Guidelines on the quality, safety and efficacy of Ebola vaccines

(Proposed new guidelines)

NOTE:

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

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee on Biological Standardization. Written comments proposing modifications to this text MUST be received by 16 September 2016 in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Essential Medicines and Health Products (EMP). Comments may also be submitted electronically to the Responsible Officer: Dr TieQun Zhou at email: zhout@who.int.

The outcome of the deliberations of the Expert Committee on Biological Standardization will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide" (WHO/IMD/PUB/04.1).
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This document provides information and guidance on the development, production, quality control and evaluation of the safety and efficacy of candidate Ebola vaccines. It is written in the form of WHO Guidelines instead of Recommendations since there is at present no licensed Ebola vaccine and is intended to facilitate progress towards the eventual licensure of such a vaccine. Guidelines allow greater flexibility than Recommendations with respect to future developments in the field. The parts printed in small type and indented text are comments or examples that are intended to provide additional guidance for manufacturers and NRAs which may benefit from these details. To facilitate the international distribution of vaccines produced in accordance with these Guidelines, a summary protocol for recording test results has been provided in Appendix 2.
Introduction

The unprecedented scale and severity of the Ebola virus disease (EVD) epidemic in West Africa in 2014–2015 led to calls for the urgent development and licensing of an Ebola vaccine (1,2). Considerable work is now underway and there have been several international consultations on public health issues and on Ebola vaccine development, evaluation and licensing (2–4). As part of WHO’s ongoing measures to support the development of Ebola vaccines, guidance has been prepared on the scientific and regulatory considerations relating to their quality, safety and efficacy.

WHO convened an informal consultation at its headquarters in Geneva on 18–19 March 2015 – attended by scientific experts, regulatory professionals and other stakeholders involved in Ebola vaccine development, production, evaluation and licensure – to review draft guidelines prepared by a drafting group and to seek consensus on key technical and regulatory issues (5). The draft guidelines were revised in the light of comments made and underwent public consultation which resulted in a large number of comments and suggestions. This draft, together with the comments, was discussed by the Expert Committee on Biological Standardization at its meeting in October 2015. A further draft was then developed and was subjected to a second round of public consultation between January and March 2016. A working group meeting was held in Geneva on 4–5 May 2016 involving the drafting group and other experts to review the smaller number of comments received. The following text was developed as a result of these discussions. The text is written in the form of guidelines rather than recommendations since guidelines allow greater flexibility than recommendations with respect to the expected future of Ebola vaccine development, production, quality control and evaluation.

Scope

This document provides scientific and regulatory guidance for national regulatory authorities (NRAs) and vaccine manufacturers on the quality, nonclinical and clinical aspects of Ebola vaccines, and particularly those based on viral vectors which are currently at the most advanced stage of development.
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 (6,7). There is also a regional guideline available for live recombinant viral vectored vaccines (8).

Although recombinant viral vector-based 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 Ebola virus (EBOV) antigen produced in *Escherichia coli* (9), Ebola virus-like particles (VLPs) expressed from recombinant baculovirus in insect cells, and other forms of subunit vaccines.

General guidance on many nonviral vector production technologies has already been published by WHO and other sources and may provide useful information for the development and manufacture of such Ebola vaccines. Examples of references include:

- inactivated vaccines (10–12)
- protein antigens produced by recombinant DNA technology (13–16)
- DNA vaccines (17,18).

Most developmental approaches to Ebola vaccine involve recombinant DNA technology. Because EBOV has such a high case fatality rate, little or no work appears to have been undertaken in developing live attenuated Ebola vaccines, although in theory EBOV could be inactivated, as has been done for rabies vaccine (10).

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 (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 so they are not elaborated in this document. Examples of vaccines mentioned in this document are provided for information only and should not be considered as endorsements of any particular candidate vaccine.
This document should be read in conjunction with other relevant WHO guidelines such as those on nonclinical \((19,20)\) and clinical evaluation \((21)\) of vaccines, as well as relevant documents that describe the minimum requirement for an effective National Pharmacovigilance System \((22)\). Other WHO guidance, such as that for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks \((23)\), should also be consulted as appropriate.

It should be noted that there are current knowledge gaps in the scientific understanding of EVD and Ebola vaccines, which are being addressed by ongoing research and development. This document has therefore been developed in the light of knowledge available so far, particularly regarding the current most advanced Ebola vaccine candidates, and will need to be updated as new data become available from additional studies.

**General considerations**

_Ebola virus, Ebola virus disease and epidemiology_

Ebola virus \((EBOV)\) belongs 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 \((24)\). There are five distinct species of EBOV: Ebola virus Zaire \((ZEBOV)\), Sudan Ebola virus \((SUDV)\), Tai Forest Ebola virus \((TAFV)\), Reston Ebola virus \((RESTV)\) and Bundibugyo Ebola virus \((BDBV)\) \((24,25)\). Marburg virus appears to be antigenically stable and at present there is only a single species, Marburg virus \((MARV)\). 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 \((26)\). Bats are believed to be the natural reservoir of all filoviruses. EBOV and MARV cause severe haemorragic fever in humans and non-human primates alike, with high morbidity and mortality rates \((27,28)\). Outbreaks of infection with Ebola filoviruses have been noted since 1976, mainly in Central Africa, and recur at intervals. Prior to the 2014–2015 EVD outbreak in West Africa there had not been such a large-scale epidemic 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 (29,30). Patients then progress rapidly to a life-threatening disease (31). Infected persons seem to become infective only once symptoms appear, but those who survive remain infective until the virus is cleared from their blood and other body fluids. It has been reported that viable EBOV can persist in ocular fluid for at least 9 weeks following clearance of virema (32). EBOV has also been detected in the semen of males for months following recovery from EVD, which is consistent with the possible persistence of the virus within immune-privileged tissue sites in the body (33,34). Presumptive sexual transmission of EBOV from recovered individuals has also been reported (35,36). There are currently no licensed vaccines or therapeutics to prevent or treat filovirus infections. Nevertheless, individuals suffering from EVD have been treated aggressively with oral and intravenous fluids, including electrolyte replacements, to combat severe diarrhoea and dehydration, sometimes successfully surviving the infection (31).

Filoviruses are high risk agents and classified as biosafety level (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 (37). The GP is a type-1 transmembrane glycoprotein 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 (38,39). Most of the vaccines under current development are based on the EBOV GP and have been shown to confer protection from lethal EBOV challenge in animal models including, importantly, non-human primates (40,41).

**Natural immune responses to Ebola viruses**

Filovirus infection in humans elicits both cellular and humoral responses. 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 (42). 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 but overall the data are somewhat conflicting (42). It is suggested that antibodies play a significant part in protection against filovirus infection but correlates of protection have not been established and no role for cellular immunity has been demonstrated.

**Ebola vaccines currently under development**

A large number of candidate Ebola vaccines are under development. Some of these vaccines had already been in preclinical development before the 2014–2015 epidemic began and are substantially more advanced than the others. An overview of candidate Ebola vaccines that are currently at advanced stages of development is provided in Appendix 1.

The most advanced candidate vaccines are those based on live recombinant virus vector platforms (Appendix 1). Such candidate vaccines have been developed in Canada, China, Europe, Russia and the USA. Four of the most advanced platforms used to engineer these vaccines are recombinant Vesicular Stomatitis Virus (rVSV) (43), Chimpanzee Adenovirus (ChAd) (44), human Adenovirus 26 (Ad26) (45) and the Modified Vaccinia Virus Ankara strain (MVA) (46). Monovalent candidate vaccines have been constructed to express the EBOV GP of one EBOV strain, such as the Zaire strain responsible for most of the epidemic in West Africa. Others have been developed as multivalent vaccines expressing the GP of two EBOV strains and MARV as well as, in one case, the EBOV nucleoprotein. 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.

The viral vectored vaccines under development include those that are replication-incompetent in the human host or in human cells, unless the human cells have been engineered to allow their replication, as well as those that are replication-competent but likely to be highly attenuated because of their recombinant gene inserts and cell culture passage (Appendix 1). Replication-incompetent vectors include adenoviral vectors, both those derived from human adenoviruses (such as Ad26) and those derived 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 was licensed in Canada and Europe. Vectors that are replication-competent but attenuated include recombinant VSV, a negative-stranded RNA virus animal pathogen, where attenuation is due to the insertion of a recombinant heterologous gene, such as the EBOV GP, in place of the VSV glycoprotein. 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 as Ebola vaccines (43, 47, 48).

