Annex 2

Guidelines on the quality, safety and efficacy of Ebola vaccines

Guidelines 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 biological products. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Guidelines 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.

Abbreviations

Ad	human adenovirus
AESI	adverse event of special interest
AR	attack rate
ARU	attack rate in unvaccinated individuals
ARV	attack rate in vaccinated individuals
BCG	bacillus Calmette–Guérin
BDBV	Bundibugyo ebolavirus
BSL	biosafety level
CBER	Center for Biologics Evaluation and Research
CEF	chick embryo fibroblast
ChAd3	chimpanzee adenovirus type 3
DCVMN	Developing Countries Vaccine Manufacturers Network
DNA	deoxyribonucleic acid
EBOV	Ebola virus
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
ERA	environmental risk assessment
EUAL	WHO emergency use assessment and listing (procedure)
EVD	Ebola virus disease
GLP	good laboratory practice(s)
GMO	genetically modified organism
GMP	good manufacturing practice(s)
GP	glycoprotein
HIV	human immunodeficiency virus
HVAC	heating, ventilation and air conditioning
IFPMA	International Federation of Pharmaceutical Manufacturers & Associations
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

ICP	immune correlate of protection
ICS	intracellular cytokine staining
Ig	immunoglobulin
LAL	Limulus amoebocyte lysate
LVV	lentiviral vector
MARV	Marburg virus
MCB	master cell bank
MVA	modified vaccinia Ankara
NAT	nucleic acid amplification technique
NRA	national regulatory authority
PCR	polymerase chain reaction
PDL	population doubling level
qPCR	quantitative polymerase chain reaction
RDT	rapid diagnostic test
RESTV	Reston ebolavirus
RNA	ribonucleic acid
RR	relative risk
RT	reverse transcriptase
rVSV	recombinant vesicular stomatitis virus
SAE	serious adverse event
SAGE	WHO Strategic Advisory Group of Experts
SPF	specific pathogen-free
SUDV	Sudan ebolavirus
SWRCT	stepped wedge randomized cluster trial
TAFV	Tai Forest ebolavirus
TSE	transmissible spongiform encephalopathy
VLP	virus-like particle
VSV	vesicular stomatitis virus
WCB	working cell bank
ZEBOV	Zaire ebolavirus

Introduction

The unprecedented scale and severity of the Ebola virus disease (EVD) epidemic in West Africa in 2014–2016 led to calls for the urgent development and licensing of an Ebola vaccine (*1*, *2*). A considerable amount of work was subsequently undertaken over a short period of time and a series of international consultations held on related public health issues and on Ebola vaccine development, evaluation and licensing (*2–4*). The development of Ebola vaccines and implications for future immunization policy recommendations are being monitored by the WHO Strategic Advisory Group of Experts (SAGE) on Immunization (*5*). In addition, as part of ongoing WHO measures to support the development of Ebola vaccines, guidance was prepared on the scientific and regulatory considerations relating to their quality, safety and efficacy. In March 2015, WHO convened an informal consultation in Geneva attended by scientific experts, regulatory professionals and other stakeholders involved in Ebola vaccine development, production, evaluation and licensure. The purpose of this consultation was to review initial draft guidelines prepared by a drafting group, and to seek consensus on key technical and regulatory issues (*6*). The draft guidelines were revised in the light of comments made, and then underwent public consultation which resulted in a large number of further comments and suggestions. The draft guidelines, together with the comments, were discussed by the WHO Expert Committee on Biological Standardization at its meeting in October 2015. During 2016, further revisions were made following public consultations and working group discussions. One major challenge during the development of these Ebola vaccine guidelines was that they were initially prepared during the rapidly evolving epidemic situation when the need for a vaccine was most urgent. With the end of the large-scale EVD outbreak in Africa, declared by WHO in June 2016, EVD returned to its previous sporadic pattern – an epidemiological situation which made the evaluation of Ebola vaccine efficacy, and thus licensing, more challenging. Interest also shifted from the development of monovalent Ebola virus (EBOV) Zaire vaccines to multivalent preparations directed against more than one EBOV strain, as well as against the Marburg virus (MARV).

The WHO Expert Committee on Biological Standardization reviewed the draft document again in October 2016 and after extensive discussion agreed that the guidance should be extended to include multivalent Ebola vaccines and the clinical evaluation of candidate vaccines using innovative clinical trial designs. There was also a recognized need to provide guidance on how to evaluate and license Ebola vaccines subsequent to the potential licensure of one of the advanced vectored vaccines. These WHO Guidelines are the result of these discussions.

This document provides information and guidance on the development, production, quality control and evaluation of candidate Ebola vaccines in the form of WHO Guidelines rather than WHO Recommendations. This allows

for greater flexibility with respect to the expected future of Ebola vaccine development, production, quality control and evaluation. Given that this is a very dynamic field both in terms of technologies and clinical trial designs, these WHO Guidelines should be read in conjunction with other relevant recent guidelines.

A model protocol for the manufacturing and control of viral-vectored Ebola vaccines is provided in Appendix 1 of these WHO Guidelines. This protocol outlines the information that should be provided as a minimum by a manufacturer to the NRA in support of a request for the release of a vaccine for use. The protocol is not intended to apply to material intended for clinical trials. A Lot Release Certificate signed by the appropriate NRA official should be provided if requested by a manufacturer, and should certify whether or not the lot of vaccine in question meets all national requirements and/or Part A of these WHO Guidelines. The purpose of this is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided in Appendix 2.

Purpose and scope

These WHO Guidelines provide scientific and regulatory guidance for national regulatory authorities (NRAs) and vaccine manufacturers on the quality, nonclinical and clinical aspects of Ebola vaccines relevant to marketing authorizations. In particular, the document deals with Ebola vaccines based on viral vectors, which are currently at the most advanced stage of development and for which no specific WHO guidance is available. The document also discusses opportunities to accelerate vaccine development and product availability during a public health emergency.

The document does not address access programmes or regulatory pathways for making investigational Ebola vaccines available for situations where their use is not primarily intended to obtain safety and efficacy or effectiveness information.

Although recombinant viral-vectored Ebola vaccines are the main category of vaccine considered in this document, some aspects of the guidance provided are relevant to other approaches. General guidance on other technologies relevant to Ebola vaccine development has been published elsewhere by WHO, including guidance on:

- inactivated vaccines (*7*–*9*)
- protein antigens produced by recombinant technology (*10*–*13*)
- DNA vaccines (*14*, *15*).

In the past 10 years, WHO has convened two consultations to consider the development, production and evaluation of viral-vectored vaccines in general,

and the reports of those meetings provide useful discussion and opinions on the quality, safety and efficacy aspects of such vaccines (*16*, *17*). A regional guideline is also available for live recombinant viral-vectored vaccines (*18*).

Although recombinant viral-vectored Ebola vaccines are by far the most advanced candidates, other approaches to the development of Ebola vaccines are also being investigated. These include different production platforms, such as recombinant DNA vaccines expressing an EBOV antigen produced in *Escherichia coli* (*19*), Ebola virus-like particles (VLPs) expressed from recombinant baculovirus in insect cells, and other forms of subunit vaccines. Most developmental approaches to Ebola vaccines involve recombinant DNA technology.

Part A of this document focuses on the development, manufacturing and quality control issues relevant to viral-vectored vaccines against EBOV. Although the key principles related to nonclinical development (Part B) and clinical development (Part C) may apply to vaccine approaches other than those based on viral vectors, special considerations and guidance would be required for such products – and they are therefore not elaborated upon in this document. Any mention of specific vaccines is for information only and should not be considered as an endorsement of a particular candidate.

Parts A, B and C provide guidance in general terms on the full quality, nonclinical and clinical requirements for a license submission for viral-vectored Ebola vaccines. The document also considers the principles which may be applied to product development, manufacturing and control – and to nonclinical and clinical evaluation – during a public health emergency to allow for the rapid introduction of an Ebola vaccine. Wherever appropriate, discussions on the minimum dataset required are highlighted and aspects of vaccine development which may be accelerated during a public health emergency are indicated. These context-specific discussions and indications are shown as indented smaller text in Parts A, B and C. In addition, special considerations regarding the quality requirements at different stages of clinical development are discussed in sections A.2.4, A.3.6, A.3.7 and A.3.8.

These WHO Guidelines should be read in conjunction with other relevant WHO guidelines such as those on nonclinical (*20*, *21*) and clinical (*22*) evaluation of vaccines, as well as relevant documents that describe the minimum requirements for an effective National Pharmacovigilance System (*23*). Other WHO guidance, such as that on the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*24*), should also be consulted as appropriate.

It should be noted that there remain knowledge gaps in the scientific understanding of EVD and Ebola vaccines which are being addressed by ongoing research and development. This document has been developed in the light of the available knowledge to date, and with regard to the currently most advanced candidate Ebola vaccines.

Terminology

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

Adventitious agents: contaminating microorganisms of a cell culture or source materials, including bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, *Rickettsia*, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents and viruses that have been unintentionally introduced into the manufacturing process of a biological product.

Adverse event of special interest (AESI): an adverse event (serious or non-serious) that is of scientific and medical concern specific to the sponsor's product or programme, and for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to be characterized and understood. Depending on the nature of the event, rapid communication by the trial sponsor to other parties (for example, regulators) might also be warranted.

Attenuated virus: a strain of virus in which pathogenicity has been reduced so that the virus strain will initiate an immune response without producing the disease.

Benefit–risk assessment: a decision-making process for evaluating whether or not the benefits of a given medicinal product outweigh the risks. Benefits and risks need to be identified from all parts of a dossier – that is, the quality, nonclinical and clinical data – and integrated into the overall assessment.

Candidate vaccine: an investigational vaccine which is in the research and clinical development stages and has not been granted marketing authorization or licensure by a regulatory agency.

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

Cell substrate: cells used to manufacture a biological product.

Expression construct: an expression vector containing the genetic coding sequence of the recombinant protein.

Expression system: the host cell containing the expression construct and the cell culture process that is capable of expressing protein encoded by the expression construct.

Final bulk: a formulated vaccine preparation from which the final containers are filled. If applicable, the final bulk may be prepared from one or more monovalent antigen bulks and, in this case, mixing should result in a uniform preparation to ensure that final containers are homogenous.

Final lot: a collection of sealed final containers of formulated vaccine that is homogeneous with respect to the risk of contamination during the filling

process. A final lot must therefore have been filled from a single vessel of final bulk or prepared in one working session.

Heterologous gene: a transgene from the disease-causing organism that is integrated into the genomic sequence of the viral vector.

Immune correlate of protection (ICP): an immunological response that correlates with vaccine-induced protection from disease and is considered predictive of clinical efficacy. The ICP may be mechanistic (that is, causative for protection) or it may be non-mechanistic (that is, an immune response that is present in persons protected by vaccination but that is not the cause of protection).

Immunogenicity: the capacity of a vaccine to elicit a measurable immune response.

Marketing authorization: a formal authorization for a medicine (including vaccines) to be marketed. Once an NRA approves a marketing authorization application for a new medicine, the medicine may be marketed and may be available for physicians to prescribe and/or for public health use (also referred to as product licensing, product authorization or product registration).

Master cell bank (MCB): a quantity of well-characterized cells of animal or other origin, derived from a cell seed at a specific population doubling level (PDL) 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. In some cases, such as genetically engineered cells, the MCB may be prepared from a selected cell clone established under defined conditions. Frequently, however, the MCB is not clonal. It is considered best practice for the MCB to be used to derive working cell banks.

Monovalent vaccine: a vaccine containing immunizing antigen, or a gene encoding an immunizing agent, against a single strain or type of disease agent.

Platform technology: a production technology in which different viral-vectored vaccines are produced by incorporating heterologous genes for different proteins into an identical viral vector backbone.

Multivalent vaccine: a vaccine containing a mixture of more than one immunizing antigen or genes encoding several immunizing agents active against more than one strain or type of disease agent.

Pooled virus harvest: a homogeneous pool of two or more single virus harvests.

Public health emergency: an extraordinary event that is determined, as provided in the International Health Regulations (*25*), to: (a) constitute a public health risk to other States through the international spread of disease; and (b) potentially require a coordinated international response.

Seed lot: a system according to which successive batches of viral-vectored vaccine are derived from the same virus master seed lot of viral vector at a given

passage level. For routine production, a virus working seed lot is prepared from the virus master seed lot. The final product is derived from the virus working seed lot and has not undergone more passages from the virus master seed lot than the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

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

Vaccine efficacy: measures direct protection (that is, protection induced by vaccination in the vaccinated population sample). Vaccine efficacy is most commonly a measure of the proportionate reduction in disease attack rate (AR) between the control group that did not receive vaccination against the infectious disease under study (ARU) and the vaccinated group (ARV). Vaccine efficacy (expressed as a percentage) can be calculated from the relative risk (RR = ARV/ARU) of disease when comparing the vaccinated group to the unvaccinated control group as [(ARU-ARV)/ARU] x 100 – that is, as (1-RR) x 100. This estimate may be referred to as absolute vaccine efficacy. Alternatively, vaccine efficacy may be defined as a measure of the proportionate reduction in disease AR in a group vaccinated with the candidate vaccine relative to a control group vaccinated with a licensed vaccine against the infectious disease under study. This estimate may be referred to as relative vaccine efficacy (*22*).

Vaccine effectiveness: an estimate of the protection conferred by vaccination. It is usually obtained by monitoring the disease to be prevented by the vaccine during routine use in a specific population. Vaccine effectiveness measures both direct and indirect protection (for example, the estimate may in part reflect protection of unvaccinated persons secondary to the effect of use of the vaccine in the vaccinated population) (*22*). Evidence for vaccine effectiveness may also be derived from challenge-protection studies conducted in animal models or from a vaccine-induced immune response (for example, pre-specified antibody threshold induced by the vaccine in vaccinated persons).

Virus master seed: a collection of appropriate containers whose contents are of uniform composition, stored under defined conditions. Each container represents an aliquot of a single pool of virus vector particles of defined passage from which the virus working seed is derived.

Virus pre-master seed: a single pool of virus vector particles of defined passage from which the virus master seed is derived.

Virus working seed: a collection of appropriate containers whose contents are of uniform composition, stored under defined conditions. Each container represents an aliquot of a single pool of virus vector particles of defined passage derived directly from the virus master seed lot and which is the starting material for individual manufacturing batches of viral-vectored vaccine product.

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

General considerations

Ebola viruses, Ebola virus disease and epidemiology

Ebola viruses belong to the *Filoviridae* family of filamentous, negative-stranded RNA, enveloped viruses consisting of three genera: Ebola virus, Marburg virus and Cueva virus – the latter being a pathogen of bats in Spain (*26*). There are five distinct species of EBOV: Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SUDV), Tai Forest ebolavirus (TAFV), Reston ebolavirus (RESTV) and Bundibugyo ebolavirus (BDBV) (*26*, *27*). Marburg virus (MARV) appears to be antigenically stable and at present there is only a single species. The first recognized MARV outbreak in humans was in 1967 and was linked to infected monkeys imported from Uganda that infected laboratory workers in Marburg and Belgrade (*28*). Bats are believed to be the natural reservoir of all filoviruses. EBOV and MARV cause severe haemorrhagic fever in humans and non-human primates alike, with high morbidity and mortality rates (*29*, *30*). Outbreaks of infection with Ebola filoviruses have been noted since 1976, mainly in Central Africa, and recur at intervals. Prior to the 2014–2016 EVD epidemic in West Africa there had not been such a large-scale outbreak and the disease had not been recorded in West Africa, apart from a single infection with TAFV.

The incubation period following infection with EBOV and prior to the onset of symptoms is believed to be approximately 2–21 days, with initial symptoms being similar to diseases such as influenza or malaria (*31*, *32*). Patients then progress rapidly to a life-threatening disease (*33*). From a practical perspective, infected individuals rarely if ever become infective before symptoms appear, but those who survive remain infective until the virus is cleared from their blood and other bodily fluids. It has been reported that viable EBOV can persist in ocular fluid for at least 9 weeks following clearance of viraemia (*34*). EBOV has also been detected in semen for months following recovery from EVD, which is consistent with the possible persistence of the virus within immune-privileged tissue sites in the body (*35*, *36*). Presumptive sexual transmission of EBOV from recovered individuals has also been reported (*37*, *38*). Individuals suffering from EVD have been treated aggressively with oral and intravenous fluids, including electrolyte replacements, to combat severe diarrhoea and dehydration, with some surviving the infection (*33*).

Filoviruses are high-risk agents classified as biosafety level 4 (BSL-4) pathogens. They consist of a non-segmented RNA genome of approximately 19 kb containing 7 genes encoding viral proteins VP24, VP30, VP35, VP40, a nucleoprotein, a glycoprotein (GP) and a polymerase (39). The GP is a type-1 transmembrane GP that is cleaved into disulphide-linked GP1 and GP2 subunits. The mature GP forms homotrimers that are presented as spikes on the surface of infected cells and virions, and is responsible for receptor binding, viral entry and, most likely, immunity (*40*, *41*). Most of the vaccines currently under development are based on the EBOV GP and have been shown to confer protection from lethal EBOV challenge in animal models – including, importantly, in non-human primates (*42*, *43*).

