Annex 2

Guidelines on the quality, safety and efficacy of plasmid DNA vaccines

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

Abbreviations

DCVMN	Developing Countries Vaccine Manufacturers Network
DNA	deoxyribonucleic acid
GCP	good clinical practice
GMO	genetically modified organism
GMP	good manufacturing practice(s)
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HPV	human papillomavirus
IFPMA	International Federation of Pharmaceutical Manufacturers & Associations
INN	international nonproprietary name
IS	international standard(s)
IU	International Unit(s)
LVV	lentiviral vector
MCB	master cell bank
mRNA	messenger RNA
NCL	national control laboratory
NRA	national regulatory authority
PCR	polymerase chain reaction
R&D Blueprint	WHO Blueprint for Research and Development: Responding to Public Health Emergencies of International Concern
rDNA	recombinant DNA
RNA	ribonucleic acid
SARS	severe acute respiratory syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
WCB	working cell bank
WNV	West Nile virus

Introduction

Vaccination involves stimulating the immune system of an individual with an infectious agent, or component(s) of an infectious agent, that has been modified in such a way as to ensure that the vaccine does not cause disease or undue harm to the recipient. Efficacious vaccination ensures that when the individual is confronted with that particular infectious agent, their immune system can respond adequately to control it before it causes overt disease. For more than a hundred years, vaccination has been achieved by one of two basic approaches:

- administering attenuated microorganisms that replicate within the vaccine recipient without causing disease and synthesize the appropriate immunogens that subsequently stimulate the immune system; or
- administering pathogen-specific antigens against which the immune system will react directly.

Since the 1990s, a novel third approach to vaccination against a broad array of target antigens and diseases has been in development. This technology involves the direct administration of plasmid deoxyribonucleic acid (DNA) containing the gene encoding the immunogen against which an immune response is sought, leading to the in-situ production of the target immunogen(s) in the vaccine recipient. Such vaccines are referred to as "plasmid DNA vaccines" or simply "DNA vaccines" (with both terms used interchangeably throughout these Guidelines). This approach offers a combination of potential advantages, including the stimulation of both B-cell and T-cell responses, stability of the vaccine across a broad temperature range, absence of infectivity of the immunogen itself, the speed with which the vaccine can be constructed (for example, in the face of an epidemic or pandemic) and the relative ease and generic nature of large-scale manufacture. It may be feasible to produce the same DNA vaccine in different facilities in different countries to facilitate accessibility and availability of the vaccine during routine immunization or in outbreak settings, thus ensuring a more stable supply of vaccine. Furthermore, DNA vaccines can be more stable than other more common vaccine types and may therefore, depending on their formulation, be stored and efficiently delivered in the absence of a cold chain. DNA vaccines do not generate anti-vector immunity or off-target acquired immunity to DNA in the vaccine recipient. DNA vaccines are not designed to be infectious and the target infectious pathogen is not used in their construction or production. However, the manufacturing of such vaccines in bacteria may require appropriate biosafety containment in accordance with local regulations. Although chromosomal integration of the plasmid DNA was initially a major theoretical concern, the data obtained to date have not

borne out this concern. In summary, DNA vaccines can be viewed as a platform technology in which the gene insert can be readily changed without necessarily having to change the manufacturing or control of the resulting new product (with the exception of the immunogen-specific tests for identity and potency). Numerous scientific publications have addressed the potential benefits of DNA vaccination (*1–10*).

Immune responses in animal models have been obtained using genes from a variety of infectious agents including influenza virus, hepatitis B virus, human immunodeficiency virus (HIV), human papillomavirus (HPV), Marburg virus, Middle East respiratory syndrome coronavirus (MERS-CoV), rabies virus, Severe Acute Respiratory Syndrome (SARS) virus, SARS-coronavirus-2 (SARS-CoV-2), West Nile virus (WNV), Zika virus, plasmodia, mycoplasmas and others (*10–12*). In many cases, protection from disease in animal models has also been demonstrated. In addition to infectious diseases, plasmid DNAs have also been studied in clinical trials for the treatment of cancer, as well as autoimmune and allergic diseases such as peanut allergy (*13–19*). The development of plasmid DNA therapies for HPV infection is currently the subject of clinical investigations in humans and provides another example of the potential applications of this technology. The value and advantages of plasmid DNA products need to be assessed on a case-by-case basis; their utility will depend upon: (a) the nature of the organism being vaccinated against or the targeted disease; (b) the nature of the immunogen or activity of the gene insert; (c) the type of immune response required for effectiveness; and (d) the delivery system and route of administration.

The development and application of DNA vaccines continues to progress. Since the WHO Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines were adopted by the Expert Committee for Biological Standardization in 2005 (*20*), many clinical trials of DNA vaccines have taken place and considerable experience in their manufacture and control has accrued. The current revision reflects this experience, especially in relation to data derived from nonclinical and clinical safety testing, which address many of the concerns expressed in previous versions of these Guidelines. The control of DNA vaccines should continue to be approached in a flexible manner to enable further modifications as more experience is gained in their production and use, and as other components or delivery systems are included. The intention of the current document is to provide a scientifically sound basis for the consistent manufacture and control of DNA vaccines for human use to ensure their continued safety and efficacy following licensure. Given the potential of DNA vaccination as a platform technology for addressing priority pathogens during public health emergencies (*21–26*), international regulatory convergence for DNA vaccines is needed. This document provides up-to-date guiding principles

for evaluating the quality, safety and efficacy of DNA vaccines for human use. It is worth noting that while plasmid DNAs are generated using recombinant DNA (rDNA) technology, existing guidelines specific to rDNA products generally do not apply to DNA vaccines, as such guidelines are intended to cover the manufacture of biotherapeutic proteins generated in cell lines.