The need for careful clinical studies with candidate vaccines in the target population will be of paramount importance and a proposed “target product profile”, setting out optimal and minimal criteria for Ebola vaccines for use in epidemic or endemic settings, has been developed by the Wellcome Group (3). WHO has developed a document, *Ebola virus disease (EVD) vaccine target product profile*, which provides guidance on WHO’s preferences for Ebola vaccines of two categories (for reactive use and prophylactic use) (49). Encouraging results on the immunogenicity and safety, as well as on clinical efficacy based on disease endpoints, have already been generated (30) and their evaluation in larger phase 2 and phase 3 trials are ongoing. The results of one human clinical study (50) using the ChAd3 vectored vaccine encoding the Zaire strain GP showed no serious safety concerns at the doses used, but immune responses were less than those reported for the same vaccine in non-human primates protected against filovirus challenge, even at the highest dose used. This is not surprising as higher immune responses at the same dose of vaccine in non-human primates than in humans has been seen with other vectors and other immunogens. However, the ChAd3 vectored vaccine, like the candidate rVSV EBOV vaccine (51), is reported to have induced in some cases transient fever at the top dose used in humans. The prophylactic window for non-replicating viral vectored vaccines is as yet unclear but pyrogenicity problems might be overcome by using a prime-boost approach (50). Various two-dose schedules of ChAd3/MVA and Ad26/MVA have been evaluated in the clinic and this approach may also be relevant where long-term protection is required. Data from phase 1 studies of the rVSV-ZEBOV vaccine in several locations, including Canada, Gabon, Germany, Kenya, Switzerland and the USA, show that pain at the injection site
Was common, as were systemic symptoms such as fever and malaise, generally lasting up to 3
days. Administration of rVSV vaccine resulted in viraemia that is detectable by polymerase
chain reaction (PCR) during the first and sometimes second week after vaccination. The vaccine
virus was also detected by PCR in the urine and saliva of a minority of the recipients. No serious
vaccine-related adverse reactions have been reported so far for rVSV vaccine from these studies,
although an unexpected safety signal was detected. Mild-to-moderate and generally short-lived
arthritis or arthralgia developed during the second week following immunization in a minority
(20%) of recipients at one site in particular (52). Fewer of these joint-related adverse events
(5%) occurred at other investigational sites. Although general rVSV vaccine reactogenicity was
found to be dose-related, the arthritis/arthralgia events were not linked with dose; they occurred at
similar frequencies in recipients receiving the lowest and highest doses. This is believed to be
due to the chimeric VSV vaccine virus, like EBOV itself, showing a tropism for joints. Ongoing
phase 2 and phase 3 clinical studies are expected to provide further safety data, especially in
African settings. Preliminary data from adaptive trials design clinical studies (ring vaccination)
using the rVSV vaccine have shown encouraging efficacy (53). However, the epidemiological
situation has now changed significantly. Using strict infection control and public health
measures, the EBOV epidemic has been eliminated. WHO declared Sierra Leone free of EBOV
transmission in March 2016, and declared Guinea and Liberia free of EBOV transmission in
June 2016, bringing to an end the Ebola outbreak in the three African countries mainly affected
(54). The assessment of EBOV vaccine efficacy will therefore now be more challenging.

Special considerations

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 whether the product is intended for
use in a clinical trial as a licensed product or whether the product is 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 resulting
in contamination of the product with unwanted components such as microorganisms, viruses 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 benefit and risk 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 be due to exploiting the knowledge gained from similar products manufactured with the same vector backbone (i.e. platform technology). Where it can be reasonably argued, and shown with data (where available), that the manufacture and control of the Ebola vaccine-specific virus vector behaves similarly with respect to process and product characterization to a previously-developed product based on the same platform technology, several aspects of manufacture and control could be based on the more fully developed product with only confirmatory information required for the EBOV-specific insert. This principle is especially applicable during the phase of clinical trial development. For licensure, product-specific data will be required but such supportive platform-derived data may decrease the requirement for some product data if it can be shown that the benefit–risk assessment remains positive. Such an approach should be discussed with the NRA in advance of the licence submission.
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 will hinder the availability of the product (e.g. by being on the critical path for product availability, or by using large quantities of scarce material that is required for clinical purposes). These deferrals would be identified on a case-by-case basis and should be discussed with the NRA.

However, even if the nature of a public health emergency affects the benefit–risk balance in such a way as to justify accelerated development and authorization of a vaccine, the marketing authorization holder would retain an obligation to complete full development work and submit the full data to the NRA as soon as they become available, even if this happens after product approval through an accelerated mechanism.

Similar considerations apply to nonclinical evaluation of candidate Ebola vaccines. For nonclinical evaluation during a public health emergency, it is paramount to determine a minimum nonclinical package that is reasonable to support initiation of early phase 1 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 instance, 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, limited toxicity data should be obtained, focusing on unexpected consequences that could result from the indirect and direct effects related to vaccination.

In general, the use of a minimum 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 appropriate for a particular vaccine should be submitted, or the application should be otherwise adequately justified.
Clinical development of an EVD 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 conduct.

The main text of Parts A, B and C of these guidelines considers in general terms the full quality, nonclinical and clinical requirements for a license submission for viral vectored vaccines. Some aspects of the nonclinical and clinical evaluation guidance (Parts B and C) may also apply to vaccine approaches other than those based on viral vectors. Additionally, the text considers the principles which may be applied to product development, manufacturing and control, as well as to nonclinical and clinical evaluation, during a public health emergency to allow the rapid introduction of an Ebola vaccine. Wherever appropriate, recommendations on the minimum dataset required are highlighted and aspects of vaccine development which may be accelerated during a public health emergency are indicated. These are found in context-specific examples shown in indented text within Parts A, B and C. Considerations of the quality requirements at different stages of clinical development are discussed in the section entitled “Special considerations” in Part A.

A WHO Emergency Use Assessment and Listing Procedure (EUAL) (55) has also been developed to expedite the availability of vaccines needed in situations of public health emergency of (usually) international concern.

**Terminology**

The definitions given below apply to the terms used in these guidelines. They 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, 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 (e.g. regulators) might also be warranted.

Attenuated virus: a strain of virus of which the 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 on 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 – i.e. the quality, nonclinical and clinical data – to be 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: the expression vector containing the 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: the 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: 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 (i.e. causative for protection) or it may be non-mechanistic (i.e. non-causative, 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 (MA): a formal authorization for a medicine to be marketed. Once an NRA approves a market authorization application for a new medicine, the medicine may be marketed and may be available for physicians to prescribe (also referred to as product licence or product authorization).

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.

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

**Pooled virus harvest:** a homogeneous pool of several single virus harvests.

**Public health emergency:** an extraordinary event that is determined, as provided in the International Health Regulations (56), (i) to constitute a public health risk to other States through the international spread of disease, and (ii) to potentially require a coordinated international response.

**Seed lot:** a seed lot system is a system according to which successive batches of viral vector 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. The origin and the passage history of the virus master seed lot and the virus working seed lot are recorded.

**Single virus harvest:** viral vector from a single culture after separation from production cells but before purification.

**Vaccine efficacy:** an estimate of the reduction in the chance or odds ratio of developing clinical disease after vaccination relative to the chance or odds ratio when not vaccinated against the disease to be prevented. Vaccine efficacy measures direct protection (i.e. protection induced by vaccination in the vaccinated population sample).
Vaccine effectiveness: an estimate of the protection conferred by vaccination in a specified population. Vaccine effectiveness measures both direct and indirect protection (e.g. the estimate may reflect in part protection of nonvaccinated persons secondary to the effect of the vaccine in the vaccinated population). Vaccine effectiveness may also be inferred from a vaccine-induced immune response (e.g. 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 and from which the virus working 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.

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

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

The replication abilities of the lead viral vectored vaccines are summarized in Appendix 1. 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 which have had specific genetic elements removed and
which rely on these elements being supplied by the production cell line (i.e. the adenovirus vectors). 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 general manufacturing requirements contained in WHO’s Good manufacturing practices for pharmaceutical products: main principles (57) and Good manufacturing practices for biological products (58) 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. The principles presented in WHO’s Laboratory biosafety manual (59) should be followed to justify this classification. Approval for the classification should be sought from the relevant authority in the country/region where the manufacturing facility is located.