Natural immune responses to Ebola viruses

Filovirus infection in humans elicits innate, cellular and humoral responses. Immunoglobulin M (IgM) and IgG antibodies have been reported to develop early in infected patients who survive, whereas fatal cases are associated with immune dysregulation and high viraemia (*44*). Some cross-reactive immune responses across the five EBOV species have been reported (*45*). Cellular responses can also be detected. The generation of neutralizing antibodies during filovirus infection and the passive transfer of neutralizing monoclonal antibodies or monkey convalescent immunoglobulin preparations have been shown to sometimes protect non-human primates against lethal filovirus challenge – though overall the data are somewhat conflicting (*44*, *46*). Data suggest that antibodies play a significant role in protection against filovirus infection but correlates of protection have not been established and the importance of cellular immunity is uncertain (*47*, *48*).

Ebola vaccines development

A large number of candidate Ebola vaccines are under development. Some of these vaccines had already been in preclinical development prior to the 2014–2016 EVD epidemic and are significantly more advanced than the others. To date, several candidate vaccines (including monovalent, bivalent and multivalent candidate vaccines) have undergone or are undergoing clinical development at different trial phases. The Phase III trial for a recombinant vesicular stomatitis virus (rVSV)-vectored candidate vaccine (rVSVΔG-ZEBOV-GP), undertaken in Guinea, is the only study that has reported clinical efficacy and effectiveness for any candidate Ebola vaccine. This candidate vaccine was granted access to the Priority Medicine (PRIME) scheme by the European Medicines Agency, and Breakthrough Therapy designation by the United States Food and Drug Administration (*5*). Examples of Ebola vaccines

currently under clinical development are provided in a WHO Working Group background paper (*49*).

The most advanced Ebola vaccines are based on live recombinant virus vector platforms. Such vaccines have been developed in Canada, China, Europe, Russia and the USA. Five of the most advanced platforms used to engineer these vaccines are rVSV (*50*, *51*), chimpanzee adenovirus type 3 (ChAd3) (*52*), human adenovirus type 26 (Ad26) (*53*), human adenovirus type 5 (Ad5) (*51*, *54*) and the modified vaccinia Ankara (MVA) strain (*55*). To date, the virus vectors have been produced in a wide variety of cell lines including PER.C6 (Ad26.ZEBOV), chick embryo fibroblasts (MVA-BN-Filo), Procell-92.S (ChAd3-EBOZ), Vero (rVSV-ZEBOV) and HEK 293 (Ad5-EBOV). Monovalent candidate vaccines have been constructed to express the EBOV GP of one EBOV strain, such as the Zaire strain responsible for the epidemic in West Africa. Others have been developed as multivalent vaccines expressing the GP of more than one EBOV strain and/or MARV and/or the TAFV nucleoprotein. Multivalent vaccines have also been produced by blending monovalent bulks expressing glycoproteins from different EBOV and/or MARV strains. These candidates are currently under study in non-human primates and in humans, either as single vaccines or for use in heterologous prime-boost vaccine schedules where priming is done with one vaccine and boosting with another – as for example, Ad26.ZEBOV/MVA-BN-Filo (*56*, *57*) and rVSV/Ad5 (*51*).

The viral-vectored vaccines under development include those that are replication-incompetent in the human host or in human cells as well as those that are replication-competent but likely to be highly attenuated because of their recombinant gene inserts and cell culture passage. Replication-incompetent vectors include adenoviral vectors derived both from human adenoviruses (such as Ad26 and Ad5) and from non-human primate adenoviruses (such as ChAd3), as well as MVA. MVA is a highly attenuated vaccinia strain, derived by more than 500 passages in hens' eggs. The non-recombinant MVA was used as a human smallpox vaccine in Germany in the 1970s and a derivative has now been licensed for use in a future smallpox emergency in Canada and Europe. Vectors that are replication-competent but attenuated include rVSV (a negative-stranded RNA virus animal pathogen) in which attenuation is due to the insertion of a recombinant heterologous gene such as the EBOV GP in place of the VSV GP. These viral-vector platforms have been used to produce other investigational products – including gene therapy products, and both prophylactic and therapeutic vaccines – and data from their quality, nonclinical and clinical evaluations provide supporting safety data for their use in Ebola vaccine production (*50*, *58*, *59*).

The need for careful clinical studies using candidate vaccines in the target population will be of paramount importance. WHO has developed

a document – *Ebola virus disease (EVD) vaccine target product profile* (*60*) – which provides guidance on WHO preferred options in relation to two categories of Ebola vaccine (reactive use and prophylactic use). Encouraging results on the immunogenicity and safety of these candidate options, as well as on their clinical efficacy based on disease end-points, have already been generated and their evaluation in larger Phase II and Phase III trials is ongoing. This includes novel trial design clinical studies (ring vaccination) using the rVSV-ZEBOV vaccine (*32*, *54*, *61–64*). A prime-boost approach, using a two-dose schedule with different vector vaccines, is also being explored. Boosting of Ad26.ZEBOV responses by MVA-BN-Filo resulted in sustained elevation of specific immunity with no vaccine-related serious adverse responses reported (*56*, *57*). Administration of rVSV-ZEBOV vaccine resulted in low-level viraemia detectable by polymerase chain reaction (PCR) during the first and sometimes second week after vaccination (*63*). The vaccine virus was also detected by PCR in the urine and saliva of a minority of the recipients. An unexpected safety signal was detected in one study when mild-to-moderate and generally short-lived arthritis developed during the second week following immunization in a minority of recipients and at one site in particular (*63*). In subsequent studies in healthy North American and European adults which carefully assessed joint-related adverse events, transient post-vaccination arthritis was noted in approximately 5% of vaccine recipients (*65*, *66*). However, the epidemiological situation has now changed significantly. Using strict infection control and public health measures, the EBOV epidemic has been ended – though there will still be a risk of new Ebola cases or clusters occurring through, for example, sexual transmission or new introduction of the virus into the human population. WHO declared Sierra Leone free of EBOV transmission in March 2016 and Guinea and Liberia free of EBOV transmission in June 2016, bringing to an end the large-scale Ebola outbreak in the three African countries mainly affected (*67*). In the absence of ongoing disease transmission, the assessment of Ebola vaccine efficacy will now be more challenging. Nevertheless, it is expected that current clinical trials of candidate vaccines will provide key data on safety, reactogenicity and immunogenicity to inform licensure.

Accelerated availability of vaccines during a public health emergency – general principles

The quality of a vaccine must always be taken into account during the process of evaluating whether the benefit derived from its administration is greater than any risks which might be associated with its use. This is a principle by which all pharmaceuticals, whether they are chemical or biological, medicine or vaccine, are evaluated to decide whether they should be made available for use or not. The principle applies equally to a product intended for use in a clinical trial or

as a licensed product, or to be made available through emergency procedures. In addition, there is an obligation to provide full assurance that the vaccine will not cause harm to the recipient due to a failure of manufacture and control that results in contamination of the product with unwanted components such as microorganisms or toxic materials. This requirement is absolute, regardless of the stage of development of the product or the urgency of the need for its availability.

Beyond this, the process and product characterization requirements will depend on the prevailing clinical situation and the urgency of need for the product. However, it is generally accepted that in order to gain marketing authorization for a vaccine the usual standards for quality development, manufacture and control will apply. During the assessment of a marketing authorization application, the balance of benefits and risks of the vaccine to the intended population is taken into consideration and must be found to be positive if the product is to be granted marketing approval. The specific findings related to the assessment of product quality are taken into account in this benefit–risk assessment.

It is not possible to provide a "road map" of the minimum process and product characterization and control requirements for a viral-vectored vaccine against EVD, or against any other disease with the potential to cause a public health emergency, since the requirements will be partially dependent on the ongoing epidemic situation in the affected countries.

In the case of viral-vectored vaccines, many of the opportunities to accelerate development and product availability during a public health emergency are likely to involve exploiting the knowledge gained from similar products manufactured with the same vector backbone (that is, platform technology). If a new vaccine is based on a well-characterized platform technology, then key aspects of manufacture and control (but not stability) can be based on the specific platform with only confirmatory information required for the new vaccine. This principle is especially applicable during the phase of clinical trial development. For licensure, product-specific data will be required but supportive platform-derived data may decrease the requirement for some product data if it can be shown that the benefit–risk assessment remains positive. Scientific advice should be sought from relevant regulatory authorities.

During product development, it might be possible to defer certain tests and development procedures provided it can be justified that their deferral does not affect product safety – and if it can also be argued that performing the tests or development procedures would hinder the availability of the product (for example, where performing the tests are on the critical path for product availability, or where large quantities of scarce material required for clinical purposes would need to be used). Such deferrals should be identified on a case-by-case basis and discussed with the NRA.

In some cases, even if the nature of a public health emergency affects the benefit–risk balance in such a way as to justify the accelerated development and approval of a vaccine for use in a public health emergency, the manufacturer would still be responsible for completing the full development work to the same standard required for a new vaccine under non-emergency conditions should it be decided to subsequently submit the product for full licensure. The required supplementary data and timelines for submission should be agreed between the applicant and the NRA.

Similar considerations apply to the nonclinical evaluation of candidate Ebola vaccines. For nonclinical evaluation during a public health emergency, it is paramount to determine a minimum nonclinical package (see section B.4) that can reasonably support initiation of early Phase I clinical trials. This should take into account the characteristics and novelty of candidate vaccines and the supportive information derived from the platform technology on which the vaccine is based. For example, the presence of nonclinical data and/or clinical experience gained with the same vector may support the omission of a specific safety test or toxicity testing programme. For a candidate vaccine derived from a novel platform, a certain amount of toxicity data (see section B.4) should at a minimum be obtained, and should focus on unexpected direct and indirect consequences that might result from vaccination.

In general, the use of a minimum safety package during nonclinical evaluation should be backed up by the continuous assessment of additional data collected during clinical development. At the time of the licensing application, the complete nonclinical programme data appropriate for a particular vaccine should be submitted, or the application should be otherwise adequately justified.

Clinical development of an Ebola vaccine in the setting of an outbreak is complex, and close collaboration between public health authorities, NRAs, the community, clinical investigators and the vaccine developer is essential to ensure that studies will meet authorization requirements, including requirements for ethical study conduct.

A WHO emergency use assessment and listing (EUAL) procedure (*68*) has been developed to expedite the availability of unlicensed vaccines needed during a public health emergency of (usually) international concern.

Part A. Guidelines on the development, manufacture and control of Ebola vaccines

At the time of writing this document, no WHO guidance on viral-vectored vaccines was available. Consequently, this section focuses on issues relevant to the development, manufacturing and quality control steps leading to the licensing of such vaccines developed to protect against EVD.

The lead viral-vectored vaccines and their replication abilities are summarized in a WHO document (*49*). The relevance of aspects of the guidance provided in this document should be considered with respect to the replication status of the products. For example, tests for reversion to competency apply to replication-incompetent viral vectors where genes required for replication are not present in the vector. On the other hand, for replication-competent viral-vectored vaccines, the level of attenuation of the parent and recombinant viral vectors should be considered.

A.1 General manufacturing guidelines

The WHO Target Product Profile (*60*) prioritizes the development of multivalent vaccines from 2016 onwards and seeks at a minimum coverage for MARV and for both Zaire and Sudan species of EBOV.

The general manufacturing requirements contained in the WHO good manufacturing practices for pharmaceutical products: main principles (*69*) and WHO good manufacturing practices for biological products (*70*) should apply to the design, establishment, operation, control and maintenance of manufacturing facilities for recombinant Ebola vaccines.

Quality control during the manufacturing process relies on the implementation of quality systems, such as good manufacturing practice (GMP), to ensure the production of consistent vaccine lots with characteristics similar to those of lots shown to be safe and effective in clinical trials. Throughout the process, a number of in-process control tests should be established (with acceptable limits) to allow quality to be monitored for each lot from the beginning to the end of production. It is important to note that most release specifications are product specific and should be agreed with the NRA as part of the clinical trial or marketing authorization.

Manufacturers should present a risk assessment regarding the biosafety level of their manufacturing facility and of the vaccine product. The principles presented in the WHO *Laboratory biosafety manual* (*71*) should be followed to justify the classification. Approval for the classification should be sought from the relevant authority in the country/region in which the manufacturing facility is located.

A.1.1 International reference materials

The highly pathogenic nature of EBOV raises particular concerns for the preparation of international reference materials as they must be both safe for use and representative of clinical samples to be analysed. Generally, plasma reference preparations are used for the standardization of assays for evaluating immune response, and artificial RNA viruses containing part of the EBOV genome are used for the standardization of nucleic acid assays for assessing viraemia.

Plasma from a recovered repatriated patient who contracted Ebola in West Africa one month before the plasma was collected was established by the 2015 WHO Expert Committee on Biological Standardization as the First WHO Reference Reagent for Ebola virus antibodies, with an assigned unitage of 1 U/ml (*72*). As the reference material resulted from a natural infection it is likely to have relevant antibody specificities. It is considered to be of acceptable safety for three reasons: (a) the patient was fully recovered clinically; (b) the plasma was negative for EBOV nucleic acid in PCR assays performed in various laboratories; and (c) the plasma was treated with solvent/detergent (an established method used in the blood products industry for decades for the inactivation of enveloped viruses).

Following evaluation and characterization of candidate materials (*73*, *74*), the First WHO International Standard for Ebola virus antibodies (assigned unitage = 1.5 IU/ml) and the First WHO Reference Panel for Ebola virus antibodies were established by the 2017 WHO Expert Committee on Biological Standardization. The First WHO International Standard for Ebola virus antibodies is intended for standardizing assays used in the detection and quantitation of EBOV antibodies. It is not intended to be used to set a protective threshold, which is currently unknown (see section C). The First WHO Reference Reagent for Ebola virus antibodies and the First WHO Reference Panel for Ebola virus antibodies can be used in the assessment of factors that affect assay variability (*75*).

Following evaluation and characterization of candidate materials (76, 77), two EBOV RNA preparations were also established as reference reagents by the 2015 WHO Expert Committee on Biological Standardization for use in the standardization of nucleic acid amplification technique (NAT)-based assays. One of these materials (Ebola NP-VP35-GP-LVV) consists of the RNA encoding the nucleoprotein VP35 and GP genes and is intended for use in standardizing assays directed at these genes only. The second (Ebola VP40-L-LVV) consists of the RNA encoding the VP40 and L genes and again is intended to standardize assays directed only at these genes. Both preparations are packaged in non-replicating lentiviral vectors (LVVs) with the EBOV genes incorporating mutations that make them inactive. Collectively the two materials were established as the First WHO reference reagents for Ebola virus RNA for NAT-based assays with assigned unitages of 7.5 $\log_{10}$ U/ml and 7.7 $\log_{10}$ U/ml respectively.

The First WHO Reference Panel for Ebola virus VP40 antigen was established by the 2016 WHO Expert Committee on Biological Standardization (*78*). The panel consists of different recombinant VP40 antigens and may be suitable for the evaluation and quality control of Ebola antigen assays based on VP40 detection.

All the reference materials listed above are available from the National Institute for Biological Standards and Control, Potters Bar, the United Kingdom.

For the latest list of appropriate WHO international standards and reference materials, the WHO Catalogue of International Reference Preparations (*79*) should be consulted.

A.2 Control of source materials

A.2.1 Viral vector

A.2.1.1 Virus master and working seeds

The use of any viral vector should be based on a master and working seed lot system, analogous to the cell banking system used for production cells described below in section A.2.2.

The rationale behind the development of the viral-vectored vaccine should be described. The origin of all genetic components of the vaccine and their function should be specified to allow for a clear overall understanding of the functionality of the vaccine and of how it is attenuated, or made replication-incompetent by genetic engineering. All intended and unintended genetic modifications such as site-specific mutations, insertions, deletions and/or rearrangements to any component should be detailed in comparison with their natural counterparts. For a vaccine construct that incorporates genetic elements to control the expression of a transgene – for example, in a tissue-specific manner – evidence should be provided on product characterization and control to demonstrate such specificity. RNA editing should be discussed if relevant.

All of the steps from the derivation of material that ultimately resulted in the candidate vaccine to the virus master seed level should be described. A diagrammatic description of the components used during vaccine development should be provided and annotated. The method of construction of the viral-vectored vaccine should be described and the final construct should be genetically characterized according to the principles discussed in this section.

The cloning strategy should ensure that if any antibiotic resistance genes are used during the development of the initial genetic construct, these are absent from the viral vaccine seed.

The nucleotide sequence of the gene insert and of adjacent segments of the vector should be provided, along with restriction-enzyme mapping of the vector containing the gene insert. The genetic stability of the vector with the recombinant construct should be demonstrated. The stability of a recombinant vector should be assessed by comparing the sequence of the vector at the level of a virus pre-master seed or virus master seed to its sequence at, or preferably beyond, the anticipated maximum passage level. The comparison should demonstrate that no changes occur in regions involved in attenuation (where known) or replication deficiency. Any modifications to the sequence of the heterologous insert should be investigated and demonstrated to have no impact

on the resulting amino acid sequence (that is, it should be a conservative change) or on the antigenic characteristics of the vaccine.

A.2.1.2 Tests on virus master seed and virus working seed

The virus master seed should be characterized as fully as possible. If this characterization is limited (for example, because of limited quantities of material) then the virus working seed should be fully characterized in addition to the limited characterization of the virus master seed. It should be noted that it would not be feasible to manufacture from the virus master seed in these circumstances.

Virus master seed characterization will include a description of the genetic and phenotypic properties of the vaccine vector. This should include a comparison with the parental vector – which is particularly important where vector modification might affect attenuation or replication competency, pathogenicity, and tissue tropism or species specificity of the vaccine vector compared with the parental vector.

