and non-replicating rotavirus vaccines.

The guidance provided in this section 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 (57), if applicable. In addition, the WHO guidelines on DNA and RNA vaccines (14,15) and the regional documents on live recombinant viral-vectored vaccines (58) 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). As such, protection against a challenge with human rotavirus would be the preferable readout of protection. Small animals such as mice or rabbits (60-62) are not susceptible to infection with human rotavirus strains although they can be used for studies of immune responses to vaccine strains and are used in vaccine development. Although the gnotobiotic piglets are well-known to be susceptible to human rotavirus infections and able to develop diarrhea upon challenge with human rotaviruses (63-65), use of such large animals is limited for practical reasons, including high cost, limited accessibility, as well as a need for specialized equipment, facilities and staff. Further research is encouraged to develop a suitable animal model that can be economic, tractable and commonly used in a laboratory setting. No recommendation on the animal challenge-protection studies can be made at this point of time.

Primary pharmacodynamics (immunogenicity) studies should be carried out in relevant species (e.g. mice, rats, guinea pigs, 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, the ROA and its putative mechanism(s) of action, the immunological parameters to be measured may include the humoral, cellular, and functional immune responses, as appropriate (e.g. IgG and IgA antibodies, B cells, or T cells, in the circulation and in the fecal specimens), to each rotavirus antigen included in the vaccine. Given the importance of heterotypic immunity witnessed for live oral rotavirus vaccines, it is recommended that studies that evaluate immune function include an evaluation of immune responses to diverse types of human rotaviruse. It is essential that the analytic methods employed for these studies should demonstrate their suitability 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 inactivated rotavirus vaccine) is formulated with an adjuvant, it is important that the studies evaluate vaccine formulations with and without the adjuvant(s), to justify the inclusion of the adjuvant(s) in the vaccine formulation (57). For a new combination vaccine that is designed to contain the rotavirus antigen(s) and other antigens derived from other infectious diseases, immune interference is a pertinent issue and should be addressed adequately in animals.

B.2 Pharmacokinetics

Studies to determine serum or tissue concentrations of vaccine components are normally not needed. However, the understanding of distribution, quantity, and clearance of the administered vaccine components following administration can be helpful in case of using novel adjuvants, new formulations, alternative routes of administration, or novel vectors (17, 57, 58).

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 (57), as applicable. Also, in line with the Regulation for replacement, reduction, and refinement of animals (66), the assessment of local tolerance, single-dose toxic effects, and safety pharmacology endpoints, where appropriate (57), should be incorporated in the design of a repeated dose toxicity study.

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 quality attributes, including impurity profile.

The use of live oral rotavirus vaccines has been associated with a small (or rare) 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 needed if the live oral rotavirus vaccine candidates have never been passaged in tissues of the central nervous system. Similarly, the examination of reproductive and developmental toxicity is not relevant to rotavirus vaccines, since the vaccination of humans with rotavirus 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 (57).

B.4 Environmental risk assessment

The live oral rotavirus vaccine or the replicating rotavirus vaccine that is based on GMO poses a potential risk of spread to a third party, i.e. unvaccinated humans and/or animals, if the vaccine organism is substantially shown to be 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 vaccine organisms 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 (67). General guidance on vaccine clinical development programmes is provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (18) and is not repeated here.

This section addresses 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 types. The guidance is generally applicable to candidate rotavirus vaccines but there are some specific considerations according to the route of administration (i.e. oral or parenteral) and the vaccine construct (i.e. live attenuated, live reassortant or non-live vaccines). The guidance assumes that candidate rotavirus vaccines will be intended for the prevention of RVGE in infancy and that the aim will be to generate data to support administration of the first dose as early in life as possible.

C.2 Safety and immunogenicity studies

In the initial studies that explore the safety and immunogenicity of the candidate vaccine, and regardless of the route of administration, sera obtained from vaccinees may be assayed to determine:

- Serum neutralizing antibody (SNA) titres using a plaque reduction neutralisation test (PRNT) that uses a defined percentage reduction endpoint with results reported as PRNT titres or SNA determined using an enzyme immunoassay
- Serum rotavirus-specific IgG and IgA

For live candidate vaccines that are developed 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 the vaccinees should be assessed during the clinical development programme (see section C.6). Furthermore, the sponsor should develop a method to differentiate the vaccine strain from wild-type strains in faeces to facilitate case detection in efficacy studies.

C.3 Dose and regimen

There is no established immune correlate of protection for 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 suffice 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. If the candidate vaccine is administered orally, sponsors may also consider attempting to document rotavirus-specific IgA in faeces and/or sera.

Consideration should be given to the need for, and feasibility of, a dose-finding study in infants with selected regimens that has an endpoint of RVGE.

C.4 Vaccine efficacy against RVGE

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

Due to the widespread recommendations for use of the licensed vaccines for prevention of RVGE in infancy, and due to the observed efficacy and effectiveness of these vaccines, it is not expected that placebo-controlled clinical efficacy studies are 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 vaccine for which efficacy was established in a placebo-controlled study.