A.1.1 International reference materials

Plasma from a recovered repatriated patient who contracted Ebola in West Africa has been established by the Expert Committee on Biological Standardization (ECBS) as the first international reference reagent for Ebola antibodies and has been assigned a unitage of 1 unit/mL. The highly pathogenic nature of EBOV raises particular concerns for the preparation of international reference materials as they must both be safe in use and also resemble the natural material to be analysed very closely if they are to be commutable. The plasma was from a natural infection and therefore is likely to have all relevant specificities. It is considered of acceptable
safety for three reasons: the patient was fully recovered clinically, the plasma was negative for
EBOV nucleic acid in a range of PCR assays, and the plasma was treated with solvent/detergent
(an established method for the inactivation of enveloped viruses used in the blood products
industry for decades).

Two EBOV RNA preparations have been established by the ECBS for the standardization of
nucleic acid assays. The first, EBOV RNA NP-VP35-GP WHO Reference Reagent, consists of
the RNA encoding the NP, VP35 and GP genes and is to be used to standardize assays directed at
these genes only. The second, EBOV RNA VP40-L WHO Reference Reagent, consists of the
RNA encoding the VP40 and L genes and is intended to standardize assays directed at these
genes only. Both preparations are packaged in non-replicating lentiviral vectors (LVV); the
EBOV genes also include mutations to make them inactive. The two Ebola RNA preparations
are called “WHO 1st International Reference Panel for Ebola NAT” with 7.5 Log10 units/mL
assigned for Ebola NP-VP35-GP-LVV and 7.7 Log10 units/mL for Ebola VP40-L-LVV.
The reference materials listed above are available from the National Institute for Biological
Standards and Control, Potters Bar, United Kingdom. The WHO catalogue of international
biological standards should be consulted for the latest list of appropriate WHO International
Standards and reference materials (60).

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 vectors should be based on a seed lot system, analogous to the cell banking
system used for production cells described below.

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; overall,
this should allow for a clear understanding of the functionality of the vaccine and how it is
attenuated or made replication-incompetent where this is the result of 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 in, for instance, a temporal or tissue-specific manner, evidence should
be provided on product characterization and control to demonstrate such specificity. RNA
editing should be discussed if relevant.

All steps from 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. Cloning 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 use of stably integrated clones may avoid the need for selective markers (e.g. antibiotics)
during production. Therefore, cloning strategies such as transfection (e.g. lipofection),
electroporation or TA cloning which do not rely on selection markers, are preferred. If they are
used, the impact of selection markers during screening and development and remaining in the
final product should be carefully evaluated. In this respect, the presence of antibiotic resistance
genes in the vaccine vector should be avoided if possible.

A full description of the biological characteristics of the recombinant viral vectors should be
given. The culture conditions used to promote the growth of the cloned vector in the production
cells should be described in detail. The description should include the construction, genetics and
structure of the viral vector, and the origin and identification of the vector and gene that are
being cloned.

The nucleotide sequence of the gene insert and of adjacent segments of the vector, and
restriction-enzyme mapping of the vector containing the gene insert, should be provided.
Genetic stability of the vector with the recombinant construct should be demonstrated. The
stability of a recombinant vector should be assessed by comparing the structure or sequence of
the vector at the level of a pre-seed or virus master seed to its structure or sequence at, or
preferably beyond, the anticipated maximum passage level. The structure and sequence should
ensure that no changes occur to 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 (i.e. it should be a conservative change) or to 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 (e.g. because of limited quantities of material), 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 and 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 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 detection of revertants (including the emergence of replication-competent vectors from replication-incompetent vectors during passage). However, other studies including antigenic analysis, infectivity titre and in vitro yield should 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 (e.g. 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.

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 safety of the product – especially with regard to adventitious agents (23), replication competence, attenuation and other phenotypes, stability and suitable genetic sequence – must be established prior to its use.

**A.2.2 Cell substrates**

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

**A.2.2.1 Master and working cell banks (MCBs and WCBs)**

The cell banks should conform to the *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks* (23).

An appropriate history of the cell bank should be provided. This should include the 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. Information should be given on the testing for adventitious agents and on gene homogeneity for the MCB and WCB.
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 structure or 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, etc. 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 the WCB, and through the 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, 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. Tissue 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/animals should be adequately quarantined for an appropriate period of time prior to use for the preparation of cells.

Information on 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 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.2.2.1 Tests on cell substrates MCB and WCB

MCBs and WCBs should be tested for the absence of bacterial, fungal, and mycoplasmal and viral contamination by appropriate tests, as specified in Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (23), or by a method approved by the NRA to demonstrate that the MCB and WCB are not contaminated with adventitious agents.

Rapid sterility methods to demonstrate absence of bacteria and fungi as well as nucleic acid amplification techniques (NATs) alone or in combination with cell culture, with an appropriate detection method, might be used as an alternative to one or both of the compendial mycoplasmal detection methods after suitable validation and agreement from the NRA (23).

The cell bank should be tested for tumorigenicity if it is of mammalian origin, as described in Section B of the Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (23). The tumorigenic potential of the cell banks(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, especially with regard to adventitious agents (9), must be established prior to its use.

A.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, mycoplasma 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 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 to pharmacopoeial testing for
bacterial, fungal and mycoplasma in the control (if relevant) and production cultures. Mycoplasma
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 (SPF) eggs. After preparation, the CEF cells should be tested for bacterial,
fungal and mycoplasma contamination, for viral adventitious agents by in vitro assay using three
cell lines, including avian and human cells (such as CEF, MRC-5 and Vero cells), and for viral
adventitious agents by in vivo assay using mice and embryonated eggs, for Avian Leucosis Virus
contamination and for the presence of retroviruses by measuring the RT-activity.

A.2.3 Cell culture medium
If serum is used for the propagation of cells, it should be tested to demonstrate absence of
bacteria, fungi and mycoplasmas – as specified in the requirements given in Part A, sections 5.2
(61) and 5.3 (62) of the WHO General requirements for the sterility of biological substances –
and freedom from adventitious viruses.

Detailed guidelines for detecting bovine viruses in serum for establishing MCB and WCB are
given in Appendix 1 of WHO’s Recommendations for the evaluation of animal cell cultures as
substrates for the manufacture of biological medicinal products and for the characterization of
cell banks (23) and should be applied as appropriate. The guidelines for detecting bovine viruses
in serum for establishing the cell banks 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, recognizing that some viruses are relatively
resistant to gamma irradiation. Whatever the process used, the validation study has to determine
the consistency and effectiveness of the viral inactivation process while maintaining serum
performance. The use of non-inactivated serum should be justified and is not advised without
strong justification. The non-inactivated serum must meet the same criteria as the inactivated serum when tested for sterility and absence of mycoplasma 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* (63).

Bovine or porcine trypsin used for preparing 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* (63).

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 the component units of each batch. The irradiation dose must be low enough so that the biological properties of the reagents are retained while being high enough to reduce virological risk. Consequently, irradiation cannot be considered a sterilizing process (23). 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 (23).

Human serum should not be used.

If human serum albumin is used at any stage of product manufacture, the NRA should be consulted regarding the requirements, as these may differ from country to country. At a minimum, it should meet the *WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives* (64). 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 (63).

Penicillin and other beta-lactams should not be used at any stage of the manufacture because they are highly sensitizing substances.

Other antibiotics may be used in the manufacture provided that the quantity present in the final lot is acceptable to the NRA.

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

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

Early-phase clinical and nonclinical studies are generally supplied with product for which knowledge of the manufacture and control is expected to be fairly rudimentary since few batches will have been manufactured and analytical methods will be in early stages of development. The material is required for the provision of early safety and proof-of-concept studies, as well as to begin 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 (65).

The majority of the data to be provided to the NRA before the human studies can begin will concern the derivation and safety of the viral vector product and the production cell line, and 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, and the genetic stability to, or preferably beyond, the anticipated maximum passage level in manufacture should be given.
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, although 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 in the production of product for clinical use must be fully tested to ensure the absence of bacteria, fungi, mycoplasmas and adventitious viruses. Where applicable, freedom from TSEs must also be addressed. 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 to be 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 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 to the production cell culture. Cells should be derived from the same expansion series but no virus vector should be added to the control cells. 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 harvests. The maximum number of single harvests that may be pooled should be defined on the basis of validation studies.