Genetic characterization will involve nucleotide sequence analysis of the vaccine vector. Restriction mapping, southern blotting, PCR analysis or DNA fingerprinting will also be useful adjuncts. Individual elements involved in the expression of the heterologous gene(s) (including relevant junction regions) should be described and delineated.

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

Phenotypic characterization should focus on the markers for attenuation/modification and expression of the heterologous antigen(s), and should generally be performed in vitro under conditions that allow for the detection of revertants (including the emergence of replication-competent vectors from replication-incompetent vectors during passage). However, other studies including antigenic analysis, infectivity titre, ratio of genome copies to infectious units (for replicating vectors) and in vitro yield should also form part of the characterization. For replicating vectors, in vivo growth characteristics in a suitable animal model may also be informative and should be performed if justified. For some vectors (for example, adenoviral vectors), particle number should be measured in addition to infectivity titre.

A subset of the above studies should be applied to the virus working seed lot and justification for the chosen subset should be provided.

Information should be given on the testing carried out for adventitious agents.

> During a public health emergency it is anticipated that the majority of the above information should be available and submitted in full for evaluation since it is essential to demonstrate the suitability and safety of the product.

It may be justified to initiate clinical trials using a product which is manufactured prior to establishment of the seed banking system. In such a case, the suitability and safety of the product must be established prior to its use – especially with regard to adventitious agents (*24*), replication competence, attenuation and other phenotypic characteristics, stability and suitable genetic sequence.

A.2.2 Cell substrates

The cell substrate for the manufacture of Ebola vaccine should be based on a cell banking system or on controlled primary cells.

A.2.2.1 Cell banks and primary cells

A.2.2.1.1 *Master and working cell banks (MCBs and WCBs)*

The cell banks should conform to 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 (*24*).

An appropriate history of the cell bank should be provided. This should include information on its origin, identification, development manipulations and characteristics for the purposes of the vaccine. Full details of the construction of packaging cell lines should be given, including the nature and identity of the helper viral nucleic acid and its encoded proteins/functions. If available, information on the chromosomal location of the helper viral nucleic acid should also be provided.

Genetic stability of the cell lines should be demonstrated. The stability of a production cell line should be assessed by comparing the critical regions of the cell line (and flanking regions) at the level of a pre-cell or master cell to its sequence at or beyond the anticipated maximum passage level. Stability studies should also be performed to confirm cell viability after retrieval from storage, maintenance of the expression system, and so on. These studies may be performed as part of routine use in production or may include samples taken specifically for this purpose.

With regard to cell cultures, the maximum number of passages (or population doublings) allowable from the MCB through to the WCB, and through production in cells should be defined on the basis of the stability data generated above, and should be approved by the NRA.

A.2.2.1.2 *Primary cells*

Primary cells are used within the first passage after establishment from the original tissue, and so it is not possible to carry out extensive characterization of the cells prior to their use. Therefore additional emphasis is placed on the origin of the tissues from which the cell line is derived. Tissues should be derived

from healthy animals/embryonated eggs subjected to veterinary and laboratory monitoring to certify the absence of pathogenic agents. Whenever possible, donor animals/embryonated eggs should be obtained from closed, specific-pathogen-free colonies or flocks. Animals used as tissue donors should not have been used previously for experimental studies. Birds and other animals should be adequately quarantined for an appropriate period of time prior to use for the preparation of cells.

Information on the materials and components used for the preparation of primary cell substrates should be provided, including the identity and source of all reagents of human or animal origin. A description of the testing performed on components of animal origin to certify the absence of detectable contaminants and adventitious agents should be included.

The methods used for the isolation of cells from tissue, establishment of primary cell cultures and maintenance of cultures should be described.

A.2.2.2 Testing of cell banks and primary cells

A.2.2.2.1 *Tests on MCBs and WCBs*

MCBs and WCBs should be tested for the absence of bacterial, fungal, mycoplasmal and viral contamination by appropriate tests, as specified in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*24*), or by a method approved by the NRA, to demonstrate that they are not contaminated with adventitious agents.

Rapid sterility methods to demonstrate the absence of bacteria and fungi, as well as NAT-based assays alone or in combination with cell culture, may be used as an alternative to one or both of the compendial mycoplasmal detection methods after suitable validation and agreement from the NRA (*24*).

The cell bank should be tested for tumorigenicity if it is of mammalian origin, as described in Part B 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 (*24*). The tumorigenic potential of the cell bank(s) should be described and strategies to mitigate risks that might be associated with this biological property should be described and justified.

> During a public health emergency, it is anticipated that the majority of the above information should be available and submitted for evaluation since it is essential to demonstrate the suitability and safety of the product. However, it may be justified to initiate clinical trials using a product which is manufactured prior to establishment of the cell banking system. In such a case, the suitability and safety of the product must be established prior to its use, especially with regard to adventitious agents (*24*).

A.2.2.2.2 *Tests on primary cells*

The nature of primary cells precludes extensive testing and characterization before use. Testing to demonstrate the absence of adventitious agents (bacteria, fungi, mycoplasmas and viruses) is therefore conducted concurrently, and should include, where relevant, the observation of control (uninfected) cultures during parallel fermentations to the production runs. The inoculation of culture fluid from production cultures and (where available) control cultures into various susceptible indicator cell cultures capable of detecting a wide range of relevant viruses (followed by examination for cytopathic changes and testing for the presence of haemadsorbing viruses) should also be performed routinely for batch release. In addition, pharmacopoeial testing for bacteria, fungi and mycoplasmas in the production cultures and (if relevant) control cultures should be conducted. Mycoplasmas and specific viruses of notable concern may also be tested for by additional methods such as PCR.

In the specific case of chick embryo fibroblasts (CEFs), the tissue should be sourced from specific-pathogen-free eggs. After preparation, the CEF cells should be tested for: (a) bacterial, fungal and mycoplasmal contamination; (b) viral adventitious agents by in vitro assay using three cell lines, including avian and human cells (such as CEF, MRC-5 and Vero); (c) viral adventitious agents by in vivo assay using mice and embryonated eggs; (d) avian leukosis virus contamination; and (e) the presence of retroviruses by measuring reverse transcriptase (RT) activity. Testing should take into consideration that CEF cells are expected to be positive for RT activity due to the presence of endogenous avian retroviral elements not associated with infectious retroviruses. It may be necessary to use an amplification strategy (for example, co-culturing of RT-positive fluids on an RT-negative, retrovirus-sensitive cell line) to determine whether a positive RT result can be attributed to the presence of an infectious retroviral agent.

A.2.3 Source materials used for cell culture and virus propagation

If serum is used for the propagation of cells it should be tested to demonstrate the absence of bacteria, fungi and mycoplasmas, as specified in the requirements given in Part A – section 5.2 (*80*) and section 5.3 (*81*) – of the WHO General requirements for the sterility of biological substances. Testing should also be conducted to demonstrate freedom from adventitious viruses.

Detailed guidance on detecting bovine viruses in serum used to establish MCBs and WCBs is provided in Appendix 1 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*24*) and should be applied as appropriate. This same guidance may also be applicable to production cell cultures. As an additional monitor of quality, sera may be

examined for endotoxin. Gamma irradiation may be used to inactivate potential contaminant viruses, while recognizing that some viruses are relatively resistant to gamma irradiation. Whichever viral inactivation process is used, a validation study must be conducted to determine its consistency and effectiveness while still maintaining serum performance. The use of non-inactivated serum should be justified and is not advised without strong justification. Any non-inactivated serum must meet the same criteria as inactivated serum when tested for sterility and absence of mycoplasmal and viral contaminants.

The source(s) of animal components used in culture medium should be approved by the NRA. These components should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*82*).

Bovine or porcine trypsin used to prepare cell cultures should be tested and found free of bacteria, fungi, mycoplasmas and adventitious 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* (*82*).

In some countries, irradiation is used to inactivate potential contaminant viruses in trypsin. If irradiation is used, it is important to ensure that a reproducible dose is delivered to all batches and to the component units of each batch. The irradiation dose must be low enough for the biological properties of the reagents to be retained while being high enough to reduce virological risk. Consequently, irradiation cannot be considered a sterilizing process (*24*). The irradiation method should be validated and approved by the NRA.

Recombinant trypsin is available and should be considered – however, it should not be assumed to be free of risk of contamination and should be subject to the usual considerations for any reagent of biological origin (*24*).

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 for this, 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 (*83*). In addition, human albumin and materials of animal origin should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*82*). Recombinant human serum albumin is available and should be considered as a substitute for the plasma-derived product.

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 at any stage of manufacture, provided that the quantity present in the final product is acceptable to the NRA.

Non-toxic pH indicators may be added (for example, phenol red at a concentration of 0.002%). Only substances that have been approved by the NRA may be added.

A.2.4 Special considerations for the development and testing of the viral vector and production cell lines

Early-phase nonclinical and clinical studies are generally supplied with product for which the level of knowledge of manufacture and control is expected to be quite rudimentary since few batches will have been manufactured and analytical methods will be in the early stages of development. The provision of material is required for early safety and proof-of-concept studies, as well as to initiate the dose-finding evaluation. Product will be tested initially in animals and then in a small number of human subjects in a well-controlled environment. This is the normal situation when there is no public health emergency and, in these circumstances, guidance on the quality requirements for investigational medicinal products in clinical trials is available (*84*).

Most data to be provided to the NRA before human studies can begin will concern the derivation and safety of the viral vector and the production cell line. The data will aim to show that the product and production system are well designed, the function of each genetic element is known and its inclusion in the product or cell line is justified. It should be confirmed that the expected elements are present in the product and cell line and that the final structure of the product is as predicted. A full description of the origin and construction of the genetic components of the viral vector and cell line should be provided, along with data on genetic stability up to (or preferably beyond) the anticipated maximum passage level in manufacture. Ideally, a virus master seed/virus working seed for the viral vector and MCB/WCB for the production cell line should be prepared early in the development of the product – though it is acknowledged that this may not be practical in the initial stages. Testing of the seed lots and cell banks at the time of their establishment should confirm comparability to the parental material. Any starting material (viral seeds and production cell lines) used to manufacture product for clinical use must be fully tested to ensure the absence of bacteria, fungi, mycoplasmas and adventitious viruses (*24*, *80*). Where applicable, freedom from TSEs must also be addressed (*82*). The potential for tumorigenicity of the cell line should also be tested and should meet current regulatory standards if it is of mammalian origin. All reagents used in the manufacture of the virus seed or cell lines (including cell culture solutions) should be tested and characterized as being of adequate quality, particularly regarding freedom from adventitious agents.

A.3 Control of Ebola vaccine production

A.3.1 Manufacture and purification

The manufacture of monovalent vaccine vectors starts with the amplification of the vaccine vector seed stock in a suitable cell line. The number of passages between the virus working seed lot and viral-vectored vaccine product should be kept to a minimum and should not exceed the number used for production of the vaccine shown in clinical studies to be satisfactory, unless otherwise justified and authorized.

If applicable to the vector platform, a control cell culture should be maintained simultaneously and in parallel with the production cell culture. Cells should be derived from the same expansion series but no virus vector should be added to the control cells. The growth medium and supplements used in culturing should be identical for the production cell culture and control cell culture. All other manipulations should be as similar as possible.

After harvesting of the culture product, the purification procedure can be applied to a single harvest or to a pool of single monovalent harvests. The maximum number of single harvests that may be pooled should be defined on the basis of validation studies.

Multivalent vaccines are generally prepared by combining batches of purified monovalent bulk that contain more than one EBOV strain and/or MARV strain. However, if the vaccine consists of a single vector containing genes encoding multiple antigens, then the recommendations for monovalent bulk manufacturing should be followed, but testing should take into account the multivalent identity and potency of the product.

By the time a marketing authorization application is submitted the manufacturing process should be adequately validated by demonstrating that a sufficient number of commercial-scale batches can be manufactured routinely under a state of control by meeting predetermined in-process controls, critical process parameters and lot release specifications. Any materials added during the purification process should be documented and their removal should be adequately validated or residual amounts tested for, as appropriate. Validation should also demonstrate that the manufacturing facility and equipment have been qualified, cleaning of product contact surfaces is adequate, and critical process steps (such as sterile filtrations and aseptic operations) have been validated.

The purified viral vector bulk and intermediates should be maintained under conditions shown by the manufacturer to ensure the retaining of the desired biological activity. Hold times should be defined.

During early clinical trials it is unlikely that there will be data from sufficient batches to validate/qualify product manufacture. However, as development progresses, data should be obtained from subsequent manufacture

and should be used in support of an eventual application for commercial supply of the product.

> During a public health emergency, on a case-by-case basis, some requirements of process validation may be abbreviated provided it can be demonstrated that the product will remain safe and well controlled. For example, if platform-specific data have demonstrated that scale-up for a vector is independent of the specific heterologous insert, this information may be used to justify fewer full-scale batches with the EBOV gene insert and a greater reliance on pre-validation and pilot-plant-scale batches. Validation data from the manufacture of platform-related products may provide useful supportive information, particularly in the identification of critical parameters.
>
> Since it is likely that there will initially be insufficient time to generate full validation data during an emergency situation, as much information as possible on the control of each batch should be presented to the NRA as supporting evidence that batch manufacture is sufficiently controlled. However, manufacturers should agree on the strategy with the NRA before relying on platform-specific validation data.

In addition to control during manufacture, the products should be adequately characterized by the stage of development. These attributes facilitate understanding of the biology of the candidate vaccine and assessment of the impact of any changes in manufacturing that are introduced as development advances or following licensure. Assessing the immunogenicity of the product, when relevant, should also be included in the characterization programme (for example, as part of the nonclinical pharmacodynamic evaluation).

A.3.1.1 Tests on control cell cultures (if applicable)

When control cells are included in the manufacturing process due to limitations on the testing of primary cells or viral harvests, or when their inclusion is required by the NRA, the following procedures should be followed. From the cells used to prepare cultures for vaccine production, a fraction equivalent to at least 5% of the total or 500 ml of cell suspension or 100 million cells should be used to prepare uninfected control cell cultures.

These control cultures should be observed microscopically for cytopathic and morphological changes attributable to the presence of adventitious agents for at least 14 days (at a temperature of 35–37 °C) after the day of inoculation of the production cultures, or until the time of final virus harvest, whichever comes last. At the end of the observation period, supernatant fluids collected from the control culture should be tested for the presence of adventitious agents, as described below. Samples that are not tested immediately should be stored at −60 °C or lower until such tests can be conducted.

If testing the control cultures for adventitious agents yields a positive result, the harvest of virus from the parallel vaccine-virus-infected cultures should not be used for production.

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

A.3.1.1.1 *Tests for haemadsorbing viruses*

At the end of the observation period a fraction of control cells comprising not less than 25% of the total should be tested for the presence of haemadsorbing viruses, using guinea-pig red blood cells. If the red blood cells have been stored prior to use in the haemadsorption assay, the duration of storage should not have exceeded 7 days and the temperature of storage should have been in the range of 2–8 °C.

In some countries the NRA requires that additional tests for haemadsorbing viruses are performed using other red blood cells, including human (blood group O), monkey and/or chicken (or other avian species). All haemadsorption tests should be read after incubation for 30 minutes at 0–4 °C, and again after further incubation for 30 minutes at 20–25 °C. Tests using monkey red blood cells should be read once more after additional incubation for 30 minutes at 34–37 °C.

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.

A.3.1.1.2 *Tests for other adventitious agents*

At the end of the observation period, a sample of the pooled fluid and/or cell lysate from each group of control cell cultures should be tested for adventitious agents. For this purpose, an aliquot of each pool should be tested in cells of the same species used for the production of virus, but not cultures derived directly from the production cell expansion series for the batch which is subject to the test. If primary cells are used for production then a different batch of that primary cell type should be used for the test than was used for production. Samples of each pool should also be tested in human cells and in a simian kidney cell line. At least one culture vessel of each kind of cell culture should remain uninoculated as a control.

The inoculated cultures should be incubated at the appropriate growth temperature and should be observed for cytopathic effects for a period of at least 14 days.

Some NRAs require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least an additional 7 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.

A.3.2 Single virus harvest

The method of harvesting the vaccine vector should be described and the titre of virus ascertained. A reference preparation should be included to validate the titration assay. Minimum acceptable titres should be established for a single virus harvest or pooled single harvests.

The integrity of the integrated heterologous gene should be confirmed. An expression assay method should be described and should be performed on production harvest material or downstream (for example, on purified final bulk). A Western blot analysis or other method for confirming that the integrated gene is present and expressed should be included in the testing of every batch.

A.3.2.1 Control tests on single virus harvest

Tests for adventitious agents should be performed on each single virus harvest according to the relevant parts of section B.11 of the WHO Recommendations for the evaluation of animal cells as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*24*). Additional testing for adventitious viruses may be performed using validated NAT-based assays.

New molecular methods with broad detection capabilities are being developed for adventitious agent detection. These methods include: (a) degenerate NAT-based assays for whole virus families, with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (b) NAT-based assays using random primers followed by analysis of the amplicons on large oligonucleotide micro-arrays of conserved viral sequencing or by digital subtraction of expressed sequences; and (c) high-throughput sequencing. 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 agreement from the NRA.

Single or pooled virus harvests should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, as specified in the requirements given in Part A – section 5.2 (*80*) and section 5.3 (*81*) – of the WHO General requirements for the sterility of biological substances.