Purpose and scope

These revised WHO Guidelines focus on the quality control of vaccines based on biologically manufactured bacterial plasmid DNA intended for use in humans. Nonclinical and clinical aspects are also briefly described. As the general principles that apply to other vaccines also apply to DNA vaccines, only notable differences or additions are discussed below. The purpose of this document is to provide guidance on:

- appropriate methods for the control of the manufacture and characterization of plasmid DNA vaccines;
- appropriate approaches to the nonclinical and clinical testing of plasmid DNA vaccines; and
- information specific to plasmid DNA vaccines that may be expected to be included in submissions by manufacturers to national regulatory authorities (NRAs) in support of applications for the authorization of clinical trials and for marketing authorization/licensure.

The main changes made to the previously published WHO Guidelines (*20*) include:

- updating the Introduction with additional data, including citations for nonclinical and clinical data that collectively address many historical safety concerns;
- restricting the scope to preventive DNA vaccines against infectious diseases;
- updating the quality section (Part A) to make it more consistent with current practices and with other WHO guidelines;
- extensively revising the nonclinical section (Part B) to include references to general WHO guidelines adopted since the previous version and to better focus on a number of specific issues;
- adding a clinical section (Part C) that also includes references to recently revised general WHO guidelines and that also focuses on a number of specific issues;

- adding a section on specific guidance to NRAs (Part D); and
- adding a model summary protocol for the manufacturing and control of plasmid DNA vaccines (Appendix 1) and a model NRA/NCL Lot Release Certificate for plasmid DNA vaccines (Appendix 2).

These WHO Guidelines thus provide guidance on the quality, nonclinical and clinical aspects of DNA vaccines (including plasmids encoding adjuvant molecules, if present) intended to prevent infectious diseases in humans. Plasmid DNA vaccines intended for veterinary use fall outside the scope of this document.

The active constituent of a DNA vaccine is a DNA plasmid (or plasmids) into which the gene(s) encoding the desired immunogen(s) is inserted and prepared in purified plasmid preparations to be administered in vivo. Typically, these plasmids possess DNA sequences necessary for selection and replication in bacteria. In addition, they contain eukaryotic promoters and enhancers as well as transcription termination/polyadenylation sequences to effect gene expression in vaccine recipients, and may also contain or encode immunomodulatory elements.

In these Guidelines, vaccines are defined as biological medicines for the prevention of infectious diseases. As a result, plasmid DNA products developed for therapeutic use against diseases such as cancer (where the plasmid may encode a viral or tumour antigen, as well as immunomodulatory proteins), autoimmune or allergic diseases are not within the scope of these Guidelines. However, the manufacture and quality control of plasmid DNA for these indications may be essentially identical. Consequently, the section on quality (Part A) of these Guidelines may also be applicable to DNA plasmid products intended for therapeutic use. Likewise, although the use of plasmid DNA to express monoclonal antibodies for preventive post-exposure prophylaxis or for therapeutic purposes is outside the scope of these Guidelines, Part A may be applicable. The detailed design of relevant nonclinical and clinical testing should consider the proposed use of the DNA plasmid(s) and the risk–benefit situation. Plasmid DNA for use in gene therapy, plasmid DNA derived in eukaryotic cells, viral replicons, bacterial cells acting as carriers for a plasmid DNA encoding a relevant antigen, and nucleic acid vaccines made entirely by chemical means are all outside the scope of these Guidelines.

The current document is also unlikely to be applicable to vaccines based on ribonucleic acid (RNA) as different requirements are likely to apply to the quality, nonclinical and clinical testing of this type of vaccine or immunotherapeutic.

The guidance provided in these Guidelines will be relevant to the DNA vaccine at the time of application for marketing authorization. Nevertheless,

some relevant information is provided regarding candidate vaccine products in development; in any case, the respective NRA should be consulted prior to clinical development on a case-by-case basis (*27–29*).

It is recognized that products that blur the current distinctions made between viral vectors, cell therapy and nucleic acid vaccines are likely to emerge (for example, RNA replicons). Other developments that will likely complicate the regulatory evaluation of nucleic acid vaccines are also foreseen (for example, self-amplifying molecules). However, at the present time, such developments remain outside the scope of these Guidelines.

Terminology

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

Adjuvants: substances that are intended to enhance relevant immune response and subsequent clinical efficacy of the vaccine (*28*).

Bulk purified plasmid (bulk): the purified plasmid before final formulation. It is obtained from one or more harvests, kept in one or more containers designated as a single homogeneous production batch and used in the preparation of the final dosage form (final formulated vaccine).

Candidate vaccine: a vaccine under investigation and not yet licensed, and regarded in national regulations as separate and distinct from other candidate and licensed vaccines (*29*).

Cell bank: a collection of vials of cells of uniform composition derived from a single bacterial cell transformed by the plasmid encoding the desired immunogen and used for the production of a vaccine directly or via a cell bank system. Related terms used in these Guidelines are **master cell bank** and **working cell bank**.