By the time of submitting a marketing authorization application, 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 they retain 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 engineering 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, the manufacturers should agree 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 in a post-licensure setting. The immunogenicity of the product, when relevant and available, should also be included in the characterization programme (e.g. as part of the nonclinical pharmacodynamic evaluation).

A.3.1.1 Tests on control cell cultures (if applicable)
Where the NRA requires the use of control cells, the following procedures should be followed. From the cells used to prepare cultures for production of vaccine, 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 the adventitious agent testing of control cultures 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 to be
performed using red blood cells of other species, including from humans (blood group O),
monkeys and/or chickens (or other avian species). All haemadsorption tests should be read after
incubation for 30 minutes at 0–4 °C, and again after a further incubation for 30 minutes at 20–
25 °C. The test with monkey red 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.3.1.2 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 as 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, 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 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 (e.g. purified final bulk). For example, a Western blot analysis or other methods to confirm 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 harvest according to relevant parts of section B.11 of WHO’s Recommendations for the evaluation of animal cells as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (23). Additional testing for adventitious viruses may be performed using validated NATs.

New molecular methods with broad detection capabilities are being developed for adventitious agent detection. These methods include: (i) degenerate NATs for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (ii) NATs with random primers followed by analysis of the amplicons on large oligonucleotide micro-arrays of conserved viral sequencing or digital subtraction of expressed sequences; and (iii) high-throughput sequencing. These methods may be used in the future to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and 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, sections 5.2 (61) and 5.3 (62) of WHO’s 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 testing for adventitious agents may be applied with the approval of the NRA.

Provided cell banks and viral seed stocks have been comprehensively tested and released, demonstrating they are free of adventitious agents, it could be discussed (and should be agreed with the NRA) whether to evaluate the possibility of delaying in vitro testing for extraneous agents (viral pathogens and mycoplasmas) at the cell harvest or bulk substance stages or replacing it with validated PCR tests. The method of production should be taken into account when deciding 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 in the facility. Samples should be retained for testing at a later date if required.

### A.3.3 Pooled virus harvests

Depending on the unit size of production, single virus harvests may be pooled, and from these virus pools 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, sections 5.2 (61) and 5.3 (62) of WHO’s General requirements for the sterility of biological substances. Alternatively, if single virus harvests have been tested to demonstrate freedom from bacteria, fungi and mycoplasmas, these tests may be omitted on the pooled virus harvests.
A.3.4 Final bulk vaccine

The final bulk vaccine can be prepared from one or several virus pools, 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 final bulk vaccine should have been shown not to impair the potency and safety of the vaccine in the concentrations employed.

Penicillin and other beta-lactams should not be used at any stage of manufacture because of their nature as 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.

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 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.

A.3.4.1 Control tests on final bulk

The final bulk vaccine should be tested and consideration should be given to using the tests listed below, as appropriate for the individual products. Alternatively, if the final bulk will be held for a short period of time, some of the tests listed below could be performed on the final lot instead. If sufficiently justified, some tests may be performed on an earlier intermediate instead of on the final bulk. All quality-control release tests for final bulk should be validated and should be 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 for 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 (e.g. with the same vector backbone but differing in heterologous gene from
the Ebola glycoprotein) where it can be justified that the specific heterologous gene is unlikely to have an impact on the result. An example of this could 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 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 tests delays product availability for use in clinical trials, and/or that the test would use an unacceptably large volume of product which is urgently required for clinical trials, it may be possible to omit or delay the test, or replace it with one that is more acceptable to 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 final bulk vaccine should be assessed using suitable methods. The purity of the bulk should be ascertained for 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 level of host cell DNA should not exceed the maximum level 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 (23).
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, although data should 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 final bulk vaccine, subject to the agreement of the NRA.

A.3.4.1.2 Potency

Each final bulk vaccine should be tested for potency measured by a combination of the following methods.

A.3.4.1.2.1 Particle number
For relevant vectors (e.g. adenovirus vectors), the total number of virus particles per millilitre, quantitated by a technique such as qPCR or HPLC should be provided for each batch of final bulk.

A.3.4.1.2.2 Infectivity
Infectious virus titre, as a measure of active product should be tested for each batch of final bulk. 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. For relevant vectors, the particle:infectivity ratio should also be specified.

A.3.4.1.2.3 Expression of the heterologous antigen in vitro
The ability of the viral particles to express the heterologous gene should be demonstrated (e.g. by the generation of immunoblots using antigen-specific antibodies) after growth 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 final 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 (61), or by methods approved by the NRA.

A.3.4.1.5 Bacterial endotoxins

Each final 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 vector 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, in the Ebola target population, many of the subjects 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 (which should include the
demonstration of replication incompetence/attenuation as well as a discussion of why reversion to competency or loss of attenuation is prevented in future batches).

A.3.4.1.7 Preservative content (if applicable)

The final 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 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 fermentation should be aligned with production cell manufacturing procedures. Process and product characterization should ensure the comparability of product throughout development. Although some differences in product characteristics can be anticipated (sometimes intended improvements due to optimization studies, sometimes unintentional consequences of process change), these 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 adequate process validation and also because the process is likely to be undergoing optimization. However, whatever batch data (including qualitative and quantitative data) are available 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 water for injection systems, heating, ventilation and air conditioning (HVAC) systems, etc. It is anticipated that during an emergency situation, these validation criteria should be addressed adequately. 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 on the basis of other criteria such as production requirements.
During later clinical stages and at licensing submission, the manufacturing process is normally fixed and process-specific validation completed by demonstrating that several consecutive full commercial-scale batches which conform to predetermined criteria can be made.

The “Quality-by-Design” approach is not considered in this document. However, such an approach is not excluded provided that the principles discussed throughout this document are adequately addressed.

A.3.6 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 some 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 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 (57, 58, 66).

A.3.7 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 this document). 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 ICH Q2(R1) (67), 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, taking safety considerations into account, should be set for impurities and quantity. For relevant vectors, reversion to competency should be tested. A justification for the quality attributes included in the specification and the acceptance criteria for purity, impurities, quantity, potency and any other quality attributes which may be relevant to performance of the vaccine should be provided. 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 3 studies and commercial product. During the manufacture of products for initial clinical trials, not all attributes tested may have specification ranges specified 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 initiation of phase 3 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) (67). Specifications for each parameter should be justified by process capability as well as by clinical suitability. If justified, after the manufacture of additional batches of product, the sponsor should commit to revise the specifications as data on the 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 (58) 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 vaccine.

If multi-dose vaccine vials are used and these vaccines do not contain preservative, their use should be time-restricted, as is the case for reconstituted vaccines such as Bacillus Calmette-Guerin (BCG) and measles-containing vaccines (68). In addition, the multi-dose container should prevent microbial contamination of the contents after opening.

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; 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 percentage of containers rejected.

A.5.2 Appearance

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

A.5.3 Identity
A.5.4 Sterility tests for bacteria and fungi
See 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 (69).

A.5.6 Purity
Testing for purity should be performed unless it is performed on the 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 prior to testing, using the diluent approved by the NRA.

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. Similarly, a suitably validated monocyte activation test may be considered as an alternative to the 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 A.3.4.1.2.
The potency specifications for live viral vectored vaccines should be set based on both the minimum dose used to demonstrate the efficacy in clinical trials and the maximum dose shown to be safe.

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 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.6 Records
The requirements given in section 17 of WHO good manufacturing practices for biological products (58) should apply.

A.7 Retained samples
The requirements given in section 16 of WHO good manufacturing practices for biological products (58) should apply.

A.8 Labelling
The requirements given in section 14 of WHO good manufacturing practices for biological products (58) should be taken into consideration.

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 (this information should be given for neonates, children, adolescents, older persons and immunosuppressed individuals, and should be the same for a given vaccine in all parts of the world);
• the amount of active substance contained in one recommended human dose;
• the number of doses, if the product is issued in a multiple-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;
• contraindications, warnings and precautions, concomitant vaccine use, adverse events.

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 (70).

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. The current guidance on evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (71). 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 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 for 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 when the manufacturer plans to change manufacturing, to aid in assessing comparability.

For vaccine licensure, the stability and expiry date of the vaccine in its final container, 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 (71). 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 under real-time conditions.

In an emergency situation and during early clinical trials, limited stability data on the 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 the 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 (19). Further guidance can be found in WHO and national and regional documents on DNA vaccines (17,18) and live-recombinant viral vector vaccines (6–8).