For viral-vectored vaccines, due to the very high titres of the single harvests, alternatives to the classical approaches to testing for adventitious agents may be applied with the approval of the NRA.

Provided that the cell banks and viral seed stocks have been comprehensively tested and released, demonstrating that they are free of adventitious agents, the possibility of delaying in vitro testing for adventitious

agents (viral pathogens and mycoplasmas) in the cell harvest or bulk substance, or replacing it with validated PCR tests, could be evaluated subject to the agreement of the NRA. The method of production should be taken into account when deciding upon the nature of any specified viruses being sought.

Additional considerations for this approach are that no animal-derived raw materials are used during manufacture, and that the manufacturing facility operates under a GMP certificate (where applicable) with assurances that prevention of cross-contamination is well controlled within the facility. Samples should be retained for testing at a later date if required.

A.3.3 Pooled monovalent virus harvests

Single virus harvests may be pooled to form virus pools from which the final bulk vaccine will be prepared. The strategy for pooling single virus harvests should be described. All processing of the virus pool should be described in detail.

A.3.3.1 Control tests on pooled virus harvests

Virus pools should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, as specified in the requirements given in Part A – section 5.2 (*80*) and section 5.3 (*81*) – of the WHO General requirements for the sterility of biological substances. Alternatively, if single virus harvests have been tested to demonstrate freedom from bacteria, fungi and mycoplasmas then these tests may be omitted on the pooled virus harvests.

A.3.4 Monovalent bulk vaccine

The monovalent bulk vaccine can be prepared from one or several virus pools containing the same antigen, or it may be derived from a single virus harvest. Substances such as diluents or stabilizers or any other excipients added during preparation of the monovalent bulk or the final bulk vaccine should have been shown not to impair the potency and safety of the vaccine in the concentrations used.

A.3.4.1 Control tests on monovalent bulk

The monovalent bulk vaccine should be tested and consideration given to using the tests listed below for the individual products as appropriate. Alternatively, if the monovalent bulk will be held for only a short period of time, some of the tests listed below could – if appropriate – be performed instead on the final bulk or final lot. If sufficiently justified, some of the tests may be performed on an earlier intermediate instead of on the monovalent bulk. All quality-control release tests for monovalent bulk should be validated and shown to be suitable for the intended purpose. Assay validation or qualification should be appropriate

for the stage of the development life-cycle. Additional tests on intermediates during the purification process may be used to monitor consistency and safety.

> During an emergency situation it is anticipated that critical assays would be fully validated. Specifications should also be given for each critical parameter. Qualification or validation, as well as specifications for some assays, may be based on related products (for example, products with the same vector backbone but differing in heterologous gene from the Ebola GP gene) where it can be justified that the specific heterologous gene used is unlikely to have an impact on the result. An example of this would be particle quantification by qPCR where the probe is demonstrated to be a non-EBOV sequence in the vector.
>
> With appropriate justification, validation for non-critical assays could be completed after product approval, provided that assay verification adequately demonstrates that the assay is fit for purpose and under control.
>
> Similarly, if adequately justified, not all of the proposed assays may need to be completed for clinical trial batch release. If it can be justified that product safety and potency are not compromised, that completion of the test(s) would delay product availability for use in clinical trials, and/or that the test(s) would use up an unacceptably large volume of the product urgently required for clinical trials, it may be possible to omit or delay the test, or replace it with one that is more acceptable in terms of the overall aims of the clinical trials in an emergency situation.
>
> However, all of the approaches discussed above should be agreed with the NRA on a case-by-case basis.

A.3.4.1.1 ***Purity***

The degree of purity of each monovalent bulk vaccine should be assessed using suitable methods. This should include testing for the presence of fragments, aggregates or empty particles of the product, as well as for contamination by residual cellular proteins. Residual cellular DNA levels should also be assessed when non-primary cell substrates are used for production. The content and size of host cell DNA should not exceed the maximum levels agreed with the NRA, taking into consideration issues such as those discussed in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*24*).

Process additives should also be controlled. In particular, if any antibiotics are added during vaccine production, the residual antibiotic content should be determined and should be within limits approved by the NRA.

> In a public health emergency, theoretical calculations to determine residual levels of process contaminants (except DNA and proteins) may be acceptable at the time of licensure – data should however be submitted as soon as possible post-licensure.

These tests may be omitted for routine lot release upon demonstration that the process consistently clears the residuals from the monovalent bulk vaccine, subject to the agreement of the NRA.

A.3.4.1.2 *Potency*

Each monovalent bulk vaccine should be tested for potency using a combination of the following methods.

Particle number

For relevant vectors (for example, adenovirus vectors) the total number of virus particles per millilitre, quantitated by techniques such as qPCR or high-performance liquid chromatography, should be determined for each batch of monovalent bulk.

Infectivity

The infectious virus titre for each batch of monovalent bulk should be determined as a measure of active product. Direct methods such as a plaque-forming assay or indirect methods such as qPCR (if suitably correlated with a direct measure of infectivity) could be considered. The particle/infectivity ratio should also be specified.

Expression of the heterologous antigen in vitro

The ability of the viral particles to express the heterologous gene should be demonstrated (for example, by the generation of immunoblots using antigen-specific antibodies) following amplification of the vector in a suitable cell line.

A.3.4.1.3 *Identity*

Tests used for assessing relevant properties of the viral vector – such as antigen expression, restriction analysis, PCR with a specific probe or sequencing – will generally be suitable for assessing the identity of the product.

A.3.4.1.4 *Sterility or bioburden tests for bacteria and fungi*

Each monovalent bulk should be tested for bacterial and fungal bioburden or sterility. Bioburden testing should be justified in terms of product safety. Sterility testing should be as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (*80*), or by methods approved by the NRA.

A.3.4.1.5 *Bacterial endotoxins*

Each monovalent bulk should be tested for bacterial endotoxins. At the concentration of the final formulation of the vaccine, the total amount of residual endotoxins should not exceed that found in vaccine lots shown to be safe in clinical trials or the amount found in other lots used to support licensing. The test may be omitted once production consistency has been demonstrated after agreement from the NRA.

A.3.4.1.6 *Reversion to replication competency or loss of attenuation*

The viral-vectored Ebola vaccines under development are either replication-incompetent in human cells or adequately attenuated to prevent disease symptoms related to the viral vector backbone. Although manufacturers generally provide theoretical justifications for why reversion to competency or virulence is unlikely to occur, low levels of viral particles may emerge that have gained the complementing gene from the production cell line by an unknown or poorly characterized mechanism. These viral particles are considered to be an impurity – it is not known whether they represent a safety concern. It should also be taken into account that many individuals within the Ebola target population could be immunocompromised. Consequently, it should be shown that the product is still replication-incompetent or fully attenuated (whichever is relevant) in initial batches of the product. After demonstrating this, it may be possible to omit such tests in future batches provided a sufficient justification is made. Such justification should include the demonstration of replication incompetence/attenuation, and discussion of why reversion to competency or loss of attenuation will not occur in future batches.

A.3.4.1.7 *Preservative content (if applicable)*

The monovalent bulk may be tested for the presence of preservative, if added. The method used and the permitted concentration should be approved by the NRA.

A.3.5 Final bulk vaccine

To manufacture the final bulk vaccine, appropriate quantities of different monovalent bulk vaccines should be pooled, mixed and formulated (if required) to form an homogeneous solution. The final bulk can be made up of one or more batches of a single monovalent vaccine, to give a monovalent vaccine product or alternatively, batches of several different monovalent bulks may be mixed to yield a multivalent vaccine.

For multi-dose preparations, the need for effective antimicrobial preservation should be evaluated, taking into account possible contamination during use and the maximum recommended period of use after opening the container or after reconstitution of the vaccine. If an antimicrobial preservative

is used, it should not impair the safety or potency of the vaccine; the intended concentration of the preservative should be justified and its effectiveness should be validated (*85*).

A.3.5.1 Control tests on final bulk vaccine

The following tests should be performed on the final bulk vaccine, unless otherwise justified and agreed with the NRA.

A.3.5.1.1 *Identity*

See section A.3.4.1.3.

A.3.5.1.2 *Antimicrobial preservative*

Where applicable, the amount of antimicrobial preservative should be determined by a suitable chemical method.

A.3.5.1.3 *Sterility tests for bacteria and fungi*

See section A.3.4.1.4.

A.3.6 Special considerations for manufacture and validation

It is acknowledged that the fermentation and downstream processes might undergo considerable optimization after the initial clinical batches are produced. Where control cells are grown in parallel to production cells, their raw materials and fermentation should be aligned with production cell manufacturing procedures. Process and product characterization should ensure the comparability of product throughout development. Some changes in product characteristics can be anticipated (for example, intended improvements due to optimization studies, or unintended changes due to a process change). All such changes should be identified and presented in clinical trial submissions or during an application for a product licence and the implications of the change should be discussed. It is not expected that process consistency will be demonstrated during early clinical development, partly because insufficient batches will have been produced to allow for adequate process validation and also because the process is likely to be undergoing optimization. However, all available batch data (including qualitative and quantitative data) should be presented. The product must be demonstrated to be free from contaminants and sufficiently characterized to allow bridging to later clinical material and commercial product. Process validation should address safety issues such as aseptic operations, sterile filtrations, cleaning validations, environmental control of facilities and validation of process utilities – such as heating, ventilation and air conditioning (HVAC) systems, and water for injection systems.

It is expected that during an emergency situation these validation criteria would be adequately addressed.

During early development, validation of pooling of single viral harvests may not have been completed and so the number of harvests pooled should be defined based on other criteria such as production requirements.

During later clinical stages and at licensing submission, the manufacturing process is normally firmly established and process-specific validation completed by demonstrating that several consecutive full-scale commercial batches can be made that conform to predetermined criteria.

Although the "Quality-by-Design" approach is not considered in these WHO Guidelines, such an approach is not excluded provided that the principles discussed throughout this document are adequately addressed.

A.3.7 Special considerations for Good Manufacturing Practice

The principles of GMP should be adhered to during the manufacture of product for clinical studies – even during a public health emergency. This may be particularly important if some normal elements of development or control have been omitted because of the urgent need for product. For example, if certain testing is to be omitted on the basis that the test is also conducted on an upstream intermediate, it is essential that the process is operated under full control. Validation and specifications are likely to be provisional during the manufacture of product for clinical trials, and additionally the process is not likely to be well understood since only a limited number of batches will have been produced. Therefore, it becomes essential that the principles of GMP, as laid down for the manufacture of investigational medicinal products, are followed (*69*, *70*, *86*).

A.3.8 Special considerations for analytical procedures and specifications

Testing of critical intermediates and of the final product, as well as in-process control testing, should primarily confirm product safety for early clinical trial batches. In this regard, tests for bioburden/sterility, endotoxin and freedom from adventitious agents should be fully developed and validated and should be applied to each batch (although some flexibility towards adventitious virus testing is also discussed in these WHO Guidelines). Other tests may not be fully validated. However, even from an early clinical phase, assay verification should have been performed. This is likely to fall short of the full validation requirements detailed in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline Q2(R1) (*87*), but should nevertheless give an indication that each method is fit for purpose.

Tests for safety, quantity, potency, identity and purity are mandatory. Upper limits should be set for quantity of impurities, taking safety considerations

into account. For relevant virus vectors, reversion to competency should be tested for. A justification should be provided for the quality attributes included in the specification and for the acceptance criteria for purity, impurities, quantity, potency and any other quality attributes which may be relevant to vaccine performance. The justification should be based on relevant development data, the batches used in nonclinical and/or clinical studies, and data from stability studies. It is acknowledged that during early clinical development, the acceptance criteria may be wider than the final specification for product intended for Phase III studies and for commercial product. During the manufacture of products for initial clinical trials, not all attributes tested may have established specification ranges since insufficient batches may have been made to know what an acceptable range is. Nor at this time is a clinically meaningful range always known. However, as the clinical programme continues – and certainly by the time of initiation of Phase III trials – specification ranges should be set for each attribute.

Product characteristics that are not completely defined in the early stages of development, or for which the available data are too limited to establish relevant acceptance criteria, should also be recorded. As a consequence, such product characteristics could be included in the specification without predefined acceptance limits. At the initial stages of development, testing may not be required to determine residual levels of process contaminants (except DNA and proteins) if sufficient justification can be provided by theoretical calculation. However, data to confirm the calculations should be provided prior to the licensing application.

For later-stage clinical trials, it is expected that all analytical procedures would be validated according to the principles set out in ICH Q2(R1) (*87*). Specifications for each parameter should be justified by process capability as well as by clinical suitability. If justified, following the manufacture of additional batches of product, the sponsor should commit to revise the specifications as data on process capability are accumulated.

> During a public health emergency, data on clinical suitability are likely to be limited and should be taken into account to the extent that they are available.

A.4 Filling and containers

The general requirements concerning filling and containers given in WHO good manufacturing practices for biological products (*70*) should apply to vaccine filled in the final form.

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

If multi-dose vaccine vials are used and these vaccines do not contain preservative then their use should be time-restricted, as is the case for reconstituted vaccines such as bacillus Calmette–Guérin (BCG) and measles-containing vaccines (*85*). In addition, the multi-dose container should prevent microbial contamination of the contents after opening. The extractable volume of multi-dose vials should be validated.

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

A.5 Control tests on final lot

Samples should be taken from each final vaccine lot – which may be monovalent or multivalent. These samples must fulfil the requirements of this section. All tests and specifications should be 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.

A.5.1 Inspection of containers

Every container in each final lot should be inspected visually or mechanically. Those showing abnormalities should be discarded and each relevant abnormality should be recorded. A limit should be established for the maximum number of containers which can be discarded before investigation of the cause; potentially resulting in batch failure.

A.5.2 Appearance

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

A.5.3 Identity

See section A.3.4.1.3. For multivalent vaccine each antigen component should be identified.

A.5.4 Sterility tests for bacteria and fungi

See section A.3.4.1.4.

A.5.5 General safety test (innocuity)

The need to test the final lots of the Ebola vaccine for unexpected toxicity (also known as abnormal toxicity) should be discussed and agreed with the NRA.

Some countries no longer require this test (*88*, *89*).

A.5.6 Purity

Testing for purity should be performed unless it is performed on the monovalent bulk or final bulk vaccine. However, limited purity testing of the final lot may be required even if purity is tested on the final bulk vaccine if, after taking the manufacturing process and nature of the vector into consideration, it is considered possible that the purity may have changed. This should be considered on a case-by-case basis.

A.5.7 pH and osmolality

The pH and osmolality values of each final lot of containers should be tested. Lyophilized products should be reconstituted with the appropriate diluent prior to testing.

A.5.8 Test for pyrogenic substances

Each final lot should be tested for pyrogenic substances through intravenous injection into rabbits. A Limulus amoebocyte lysate (LAL) test may be used in lieu of the rabbit pyrogen test if it has been validated and the presence of non-endotoxin pyrogens has been ruled out. A suitably validated monocyte-activation test may also be considered as an alternative to the rabbit pyrogen test. The endotoxin content or pyrogenic activity should be consistent with levels found to be acceptable in vaccine lots used in clinical trials and should be approved by the NRA.

A.5.9 Potency, particle number and infectivity

See section A.3.4.1.2.

The potency specifications for live viral-vectored vaccines should be set based on the minimum dose used to demonstrate efficacy or effectiveness in human clinical trials and/or challenge studies with a suitable non-human preclinical model plus human immunogenicity data. An upper limit should also be defined based on available human safety data. For multivalent vaccines it may be necessary to perform this test on the monovalent bulks instead if analytical methods cannot distinguish between the different monovalent vaccines in the final lot.

A.5.10 Extractable volume

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

A.5.11 Aggregates/particle size

Since virus particles are susceptible to aggregation, each final lot should be examined for particle size/aggregate content at lot release and at end of shelf-life unless it can be shown that the test is not necessary.

A.5.12 Preservatives (if applicable)

Each final lot should be tested for the presence of preservative, if added.

A.5.13 Residual moisture (if applicable)

For freeze-dried final product, the residual moisture should be shown to be within acceptable limits.

A.5.14 Reconstitution time (if applicable)

For freeze-dried final product, the reconstitution time of the product should conform to specification.

A.6 Records

The requirements given in section 17 of WHO good manufacturing practices for biological products (*70*) should apply.

A.7 Retained samples

The requirements given in section 16 of WHO good manufacturing practices for biological products (70) should apply.

A.8 Labelling

The requirements given in section 14 of WHO good manufacturing practices for biological products (*70*) should apply.

The label on the carton, the container or the leaflet accompanying the container should state:

- the name of the vaccine;
- the lot number;
- the nature of the cells used to grow the viral vector;
- the volume of one recommended human dose, the immunization schedule and the recommended routes of administration;
- the amount of active substance(s) contained in one recommended human dose;

- the number of doses, if the product is issued in a multi-dose container;
- the name and maximum quantity of any antibiotic present in the vaccine;
- the name and concentration of any preservative added;
- the temperature recommended during storage and transport;
- the expiry/retest date;
- any special dosing schedules; and
- contraindications, warnings and precautions, concomitant vaccine use advice, and potential adverse reactions.

Labelling should conform to the national requirements of the region in which the vaccine will be used.

A.9 Distribution and transport

Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (*90*).

Efforts should be made to ensure that shipping conditions are such as to maintain the vaccine in an appropriate environment. Temperature indicators should be packaged with each vaccine shipment to monitor fluctuations in temperature during transportation.