DNA vaccine (or plasmid DNA vaccine): a vaccine in which the active constituent is a DNA plasmid (or plasmids) into which the gene(s) encoding the desired immunogen(s) is inserted and prepared in purified plasmid preparations to be administered in vivo. Typically, these plasmids possess DNA sequences necessary for selection and replication in bacteria. In addition, they contain eukaryotic promoters and enhancers as well as transcription termination/polyadenylation sequences to effect gene expression in vaccine recipients; they may also contain or encode immunomodulatory elements.

Final lot: a collection of sealed final containers that is homogeneous with respect to the composition of the product and the avoidance of contamination during filling. A final lot must therefore have been filled from a formulated bulk in one continuous working session.

Final product: a finished dosage form (for example, suspension or lyophilized cake) that contains an active ingredient, generally but not necessarily in association with inactive ingredients (excipients) or adjuvants. Also referred to as "finished product" or "drug product" in other documents.

F**ormulated bulk**: an intermediate in the drug product manufacturing process, consisting of the final formulation of antigens, adjuvants and excipients at the concentrations to be filled into primary containers.

Good manufacturing practice (GMP): a system that ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

Heterologous prime-boost: DNA vaccines have often been investigated in combination with another vaccine type (such as a viral-vectored vaccine or a protein subunit vaccine) in a regimen in which one vaccine is given in a priming dose series and the other vaccine (or a combination of the two vaccines) is administered as a booster.

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

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

Master cell bank (MCB): a bank of a cell substrate from which all subsequent **cell banks** used for vaccine production will be derived; the MCB represents a well-characterized collection of cells derived from a single cell.

Plasmid: a circular extrachromosomal bacterial DNA element that undergoes autonomous replication in bacterial cells. It usually carries a number of genes, typically one of which confers resistance to various antibiotics or other selection markers; such resistance or selection markers are used to discriminate between organisms that contain the plasmid and those that do not.

Plasmid DNA vaccine (or DNA vaccine): see **DNA vaccine** above.

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

Working cell bank (WCB): a cell bank derived by propagation of cells from a **master cell bank** under defined conditions and used to initiate production of cell cultures on a lot-by-lot basis; a WCB is also referred to as a "manufacturer's working cell bank" in other documents.

General considerations

Background

DNA vaccine technology involves the direct administration of plasmid DNA containing a gene(s) which encodes an immunogen(s) against which an immune response is sought, leading to the in situ production of the target immunogen(s) in the vaccine recipient. DNA vaccines are able to generate functional antibodies and both CD4+ and CD8+ T-cell responses. The ability to generate MHC-Class I restricted CD8+ T-cells (cytolytic T lymphocytes), which generally are not induced following the administration of proteins or inactivated viruses, may be important for key responses against certain pathogens, as well as enabling cross-strain responses when many antibody responses are strain specific. Because the encoded protein is synthesized in vivo by the vaccine recipient following administration, DNA vaccines can encode membrane-bound proteins (such as full-length HIV Env gp160) instead of solely encoding soluble proteins (such as gp120) (*30*). This can be important because key neutralizing epitopes (including, in the case of HIV, broadly neutralizing epitopes against more than one HIV strain) are located in protein regions that would be excluded, or not formed in a monomeric truncated soluble version. Unlike certain other vectored vaccines (such as viral vectored vaccines, which may be used in a heterologous prime-boost regimen with DNA vaccines), DNA vaccines do not stimulate adaptive immune responses against the vector (plasmid backbone) – though the DNA itself can stimulate certain innate immune responses (*31*). In other words, such vaccines do not generate anti-vector immunity that could otherwise blunt antigen-specific responses following multiple administrations.

Theoretically, DNA vaccines would be ideal for use in boosting immune responses as they could be used repeatedly (and for different purposes) because they do not generate anti-vector immune responses. However, existing data demonstrate that DNA vaccines excel at priming immune responses. These primed immune responses are boosted by the subsequent delivery of a heterologous vaccine (for example, a protein antigen or a different gene-based vector) such that the resulting immune responses are often more potent than if either modality is used alone for both prime and boost or if the DNA vaccine is given second (*32–39*). In some cases, the priming immune response to a DNA vaccine is only revealed once a heterologous boost has been administered (*40*, *41*). The responses to the heterologous boost may be amplified compared to the responses to a homologous regimen of the booster vaccine (*42–45*). The DNA prime can also modulate the type of immune response observed following the heterologous boost; an effect not seen with the booster vaccine alone (*38*, *41*, *45*). In other cases, robust responses to the DNA vaccine alone can be observed (*46*). Clearly, the nature of the immune response will depend upon the immunogen expressed and the immunomodulatory elements in the design

or formulation of the DNA vaccine, as well as on the method of delivery (*47*). Evaluation of the contribution of the DNA vaccine to the immunogenicity of any given vaccination regimen may best be assessed by the ultimate immune responses of the regimen as a whole in comparison with a regimen that does not include DNA vaccination. This is not to suggest that immune responses to the plasmid DNA prime should not also be evaluated, but rather that the priming response may best be assessed in the context of the boosted response.