The nonclinical safety evaluation, whenever necessary, should yield sufficient information to conclude 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 on the minimum dataset required are also highlighted.

B.2 Product characterization and process development

It is critical that vaccine production processes are standardized and appropriately controlled to ensure consistency in manufacturing and the collection of nonclinical data that may suggest potential safety and efficacy in humans. The extent of process validation may vary with the stage of product development. The vaccine lots produced for nonclinical 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 for stability of the vector genome should be defined as far as is practical. Phenotypic markers are useful for 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 in section B.4 below.

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 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 should be recognized that conduct of 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. 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), 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 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 1 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 endpoints in subsequent clinical trials.
As normally expected in any challenge-protection animal study, the endpoints used to define protection should correlate with the desired effect in humans, which is typically a survival benefit or attenuation of severe disease reflected by viral shedding, body weight changes and other relevant clinical signs. Other key characteristics of the experimental design include use of appropriate challenge virus strains, dose(s) and route of challenge. Preferably, testing of different exposure doses may be considered instead of using the historical 1000 pfu dose alone. However, the studies with a 50% lethal dose may not be feasible because of technical concerns and costs.

The collection of challenge-protection data should take account of the proposed indication for use – i.e. pre-exposure versus post-exposure prophylaxis against Ebola. Appropriate timing of challenge should be an important consideration. For pre-exposure prophylaxis, animals are usually challenged at the time when the peak level of vaccine response (e.g. peak antibody titres) developed post-vaccination. It would also be informative for various strategies of public-health use of the vaccine to challenge the animals at other times (e.g. before peak or after the immune responses wane) when feasible. For post-exposure prophylaxis, challenge at various timepoints should be considered.

**Use of challenge-protection animal study to support licensure**

In some circumstances where demonstration of 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 (e.g. by inferring animal challenge results to humans). If this is pursued and agreed by the relevant NRA, the study should be adequately designed to generate reliable data to infer effectiveness to humans (see section C.1.2).

Specific considerations may include use of the most appropriate animal species (i.e. the non-human primates), vaccinating animals with an appropriate range of doses of the vaccine so that the level of immune response developed in animals (e.g. range of relevant antibody titres) can match that in humans. The compliance with good laboratory practices (GLP) is a big advantage and is encouraged; however, it is understood that compliance with GLPs 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.
Standardization of non-human primate challenge models (challenge dose, strain of animal, challenge material, including virus variant and passage number, challenge route, criteria for animal euthanasia, and standardized data collection and reporting, etc.) should be considered as an important need when different candidate Ebola vaccines are compared for the purpose of supporting licensure. Current thinking on this issue can be found in one recent publication (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 in both quantitative and qualitative relationship to the proposed use in human beings.

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

Either during public a health emergency or in a normal situation, immunogenicity data derived from a relevant species responsive to the vaccine antigen for desired immune responses are an expected minimum requirement prior to starting phase 1 clinical trials. Alternatively, strong supportive data generated from the same platform technology (e.g. the same vector and manufacturing process, but expressing different vaccine antigens) may be considered sufficient for phase 1 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 examination of phenotypic and functional characterization of CD8+ and CD4+ T cell responses using sensitive and highly specific assays such as ELISpot and intracellular cytokine staining (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+ 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, assessment of immunogenicity parameters in proof-of-concept challenge studies may allow for the establishment of any 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 during the development of the product.

B.4 Nonclinical safety studies (toxicity testing)

In a normal situation, safety assessment including repeat-dose toxicity and local tolerance studies is required for all new candidate vaccines. In general, these studies will have been completed and analysed prior to initiation of phase 1 clinical trials. Additional safety testing may be necessary depending on the property of the candidate vaccines. For a replicating recombinant vaccine vector with neurovirulent potential, neurovirulence testing in a relevant animal species is an important consideration and should be conducted before proceeding to humans.

During a public health emergency, data on acute toxicity, including the immediate effect on survival and vital physiological functions, and submission of draft, unaudited toxicity study reports may be sufficient to support proceeding to phase 1 clinical trials with novel platform/candidate vaccine. Omission of toxicity studies may be possible if there is adequate platform toxicology data and clinical safety experience (see B.1). For instance, for the viral vectored vaccines that this document focuses on, toxicity studies were not required during the 2014–2015 Ebola epidemic.

Such a limited dataset should be of good quality – i.e. 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 later on when they become available, including any delayed effect that is observed at later timepoints in repeat-dose toxicity studies, histopathological data, and the final signed audited reports. Early discussion with NRAs where the phase I clinical trials are to be conducted is encouraged.
Since the 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. Important consideration should be given to vaccine administration that enables the exposure of pregnant animals to a vaccine response in the early phase of implantation/organogenesis. For a replicating recombinant vaccine vector that may have a direct effect on the embryo/foetus, the dosing regimen should ensure a sufficient level of vaccine vector in the blood that is exposed to pregnant animals.

The requirement for a developmental toxicity study is an important issue for consideration, depending on the level of threats or control of the disease. During the 2014–2015 Ebola outbreak, large-scale phase 3 efficacy trials were approved in endemic countries without intentionally enrolling pregnant women. With decreasing numbers of cases as the 2014–2015 outbreak 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 virus has one of the following characteristics: it is novel in nature and there are no existing biodistribution data from the platform; there is a likelihood of altered infectivity and tissue tropism due to recombination; or 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 early in the development of this class of product.

The WHO Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated) (72) provide advice in this aspect which may 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 – i.e. unvaccinated humans or domestic animals following human administration. In case of replicative-deficient recombinant viral vector, no shedding experiment is required. For future candidate novel live recombinant vaccines based on a genetically modified organism, 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 of Ebola vaccines have to 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 common to all programmes should be: (i) the standards for demonstration of safety and effectiveness are the same as for other vaccines, and (ii) clinical studies are conducted in accordance with the principles described in WHO’s Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (73) and Guidelines on clinical evaluation of vaccines: regulatory expectations (21).

As for all vaccines, phase 1 and 2 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 3 studies. For instance, in the face of an outbreak, without available preventive vaccines, compressed timelines for clinical development may be achieved by initiating phase 3 studies on the basis of interim safety and immunogenicity data from earlier-phase studies rather than on data from final study
reports. Clinical development of an EVD 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.

For detailed considerations regarding approaches and design of studies to demonstrate vaccine effectiveness, see section C.1.2.

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 ensure study integrity. Meetings between sponsors and the relevant NRA at critical timepoints during clinical development, as well as to discuss scientific and medical questions that may arise at any time during the course of an investigation, should be encouraged.

C.1.1 Study population

Study population characteristics (e.g. demographics, location, underlying medical conditions, Ebola immune status) may vary by phase of clinical development and are discussed in detail in section C.2. Specific considerations for the evaluation of Ebola vaccines in the paediatric population are discussed in section C.5.2.

Inclusion and exclusion criteria should be defined for each study planned. Exclusion criteria may include previous receipt of an Ebola vaccine and possible previous exposure to wild-type EBOV. Consideration should be given to excluding subjects with immunodeficiency or immunosuppression, particularly for 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 persons who may have an increased risk of significant adverse reactions and persons whose underlying conditions may make it difficult to interpret the safety data. For example, an investigational recombinant VSV-vectored Ebola vaccine has been associated with arthritis. Thus, consideration should be given to excluding persons with arthritis or related conditions (active or past medical history) from participating in studies of this particular vaccine, taking into account participants' risk of contracting Ebola. Thus, considerations for exclusion would differ for studies of healthy volunteers with 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 in 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 1 study of healthy volunteers not at risk of EVD.

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 baseline health status. 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 be facilitated by knowledge of background rates of events in the relevant general population.

C.1.2 Demonstration of effectiveness of Ebola vaccine candidates

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 (i.e. protection induced by vaccination in the vaccinated population sample). The best estimates of vaccine efficacy come from randomized controlled clinical trials. However, estimates of vaccine efficacy from observational studies that are carefully designed to minimize bias may also provide valuable estimates of vaccine efficacy.