A.10 Stability testing, storage and expiry date

A.10.1 Stability testing

Adequate stability studies form an essential part of vaccine development. Guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (*91*). Stability testing should be performed at different stages of production, namely: on single harvests or single harvest pools (if the process is held up for a period of time, which may affect product attributes at these points); final monovalent bulk; final bulk; whenever materials are stored for a period of time before further processing (which may affect product attributes); and final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. A shelf-life should be established and assigned to all in-process materials during vaccine production, and particularly to the vaccine intermediates.

Accelerated stability tests may be undertaken to give additional information on the overall characteristics of a vaccine, and may also be useful in assessing comparability when the manufacturer plans to make changes to manufacturing.

For vaccine licensure, the stability and expiry date of the vaccine in its final container, when maintained at the recommended storage temperature, should be demonstrated to the satisfaction of the NRA using final containers from at least three final lots made from different vaccine bulks. During clinical trials, fewer data are likely to be available. However, the stability of the vaccine under the proposed storage conditions should be demonstrated for at least the expected duration of the clinical trial.

Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (*91*). Data should be provided to the NRA according to local regulatory requirements.

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

In-use stability should also be specified and justified with adequate data generated under real-time conditions.

> In an emergency situation and during early clinical trials, limited stability data on the monovalent or final bulk vaccine and finished product may be acceptable to preserve scarce stocks of product for use in clinical trials, or if there is insufficient time to generate real-time stability data. Data from one batch of bulk and final product may be sufficient initially but this should be supplemented with data from at least two more batches of bulk and final product as material that is surplus to clinical trial requirements becomes available.
>
> Even if limited stability data are available, it is preferable to provide an expiry or retest date on the immediate product label since this provides important information to the user. If this goes beyond the available real-time data, accelerated stability data should be available to help support the proposed extrapolation to the shelf-life, and the clinical trial sites should be able to demonstrate a robust system for recalling the product if real-time data do not support the extrapolated shelf-life. In exceptional circumstances, the rationale for omitting this information from the label may be discussed with NRAs.

A.10.2 Storage conditions

Storage conditions should be fully validated. The vaccine should have been shown to maintain its potency for a period equal to that from the date of release to the expiry date. During clinical trials, this period should ideally be at least equal to the expected duration of the clinical trial.

A.10.3 Expiry date

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

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals are inoculated.

Part B. Nonclinical evaluation of Ebola vaccines

B.1 General remarks

The design, conduct and analysis of nonclinical studies should be based on the WHO Guidelines on nonclinical evaluation of vaccines (*20*). Further guidance can be found in WHO and national and regional documents on DNA vaccines (*14*, *15*) and live recombinant viral-vectored vaccines (*16–18*).

The nonclinical safety evaluation, whenever necessary, should yield sufficient information to demonstrate that the candidate vaccine is reasonably safe for use in humans.

The following sections describe the types of nonclinical information that should be submitted to support the licensing of a new Ebola vaccine. Wherever appropriate, recommendations are also made on the minimum dataset required.

B.2 Product characterization and process development

It is vitally important that vaccine production processes are standardized and appropriately controlled to ensure consistency in manufacturing. The extent of process validation may vary with the stage of product development. The vaccine lots produced for nonclinical good laboratory practice (GLP) safety studies should be manufactured with production process, formulation and release specifications similar to those of the lots intended for clinical use. Supporting stability data generated under conditions of use should be provided.

For a live viral-vectored vaccine, the degree of attenuation and the stability of the phenotype should be evaluated. The critical genetic and phenotypic markers of stability of the vector genome should as far as is practical be defined. Phenotypic markers are useful for the detection of reversion events and may include, though are not restricted to, vector replication efficiency, induction of viraemia and level of virulence, and neurovirulence. The need for neurovirulence testing is discussed below in section B.4.

B.3 Pharmacodynamic studies

B.3.1 Challenge-protection studies

In the past, rodents (mouse, guinea-pig) and non-human primates (cynomolgus or rhesus macaques) have been used to study the pathogenesis of EBOV infection and the mechanism of immune protection. Rodent models are frequently used to provide initial evidence for the immunogenicity or efficacy of candidate vaccines. However, non-human primates display natural susceptibility to EBOV infection and similarity in genetics, morphology and immunology with humans, and more closely mimic EVD observed in humans. As a consequence, the non-human primate models are particularly useful for proof-of-concept challenge studies and characterization of the mechanism of protection. It is expected that proof-of-concept data be collected for each virus strain included in the candidate vaccines.

It should be noted that conducting proof-of-concept challenge studies with wild-type EBOV requires a BSL-4 containment facility. The same requirement may apply to running virus-neutralization assays when wild-type EBOV is used to evaluate vaccine immunogenicity and to evaluate serology samples obtained from animals after EBOV challenge. A BSL-2 facility is sufficient to contain animals until the time of challenge, to run other immunological assays – such as enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot (ELISpot) and intracellular cytokine staining (ICS) – without involvement of a wild-type EBOV, or to manufacture a genetically modified organism (GMO).

Due to limited availability of BSL-4 laboratories, the proof-of-concept challenge studies will generally be small. Nonetheless, these studies are of higher predictive value than immunogenicity studies for forecasting vaccine performance in humans. The parallel assessment of vaccine immunogenicity and efficacy (protection from EVD) in proof-of-concept challenge studies may permit the establishment of an immune correlate of protection (ICP) and an understanding of the underlying protective mechanism.

> Either during a public health emergency or in a normal situation, the challenge studies are not required prior to initiating Phase I clinical trials. However, it is nevertheless desirable for proof-of-concept challenge studies to be conducted early during product development since these studies, in combination with immunogenicity assessment, could provide important information regarding an ICP and protective mechanism, which would assist in the selection of immunological end-points in subsequent clinical trials.

The design of challenge-protection studies should take into account the planned posology for a specific route of administration and valency of candidate vaccines. For a multivalent candidate vaccine intended to induce durable protective immunity, a heterologous prime-boost regimen may need to

be considered. The protective activity of the vaccine with respect to each of the Ebola strains targeted should be assessed.

As in any challenge-protection animal study, the end-points used to define protection should normally correlate with the desired effect in humans – typically a survival benefit or attenuation of severe disease indicators such as viral shedding, body weight changes and other relevant clinical signs. Other key characteristics of the experimental design include the use of appropriate challenge virus strains, dose(s) and route of challenge. The challenge dose should be sufficiently high to produce an appropriate degree of lethality in the control group of animals so that the vaccine protective effect can be shown with adequate statistical power. For example, doses of 100–1000 plaque-forming units (PFU) have been used (92).

The collection of challenge-protection data should take account of the proposed indication for use – that is, pre-exposure versus post-exposure prophylaxis against EVD. Appropriate timing of the challenge is another important consideration. For pre-exposure prophylaxis, animals are usually challenged at the time when the peak level of vaccine response (for example, peak antibody titres) has developed post-vaccination. Where feasible, it would also be informative for various public health vaccine strategies to challenge animals at other times (for example, before the peak response or after the immune responses have waned). For post-exposure prophylaxis, challenge at various time points should be considered.

B.3.1.1 Use of a challenge-protection animal study to support licensure

In some circumstances in which demonstrating vaccine efficacy in clinical trials is not feasible – due to low rates of EVD or absence of an EVD outbreak, or when a human ICP has not been established for a vaccine – manufacturers may propose an alternative approach to estimating vaccine effectiveness to support licensing (for example, by inferring animal challenge results to humans). If this course is pursued – and agreed to by the relevant NRA – the study should be adequately designed to generate reliable data for inferring effectiveness in humans (see section C.2.5).

Beyond the key design elements discussed above, further considerations may include the use of non-human primates, vaccinating animals with an appropriate range of doses of the vaccine so that the level of immune response developed in animals (for example, range of relevant antibody titres) can match that in humans. Compliance with GLP also brings significant advantages and is encouraged. However, it is acknowledged that compliance with GLP may not be possible in BSL-4 laboratories. Consequently, well-controlled and well-documented non-GLP studies are also acceptable. The use of good documentation practices to ensure data integrity is required.

The standardization of non-human primate challenge models is important for generating reproducible and relevant data for the purpose of supporting licensure, especially when different candidate Ebola vaccines are compared. Relevant aspects here include species and age of animals, challenge material (including virus strain/variant and passage number), challenge route, challenge dose, criteria for animal euthanasia, and standardized data collection and reporting. Further current thinking on this issue can be found elsewhere (*4*).

B.3.2 Immunogenicity studies

Immunogenicity studies in animal models can generate important information on the immunological properties of the candidate vaccine. These studies should evaluate immune responses both quantitatively and qualitatively as per intended posology. The immune responses to each of the Ebola strains in a multivalent vaccine should be assessed, including any potential immunological interference between strains. Data on cross-neutralizing antibodies and cross-reactivity should be obtained for monovalent and multivalent vaccines through the use of heterologous viruses.

Such studies can provide evidence for the appropriateness of the vaccine dose, the number of doses, dosing interval and dose–response relationship.

> Either during a public health emergency or in a normal situation, immunogenicity data derived from a relevant species responsive to the vaccine antigen in terms of desired immune responses are an expected minimum requirement prior to starting Phase I clinical trials. Alternatively, strong supportive data generated from the same platform technology (for example, the same vector and manufacturing process, but expressing different vaccine antigens) may be considered sufficient for Phase I trial initiation.

Immunogenicity should be measured as humoral, cellular or functional immune responses, as appropriate to each of the intended protective antigens and to the antigens of the vector used. For several leading candidate vaccines using Ebola GP as a sole protective antigen, antigen-specific ELISA (which measures the quantity of serum GP-specific IgG antibodies) has been routinely used to characterize the humoral response. Evaluation of cellular responses should include the phenotypic and functional characterization of CD8+ and CD4+ T cell responses using sensitive and highly specific assays such as ELISpot and ICS by multiparameter flow cytometry. The functional activity of immune responses may be measured in vitro in neutralization assays using either wild-type virus or pseudovirion virus. More extensive analyses may include examination of Th1 and Th2 responses, the kinetics and duration of CD8+ and CD4+ T cells and antibody responses, as well as assessment of the quality or fine specificity of the antibody response.

As discussed in section B.3.1, the assessment of immunogenicity parameters in proof-of-concept challenge studies may allow for the establishment of a correlation between an antibody or other immune response (such as cellular immunity or cytokine response) and the level of protection from disease or death, or for understanding the underlying protective mechanisms. These key data may be expected to be generated during the development of the product.

Assessment of immunogenicity against multiple EBOV types should be performed for multivalent vaccines and should also be considered for monovalent vaccines.

B.4 Nonclinical safety studies (toxicity testing)

A safety assessment, including repeat-dose toxicity and local-tolerance studies, is generally required for all new candidate vaccines, unless otherwise adequately justified (*20*). In general, these studies will have been completed and analysed prior to the initiation of Phase I clinical trials. Additional safety testing may be necessary depending on the properties of the candidate vaccines. For a replicating recombinant vaccine vector with neurovirulent potential, neurovirulence testing in an animal species acceptable to the relevant NRA is an important consideration and should be conducted before proceeding to trials in humans.

> During a public health emergency, interim data from ongoing toxicity studies (including on the immediate effect on survival and vital physiological functions) and the submission of draft unaudited toxicity study reports may be sufficient to support proceeding to Phase I clinical trials with a novel platform/candidate vaccine.
>
> As in a normal non-emergency situation, the omission of toxicity studies may be possible if there are adequate platform toxicology data and clinical safety experience. For example, for the viral-vectored vaccines that this document focuses on, toxicity studies were not required during the 2014–2016 EVD epidemic.
>
> Such a limited dataset should be of good quality – that is, it should be generated from a relevant animal species and should follow GLP principles.
>
> Since the use of a reduced toxicity dataset during a public health emergency provides less certainty about the safety of the product, additional data should be submitted once they become available, including data on any delayed effect observed at later time points in repeat-dose toxicity studies, histopathological data and the final signed audited reports. Early discussion with NRAs in the countries where the Phase I clinical trials are to be conducted is encouraged.

Since Ebola vaccines are also beneficial for women of childbearing potential, a reproductive-toxicity study will need to be conducted at an appropriate point during product development. Serious consideration should be given to vaccine administration that results in the exposure of pregnant animals to a vaccine response during the early phase of implantation/organogenesis. For a replicating recombinant vaccine vector that may have a direct effect on the embryo/fetus, the dosing regimen should ensure a sufficient level of vaccine vector in the blood of exposed pregnant animals.

> The requirement for a developmental toxicity study is an important issue for consideration, depending on the level of threat or control of the disease. During the 2014–2016 EVD epidemic, large-scale Phase III efficacy trials were approved in endemic countries without intentionally enrolling pregnant women. With decreasing numbers of cases as the 2014–2016 epidemic was brought under control, the local NRAs required that developmental toxicity data be made available to support the enrolment of pregnant women.

B.5 Pharmacokinetic (biodistribution) studies

Classic pharmacokinetic studies with live viral-vectored vaccines are normally not required. However, a biodistribution study in a relevant species should generally be considered if the recombinant viral vector has any of the following characteristics: (a) it is a novel viral vector or a known vector with a novel envelope and there are no existing biodistribution data for the platform; (b) there is a likelihood of altered infectivity and tissue tropism due to recombination; or (c) a novel route of administration and formulation is to be used.

B.6 Environmental risk

The use of Ebola vaccines based on recombinant viral vectors could result in the release of recombinant microorganisms into the environment. Some countries have legislation covering environmental and other concerns related to the use of live vaccines derived by recombinant DNA technology since they may be considered as GMOs, and an environmental risk assessment (ERA) must be submitted with any application to market these products. The specifics of the ERA assessment within each country/region vary. Manufacturers are encouraged to start a dialogue with the responsible authorities, including regulatory authorities in countries where clinical trials are planned, early in the development of this class of product.

The WHO Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated) (*93*) provide advice in this respect that may also be useful in the case of Ebola vaccines.

The primary environmental risk of a replicating recombinant vaccine vector relates to vaccine vector shedding and shedding-based transmission to third parties – that is, to unvaccinated humans or domestic animals following human administration. In the case of a replication-incompetent recombinant viral vector, no shedding experiment is required. For future candidate novel live recombinant vaccines based on a GMO, an ERA of the possible shedding of the vaccine organisms following administration is required as part of the preclinical evaluation.

Part C. Clinical evaluation of Ebola vaccines

C.1 General considerations

Clinical development programmes for Ebola vaccines must take into account the epidemiology of the disease, the infrastructure for conducting clinical trials in affected areas and the regulatory frameworks of particular NRAs. However, key points that should be common to all such programmes are: (a) the standards for demonstrating Ebola vaccine safety and effectiveness are the same as for other vaccines; and (b) clinical studies are to be conducted in accordance with the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (*94*) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*22*).

As for all vaccines, close monitoring of studies by an independent data monitoring committee (if warranted), the ethics committee(s) and the sponsor should help to ensure study integrity. Meetings between sponsors and the relevant NRA at critical time points during clinical development should be encouraged, as well as meetings to discuss scientific and medical questions that may arise at any time during an investigation.

C.1.1 Study population

Study population characteristics (for example, demographics, location, underlying medical conditions and Ebola immune status) may vary by phase of clinical development, as further discussed in section C.1.2. Specific considerations for the evaluation of Ebola vaccines in the paediatric population are discussed in section C.7.2.

Inclusion and exclusion criteria for participants should be defined for each study planned. Exclusion criteria may include previous receipt of an Ebola vaccine and possible previous contact with a person with EVD. Consideration should be given to excluding subjects at risk of loss to follow-up (for example, individuals not planning to live in the area for the duration of safety follow-up), as well as immunodeficient or immunosuppressed subjects, particularly in the

case of live vaccines based on replication-competent viral vectors. Additional exclusion criteria should be based on clinical experience with the particular vaccine, with the aim of excluding individuals who may have an increased risk of significant adverse reactions, and individuals whose underlying conditions may make it difficult to interpret safety data. For example, an investigational recombinant VSV-vectored Ebola vaccine has been associated with arthritis in one study. Consideration should be given to excluding individuals with arthritis or related conditions (active or in past medical history) from participating in initial studies of this vaccine, taking into account their risk of contracting Ebola, and pending subsequent determination of the frequency, duration and severity of this adverse event. Thus, considerations for exclusion would likely differ for studies of healthy volunteers with a low risk of exposure to EBOV and for studies conducted in the setting of an active outbreak.

> The phase of clinical development and circumstances of the study should also be considered when developing inclusion and exclusion criteria. For example, a later-phase study being conducted in an emergency situation in a population at high risk of EVD would probably have fewer exclusion criteria than a Phase I study of healthy volunteers not at risk of EVD. The phases of clinical development are described below in section C.1.2.

Pre-vaccination sera should be collected, at least in early-phase trials, to assess pre-existing antibodies to EBOV and vaccine vector viruses, as well as to assess aspects of baseline health status. The laboratory values expected for the study population and any exclusion criteria should be specified in the study protocol. Stored pre-vaccination serum may also be useful in the assessment of certain post-vaccination adverse events that may occur. Assessment of possible causal associations between vaccination and adverse events can also be facilitated by knowledge of the background rates of events in the relevant general population.

C.1.2 Phases of clinical development

The phases of vaccine clinical development are typically a continuum from Phase I, which often includes the first-in-human clinical trials carried out primarily to assess safety and preliminary immunogenicity, to Phase II to further describe safety and dose relationship to immunogenicity, and then to Phase III pivotal studies to demonstrate the safety and effectiveness of a product in support of licensure.