However, this approach would require that the same primary endpoint is applicable to the candidate and reference (licensed) vaccine and that a robust and well-justified non-inferiority margin can be determined. There are several potential difficulties with such an approach, which include, but are not limited to, the following considerations both for study design and for determining an appropriate non-inferiority margin:

The primary analyses of efficacy of the licensed live, oral vaccines concerned protection against RVGE due to the rotavirus type(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 types as possible. A study that aims to show non-inferiority for efficacy against RVGE due to rotavirus type(s) for which the efficacy of the licensed vaccine is not known or is estimated to be sub-optimal is not an appropriate basis for licensure.

Secondary analyses in the efficacy studies for licensed vaccines examined prevention of RVGE due to any rotavirus type as well as efficacy against specific rotavirus types included in the vaccine and types 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 that compares the efficacy of a candidate and reference vaccine against RVGE due to any rotavirus type and/or against selected rotavirus types.

The placebo-controlled efficacy studies conducted with the 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 between the initial 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 (e.g. general health and level of nutrition) and with concomitant infections (e.g. helminthic infections). Therefore, it is not possible to select a valid non-inferiority margin for a comparative efficacy study performed in a population that is different to that included in any one placebo-controlled study that was conducted with the reference vaccine.

There is also the issue of change in background factors with time. For example, the factors that led to the geographical variation in vaccine efficacy observed in the prior placebo-controlled studies with licensed vaccines are unlikely to apply to a similar extent to a population enrolled into a prospective comparative efficacy study in the same geographical location(s) at later time. This adds to the many difficulties of identifying a relevant and robust non-inferiority margin.

Due to these issues, amongst others, it is recommended that the primary objective of comparative vaccine efficacy studies is to demonstrate superiority in prevention of RVGE for a candidate vaccine (regardless of construct and route of administration) to a licensed vaccine for which absolute vaccine efficacy against RVGE due to vaccine strains has been documented. In this setting, all infants randomized to the control group will still receive a licensed vaccine that is currently standard of care. Since study success is based on superiority in preventing RVGE, it does not matter if the efficacy of the licensed vaccine is not known or is estimated to be sub-optimal against certain rotavirus types and/or in certain populations.

The primary endpoint for such a study will depend on the composition of the candidate vaccine and what is expected from it in terms of rotavirus type-specific protection against RVGE. Thus, if the vaccine is designed to provide protection against specific rotavirus types, the primary endpoint could be RVGE due to these rotavirus types, with a secondary analysis based on all RVGE.

However, if it is anticipated that the candidate vaccine can confer protection against a very broad range of rotavirus types, the primary endpoint could be RVGE due to any rotavirus type with secondary analyses of efficacy against specific rotavirus types.

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 includes 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 kinetic 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 efficacy against mild/moderate vs. 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 over at least one year from the last dose of vaccine.

Hospitalization is not appropriate for defining a case and/or its severity because 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 healthcare professionals could be designated as secondary or exploratory endpoints.

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 there was no preliminary efficacy study conducted with the candidate vaccine (i.e. 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 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 made public. 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

Live rotavirus vaccines have been incorporated into routine childhood immunization programs based on the 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 into pivotal efficacy studies.

C.6 Vaccine safety

Due to the experience with an initial reassortant rotavirus vaccine, the live orally-administered rotavirus vaccines that were developed subsequently underwent pre-licensure assessments of the risk for vaccine-attributable intussusception. These studies provided an estimate of the relative and absolute risk compared to placebo together with 95% confidence intervals that gave an idea of the degree of risk that could not be excluded. Post-marketing safety surveillance followed, suggesting that the risk of vaccine-associated intussusception is far outweighed by the benefit in terms of prevention of RVGE in infants.

It is no longer possible to conduct such pre-licensure, placebo-controlled studies. Furthermore, 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 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 program should include an assessment of the risk for transmission of the vaccine virus(s), 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). If the vaccine is likely to be used in regions where there are substantial numbers of HIV-infected infants, sponsors should consider conducting studies that assess safety, immunogenicity and risk of transmission in this specific sub-population.

WHO published specific guidance on post-marketing surveillance of rotavirus vaccine safety (68) which 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 (69) 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 has 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 and 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, and reagents from the manufacturer to be used for lot release until the international or national standard preparation 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 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, 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 Guidelines. 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 vaccines.

A model NRA/NCL Lot Release Certificate is provided below in Appendix 2.