Vaccine effectiveness is an estimate of the protection conferred by vaccination in a specified population. It may measure both direct and indirect protection (e.g. the estimate may reflect in part protection of nonvaccinated persons 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. Vaccine effectiveness may also be inferred from a vaccine-induced immune response (e.g. pre-specified antibody threshold induced by the vaccine in vaccinated persons) or from animal efficacy data.
For any preventive vaccine, the most direct approach to demonstrate effectiveness is based on clinical endpoint efficacy trials showing protection against disease, or alternatively, based on clinical trials evaluating a scientifically well-established immune correlate of protection (ICP) (e.g. antibody response data). For diseases like EVD, for which there is not a well-established correlation of protection, if disease incidence is too low to feasibly conduct clinical endpoint efficacy studies, evidence for effectiveness may be derived from animal efficacy data and/or clinical immunogenicity data. Central to approaches based on animal efficacy data or immunogenicity clinical endpoints is that the endpoints are likely to predict clinical benefit. In the case of EVD, protection of humans against EVD would be the predicted clinical benefit.

The incidence of EVD will be a primary determinant of the approach to evaluate vaccine effectiveness and the design of phase 3 studies. Direct assessment of vaccine efficacy in randomized controlled trials requires a sufficiently high disease incidence. In settings with relatively low disease incidence, vaccine efficacy study designs – such as ring vaccination in which persons at highest risk of infection are recruited – may be considered in order to maximize statistical power (74). Critical aspects of clinical endpoint efficacy trials, including ring vaccination trials, include: an appropriate control group; appropriate methods for randomization; masking procedures; a pre-specified primary endpoint (e.g. EVD confirmed by PCR); pre-specified important secondary endpoints (e.g. EVD not laboratory confirmed); pre-specified, detailed clinical case definitions for the primary endpoint; validated diagnostic assays to support the pivotal efficacy analyses; unbiased case ascertainment methods; and adherence to relevant statistical principles. Specific considerations regarding the design of clinical endpoint efficacy trials and diagnostic tests for EVD are discussed in sections C.2.3.1 and C.4.1, respectively.

Consideration should be given to the establishment of an independent data monitoring committee for clinical endpoint efficacy trials of Ebola vaccines in order to advise the sponsor regarding the continuing validity and scientific merit of the trial.

Clinical endpoint efficacy trials provide an opportunity to identify a serological correlate of protection. Derivation of a serological correlate of protection is facilitated by the availability of serum samples from a relatively large number of protected trial participants as well as vaccinated
participants who develop disease. Thus, for all Ebola vaccine clinical disease endpoint efficacy trials, pre- and post-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 (75-80). Consideration should also be given to 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 endpoint efficacy trial, immunogenicity data from phase 2 and phase 3 studies are critical to alternative approaches to demonstrate vaccine effectiveness. As ICP, including potential antibody thresholds associated with protection, 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 (81).

Potentially important immunogenicity endpoints include Ebola virus IgG ELISA antibody titre and presence/levels of EBOV neutralizing antibody. Endpoints evaluating T-cell mediated responses following vaccination may also be considered. Specific considerations regarding immunological assays, including assay validation, are discussed in section C.4.

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 persons initially seropositive. Seroconversion is typically based on achieving a measurable antibody level post-vaccination in persons who were initially seronegative. A detailed justification for the definition should be provided, including reference to serological data from non-human primate challenge studies. The definition of seroresponse may differ across Ebola vaccines and assays. Serological endpoints and evaluation criteria should be determined.
following input from, and agreement by, the NRA before study unblinding and serological analysis.

In the absence of an accepted, scientifically-established ICP, the ability to approve a vaccine on the basis of human immune response data and/or efficacy data from animal studies will depend on the specific regulatory frameworks of the relevant NRA. Specific regulatory requirements associated with such provisions (e.g. criteria for animal models, post-licensure studies to verify clinical benefit, requirements for pre-licensure safety and immunogenicity studies in humans) must be adhered to.

Considerations regarding evaluation of durability of vaccine-induced protection are discussed in section C.2.3.2.

C.1.3 Safety evaluation of Ebola vaccine candidates

Sponsors must comply with the adverse event reporting requirements of the relevant NRA and the independent ethics committee(s). Templates of forms used to monitor and document adverse events should be provided with each protocol. Sponsors are encouraged to initiate an 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 in determining the size of the safety database.

While the exact definition of a serious adverse event (SAE) may vary somewhat across different NRAs, the International Conference on Harmonisation E2A Guideline defines a serious adverse event 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 (82). WHO considers an adverse event following immunization (AEFI) as “serious” if it meets any of these criteria or if it requires intervention to prevent permanent impairment or damage (83).
Monitoring serious adverse events

All participants in pre-licensure clinical trials of Ebola vaccines should be closely and actively monitored (e.g. 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 also should be incorporated. A longer-term safety follow-up period (e.g. through 12 months following the last vaccination) for assessment of SAEs may be warranted for some vaccines (e.g. vaccines containing novel adjuvants). Long-term safety follow-up (i.e. through 6–12 months post-vaccination) may be accomplished by telephone follow-up or other methods appropriate for the setting.

Monitoring adverse events of special interest

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

Monitoring adverse events

All study participants also should 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 all unsolicited adverse events may be monitored for relatively short periods post-vaccination (e.g. 21 days, or 42 days for live replicating viral vaccines), monitoring for new-onset chronic medical conditions for a longer period (e.g. 6–12 months) may be useful for detection of unexpected safety signals.

In phase 1 and 2 studies, all participants should be monitored for pre-specified, solicited local and systemic adverse reactions at specified timepoints, for a specified period following vaccination (e.g. daily for at least 7 days; longer if warranted based on vaccine characteristics and available preclinical and clinical data). In phase 3 studies, it may be acceptable to monitor actively only a subset of participants (e.g. several hundred per group) for common, non-serious local and systemic adverse reactions.
Specific safety monitoring methods should be tailored to the specific study population (e.g., children, adults, pregnant women, persons in areas where EVD is endemic) and with consideration given to adverse events known to be associated with a particular vaccine (e.g., fever, arthralgia, and arthritis have been observed in some Ebola vaccine studies). Study protocols should specify methods for monitoring and documenting adverse events, including: use of standardized subject diaries and case report forms; procedures to inquire about adverse events at study visits; severity grading scales; definitions for adverse event categories (e.g., serious, new-onset chronic medical condition; AESI); and requirements for prompt reporting of 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 hematologic 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/stoping rules for SAEs overall. Particularly for any trials involving children and large-scale, later-phase trials, consideration should be given to the establishment of an independent data monitoring committee to advise the sponsor regarding the continuing safety of trial participants and those to be recruited into the trial.

Other aspects of safety that should be addressed in the protocol include assessment of shedding and the potential for secondary transmission for replicating or potentially replicating competent live virus-vectored vaccines, at least in early-phase studies, as well as procedures to minimize the risks of Ebola virus transmission to study personnel involved in clinical endpoint efficacy studies.

C.1.4 Ethical considerations
Compliance with good clinical practice standards (21,74) 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 this committee’s approval 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. In some countries, the informed consent process may need to be more specifically tailored to take into account 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 (e.g. 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.5.2 for considerations regarding initiation of clinical studies in the paediatric population.

C.2 Phases of clinical development and trial design

The phases of vaccine clinical development are typically a continuum from phase 1 trials, which are often the first-in-human clinical trials carried out primarily to assess safety and preliminary immunogenicity, to phase 2 to further describe safety and dose relationship to immunogenicity, and then to phase 3 with pivotal studies to demonstrate the safety and effectiveness of a product in support of licensure.

In the setting of an outbreak, the evaluation of investigational Ebola vaccines should adhere to the principles of this phased approach although the intervals between phases of evaluation may be compressed and overlapping. Adaptive elements can be pre-specified in the phase 2 and phase 3 designs to expedite trial decisions based on interim safety and effectiveness data.

C.2.1 Phase 1 studies
The primary purpose of phase 1 vaccine studies is to obtain preliminary safety and immunogenicity data. For Ebola vaccines, these studies generally would be first conducted in a small number (e.g. < 100) of healthy adult volunteers previously unexposed and at low risk of EVD.

However, in the face of an outbreak, NRAs may consider larger phase 1 clinical studies to increase the early safety and immunogenicity database, as well as study populations similar to the eventual target population, facilitating timely initiation of phase 2 clinical studies.

The design of phase 1 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 dose-escalation by a sequential design whereby subjects enrolled in lower-dose cohorts are closely monitored for safety for a defined period of time (e.g. 1–2 weeks or as appropriate for the characteristics of the vaccine) and these data are reviewed before subsequent enrolment of subjects in successively higher dose cohorts. All study participants should be actively and closely monitored for safety.