> As for all vaccines, Phase I and Phase II studies of investigational Ebola vaccines are expected to provide initial safety and immunogenicity data, and to assess the optimal dose. The epidemiology of the disease is likely to have a major impact on the timing and design of Phase III studies. In the face of an outbreak, without available preventive vaccines, vaccine

evaluation should adhere to the principles of this phased approach but intervals between phases of evaluation may be compressed and overlapping. For example, compressed timelines for clinical development may be achieved by initiating Phase III studies based on interim safety and immunogenicity data from earlier-phase studies rather than on data from final study reports. Clinical development of an Ebola vaccine in the setting of an outbreak is complex. Close collaboration between public health authorities, NRAs, the community, clinical investigators and the vaccine developer is essential to ensure that studies will meet licensure requirements, including requirements for ethical conduct. Phase II and Phase III clinical trials may be designed with prospectively planned adaptive features that allow for changes in design or analyses based on examination of the accumulated data at pre-specified interim points in the trial. Such adaptive features may make trials more efficient. For detailed considerations regarding approaches and the designing of studies to demonstrate vaccine effectiveness see section C.2.

C.1.2.1 Phase I studies

The primary purpose of Phase I vaccine studies is to obtain preliminary safety and immunogenicity data. For Ebola vaccines, these studies would generally be first conducted in a small number (for example, < 100) of healthy adult volunteers previously unexposed to EBOV and at low risk of EVD.

However, in the face of an outbreak, NRAs may consider larger Phase I clinical studies (for example, by enrolling more sites) to increase the early safety and immunogenicity database, as well as the use of study populations similar to the eventual target population, thus facilitating timely initiation of Phase II clinical studies.

The design of Phase I studies can be uncontrolled and open label or may include a placebo control. When possible, the concomitant use of other vaccines should be avoided to optimize the safety evaluation. The study design may include sequential dose-escalation whereby subjects enrolled in lower-dose cohorts are closely monitored for safety for a defined period (for example, 1–2 weeks or as appropriate for the characteristics of the vaccine) and the resulting data are reviewed before subsequent enrolment of additional subjects in successively higher-dose cohorts. All study participants should be actively and closely monitored for safety.

C.1.2.2 Phase II studies

Phase II studies are initiated once satisfactory safety and immunogenicity data from Phase I studies are available. In the absence of safety concerns from short-term post-vaccination follow-up in Phase I studies (for example, 7 days or as

appropriate for the specific vaccine), development may in some cases proceed to Phase II studies in parallel with the continued collection of longer-term safety data from Phase I studies. Phase II studies provide further information on safety and immunogenicity to determine the optimal dose and dosing regimen, and to support initiation of Phase III studies. Phase II studies typically involve up to several hundred subjects and are frequently randomized, double-blind and controlled. The comparator is usually an inert placebo or a control vaccine that provides protection against disease unrelated to EVD. Phase II trials should be of sufficient size to test hypotheses on dose and dosing regimen. Phase II studies should be conducted in the proposed target population or in a population similar to the target population in terms of demographic and ethnic factors, and other factors that might impact on vaccine effectiveness or safety (for example, concomitant infections). Detailed safety and immunogenicity data should be obtained in Phase II studies.

C.1.2.3 Phase III studies

Large-scale Phase III clinical studies involve more-extensive testing to provide a rigorous assessment of vaccine effectiveness that may include direct evaluation of efficacy in protecting against clinical disease, expanded safety evaluation and opportunities to potentially identify an ICP. Definitions of vaccine effectiveness and vaccine efficacy are provided in section C.2.1. Phase III clinical trials may also permit clinical evaluations of lot-to-lot manufacturing consistency. The target population for Phase III clinical trials with candidate Ebola vaccines should consist of individuals at high risk for the disease (that is, populations residing in EVD outbreak areas, relevant health-care providers, laboratory personnel or first responders). The design of Phase III effectiveness studies must be of adequate scientific rigour to support effectiveness claims, while adhering to ethical standards. Ideally, effectiveness is evaluated in randomized, double-blind, well-controlled trials with a parallel control group receiving an inert placebo such as saline injection or a vaccine that provides protection against another disease. In some settings, the balance between scientific rigour and ethical standards may preclude the use of a placebo group – for example, if there is an existing efficacious Ebola vaccine that those in the trial might be eligible to receive. Ethical considerations for the use of placebos in vaccine research, including in circumstances in which an efficacious vaccine is already available, are discussed in the WHO meeting report *Expert consultation on the use of placebos in vaccine trials* (95). As discussed in section C.2 below, other study designs for obtaining effectiveness data for candidate Ebola vaccines may be considered if a placebo-controlled trial is not considered ethical or is not feasible.

To demonstrate vaccine effectiveness, Phase III trials may be based on a disease end-point or, as described in section C.2, they may be based on

the attainment of a level of an immune marker predictive of protection. The incidence of EVD and ethical considerations will be primary determinants of the approach used to evaluate vaccine effectiveness and the design of clinical end-point efficacy studies, as also discussed in more detail in section C.2. For many disease end-point clinical efficacy study designs, large sample sizes may be needed, particularly if the incidence of the disease in the study population is expected to be low or to decline during the study period. Adequate statistical justification of the size and duration of the trial should be provided, and trial end-points and criteria for trial success specified prior to initiation of the study. Plans should be included to monitor the conduct of the trial, taking into consideration the potential for changes in disease incidence which may necessitate trial design modification. It is important that some attempt should be made to define an ICP as part of efficacy studies. For such an evaluation to be clinically meaningful, validated standardized assays are essential.

Clear and definite evidence that the vaccine is safe and effective is required for regulatory decision-making. Discussions should be held with relevant NRAs on the study design and on plans for conducting the study and analysing its results at the early conceptual stage of the Phase III study, and agreement reached with the NRAs prior to trial initiation. Close consultation with local community leaders, health policy-makers and ethics committee(s) in EVD outbreak regions where efficacy studies are planned is also crucial.

C.2 Demonstration of effectiveness of candidate Ebola vaccines

C.2.1 Definitions of effectiveness and efficacy

It is important to distinguish vaccine effectiveness from vaccine efficacy. Vaccine efficacy is an estimate of the reduction in the incidence of clinical disease observed in a vaccinated group relative to the incidence of disease in a group not vaccinated against the disease to be prevented. Vaccine efficacy measures direct protection (that is, protection induced by vaccination in the vaccinated population sample). The best estimates of vaccine efficacy come from randomized controlled clinical trials.

Vaccine effectiveness is an estimate of the protection conferred by vaccination. It is usually obtained by monitoring the disease to be prevented by the vaccine during routine use in a specific population. It may measure both direct and indirect protection (for example, the estimate may reflect in part the protection of non-vaccinated people secondary to the effect of the vaccine in the vaccinated population). Thus, the term vaccine effectiveness may be used broadly to encompass vaccine efficacy (direct protection) as well as indirect protection. Evidence for vaccine effectiveness may be derived from challenge-protection studies conducted in animal models or from a vaccine-induced

immune response (for example, pre-specified antibody threshold induced by the vaccine in vaccinated people).

For any preventive vaccine, the most direct approach for demonstrating effectiveness is based on clinical end-point efficacy trials showing protection against disease, or alternatively, based on clinical trials evaluating a scientifically well-established ICP (for example, antibody response).

C.2.2 Immunological evaluation of Ebola vaccines

Clinical disease end-point efficacy trials provide an opportunity to identify an ICP. The derivation of an ICP is facilitated by the availability of post-vaccination serum samples from a relatively large number of protected trial participants as well as from vaccinated participants who develop disease. Thus, for all Ebola vaccine clinical disease end-point efficacy trials, post-vaccination serum samples (and preferably also pre-vaccination serum samples) would ideally be collected from all subjects, with post-vaccination sampling at regular predefined intervals throughout the study period. If this is not feasible, pre- and post-vaccination serum samples should be collected from as many subjects as possible. Ebola prevalence studies in various African countries have revealed unexpectedly high rates of baseline Ebola seropositivity in some regions, as measured by serum IgG antibodies, underscoring the importance of collecting baseline serum samples in studies conducted in these countries (*96–101*). Consideration should also be given to the collection of blood samples for the evaluation of cell-mediated immunity which may play a role in protection for some vaccines.

Even if it is not possible to identify an ICP from a clinical end-point efficacy trial, immunogenicity data from Phase II and Phase III studies are crucially important for the use of alternative approaches to assess vaccine effectiveness based on surrogate immune response end-points likely to predict protection and/or for challenge-protection studies conducted in animal models (see sections C.2.4 and C.2.5 respectively).

Potentially important immunogenicity end-points include EBOV IgG ELISA antibody titre and presence/levels of EBOV neutralizing antibody. End-points evaluating T cell mediated responses following vaccination may also be considered. Specific considerations regarding immunological assays are discussed below in section C.6.

In evaluating antibody response to vaccination, it is important to stratify analyses by baseline serostatus and to pre-specify the definition of seroresponse, and seroconversion. Seroresponse is typically based on an x-fold rise in antibody level from pre-vaccination to post-vaccination in initially seropositive individuals. Seroconversion is typically based on achieving a measurable antibody level post-vaccination in individuals who were initially

seronegative. A detailed justification for the definition of each term should be provided. The definition of seroresponse may differ for different Ebola vaccines and assays. Serological end-points and evaluation criteria should be determined following input from, and agreement by, the NRA before study un-blinding and serological analysis.

As an ICP (including potential antibody thresholds associated with protection) or a surrogate immune marker may differ for different vaccines, it is important to obtain vaccine-specific human serological data. Ideally, vaccine-specific human cellular immune response data would also be obtained (*102*). Applicability of an ICP or a surrogate immune marker will depend on specific vaccine characteristics such as antigen structure, mode of delivery, antigen processing in the vaccinee and virus serotype. For example, an ICP established for an adenovirus-vectored Ebola vaccine cannot be presumed to be applicable to a VSV-vectored Ebola vaccine given that the two vaccines present antigen differently and engender different types of protective immune responses. Similarly, Ebola vaccines that are, for example, based on VSV and adenovirus vectors and administered using a prime-boost regimen may induce different protective immune responses than Ebola vaccines based on different platforms or technologies and administered using a different regimen. As another example, an ICP or a surrogate immune marker identified for a vaccine containing a particular EBOV (for example, ZEBOV) cannot be assumed to be applicable to another vaccine containing a different EBOV (for example, SUDV).

C.2.3 Clinical disease end-point studies

C.2.3.1 General principles of clinical disease end-point studies

In general, the crucially important aspects of clinical disease end-point efficacy studies include: (a) an appropriate control group; (b) appropriate methods for randomization, as applicable; (c) masking procedures, as applicable; (d) a pre-specified primary end-point (for example, EVD confirmed by PCR); (e) pre-specified important secondary end-points (for example, EVD not laboratory confirmed); (f) pre-specified, detailed clinical case definitions for the primary end-point; (g) validated diagnostic assays to support the pivotal efficacy analyses; (h) unbiased case-ascertainment methods; and (i) adherence to relevant statistical principles. Measures to reduce potential bias are important in all trials, but particularly so for designs other than randomized, double-blind, controlled trials with a parallel control group. Specific considerations regarding the design of clinical end-point efficacy studies and diagnostic tests for EVD are discussed below in sections C.2.3.2 and C.6.1, respectively. Consideration should be given to the establishment of an independent data-monitoring committee for clinical end-point efficacy studies of Ebola vaccines in order to advise the sponsor on the continuing validity and scientific merit of the study.

C.2.3.2 Design of clinical disease end-point studies

C.2.3.2.1 *Randomized controlled trials*

The prospective randomized, double-blind, placebo-controlled trial with an EVD end-point is the gold standard for demonstrating the efficacy of any investigational Ebola vaccine(s) when no licensed efficacious vaccine is available. This design avoids potential bias in the assessment of end-points and maximizes the chance that a difference in disease incidence observed between the vaccinated and unvaccinated groups is due to a true effect of the vaccine being evaluated. The unit of randomization is usually the individual subject enrolled in the trial, although other units of randomization may be considered. While direct assessment of vaccine efficacy in randomized controlled trials provides the most definitive evidence of effectiveness, it requires a sufficiently high disease incidence and a correspondingly adequate sample size.

C.2.3.2.2 *Ring vaccination design*

In settings with relatively low disease incidence, vaccine efficacy clinical trial designs – such as ring vaccination in which people at highest risk of infection are recruited – may be considered in order to maximize statistical power (*64*, *103*).

> A novel cluster randomized controlled trial design to evaluate vaccine efficacy and effectiveness during outbreaks, the ring vaccination trial design was developed with special reference to Ebola (*101*). The approach taken to increase statistical power is to recruit those at highest risk of infection (for example, individuals who are socially or geographically connected to an index case). An important consequence of this increase in power is that this trial design has the potential to yield an estimate of vaccine efficacy within a shorter period of time and possibly with a smaller sample size, compared to more-common trial designs.
>
> A ring is a socio-geographical population group made up of the contacts and contacts of contacts of the index case. Rings are randomly assigned to immediate or delayed vaccination, with the delayed vaccination rings serving as controls. Vaccine efficacy is calculated on the basis of the relative rates of disease in the immediate and the delayed vaccination rings. An efficacy trial using ring vaccination with an investigational Ebola vaccine was conducted in Guinea in 2015 (*64*, *103*).

C.2.3.2.3 *Stepped wedge randomized cluster trial*

After licensure of an Ebola vaccine – and in some settings even before licensure – the high case-fatality rate of EVD may raise ethical concerns about non-vaccination in a parallel control group. To mitigate these concerns, a stepped wedge randomized cluster trial (SWRCT) design in which clusters of participants

are sequentially vaccinated over a number of time periods, may be considered. In this design, all participants start in the control group and, at predefined time points, a cluster of participants is vaccinated in a random order (known as "steps"). Vaccine efficacy is calculated on the basis of the relative rate of disease in the vaccinated population compared to the unvaccinated population. This design, in which all participants are vaccinated by the end of the study, may be ethically acceptable in settings where the candidate vaccine is not available simultaneously for all participants and where the use of a placebo group is considered unacceptable.

Disadvantages of the SWRCT design include difficulty in blinding, attrition in the later vaccinated clusters and the more complex analysis required. In addition, an underlying requirement for validity of an SWRCT design is that disease incidence rates must remain fairly stable throughout the trial. If disease incidence rates are not expected to remain reasonably constant during the course of the trial, data analyses may be performed separately within narrow windows of time (for example, by day or week) within which it can be assumed that disease incidence rates are stable. This time stratification will necessitate more careful recording of disease incidence rate with time. The impact of misclassification of disease incidence rate with time will need to be considered. Another issue is that SWRCT designs randomize the timing of vaccination of the clusters, which unlike most designs disallows the flexibility to move vaccination to high-risk areas that evolve while the trial is ongoing, and which could also potentially cause the SWRCT to take longer to complete compared to other trial designs (*104*).

C.2.3.2.4 *Test-negative case control design*

Once a vaccine has been deployed in a population, it may be possible to estimate vaccine effectiveness using a test-negative case control design (*105–107*). In the test-negative case control design, patients seeking health care for symptoms compatible with EVD are recruited into the study and tested for the disease. Vaccine effectiveness is estimated by comparing the odds of vaccination in subjects testing positive for Ebola (cases) to the odds of vaccination in subjects testing negative (controls).

Test-negative case control studies are relatively low cost and easy to conduct. However, controlling for potential bias in this non-randomized design is particularly challenging because vaccinated and unvaccinated individuals may have different risk factors for disease.

Test-negative case control studies are also subject to the same sources of bias and measurement error as other non-randomized studies – some of which may not be recognized or adequately adjusted for in the statistical analyses. Furthermore, it may be difficult to assure comparable disease severity across participants at study entry or to achieve complete ascertainment of vaccination

status. Potential sources of bias and limitations inherent in this design need to be carefully considered in planning study procedures and statistical analysis, as well as in interpreting the results.

C.2.4 Surrogate end-points for demonstration of effectiveness

For diseases like EVD, for which there is no well-established ICP, if disease incidence is too low to feasibly conduct clinical end-point efficacy studies then effectiveness may be based on controlled clinical studies which establish an effect on a surrogate end-point (for example, immune response) considered likely to predict clinical benefit. The surrogate end-point used to evaluate effectiveness could be derived from human studies – for example, immune responses in vaccinated individuals from Phase II and Phase III studies and/or from a comparison of antibody responses post-vaccination in protected vaccinees with those of vaccinees who contract EVD. In this scenario, immune responses such as antibody titres achieved in vaccinated non-human primates that correlate with protection from challenge may also help in determining an immunogenicity end-point likely to predict protection in humans. Some NRAs may have provisions that would allow for the licensing of an Ebola vaccine based on such an approach for demonstrating effectiveness. Specific regulatory requirements associated with such provisions (for example, post-licensure studies to verify clinical benefit, and requirements for pre-licensure clinical safety studies in humans) must be adhered to.

As discussed above in section C.2.2, a surrogate immune marker identified for a particular vaccine may not be applicable to another vaccine.