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Appendix 1

Model summary protocol for the manufacturing and control of live attenuated rotavirus vaccine (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 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 (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 cultures for virus production

Cell banks (section A.3.1)— 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 ampoules 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 ampoules: _____

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

Percentage of total cell bank ampoules tested: _____

Identification 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 ℃	_____	_____	_____	_____	_____
30–36 ℃	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Tests 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): _____

Tests for retroviruses (if applicable):

Date of test: _____

Method used: _____

Results: _____

Cell culture medium

Serum used in cell culture medium

Animal origin of serum: _____
Batch number: _____
Vendor: _____
Country of origin: _____
Certificate of freedom from TSE (yes/no): _____
Tests performed on serum: _____
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.3.2)— every submission

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: _____

Information on seed lot preparation (section A.3.2.1 & A.3.2.2)—every submission
Virus master seed lot (VMS) and virus working seed (VWS)

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.3.2.3)— 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 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 ℃	_____	_____	_____	_____	_____
30–36 ℃	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Tests 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 controls: _____

Date of staining: _____

Results: _____

Tests for adventitious agents:

Date(s) of satisfactory test(s) for freedom from adventitious agent: _____

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 survived 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 on control cell culture:

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:

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:

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 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: _____

Identity test:

Date of test: _____

Method used: _____

Results: _____

Cell cultures for vaccine production (section A.4.2)

Tests for adventitious agents

Date of examination (inoculation): _____

Results: _____

Tests for bacteria, fungi and mycoplasmas

Date and volume of sampling: _____

Volume of samples tested: _____

Tests for bacteria and fungi

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 ℃	_____	_____	_____	_____	_____
30–36 ℃	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Tests 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 controls: _____

Date of staining: _____

Results: _____

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

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

Virus serotype

Lot number of single harvest: _____
Date of virus inoculation: _____
Multiplicity of infection: _____
Incubation conditions: _____
Date of harvesting: _____
Volume harvested: _____
Date of sampling: _____
Volume of sampling: _____
Storage conditions and period: _____

Monovalent virus pool (pre-clarification)

Lot number of virus pool: _____
Date of pooling: _____
Virus single harvests pooled:

Lot number	Volume pooled
_____	_____

Volume of virus pool after pooling: _____
Date of sampling: _____
Volume of sampling: _____
Storage conditions and period: _____

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

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

Identity

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

Sterility 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 ℃	_____	_____	_____	_____	_____
30–36 ℃	_____	_____	_____	_____	_____

Negative control_____ _____ _____ _____ _____

Tests 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 controls: _____

Date of staining: _____

Results： _____

Tests for adventitious agents

Volume of samples for neutralization and testing： _____

Batch number(s) of antiser(a)um 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
Date of test: _____
Method used: _____
Reference lot no.: _____
Results: _____

Tests for consistency of virus characteristics
(Tests are performed during vaccine development and process validation, may not be required for batch release.)
Item tested: _____
Date of test: _____
Methods used: _____
Results: _____

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 final lot (section A.6)

Vaccine (section A.6.1)

Inspection of final containers

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

Identity

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

	Specification	Date test initiated	Method	Results
Sterility	_____	_____	_____	_____

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

pH

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

Residual moisture (if applicable)

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

Virus concentration

Date titration 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

Duration of exposure: _____

Temperature of exposure: _____

Date titration began and ended: _____

Method used for titration: _____

Results: _____

Total virus titre

Exposed sample: _____

Non-exposed sample: _____

Titre reduction: _____

Lot number of reference virus: _____

Lot number of other reference reagents if used: _____

Diluent used: _____

Lot number of diluent used: _____

Residual antibiotics (if applicable)

Date of test: _____

Method used: _____

Results: _____

Stabilizer (if applicable)

Date of test: _____

Method used: _____

Results: _____

Diluents (section A.6.2, if applicable)

Nature and volume: _____

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

Antacid (section A.6.2, if applicable)
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:
_____
_____
_____
_____

Extractable volume (section A.6.3, if applicable)
Extractable volume (mL): _____
The number of drops, using the approved dropper,
in a minimum of five individual final containers: _____

Certification by the manufacturer

Name of head of production and/or quality control (typed) _____

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 container, meets all national requirements and/or satisfies Part A[1] of the WHO Recommendation to assure the quality, safety and efficacy of live attenuated rotavirus vaccines (oral)[2].

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.

[1] *With the exception of provisions on distribution and transport, which the NRA may not be in a position to assess.*

[2] *WHO Technical Report Series, No. XXXX, Annex 2.*

Appendix 2

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

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 _____[1] in _____[2], whose lot numbers appear on the labels of the final containers, meet all national requirements[3] and Part A[4] of the WHO Recommendations to assure the quality, safety and efficacy of live attenuated rotavirus vaccines[5] and comply with WHO good manufacturing practices for pharmaceutical products: main principles,[6] and WHO good manufacturing practices for biological products[7] and Guidelines for independent lot release of vaccines by regulatory authorities.[8]

The release decision is based on _____[9].

The certificate may include the following information:

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

The Director of the national regulatory authority (or other appropriate authority):
Name (typed): _____
Signature: _____
Date: _____

[1] *Name of manufacturer.*

[2] *Country of origin.*

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

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

[5] *WHO Technical Report Series, No. XXX, AnnexX.*

[6] *WHO Technical Report Series, No. 986, Annex2.*

[7] *WHO Technical Report Series, No. 999, Annex2.*

[8] *WHO Technical Report Series, No. 978, Annex 2.*

[9] *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.*