As of 2020, a number of DNA vaccines had progressed as far as Phase IIb pilot efficacy trials. It is anticipated that some candidate vaccines will proceed to Phase III clinical testing. To date, the strong immune responses observed in animal models have generally not been reproduced in humans, with a few exceptions. One such exception is a DNA vaccine for WNV that generated neutralizing antibody in humans at titres that are known to be protective in horses. Furthermore, robust titres were produced in elderly humans, who generally have suboptimal immune responses to vaccines. While there is no licensed human vaccine against WNV at this time, a WNV DNA vaccine was licensed for use in horses in 2005 (*48–50*). Likewise, robust immune responses have been observed in clinical trials of DNA vaccines for Ebola/Marburg – though a different gene-based vector was advanced to efficacy testing because it required only a single vaccination as opposed to the three administrations needed for the DNA vaccines (*51–53*).

Many approaches have been tested and are being evaluated to enhance the immune response to DNA vaccines in humans. These approaches involve different intended mechanisms of increasing immunogenicity and improving efficacy, including enhancing cellular uptake, strengthening expression, modulating the immune response towards a more favourable profile or optimizing adjuvant effects. Examples of such approaches include:

1. Optimization of the vector itself:

    - optimizing codon usage of the gene encoding the antigen of interest (to increase expression);
    - optimizing the expressed RNA for translation, for example by eliminating cryptic splice sites or polyadenylation sites, changing the sequence to avoid secondary structures, or runs of high GC or AT base pairs;
    - using stronger promoters/enhancers;
    - incorporating signal sequences on protein antigens to facilitate presentation; and
    - encoding a variety of T-cell epitopes either instead of or in addition to a full-length protein antigen (to modulate the immune response by targeting T-cell stimulation).

2. Optimization of the formulation/delivery:

    - complexing the DNA with polymers (to enhance uptake, to improve stability after administration and uptake);
    - encapsulating the DNA on or within microparticles (to assist uptake, presentation, and stability after administration and uptake);
    - optimizing administration, for example using particle-mediated delivery (gene gun), CO2 or air injector (jet injector) or electroporation (to enhance uptake);
    - changing the route of administration, for example mucosal versus parenteral (to modulate the immune response);
    - boosting with viral vectors or protein antigen following an initial priming with plasmid DNA (to boost and/or modulate immune responses); and
    - co-administrating DNA encoding an immune stimulatory molecule (molecular adjuvant), for example a cytokine (to enhance or modulate the immune response).

To date, published data from clinical trials indicate that DNA vaccines are safe and have acceptable reactogenicity profiles (*11*, *22*, *35*, *51*, *54–56*). However, approaches to enhancing the efficacy of a DNA vaccine may raise specific safety concerns and these should be addressed in appropriate nonclinical and clinical safety studies. Whether approaches that result in enhanced expression will also increase reactogenicity remains an open question at present.

DNA vaccines have been developed for veterinary use, and their efficacy in animal target species has been observed in a number of trials. Potentially protective immune responses have been observed against many infectious agents in several target species including fish, companion animals and farm animals. Although the quality and safety considerations for veterinary vaccines may differ from those for human use, experience with veterinary DNA vaccines can provide valuable information for the control and use of human DNA vaccines. One DNA vaccine against WNV, which generates protective antibody responses, has been licensed for use in horses in the USA. In addition, a DNA vaccine against infectious hematopoietic necrosis virus, which affects both trout and salmon, was licensed in Canada in 2005 for use in salmon, while in 2016 a DNA vaccine against pancreas disease was licensed for use in salmon in several countries and is currently used in farmed salmon (*57*). This latter vaccine was evaluated for integration and long-term persistence in salmon, and the risk was found to be "orders of magnitude lower than the upper estimated integration rate calculated in the context of the worst-case scenarios" (*58*, *59*).

Further considerations

It is important to note that the method or specific device used to deliver the vaccine (for example, injector or electroporator) may be integral to achieving efficacy. Where a specific device is required, other delivery methods may not be interchanged, unless justified (*60*, *61*). The product labelling information for the vaccine will need to take this into account. Regulatory pathways for licensure of a vaccine in the context of its delivery device may vary by regulatory jurisdiction, and early discussions with the NRA are advised. If the DNA vaccine is to be marketed along with a novel device (for example, as a combination product), the NRA shall decide upon the regulatory requirements for marketing authorization/approval best suited to the needs of their country. In some jurisdictions, the vaccine and device taken together may be considered to be a combination product, with a defined regulatory pathway for marketing authorization. Whatever regulatory approach is used in the jurisdiction in which marketing authorization is being sought, it is important to recognize that the marketing authorization should reflect the device (and device parameters) used to deliver the candidate vaccine during the pivotal efficacy trial(s) and for which there is a sufficiently large safety database. Furthermore, if there is more than one vaccine in the regimen and they are produced by different manufacturers, it will be important to identify a single licensee or marketing authorization holder as is presently done for combination vaccines with antigens produced by different manufacturers in a single vaccine. While each vaccine may be licensed separately, the prescribing information needs to make clear that they are to be used in a regimen per the license.

Formulation may be crucial to the safety and effectiveness of any vaccine, but for DNA vaccines in which a transfectant, facilitator, adjuvant or plasmid-encoded adjuvant (for example, cytokine gene) is included in the formulation, special attention should be given to ensuring the use of the formulation that is demonstrated to be safe and efficacious in the pivotal efficacy and/or large safety trials.