C.2.5 Animal efficacy data for demonstration of effectiveness

If clinical end-point efficacy studies in humans are not ethical or feasible and there is no well-established ICP or surrogate immune marker likely to predict protection then evidence for effectiveness may be based on controlled challenge-protection studies conducted in an appropriate animal model (see section B.3.1) and clinical immunogenicity data. A central principle of approaches based on animal efficacy data is that the results of the animal studies establish that the vaccine is likely to produce clinical benefit in humans. Some NRAs may have provisions that would allow for the licensing of an Ebola vaccine based on such an approach for demonstrating effectiveness. Specific regulatory requirements associated with such provisions (for example, meeting certain criteria for the animal model(s), accrual of information in animals and humans to allow for selection of an effective dose in humans, pre-licensure safety studies in humans, and post-licensure studies to verify clinical benefit when such studies are feasible and ethical) must be adhered to.

C.2.6 Special considerations

C.2.6.1 Evaluation of effectiveness of candidate vaccines after initial licensure of an Ebola vaccine

Licensure of an Ebola vaccine may facilitate the evaluation of effectiveness of a new candidate Ebola vaccine if an ICP is established or a surrogate immune marker likely to predict clinical benefit is identified during development of the licensed vaccine and is considered to be applicable to new candidate vaccines. In such cases, an adequately conducted, randomized, controlled clinical trial(s) comparing the immune response, as measured by the relevant immunological parameter(s), in recipients of the candidate vaccine to that of recipients of the already licensed vaccine, using pre-specified statistical criteria, appropriate statistical methods and validated assays, could provide sufficient evidence of effectiveness to support licensure. As previously described, if the estimate of effectiveness is based on a surrogate marker likely to predict clinical benefit, approval may be subject to post-marketing requirements to verify the clinical benefit of the vaccine.

Alternatively, an Ebola vaccine may be licensed without an ICP or surrogate immune marker likely to predict protection considered to be applicable to other candidate Ebola vaccines. It may therefore be necessary to demonstrate vaccine effectiveness using other approaches (for example, animal challenge-protection studies combined with clinical immunogenicity studies). For this purpose, the animal challenge-protection studies should be adequately designed to provide reliable data, as discussed in B.3.1.

Licensure of an Ebola vaccine may make it infeasible and unethical to conduct pre-licensure clinical end-point efficacy trials with new candidate Ebola vaccines. Even conducting a comparative efficacy trial to demonstrate non-inferiority of a new candidate vaccine to the licensed vaccine would be challenging.

C.2.6.2 Evaluation of effectiveness of multivalent vaccines

For multivalent vaccines (for example, containing more than one EBOV strain, or an EBOV strain(s) and MARV) effectiveness (that is, based on clinical end-point efficacy studies, animal efficacy data and/or human immune response data) will need to be demonstrated for each strain contained in the vaccine.

C.2.6.3 Duration of immune response and protection, and need for booster vaccinations

The long duration of the 2014–2016 EVD epidemic and the potential for future exposures highlight the need to consider the durability of vaccine-induced protection and the potential need for booster doses in the evaluation of Ebola vaccines. This evaluation could be facilitated by the identification of an ICP.

Importantly, Phase II and Phase III clinical trials should attempt to identify ICPs and should evaluate the kinetics of the immune response and induction of immunological memory.

C.3 Safety evaluation of candidate Ebola vaccines

C.3.1 General considerations

Sponsors must comply with the adverse event reporting requirements of the relevant NRA and the independent ethics committee(s). Templates of the forms used to monitor and document adverse events should be provided with each protocol. Sponsors are encouraged to initiate early dialogue with the appropriate NRAs to reach agreement on the size of the safety database needed to support licensure of a particular vaccine. As with all vaccines, the size of the safety database depends in part on the characteristics of the candidate vaccine as well as on available preclinical and clinical safety data. Safety data from previous preclinical and clinical experience with related vaccines using the same platform may also be considered when determining the size of the safety database.

Safety-monitoring methods should be tailored to the specific study population (for example, children, adults, pregnant women or people living in areas where EVD is endemic), with consideration given to adverse events known to be associated with a particular vaccine – for example, in some Ebola vaccine studies, fever, arthralgia and arthritis have been observed. Study protocols should specify methods for monitoring and documenting adverse events, including: (a) use of standardized subject diaries and case report forms; (b) procedures for inquiring about adverse events at study visits; (c) severity grading scales; (d) definitions for adverse event categories – for example, serious, new-onset chronic medical condition, and adverse event of special interest (AESI); and (e) requirements for prompt reporting of serious adverse events (SAEs) to the sponsor.

In early-phase clinical studies (and at later phases if warranted), consideration may be given to pre- and post-vaccination assessment of safety laboratory parameters, including haematological and clinical chemistry evaluations. If such parameters are monitored, grading scales appropriate for the study population should be utilized.

It is also important to establish stopping rules for subsequent doses for individual study participants who experience an SAE, as well as study pausing/stopping rules for SAEs overall. Consideration should also be given to the establishment of an independent data-monitoring committee to advise the sponsor with regard to the continuing safety of trial participants and those to be recruited into the trial, particularly for any trials involving children and any large-scale later-phase trials.

Other aspects of safety that should be addressed in the study protocol include assessment of virus shedding and the potential for secondary transmission of replicating or potentially replication-competent live vaccine virus vectors, at least in early-phase studies, as well as procedures to minimize the risk of EBOV transmission to study personnel involved in clinical end-point efficacy studies.

C.3.2 Monitoring for common, solicited adverse reactions

In Phase I and Phase II studies, all participants should be monitored for pre-specified, solicited local and systemic adverse reactions at specified time points, for a specified period following vaccination (for example, daily for at least 7 days, or longer if warranted based on vaccine characteristics and available preclinical and clinical data). In Phase III studies, it may be acceptable to actively monitor only a subset of participants (for example, several hundred per group) for common, non-serious local and systemic adverse reactions. Data-collection methods may include the use of memory aids in literate populations and telephone interviews.

C.3.3 Monitoring for unsolicited adverse events

All study participants should also be monitored for unsolicited adverse events, including new-onset chronic medical conditions and exacerbation of medical conditions that may not necessarily meet the NRA's definition of serious. Whereas monitoring for all unsolicited adverse events may be conducted for relatively short periods post-vaccination (for example, 21 days, or 42 days for replicating live viral vaccines), monitoring for new-onset chronic medical conditions for a longer period (for example, 6–12 months) may be useful in detecting unexpected safety signals.

C.3.4 Monitoring for serious adverse events

While the exact definition of an SAE can vary across different NRAs, the ICH Guideline E2A defines an SAE as any untoward medical occurrence that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect (*108*). WHO considers an adverse event following immunization as "serious" if it meets any of the above criteria or if it requires intervention to prevent permanent impairment or damage (*109*).

All participants in pre-licensure clinical trials of Ebola vaccines should be closely and actively monitored (for example, with diary cards or follow-up visits) for SAEs for at least 21 days (or 42 days for replicating live viral vaccines) after each vaccination. A method to further query for SAEs over a minimum of 6 months following the last vaccination should also be incorporated into

the study protocol. A longer-term safety follow-up period for the assessment of SAEs (for example, through the 12 months following the last vaccination) may be warranted for some vaccines (for example, vaccines containing novel adjuvants). Long-term safety follow-up (that is, for 6–12 months post-vaccination) may be accomplished by telephone follow-up or other methods appropriate for the setting.

C.3.5 Monitoring for adverse events of special interest

All study participants should be monitored for any AESIs for a particular vaccine for a specified period post-vaccination (for example, 6–12 months). The period of follow-up may vary for different AESIs, depending on the anticipated window of risk.

C.4 Ethical considerations

Compliance with good clinical practice standards (*22*, *94*) provides assurance that the rights, safety and well-being of study participants are protected and study integrity is preserved. For any clinical study, a review by an independent ethics committee is mandatory and the approval of this committee must be obtained prior to study initiation. Informed consent must be given freely by every study participant and should be documented. For children participating in clinical studies, consent must be given by their parent or legal guardian. The informed consent process may need to be more specifically tailored to take into account local cultural views or practices. Child participants should be informed about the study to the extent compatible with their understanding and, if capable, should provide their assent. Participants in vaccine studies should not be exposed to unreasonable or serious risks of illness or injury. A study should be initiated and continued only if the anticipated benefits justify the risks. Low-resource communities, which are often those at greatest risk of EVD, should not be exploited in conducting research (for example, where there will be no long-term benefit to the community because the developer does not intend to seek licensure in the country where the vaccine is studied).

See section C.7.2 for considerations regarding initiation of clinical studies in the paediatric population.

C.5 Statistical considerations

C.5.1 General statistical principles

General statistical principles for clinical trials should be based on the relevant WHO document (*21*), where available, and other guidelines such as ICH E9 (*110*). Phase I studies are generally exploratory and may lack statistical power for hypothesis testing.

Phase II studies are for selecting the final optimal dose and dosing regimen and should be rigorously designed and analysed. The potential role of immunogenicity data should be taken into consideration to ensure the adequacy of data to support licensure if necessary.

Phase III studies are designed to provide robust data on vaccine effectiveness and more-extensive data on safety. The study protocols should clearly describe the procedures for randomization and blinding, primary and secondary objectives, end-points to be analysed, null and alternative hypotheses to be tested, level of type I error, sample size calculations, statistical methods for assessing each end-point, and analysis populations (per-protocol and intent-to-treat). If interim analyses for efficacy are planned, detailed information should be included in the protocol regarding the timing of interim analyses, type I error allocated to each analysis, and stopping rules. The study reports should include detailed information on subject disposition. Statistical estimates should be presented along with confidence intervals.

C.5.2 Statistical considerations for evaluating vaccine effectiveness

The effectiveness of a new Ebola vaccine is most convincingly demonstrated in a randomized, double-blind, placebo-controlled study based on an EVD end-point – though circumstances may dictate that alternative trial designs be considered. Vaccine efficacy and the corresponding confidence interval (usually 95%) should be estimated. Sample size for these trials depends on disease incidence rates in the study population, the level of vaccine efficacy considered to be clinically relevant and the chosen trial design.

Rapidly changing and/or declining incidence rates during an outbreak may need to be considered when choosing a study design. In some circumstances, designs such as cluster randomization may need to be used. For cluster-randomized trials, data should be analysed using statistical methods appropriate for the study design and study objectives. If inference will be at the usual individual level rather than the cluster level, sample size calculations and statistical analysis methods should appropriately address the within-cluster correlation, as feasible. Randomization should be carefully planned to avoid imbalance in disease risk or incidence rate between clusters randomized to be vaccinated or to serve as controls. As mentioned in section C.2.3.2.2, seeking to confine a trial to individuals at relatively high risk of EVD (as with the ring vaccination trial design) may have higher statistical power to detect vaccine efficacy than a trial in a population at lower risk of disease and, as a consequence, can potentially require a smaller sample size and achieve faster completion time compared to other study designs.

When ICPs established in animal challenge studies are being used to define immune response end-points for effectiveness evaluation or to infer

clinical benefit under other alternative licensure pathways, these studies (for example, in non-human primates) should be conducted using an appropriate dose range and an adequate number of animals such that the relationship between immune response and protection, and the protective threshold, can be estimated with satisfactory precision (see Part B).

C.5.3 Statistical considerations for evaluating vaccine safety

Safety evaluation is inherently exploratory and typically uses descriptive statistics. The calculation of p-values is sometimes useful as a flagging device applied to a large number of outcomes to detect differences that may need further evaluation. Multiplicity adjustment is not performed in order to increase the ability to detect potential signals. However, the potential for false-positive signals resulting from multiple tests must be considered prior to drawing firm conclusions.

If detection of several pre-specified SAEs is the primary focus of a large pre-licensure safety trial then multiplicity adjustment for testing a small number of hypotheses can be considered. When specific safety issues are identified during preclinical studies or early clinical trials (for example, cases of post-vaccination arthritis in clinical studies with certain viral-vectored vaccines) then prospective monitoring for related events as well as formal statistical testing should be considered.

C.6 Serological and diagnostic assays

The incubation period for EVD is 2–21 days. While patients are infectious by the time symptoms are evident, levels of virus in saliva or blood may not reach detectable levels until two or three days later. At this point in the course of infection, viral antigen can be detected by immunoassay and viral nucleic acid by a NAT-based assay. For both antigen and nucleic-acid-based tests the use of blood is preferred due to lower sensitivity of these assays with saliva. While serum IgM may also be detectable at this time, there is a risk of obtaining false-negative results so early in the course of infection. Serological testing should therefore be reserved for confirming prior infection or for evaluating vaccine responses. Isolation of EBOV in tissue culture must be performed in a high-containment laboratory, of which there are few, and this is therefore not routinely performed.

C.6.1 Diagnostic tests

All currently available EBOV NAT-based assays are based on the same principle – detection of an EBOV nucleic acid target sequence after extraction of viral nucleic acids from clinical samples, reverse transcription of RNA and in vitro amplification. The primers used in different NAT-based assays target different viral genome regions, which should be considered, particularly when used

in vaccine trials, so that infection can be distinguished from vaccination. For example, if the EBOV gene targeted by the NAT-based assay is also expressed by the vaccine, a positive result on a blood sample could mean that the subject may have EVD or it could mean that the subject is shedding vaccine virus.

Although many EBOV diagnostic kits have received approval for emergency use, this should not be taken to mean that they have been validated for non-emergency purposes, such as establishing vaccine efficacy in field trials. Assay performance parameters investigated as part of emergency-use approval often do not include more rigorous assessments, such as repeatability over the operating range, inter-assay precision or performance in the field. Appropriate RNA process controls and international reference standards became available for these assays in 2015 (see section A.1.1), which should now enable assessment of assay performance and comparison of results across different assay platforms.

Rapid diagnostic tests (RDTs) designed for EBOV antigen detection provide results more rapidly (sometimes within minutes), are easier to perform compared to NAT-based assays and do not require complex equipment (or electricity). However such tests are less sensitive than NAT-based assays and results should be confirmed by NAT-based assay where possible. As with NAT-based assays, care should be exercised when interpreting the results of RDTs using samples obtained from vaccinees, given that the antigen targeted by the kit may share homology with vaccine antigen.

C.6.2 Immunological tests

Although an ICP against EVD has not been established, myriad immunological tests have been developed. Of these, the EBOV IgG ELISA has gained the greatest acceptance based mostly on studies of experimentally vaccinated non-human primates in which high IgG levels have been linked to protection against subsequent challenge. Whether protection was via antibody detected by ELISA, or whether the presence of high levels of ELISA antibody is a marker of some other more meaningful form of immune response, is not known. In the absence of available data from humans defining an ICP (for example, data from a successful vaccine efficacy trial), an ICP may have to be established in an animal model. On the basis of data available to date, non-human primates appear to be an acceptable animal model for such an exercise, with inadequate information to support the use of other animal species.

Few EBOV immunoassays are commercially available – most reside in research laboratories where they were developed for use in preclinical or clinical trials of investigational vaccines. For this reason, most ELISAs are designed to detect antibodies against the EBOV GP – that is, the protein expressed by most investigational vaccines. There are numerous concerns about these tests and care should be taken in interpreting the data they produce. ELISA plates coated with lysates of cells expressing non-EBOV antigen that is also contained

in, or expressed by, the vaccine may be prone to yielding false-positive results. Other issues for consideration are the source of virus antigen used in the ELISA (reduced cross-recognition between virus strains), conformational changes of the antigen upon binding to the plate and antigen stability over time.

Since ELISAs are not necessarily informative of functional immunity, assays that measure virus neutralization and cell-mediated responses have been developed. The neutralization assays generally employ pseudovirions (such as VSV in which the GP gene has been replaced with that of EBOV) or lentivirus packaging systems. Consideration should be given as to whether virus-neutralizing activity detected in these in vitro assays is predictive of EBOV-neutralizing activity in vivo. It is also important to consider false positivity through the use of ELISA plates coated with non-EBOV vaccine components. For example, non-EBOV antibodies generated in response to receipt of a VSV-vectored Ebola vaccine may have an impact on the performance of VSV-based neutralization assays. This is less of a problem for neutralization assays using wild-type EBOV as the target virus but it highlights the need for careful evaluation of assay specificity as part of assay validation.

Although not well established, there is evidence supporting the importance of T cell-mediated responses in preventing EVD. In a study of an Ad5-vectored Ebola vaccine in non-human primates, depletion of CD8+ T cells in vaccinated animals before challenge abrogated protection (*111*). Several different types of tests for cell-mediated immunity have been developed, including the ELISpot and ICS tests. These tests present additional challenges, including determination of the appropriate peptide pools to be used and logistical and safety issues concerning the collection and storage of peripheral blood mononuclear cells, as well as assay validation issues.

In general, there are few published data on the performance of assays to detect immunological responses to EBOV infection or to Ebola vaccines. Where available, international standards or reference reagents (see section A.1.1) should be used to standardize assay performance, and improve comparison of results across vaccines, across studies and across different assay platforms.

C.7 Special populations

Ideally, developers of candidate Ebola vaccines will perform studies to gather data in at least some, if not all, of the relevant populations discussed below.

C.7.1 Pregnant women

Evidence from the 2014–2016 EVD epidemic suggests that EVD is associated with high rates of maternal and neonatal mortality (112). The use of Ebola vaccine in pregnant women may have potential benefits in: (a) preventing EVD in the mother and reducing maternal morbidity; (b) preventing EVD in the

early neonatal period; and (c) limiting the spread of EVD from pregnant women during labour and delivery to health-care workers in an outbreak setting (*113*).