C.2.2 Phase 2 studies

Phase 2 studies are initiated once satisfactory safety and immunogenicity data from phase 1 studies are available. In the absence of safety concerns from short-term (e.g. 7 days) post-vaccination follow-up in phase 1 studies, in some cases development may proceed to phase 2 studies in parallel with continued collection of long-term safety data from phase 1 studies. Phase 2 studies provide further information on safety and immunogenicity in order to determine the optimal dose and dosing regimen, and to support initiation of phase 3 studies. Phase 2 studies typically involve up to several hundred subjects and are frequently randomized and controlled. The comparator may be an inert placebo or a control vaccine that provides protection against disease unrelated to EVD. Phase 2 trials are often of sufficient size to test hypotheses on dose and dosing regimen. Phase 2 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. Detailed safety and immunogenicity data should be obtained in phase 2 studies.
C.2.3 Phase 3 studies

Large-scale phase 3 studies of Ebola vaccines have been designed to provide data on vaccine efficacy and safety and possibly define an ICP. Ideally, phase 3 studies are double-blind, randomized and controlled. The target population for phase 3 clinical trials with the Ebola vaccine candidates should consist of individuals at high risk for the disease (i.e. populations residing in EVD outbreak areas, relevant health-care providers, or first responders). To demonstrate vaccine effectiveness, phase 3 trials may be based on a disease endpoint or, as described in other sections, they may be based on attainment of a level of an immune marker predictive of protection.

For disease endpoint clinical efficacy studies, large sample sizes may be needed, particularly if the incidence rate 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, trial endpoints and criteria for trial success should be pre-specified prior to initiation of the study. 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.

An appropriate control is required to demonstrate the efficacy of Ebola vaccine candidates and may include an inert placebo such as saline injection or a vaccine that provides protection against another disease. Large-scale phase 3 clinical trials provide an opportunity for expanded safety and immunogenicity assessments and may permit clinical evaluations of lot-to-lot manufacturing consistency.

C.2.3.1 Trial designs

The design of an efficacy study must be of adequate scientific rigour to support efficacy claims, as well as being consistent with ethical standards. In some settings, this balance may preclude the use of a placebo group. Nevertheless, clear and definite evidence that the vaccine is safe and effective is required for regulatory decision-making. There should be discussion with relevant NRAs on the design, plan for conduct, and analysis at the early conceptual stage of the phase 3 study, and agreement with the NRA prior to trial initiation.
The prospective randomized, double-blind, placebo-controlled trial with an EVD endpoint 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 endpoints 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.

Ethical considerations for the use of placebos in vaccine research, including 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* (84). Other study designs for obtaining efficacy data for Ebola vaccine candidates may be considered if a placebo-controlled trial is not considered ethical or is not feasible. Close consultation with local community leaders and health policy-makers in the EVD outbreak regions where the efficacy study is planned is critical. Alternate study designs such as a stepped wedge randomized cluster trial (SWRCT) may be considered, especially if available vaccine is limited at the time of trial initiation and use of a placebo group is considered unacceptable. In this design, all participants start in the control group and, at predefined timepoints, a cluster of participants is vaccinated in a random order (known as “steps”). Vaccine efficacy is calculated on the basis of the relative rates of disease in the vaccinated population compared to the rate in the unvaccinated. The disadvantages of SWRCT include difficulty in blinding, attrition in the later vaccinated clusters, and the more complex analysis.

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 (75). The approach to increase statistical power is to recruit those at highest risk of infection (e.g. persons socially or geographically connected to an index case). An efficacy trial using ring vaccination with an investigational Ebola vaccine was conducted in Guinea in 2015 (53). Other study designs may be acceptable and should be discussed with the respective NRA.

C.2.3.2 Duration of immune response and protection and need for booster vaccinations
The long duration of the Ebola outbreak during 2014–2015 and the potential for future exposures highlight the need to consider durability of vaccine-induced protection and the potential need for booster doses in the evaluation of the effectiveness of Ebola vaccines. This evaluation could be facilitated by the identification of an ICP. Importantly, phase 2 and 3 clinical trials should attempt to identify ICPs and should evaluate the kinetics of the immune response and induction of immunological memory. It may be necessary after completion of efficacy trials to follow subjects into the post-licensure period or to conduct post-marketing surveillance studies to collect data on long-term protection and the need for, and timing of, booster immunization.

C.3 Statistical considerations

C.3.1 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 (85). Phase 1 studies generally are exploratory and may lack statistical power for hypothesis-testing.

Phase 2 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 3 studies are designed to provide the most compelling data on vaccine effectiveness and more extensive data on safety. The study protocols should describe clearly the procedures for randomization and blinding, primary and secondary objectives, endpoints to be analysed, null and alternative hypotheses to be tested, level of type I error, sample size calculations, statistical methods for assessing each endpoint, 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.3.2 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 endpoint. 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, as well as on the level of vaccine efficacy considered to be clinically relevant.

Rapidly changing and/or declining incidence rates during an outbreak may need to be considered when choosing a study design. In some circumstances, other 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 different groups of clusters. As mentioned in section C.2.3.1, a ring vaccination trial design may have higher statistical power to detect vaccine efficacy than other study designs.

When ICPs established in animal challenge studies are being used to define immune response endpoints for effectiveness evaluation or to infer vaccine effectiveness under other alternative licensure pathways, these studies (e.g. 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.3.3 Statistical considerations for evaluating vaccine safety

Safety evaluation is inherently exploratory, typically using 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 a few 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 (e.g. cases of post-vaccination reactive arthritis in clinical studies with certain viral vector vaccines), prospective monitoring for related events as well as formal statistical testing should be considered.

C.4 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, antigen can be detected by immunoassay and virus by either viral nucleic acid test (often called a nucleic acid amplification test, or NAT) or culture. For both antigen and nucleic acid-based tests, 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 therefore this is not routinely performed.

C.4.1 Diagnostic tests

All currently available EBOV NATs work is 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 NATs 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 Ebola virus gene targeted by the NAT is also expressed by the vaccine, a positive NAT 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 Ebola virus 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
for 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 (section A1.1), which should now enable assessment of assay performance and comparison of results across different assay platforms.

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

C.4.2 Immunological tests

An ICP against EVD has not been established. Nevertheless, myriad immunological tests have been developed. Of these, the Ebola virus IgG ELISA has gained the greatest acceptance based mostly on studies from 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 (e.g. data from a successful vaccine efficacy trial), an ICP may have to be established in a validated animal model. On the basis of data available to date, non-human primates appear to be an acceptable animal model for such an exercise; there is 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 the EBOV glycoprotein, the protein expressed by most investigational vaccines. Concerns with these tests are numerous and care should be
taken with interpretation of the data they produce. ELISA plates coated with lysates of cells expressing non-Ebola virus antigen that are 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 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 use of pseudovirions, such as VSV whose GP gene has been replaced with that of Ebola virus, or use of lentivirus packaging systems. Whether virus neutralizing activity detected in these in vitro assays is predictive of Ebola virus neutralizing activity in vivo remains to be demonstrated, as does the role of Ebola virus neutralizing antibodies in EVD and protection. The same precaution concerning false positivity through use of ELISA plates coated with non-Ebola virus vaccine components applies here too. For instance, non-Ebola virus antibodies generated in response to receipt of a VSV-based 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 adenovirus 5-based Ebola virus vaccine in non-human primates, depletion of CD8+ T cells in vaccinated animals before challenge abrogated protection; nevertheless, antibody levels did correlate with the efficacy of the vaccine (86). Several different types of tests for cell-mediated immunity have been developed, including the ELISpot and intracellular cytokine staining tests. These tests present additional challenges, including determination of appropriate peptide pools to be used, and logistical and safety issues concerning the collection and storage of peripheral blood mononuclear cells (PBMCs), as well as assay validation.

In general, there are few published data on the performance of assays to detect immunological responses to Ebola virus infection or Ebola vaccines. An International Reference Plasma
Standard (see A1.1.) is now available and should be used to standardize assay performance and to improve comparison of results across vaccines, across studies, and across different assay platforms.

C.5 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.5.1 Pregnant women

Evidence from the 2014–2015 Ebola outbreak suggests that EVD is associated with high rates of maternal and neonatal mortality (87). Use of Ebola vaccine in pregnant women may have potential benefits in: (i) preventing EVD in the mother and reducing maternal morbidity, (ii) preventing EVD in the early neonatal period, and (iii) limiting the spread of EVD from pregnant women during labour and delivery to health-care workers in an outbreak setting (88).