The current generation of DNA vaccines made from bacteria are produced biologically and are considered to be a biological product. In addition, even though the plasmid is generated by recombinant DNA technology, it should be clarified that a plasmid DNA vaccine is not an organism; thus, it is not a genetically modified organism (GMO) per se, nor is it a gene-transfer or gene-therapy product. There is a wealth of evidence that DNA vaccines to date do not persist or even biodistribute throughout the body of the vaccine recipient when delivered parenterally into muscle, subcutaneous tissue or various dermal layers (*62–70*). What does predominantly biodistribute is the immune response generated following uptake of the plasmid DNA and in situ expression of the immunogen(s), along with cross-priming from myocytes to professional

antigen-presenting cells (*71*, *72*). The local response to plasmid DNA inoculation is that cells take up the plasmid and then express the immunogen(s) encoded in the DNA vaccine and/or the nucleic acid is degraded by normal molecular mechanisms. As a consequence, the plasmid DNA clears from the injection site over time, while it is the immune response that may persist.

Structure of the Guidelines

The quality section of these Guidelines (Part A) addresses the control of the bulk purified plasmid (including control of the manufacturing process and starting materials, and characterization and control of the purified plasmid) and control of the final formulated vaccine (including formulation, control of materials used in formulation, and stability of the bulk purified plasmid and the final formulated vaccine). The appropriate use of reference materials (including international standards, once available) is also described. Whenever changes to the manufacturing process are implemented, the comparability of lots, especially to those used in pivotal studies and the commercial process, should be demonstrated.

The nonclinical and clinical sections of these Guidelines (Parts B and C respectively) reference existing general WHO guidelines, (*27–29*) while also addressing a number of issues that may apply to DNA vaccines more than to other types of vaccines. The section on nonclinical evaluation has also been made more succinct in light of additional data now available on the initial concerns raised before there was such extensive nonclinical and clinical experience with DNA vaccines. The current revision therefore also includes a section on clinical evaluation for the first time. Taken as a whole, the current nonclinical and clinical databases support the conclusion that prior concerns about integration, autoimmunity and immunopathology have not been borne out (*29*, *60–67*). To date, based on clinical experience, the observed reactogenicity appears to relate more to the delivery method than to the DNA vaccine itself, most notably in the case of electroporation or particle-mediated bombardment (*1*, *4*, *21*, *30–37*, *73–75*).

The control, nonclinical testing and clinical development of each DNA vaccine should be considered individually, and any special features of a particular candidate vaccine should be taken into account. Early consultation with the NRA will be key to assuring the efficient development of any given candidate DNA vaccine.

Part A. Guidelines on the manufacture and control of plasmid DNA vaccines

A.1 Definitions

A.1.1 International name and proper name

The international name should be "plasmid DNA vaccine". The proper name should be the equivalent of the international name in the language of the country in which the vaccine is licensed.

The use of the international name should be limited to vaccines that meet the specifications given below. Defined recombinant nucleic acids used as active substances in vaccines, whether of biological or synthetic origin, could be assigned an international nonproprietary name (INN) upon request (*76*, *77*).

A.1.2 Descriptive definition

A DNA vaccine is a sterile liquid or lyophilized vaccine preparation that contains x μg or x mg of each of one or more plasmid DNAs; the amount of each plasmid may vary from that of another plasmid in the formulation based on relative expression or immunogenicity. The DNA vaccine may be formulated with a suitable adjuvant or other excipients that might enhance uptake, expression or immunogenicity of the plasmid DNA(s) in the vaccine recipient. Such vaccines are for preventive/prophylactic use in humans.

A.2 General manufacturing guidelines

Plasmid DNA vaccines are considered to be similar to bacterial and viral vaccines produced by traditional methods in so far as adequate control of the starting materials and manufacturing process is as important as that of the final product. These Guidelines therefore place considerable emphasis on the control strategy for the manufacturing process of the vaccine, as well as on comprehensive characterization and batch and lot release of the bulk and the vaccine itself.

The general guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (*78*) and WHO good manufacturing practices for biological products (*79*) should be applied to the design, establishment, operation, control and maintenance of manufacturing facilities for DNA vaccines. The guidance also covers the control of vaccine filled in the final form, the keeping of records and retained samples (for future studies and needs), labelling, distribution and transport, and stability testing, storage and expiry date (*78*, *79*). Quality control during the manufacturing process relies on the implementation of quality systems, such as good manufacturing practices (GMP), to ensure the production of consistent commercial vaccine lots with product 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 while most release specifications are product specific, DNA vaccines, as a product class with shared characteristics, tend to meet product-class-specific specifications for many release parameters. Whatever the case, these specifications should be agreed with the NRA as part of the clinical trial or marketing authorization.

DNA vaccines for use in clinical trials should also be prepared under GMP conditions suitable for the stage of clinical development – that is, full compliance may not be possible in initial or early development when manufacturing and control procedures remain in development and may not yet be validated. However, it would be expected that validated procedures would be used in early development if they are procedures shared with other DNA vaccines made in that facility that have attained higher phases of development in which the procedures have already been validated. Appropriate attention needs to be given to the quality of all reagents used in production, including the components of the fermentation medium. Particular attention is required to the sourcing of components of animal (including human) derivation. Many of the general requirements for the quality control of biological products, such as tests for endotoxin, stability and sterility, also apply to DNA vaccines.