The following concepts should be considered when planning clinical trials in pregnant women. Details regarding such trials should be discussed with the respective NRA(s) and can also be found in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*22*). Prior to enrolling pregnant women in clinical trials, developmental toxicity studies in animal models are needed to address the potential reproductive risk of the product (see section B.4). In addition, supportive safety data from completed Phase I and Phase II clinical trials in healthy men and non-pregnant women should be available. The consent form should include information on what is known and unknown regarding the potential risks and benefits of the investigational product to both mother and infant, and should reflect available data from non-pregnant adults and nonclinical studies. A reasonable effort should be made to accurately calculate gestational age for pregnant participants prior to enrolment, taking into consideration the standard of care in the region where the clinical trial is being conducted. For studies of preventive vaccines in general (including Ebola vaccines), consideration should be given, as part of a cautious approach, to excluding women in the first trimester of pregnancy.

Safety data specific to both the pregnant mother and her fetus should be collected. Information on pregnancy-related outcomes (such as spontaneous abortion or intrauterine growth restriction) and on pregnancy-related complications (such as new-onset gestational diabetes or placenta previa) should be collected. In addition, severity scales used for the grading of adverse outcomes should be based on pregnancy-specific physiological and laboratory values, if available. Efforts should be made to monitor infants for developmental abnormalities.

C.7.2 Paediatric populations

A paediatric clinical development plan for a vaccine to protect against EVD should be considered early (prior to Phase III) and should take into account the incidence and prevalence of EVD, as well as existing therapies, in the paediatric population, including neonates. In general, enrolment of children in Ebola vaccine studies should be considered when there is sufficient evidence to support the safety of studies in the paediatric population and there is a reasonable demonstration of a sufficient prospect of direct benefit from animal and/or human adult studies to justify the risks. Scientific and ethical considerations regarding the initiation of paediatric studies of Ebola vaccines should be discussed with the relevant NRA early in clinical development. Available preclinical data and clinical data in older age groups should support the paediatric dose and regimen to be evaluated, and should guide decisions on

the potential need for incremental evaluation in older paediatric groups first, followed by younger children and possibly infants. Safety considerations will be critical when deciding upon the potential study of Ebola vaccines based on live, replication-competent viral vectors in infants younger than 1 year of age.

Whether evidence of effectiveness can be extrapolated from adults to specific paediatric age groups or from older to younger paediatric age groups will depend on the similarities between the relevant age groups with respect to factors such as the course of the disease and the immune response to vaccination. Consideration may also be given to bridging effectiveness from older to younger populations on the basis of a comparison of immune responses, as measured by a validated assay using an immune marker that is thought to predict clinical benefit. In some cases, immunological markers that are thought to contribute to protection may be used to bridge across age groups even if they are not scientifically well-established correlates of protection.

If the adult formulation of a vaccine is not suitable for certain paediatric age groups (for example, due to the large dose volume), sponsors should plan for the development of an age-appropriate paediatric formulation.

In paediatric studies, grading scales for adverse events and normal ranges for laboratory tests should be specifically tailored to the age group studied.

C.7.3 Immunocompromised individuals and individuals with underlying disease

Countries that have experienced prior Ebola outbreaks frequently have a relatively high prevalence of concomitant illnesses or conditions such as HIV/AIDS, tuberculosis, malaria and malnutrition. This prompts a number of unique considerations with respect to clinical development programmes for Ebola vaccines. Information on underlying medical conditions that may have an impact on the safety and effectiveness evaluations of a vaccine should be collected for participants in clinical trials.

The safety evaluation of investigational vaccines in immunocompromised individuals should include assessment of exacerbation of the underlying disease post-vaccination. For example, plasma HIV viral load has been shown in some studies, but not in others, to transiently increase following vaccination with influenza and pneumococcal vaccines – though without established clinical consequence. Product-specific considerations may preclude the use of some vaccines in certain populations due to unacceptable risks (for example, risk of disseminated disease following immunization of HIV-infected individuals with BCG vaccine).

The effectiveness of an Ebola vaccine may differ in countries according to the prevalence of certain underlying medical conditions. Thus, effectiveness data should be obtained in the region where the vaccine is most likely to be used.

C.8 Post-marketing surveillance

As part of preparing for marketing approval of any new Ebola vaccine, pharmacovigilance plans specific to each vaccine should be developed. Depending on the situation, these plans could be prepared/implemented by vaccine manufacturers and public health authorities in the countries where the vaccine will be used, or through cooperative efforts that could also include participation by regulators, WHO and other institutions.

According to the ICH, a pharmacovigilance plan should be prepared for any new vaccine (*114*). A first step towards the preparation of such a plan is the "safety specification" which summarizes: (a) the important identified and potential risks of the vaccine; and (b) the important missing information. The safety specification should also describe the populations that are potentially at risk for EVD (that is, the populations in which the vaccine will most likely be used) and any outstanding safety questions which warrant further investigation. The safety specification is intended to help industry, regulators and other institutions involved in the process to identify any need for specific data collection and to facilitate preparation of the pharmacovigilance plan (*114*). The safety specification is usually prepared by the sponsor (the institution submitting the vaccine for marketing authorization, which is usually, but not always, the manufacturer) during the pre-marketing phase. For products of international public health importance, such as Ebola vaccines, pharmacovigilance planning would benefit from dialogue not only with regulators but also with public health authorities, WHO and other institutions involved in the process.

In the case of vaccines for which no specific concerns have arisen, routine pharmacovigilance should be sufficient for post-approval safety monitoring. Nevertheless, for products with important identified risks, important potential risks or important missing information (which may be the case with new Ebola vaccines) the pharmacovigilance plan should consider appropriate risk-management and risk-minimization activities to address these concerns (*114*).

The strategies proposed for the identification and investigation of vaccine safety signals should be specified in the pharmacovigilance plan. These may depend, in part, on decisions made regarding the use of the vaccine(s) during epidemic and inter-epidemic periods. Specifically, pharmacovigilance activities may need to be adapted to situations in which the vaccine is recommended for: (a) well-defined and relatively small groups (for example, first responders, health-care workers and/or specific groups at high risk such as the close contacts of suspected cases); (b) large demographic groups (for example, all individuals in a certain age range or the inhabitants of a specific geographical region); or (c) the overall population of a country or region.

Ideally, the pharmacovigilance plan should permit the detection of new safety signals (a role performed mainly by spontaneous or passive reporting

systems) and confirmation of the association between the suspected event(s) and the vaccine being investigated (*115*, *116*). Currently, no effective post-marketing surveillance systems with clear protocols, tools and a mandate exist in countries affected by the 2014–2016 EVD epidemic. Thus, enhanced capacity for vaccine pharmacovigilance may be needed, in accordance with the WHO *Global vaccine safety blueprint* (*23*). This blueprint defines the need for enhanced capacity as follows:

> *Enhanced vaccine pharmacovigilance, at a minimum level, includes improved data collection, in passive surveillance, towards higher data quality and more complete data sets, but also improved collation, verification, analysis and communication by building capacity for stimulated and active surveillance. It also includes the ability to perform population-based studies and appropriate epidemiologic studies testing hypotheses by assessing relative and absolute risk ratios, when appropriate.*

The document goes on to state that:

> *Spontaneous reporting systems are insufficient to enable rapid assessment and adequate public health response to vaccine safety signals. Rapid response to vaccine safety signals is required to identify those rare instances where real adverse reactions occur, so that their impact can be minimized as they emerge. Countries where an increased level of vaccine safety activity is judged to be necessary are those where newly developed vaccines are being introduced and in countries that manufacture and use prequalified vaccines (23).*

The WHO Global Advisory Committee on Vaccine Safety (GACVS) has reviewed safety data from Phase I studies of two investigational Ebola vaccines (*117*). The adverse event profiles from these studies provide useful information for planning safety evaluations in further studies of these vaccines. Pharmacovigilance plans for the introduction of Ebola vaccines should take into account the observed safety profiles from clinical studies and should be aligned with WHO guidance.

In summary, the implementation of an adequate pharmacovigilance plan for the post-marketing evaluation of adverse events following the introduction of Ebola vaccines requires a functioning spontaneous reporting system, active surveillance systems and the ability to perform appropriate epidemiological studies to further investigate any possible association between suspected event(s) and the vaccine. Given existing limitations in countries that were affected by the 2014–2016 EVD epidemic, an enhanced capacity for pharmacovigilance may be needed in some countries, and more than one active surveillance approach may need to be implemented to achieve effective pharmacovigilance.

Part D. Guidelines for NRAs

D.1 General

The general recommendations for control laboratories given in the WHO Guidelines for national authorities on quality assurance for biological products (*118*) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (*119*) should apply after the vaccine product has been granted a marketing authorization. These recommendations specify that no new biological substance should be released until consistency of batch manufacturing and quality has been established and demonstrated. The recommendations do not apply to material for clinical trials.

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

The NRA may obtain the product-specific working reference 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 vaccines. In particular, during review of the marketing authorization dossier, 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, produced using the procedures and control methods that will be used for the marketed vaccine.

D.2 Release and certification

A vaccine lot should be released to the market only if it fulfils all national requirements and/or satisfies Part A of these WHO Guidelines (*119*). A protocol for the manufacturing and control of Ebola vaccines, based on the model protocol provided in Appendix 1 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for the release of a vaccine for use.

A Lot Release Certificate signed by the appropriate NRA official should then be provided if requested by a manufacturing establishment, and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these WHO Guidelines. The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided below in Appendix 2.

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Appendix 1

Model protocol for the manufacturing and control of viral-vectored Ebola vaccines

The following provisional protocol is intended for guidance. It indicates the information that should be provided as a minimum by the manufacturer to the NRA after the vaccine product has been granted a marketing authorization. The protocol is not intended to apply to material intended for clinical trials.

Since the development of these vaccines is incomplete at the time of writing this document, detailed requirements are not yet finalized. Consequently only the essential requirements are provided in this appendix. Information and tests may be added or omitted (if adequate justification is provided) as necessary to be in line with the marketing authorization approved by the NRA. It is therefore possible that a protocol for a specific product will differ from the model provided here. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO Guidelines on a particular product should be given in the protocol submitted.

The section concerning the final product should be accompanied by a sample of the label and a copy of the leaflet that accompanies the vaccine container. If the protocol is submitted in support of a request to permit importation, it should also be accompanied by a Lot Release Certificate from the NRA of the country in which the vaccine was produced and/or released stating that the product meets national requirements as well as Part A of these WHO Guidelines.

1. Summary information on finished product (final vaccine lot)

International name:
Commercial name:
Product licence (marketing authorization) number:
Country:
Name and address of manufacturer:
Name and address of product licence-holder if different:
Viral vector(s):
Ebola virus strain(s):
Batch number(s):
Type of container:
Number of filled containers in this final lot:
Number of doses per container:
Composition (viral vector concentration)/volume of single human dose:

Target group:
Expiry date:
Storage conditions:

2. Control of source material

2.1 Virus seeds (repeat for each monovalent vaccine component)

2.1.1 Seed banking system

- Name and identification of viral vector:
- Origin of all genetic components:
- Construction of viral vector:
- Nucleotide sequence of the transgene and flanking regions:
- Antigenic analysis, infectivity titre, in vitro yield:
- Comparison of genetic and phenotypic properties with parental vector:
- Seed bank genealogy with dates of preparation, passage number and date of coming into operation:
- Tests performed for detection of adventitious agents at all stages of development:
- Freedom from TSE agents:
- Details of animal or human components of any reagents used in the manufacture of seed banks, including culture medium:
- Genetic stability at the level of a virus pre-master seed or virus master seed to its sequence at, or preferably beyond, the anticipated maximum passage level:
- Confirmation of approval for use by manufacturer, and the basis for that approval:

2.2 Cell cultures (if applicable) (repeat for each monovalent vaccine component)

2.2.1 Cell banking system

- Name and identification of cell substrate:
- Origin and history of cell substrate:
- Details of any manipulations (including genetic manipulations) performed on the parental cell line in the preparation of the production cell line:
- Cell bank genealogy with dates of preparation, passage number and date of coming into operation:

- Confirmation of approval for use by manufacturer, and the basis for that approval:
- Tests performed for detection of adventitious agents at all stages of development:
- Test for tumorigenic potential (if of mammalian origin):
- Details of animal or human components of any reagents used in manufacture of cell banks, including culture medium:
- Freedom from TSE agents:
- Genetic stability (if genetically manipulated):

2.2.2 Primary cells (if generated)

- Source of animals and veterinary control (for example, specify if animals or eggs are sourced from closed, pathogen-free colonies):
- Name, species and identification of primary cell batches:
- Details of animal or human components of any reagents used in manufacture of cells:
- Methods of isolation of the cells:
- Tests performed for detection of adventitious agents during manufacture (may be performed on control cells if necessary):
- Freedom from TSE agents:

3. Control of vaccine production (repeat for each monovalent vaccine component)

3.1 Control of production cell cultures/control cells

3.1.1 Information on preparation

- Lot number of master cell bank:
- Lot number of working cell bank:
- Date of thawing ampoule of working cell bank:
- Passage number of production cells:
- Date of preparation of control cell cultures:
- Result of microscopic examination:

3.1.2 Tests on cell cultures or control cells

- Adventitious agents:
- Sterility (bacteria, fungi, mycoplasmas):

3.2 Viral vector harvests or pooled viral vector harvests

3.2.1 Information on manufacture

- Batch number(s):
- Date of inoculation:
- Date of harvesting:
- Lot number of virus master seed lot:
- Lot number of virus working seed lot:
- Passage level from virus working seed lot:
- Methods, date of purification if relevant:
- Volume(s), storage temperature, storage time and approved storage period:

3.2.2 Tests

- Adventitious virus tests:
- Bacteria/fungi/mycoplasmas:
- Virus titre:

3.3 Monovalent viral vector bulk

3.3.1 Information on manufacture

- Batch number(s):
- Date of formulation:
- Total volume of monovalent bulk formulated:
- Virus pools used for formulation:
- Lot number/volume added:
- Virus concentration:
- Name and concentration of added substances (for example, diluent, stabilizer if relevant):
- Volume(s), storage temperature, storage time and approved storage period:

3.3.2 Tests

- Identity:
- Purity:
- Residual HCP:
- Residual HC DNA (if non-primary cell lines):

- Potency:
  - Particle number (for adenovirus):
  - Infectious virus titre:
  - Particle-to-infectivity ratio (for adenovirus):
  - Expression of heterologous antigen in vitro:
- Replication competence (for adenovirus):
- pH:
- Preservative content (if applicable):
- Endotoxin:
- Sterility or bioburden:

3.4 Final viral vector bulk

3.4.1 Information on manufacture

- Batch number(s):
- Date of formulation:
- Total volume of final bulk formulated:
- Monovalent virus pools used for formulation:
- Lot number/volume added:
- Virus concentration:
- Name and concentration of added substances (for example, diluent, stabilizer if relevant):
- Volume(s), storage temperature, storage time and approved storage period:

3.4.2 Tests

- Identity:
- Sterility or bioburden:
- Concentration of antimicrobial agent, if relevant:

4. Filling and containers

Lot number:
Date of filling:
Type of container:
Volume of final bulk filled:
Filling volume per container:
Number of containers filled (gross):

Number of containers rejected during inspection:
Number of containers sampled:
Total number of containers (net):
Maximum period of storage approved:
Storage temperature and period:

5. Control tests on final vaccine lot

Inspection of containers (that is, inspection container integrity):
Appearance (that is, appearance of container content):
Identity:
pH and osmolality:
Potency (if feasible to measure in a multivalent system):

- Particle number (adenovirus):
- Infectious virus titre:
- Particle-to-infectivity ratio (for adenovirus):
- Expression of heterologous antigen in vitro:

General safety tests (initial batches only):
Endotoxin:
Sterility:
Extractable volume:
Aggregate/particle size:
Presence of preservative (if relevant):
Residual moisture content (for freeze-dried product):
Reconstitution time (for freeze-dried product):

6. Certification by the manufacturer

Name of Head of Production (typed) _____

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

I certify that lot no. _____ of Ebola vaccine, whose number appears on the label of the final containers, meets all national requirements and satisfies Part A[1] of the WHO Guidelines on the quality, safety and efficacy of Ebola vaccines[2] (if applicable)

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

[2] WHO Technical Report Series, No. 1011, Annex 4.

Name (typed) _____

Signature _____

Date _____

7. Certification by the NRA

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

Appendix 2

Model NRA Lot Release Certificate for viral-vectored Ebola vaccines

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

Certificate no. _____

The following lot(s) of Ebola vaccine produced by _____[1] in _____[2] whose lot numbers appear on the labels of the final containers, complies with the relevant specification in the marketing authorization and provisions for the release of biological products[3] and Part A[4] of the WHO Guidelines on the quality, safety and efficacy of Ebola vaccines[5] and comply with WHO good manufacturing practices for pharmaceutical products: main principles,[6] WHO good manufacturing practices for biological products,[7] and Guidelines for independent lot release of vaccines by regulatory authorities.[8]

The release decision is based on _____[9]

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);

[1] Name of manufacturer.

[2] Country of origin.

[3] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the 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. 1011, Annex 2.

[6] WHO Technical Report Series, No. 986, Annex 2.

[7] WHO Technical Report Series, No. 999, Annex 2.

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

[9] Evaluation of product-specific summary protocol, independent laboratory testing, and/or specific procedures laid down in a defined document, etc., as appropriate.

- type of container used;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and expiry date;
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other authority as appropriate):

Name (typed) _____
Signature _____
Date _____