The following describes a few concepts that 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 (21). 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 Part B). In addition, supportive safety data from completed phase 1 and phase 2 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 address available data from non-pregnant adults and nonclinical studies. A reasonable effort should be made to calculate gestational age accurately 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, as a cautious approach, consideration should be given 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 pregnancy-related complications (such as new-onset gestational diabetes, 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.5.2 Paediatric populations

A paediatric clinical development plan for a vaccine to protect against EVD should be considered early (prior to phase 3) 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 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 about 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 (e.g. 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.5.3 Immunocompromised persons and persons 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, prompting unique considerations with respect to the clinical development programmes for Ebola vaccines. Information on underlying medical conditions that may have an impact on the safety and effectiveness evaluation of a vaccine should be collected for participants in clinical trials.

The safety evaluation of investigational vaccines in immunocompromised persons should include assessment of exacerbation of the underlying disease post-vaccination. As an example, plasma HIV viral load has been shown in a few studies, but not in others, to increase transiently following vaccination with influenza and pneumococcal vaccines (although without established clinical consequence). Product-specific considerations may preclude the use of some vaccines in certain populations due to unacceptable risks (e.g. risk of disseminated disease following immunization of HIV-infected persons 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.6 Post-marketing surveillance

As part of the preparation 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 by cooperative efforts that could also include participation by regulators, WHO and other institutions.

According to the International Conference on Harmonisation, a pharmacovigilance plan should be prepared for any new vaccine (89). A first step towards preparation of the pharmacovigilance plan is the “safety specification”, a summary of (i) the important identified and potential risks of the vaccine, and (ii) the important missing information. The safety specification should also address populations that are potentially at risk (populations in which the vaccine will most likely be used) and 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 (89). 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 include additional actions designed to address these concerns (89).

The strategies proposed for the identification and investigation of vaccine safety signals should be specified in the pharmacovigilance plan. They may depend, in part, on decisions made regarding 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: (i) well-defined, relatively small groups (e.g. first responders, health-care workers, specific high-risk groups, including close contacts of suspected cases), (ii) large
demographic groups (e.g. all individuals in a certain age range, inhabitants of a specific geographical region), or (iii) the overall population of a country or region.

Ideally, the 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 (90, 91). Currently, no effective post-marketing surveillance systems with clear protocols, tools and a mandate exist in countries affected by the 2014–2015 Ebola epidemics. Thus, enhanced capacity for vaccine pharmacovigilance may be needed, in accordance with WHO recommendations (22). WHO’s Global Vaccine Safety 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 Global Vaccine Safety Blueprint 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” (22).

WHO is currently preparing a detailed guidance document for Ebola virus vaccines safety monitoring that should provide information regarding pharmacovigilance activities following introduction of Ebola vaccines (92). WHO’s Global Advisory Committee on Vaccine Safety (GACVS) has reviewed safety data from phase 1 studies of two investigational Ebola vaccines (93). The adverse event profiles from these studies provide useful information for planning the safety evaluation 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 future guidance from WHO. Pharmacovigilance plans for the introduction of Ebola vaccines should be aligned with guidance provided by these documents.

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

Part D. Guidelines for NRAs

D.1 General

The general recommendations for control laboratories given in WHO’s Guidelines for national authorities on quality assurance for biological products (94) and Guidelines for independent lot release of vaccines by regulatory authorities (95) 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 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 (95).

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 certificate should provide information on the date and location of manufacture of the lot, as well as on its safety, identity, quality, potency and purity. The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries, and it should be provided to importers of the vaccines.
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<table>
<thead>
<tr>
<th>Product and developer (alphabetically by company name)</th>
<th>Active substance</th>
<th>Production cell line</th>
<th>Viral replication status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad26 ZEBOV, Crucell</td>
<td>Recombinant replication-deficient human adenovirus Type 26 (Ad26) vectors expressing wild-type Ebola</td>
<td>Recombinant human embryonic retinal cells PER.C6</td>
<td>Replication-deficient in human cells due to deletion of several genetic elements. The missing genetic elements are supplied by the</td>
</tr>
<tr>
<td>Vaccine</td>
<td>Expression</td>
<td>Production Cell Line</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>MVA-BN-Filo, Crucell Bavarian Nordic</td>
<td>Modified Vaccinia Ankara (MVA) – Bavarian Nordic (BN) vaccine, (MVA-BN-Filo), expressing the SUDV GP, the EBOV GP, the MARV Musoke GP, and the Tai Forest virus (TAFV) nucleoprotein (NP)</td>
<td>Chick embryo fibroblasts (CEF)</td>
<td>Live attenuated virus. Attenuation due to successive passaging in avian cells resulting in a loss of ~10% of the genome. Replication-proficient in specific cell lines (usually avian). Does not replicate in most human cell lines/cells.</td>
</tr>
<tr>
<td>ChAd3-EBO-Z, GSK</td>
<td>Recombinant replication-deficient chimpanzee adenovirus Type 3 (ChAd3) vectors expressing wild-type Ebola virus GP from Zaire strain</td>
<td>Recombinant human Procell-92.S</td>
<td>Replication-deficient in human cells due to deletion of several genetic elements. The missing genetic elements are supplied by the production cell line.</td>
</tr>
<tr>
<td>rVSV-ZEBOV, Merck NewLink</td>
<td>Recombinant replication-competent, attenuated vesicular stomatitis virus (rVSV) vector expressing wild-type Ebola virus GP from Zaire strain</td>
<td>Vero</td>
<td>Replication competent virus. Attenuated due to addition of recombinant heterologous gene.</td>
</tr>
<tr>
<td>Ad5-EBOV, Military Academy of Medical Sciences; CanSino</td>
<td>GP antigen from Ebola Zaire strain Guinea 2014 expressed in a replicate-defective human Ad5 viral vector, lyophilized, single dose</td>
<td>Human embryonic kidney (HEK) 293SF-3F6</td>
<td>Replication deficient in human cells due to deletion of several genetic elements. The missing genetic elements are supplied by the production cell line.</td>
</tr>
<tr>
<td>Biotech. China</td>
<td>EBOV GP, Novavax</td>
<td>Ebola GP recombinant nanoparticle with/without adjuvant (Matrix-M)</td>
<td>Baculovirus expression system in Sf9 cells</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------------------------------</td>
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</tr>
</tbody>
</table>
Appendix 2. Model summary protocol for manufacturing and control of viral vectored Ebola vaccine

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, the final 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. 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 the guidelines of this document published by WHO.

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:
   - Ebola virus strain

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1 This applies after the vaccine product has been granted a Marketing Authorization.
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

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 pre-seed or virus master seed to its structure or 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)
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 applicable)

- 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

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

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/mycoplasma
Virus titre

3.3 Final viral vector bulk

3.3.1 Information on manufacture

Batch number(s):
Date of formulation:
Total volume of final bulk formulated:
Virus pools used for formulation:
Lot number/volume added:
Virus concentration:
Name and concentration of added substances
(e.g. 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

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
   Appearance
   Identity
   pH and osmolality
   Potency
   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)

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 A1 of the WHO Guidelines on the quality, safety and efficacy of Ebola vaccines (if applicable).
   Name (typed)
   Signature
   Date

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

Appendix 3. Model certificate for the release of viral vectored Ebola vaccine by NRAs

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

Lot release certificate
Certificate no.___________________

The following lot(s) of Ebola vaccine produced by __________________________ in____________________ 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 and Part A of the WHO Guidelines on the quality, safety and efficacy of Ebola vaccines and comply with WHO good manufacturing practices for pharmaceutical products: main principles, WHO good manufacturing practices for biological products, and Guidelines for independent lot release of vaccines by regulatory authorities.

The release decision is based on __________________________________________

The certificate may include the following information:

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

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

3. Name (typed) ____________________________
4. Signature ________________________________
5. Date _________________________________

1 Name of manufacturer.
2 Country of origin.
3 If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has
   nevertheless been authorized by the NRA.
4 With the exception of provisions on distribution and shipping, which the NRA may not be in a position to
   assess.
5 WHO Technical Report Series, No. XXX, Annex X.
9 Evaluation of product specific summary protocol, independent laboratory testing, and/or specific procedures laid
   down in defined document, etc., as appropriate.

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