Particular attention should be given to DNA vaccines prepared in multi-use facilities, as would be typical in initial or early clinical development. Cleaning validation would be expected even in early development for such multi-use facilities, even though such validation would normally occur later (though prior to commercial production) in a dedicated facility. One novel aspect particular to DNA vaccines is that cleaning procedures need to be verified with an assay sensitive enough to detect not only microorganisms and other biological materials that may be residual from prior manufacture, but also residual full-length plasmid DNA from prior lots of products made using the same equipment and facility. This issue of carry-over or potential for cross-contamination is a specific concern because of the amounts (often, mg quantities) in which DNA vaccines are administered. These amounts may vary from other products made using the same equipment or in the same facility due to formulation and delivery method. Manufacturers should also define the methods they use to prevent carry-over and cross-contamination.

It is recognized that the level of detail required by a regulatory authority increases as product development proceeds. During the initial phases of clinical development, the information contained in a clinical trial application should be adequate to allow for assessment of the safety risks derived from the manufacturing process. This would include, for example, the methods and results of testing of the bacterial cell banks for identity, identification and specifications

for all materials used in the process, assessment of risks from biologically sourced materials, certification or phase-appropriate GMP compliance of the manufacturing facility, a brief description of the process and tests, results of testing of the clinical trial material, and preliminary stability of the final product. As with all vaccines, the level of detail expected on the Quality (manufacturing and control) would increase for late-stage clinical trials.

Any changes made to the product composition (for example, addition of adjuvant or preservative) or to its manufacturing (process, site or scale) during the development of clinical lots should be adequately described. Depending on how the final product composition is changed (for example, addition of novel excipients) new preclinical studies might be warranted. For changes to the manufacturing process (such as scale-up or change to the purification process) the comparability of the clinical trial material to the material manufactured using the previous process should be evaluated. The comparability studies might include immunogenicity data from animal models, the results of physicochemical analyses, process and product-related impurity studies, and stability data (*80*).

A.3 Control of bulk purified plasmid (bulk)

A.3.1 General information

The overview of the development and manufacture of the plasmid(s) should include a justification for the selection of the gene(s) of interest, other gene(s) encoded in the plasmid (for example, tags, selection markers or antibiotic resistance gene), and regulatory elements used. Any gene expression optimization modifications should also be described. The nucleotide sequence of the entire plasmid should be provided.

A.3.2 Manufacture

A.3.2.1 Control of materials

The materials used in the manufacture of the bulk plasmid DNA (for example, raw materials, biological starting materials, column resins, solvents, reagents and catalysts) should be listed and information given on where each material is used in the process. Information on the quality and control of these materials should be provided.

Reference to internationally accepted pharmacopoeias or details on the specifications used should be provided.

A.3.2.1.1 *Control of source and starting materials of biological origin, including animal/human origin*

Information, including proper certification, regarding the source, manufacture and characterization of all biologically sourced materials or materials produced using biologically sourced materials should be provided. Risk assessments for

bovine spongiform encephalopathy agents should be provided if bovine materials were used at any stage. Compliance is expected with the WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (*81*).

A.3.2.1.2 *Source, history and generation of the host cell and plasmid*

Information should be provided on the bacterial host cell, including its source, phenotype and genotype. Particular attention should be given when using a host cell that is a novel strain or species of bacterium, including attention to the types of toxins they may express.

The nucleotide sequence of the entire plasmid DNA vaccine should be provided together with appropriate annotation indicating the important elements, such as the promoters/enhancers, termination sequences, drug-resistance or other marker for selection in bacteria, and bacterial origin of replication. In most cases, it is likely that the gene for the immunogen will be optimized for expression and synthesized chemically before being recombined into the plasmid DNA. As such, the gene will have a novel sequence and will not be present in any database. In contrast, if the gene is obtained from other sources such as amplification from a natural element by polymerase chain reaction (PCR) then the source of that material should be provided.

As part of characterization, a DNA sequence homology check of the plasmid against international databases (for example, the National Center for Biotechnology Information, National Institutes of Health, the USA and/or other international nucleotide databases) should be performed to investigate the presence of unintended sequences of biological significance, such as those encoding cellular growth factors, other known immunogens or viral sequences.

The identity of the plasmid after transformation into the bacterial cell to be used for production should be confirmed. While sequencing is preferred, representative restriction enzyme maps may also be useful. The candidate vaccine selected to advance in clinical development and for marketing authorization should be demonstrated to be genetically stable. Freedom from cross-contaminating plasmids should be controlled and verified.

A.3.2.1.3 *Cell bank system, characterization and testing*

The production of a plasmid DNA vaccine should be based ideally on a cell bank system involving a master cell bank (MCB) and a working cell bank (WCB).

For early-stage clinical trials, it may be appropriate to use the MCB to initiate production – though manufacturers are expected to prepare a WCB for later clinical studies. Initiating production from a well-characterized WCB is expected for commercial manufacturing.

A well-characterized bacterial cell containing the plasmid should be cloned and used to establish the MCB. The preparation of the MCB and WCB should be conducted according to GMP with appropriate precautions taken to prevent contamination. Information should be provided on the origin and storage conditions of the cell banks. Evidence for the viability of the MCB and WCB under storage and recovery conditions should also be provided by the time of application for marketing authorization. New WCBs should be fully characterized and meet established acceptance criteria. Specific phenotypic features that can form a basis for identification of the transformed cell should be described. Prior to their use, either a protocol for establishing and releasing new WCBs or information on each new WCB should be provided for regulatory review and concurrence.

The nucleotide sequence of the entire vaccine plasmid should be confirmed at the MCB and WCB stages. Production of full-length protein(s) from the plasmid should be characterized, demonstrating freedom from truncated or alternative protein products.

The genetic stability of the plasmid should be confirmed by characterizing its size and complete nucleotide sequence throughout the fermentation process.

A.3.2.2 Process development and in-process control

The developmental history of the manufacturing process should be provided. Tests and acceptance criteria for critical steps of the manufacturing process should be developed to ensure, and provide feedback on, the control of the process.

Validation of the manufacturing process should be shown to yield a product consistently meeting the predefined quality attributes, including demonstration of reproducible and consistent clearance of process and product-related contaminants to levels acceptable for the intended use in humans.

Although process validation is not generally required for a product used in early-stage clinical trials, critical steps such as aseptic processing, sterility of final product and cleaning validation (particularly when multi-product facilities or contract manufacturing organizations are used for the manufacturing) should be validated or carefully and convincingly controlled prior to initiation of manufacture of clinical materials.

A.3.3 Characterization

A.3.3.1 Characterization of bulk purified plasmid

A summary of the characterization of the bulk purified plasmid(s) should be provided in addition to in-process and lot-release testing. Rigorous characterization using a range of orthogonal chemical, physical and biological methods will be essential.

During development, the nucleotide sequence of the entire plasmid should be determined, as discussed in sections A.3.2.1.2 and A.3.2.1.3 above. Demonstration of expression of the full-length protein(s) without truncated or alternative forms should also be provided.

The immunogenicity elicited by the plasmid should be characterized. Whenever other immunomodulatory elements or genes are included, their contribution to the mode-of-action (immunogenicity) of the plasmid should also be determined in order to justify their inclusion.

Potential impurities in the purified product should be described and investigated. These potential impurities include residual host cell proteins, endotoxins, residual host cell RNA and chromosomal DNA, materials used in the manufacturing process and medium components. Data should be provided on the contaminants present in the bulk purified plasmid, with estimates given of their maximum acceptable or lowest achievable levels. For contaminants and residuals with known or potential toxic effects, a toxicological risk assessment is expected. Degraded plasmid DNA may be assessed as part of analytical procedures such as polyacrylamide gel electrophoresis, high-performance liquid chromatography (HPLC) and/or capillary electrophoresis. One important characteristic of the bulk purified plasmid that needs to be determined is the degree to which the plasmid remains supercoiled or has been partially converted to relaxed circles or linear forms.

A.3.3.2 Consistency of manufacturing

Prior to seeking marketing authorization, a number of consecutive batches should be characterized and analysed using validated methods to determine consistency of manufacture. Any differences observed between one batch and another outside the accepted range for the parameters tested should be noted. The data obtained from such studies, as well as clinical trial outcomes with various lots, should be used as the basis for justification of the chosen specifications.

During early-stage development, few lots will have been made, and demonstration of consistency may be limited. Demonstration of consistency will occur as manufacturing experience is gained during product development. Demonstration of the consistency of lots is generally performed during advanced development (when the manufacturing process has been scaled-up for commercial manufacture) but prior to submission of a licence or marketing application. Whenever changes to the manufacturing process are implemented, the comparability of lots, especially to those used in pivotal studies and the intended commercial process, should be demonstrated. Comparability protocols and strategies for demonstrating comparability are discussed in the WHO Guidelines on procedures and data requirements for changes to approved vaccines (*80*).

A.3.4 Control of bulk purified plasmid

Specifications for the critical quality attributes of identity, purity, quality and safety of the bulk purified plasmid should be established and justified. Descriptions of the analytical methods used (including assay validation information) and acceptance limits defined should be provided. A summary of the results of testing of all batches produced at commercial scale should be provided.

Early in development, the results of testing batches made in accordance with GMP and, if available, of engineering runs performed to establish manufacturing procedures should be summarized and provided.

It is recommended that the specifications for the bulk purified plasmid include, at a minimum, an assessment of the identity, purity, physical state and quantity of the plasmid, along with the endotoxin content, and sterility or bioburden of the bulk. A justification of the specifications should be provided. Specifications should also be established for stability under storage conditions.

Early in development, the specifications may be limited and have somewhat wide acceptance criteria. Not all of the tests conducted during product characterization need to be carried out on each batch of vaccine. Some tests are required only to establish the validity or acceptability of a procedure, whereas others might be performed on a limited series of batches to establish consistency of production. Thus, a comprehensive analysis of the initial commercial production batches should be undertaken to establish consistency with regard to identity, purity, quality, safety and stability; thereafter, a limited series of tests may be appropriate.

A.3.4.1 Identity

The identity of each bulk purified plasmid batch should be confirmed by an appropriate method such as PCR analysis, sequencing, restriction enzyme analysis or in vitro expression (mRNA or protein) of the gene insert of the plasmid accompanied by confirmation of the identity of the expressed antigen.

A.3.4.2 Purity

Limits based on process capability and regulatory guidance should be established for all impurities detected, and these should be identified and characterized as appropriate. The degree of contamination with host cell chromosomal DNA, RNA and proteins should be evaluated and limits established, and acceptance criteria established and specified. Comparison of the absorbance at 260 nm and 280 nm may be useful for purity assessment, for example of the extent of contamination introduced by RNA and cellular proteins. However, other suitable methods may be appropriate for purity assessment. Residual levels of medium components (including antibiotics, if applicable) and other materials from process steps should also be controlled. The analysis should include sensitive

and reliable assays for process- and product-related contaminants and strict upper limits should be specified for their content in the bulk purified plasmid. A maximum allowable limit should be established and justified. It is important that the techniques used to demonstrate purity be based on as wide a range of physicochemical properties as possible. Measuring residual levels of process- or product-related impurities as part of quality control may be discontinued after suitable processes for their removal have been adequately validated. Plans and specifications for the periodic revalidation of processes should be described. Until such processes have been validated, impurities should continue to be measured for a number of lots, as acceptable to the NRA. In the case of major changes to manufacturing, process revalidation or continued measurement for the number of lots agreed to by the NRA would be expected. Container-closure system compatibility, leachables and extractables should be assessed and discussed in the marketing authorization application.

Where multi-product facilities or contract manufacturing organizations are used for the manufacturing process, freedom from contamination with other products, especially other DNA plasmids made in the same facility, should be demonstrated to established limits or below detection.

A.3.4.3 Physical state and quantification of plasmid

The proportion of supercoiled plasmid should be determined and specifications set. Quantification of the plasmid amount is usually based upon absorbance at 260 nm. Any additional quality parameters relevant to the bulk purified plasmid should also be determined and specifications set – for example, pH or viscosity might be important for certain products to ensure stability and quality at the bulk purified plasmid stage.

A.3.4.4 Safety

Relevant safety tests should be described and may include tests for: (a) endotoxins; (b) bacterial and fungal sterility (including demonstration of lack of bactericidal or fungicidal activity of the test article); or (c) bioburden (including quantity, identification and freedom from specified unwanted organisms). Although a test for pyrogenicity may be performed if required by the NRA, animal testing should be avoided whenever alternative satisfactory testing is accepted. For ethical reasons, it is desirable to apply the 3Rs concept of "Replace Reduce Refine" to minimize the use of animals, and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation. In particular, manufacturers and regulators should take note of the decision of the WHO Expert Committee on Biological Standardization in 2018 to discontinue the inclusion of the general safety (innocuity) test in routine lot release testing

requirements for all vaccines in WHO Recommendations, Guidelines and other guidance documents for biological products (*82*). This test should therefore not be required or requested.

A.3.5 Reference materials

An in-house reference preparation should be established for use in assay standardization. Information on the reference standards or reference materials used for testing of the bulk purified plasmid should be provided by the time of application for marketing authorization. A suitable batch (that is, one that has been clinically evaluated) should be fully characterized in terms of its chemical composition, purity, biological activity and complete sequence, and retained for use as a chemical and biological reference material. A plan for replacing the initial reference material upon exhaustion should be agreed with the NRA.

In early development, an engineering run batch or a batch from which the lot of DNA vaccine tested in the pivotal nonclinical studies was produced may be used until a suitable clinical trial lot has been identified and characterized for use in advanced development and commercial manufacture.

In future, international standards (IS) expressed in International Units (IU) may be prepared by a WHO collaborating centre. When such IS become available it will be important to compare the internal reference material against the IS so that IU may be assigned, and in order to fully validate the quality control tests or assays. By using this approach, comparisons can also be made in a more reliable and less variable way whenever new reference materials need to be prepared.

A.3.6 Stability

The stability assessment should be in compliance with the WHO Guidelines on stability evaluation of vaccines (*83*). The types of studies conducted, the protocols used and the results of the studies should be summarized in an appropriate format such as tables, graphs or a narrative document. The summary should include results and conclusions regarding appropriate storage conditions and shelf-life. Stability data to support the shelf-life of the bulk and any future extension of it should be derived from long-term, real-time stability studies under actual conditions.

Limited stability information would be expected during initial clinical development. For example, some regulators accept three months of real-time stability at the time of application for clinical trial authorization, but this should be agreed with the NRA. Lots should be labelled with a retest or re-pass date, if required by the NRA.

A.4 Control of final formulated vaccine (vaccine)

A.4.1 Composition

The final composition of the vaccine should be described. If it is required for established safety and efficacy that the vaccine needs to be delivered by a specific method or device then this should also be described.

A.4.2 Manufacture

A flowchart should be provided illustrating the manufacturing steps from the bulk purified plasmid to the final formulated vaccine. This flowchart should include all steps (that is, unit operations), identification of materials and in-process and quality control tests. In some cases, this may involve simple dilution of the purified bulk, while in other cases a more complex formulation may be involved including the combining of purified bulks of more than one plasmid. A narrative description of each process step depicted in the flowchart should be provided. Information should be included on, for example, scale of production, buffers and other additives, major equipment and process controls, including in-process tests and critical process operational parameters with acceptance criteria. In the case of simple dilution or no further formulation of the bulk purified plasmid other than filling into final containers for the final formulated vaccine, some quality control tests performed on the bulk purified plasmid may suffice as control for the final formulated vaccine.

A.4.3 Control of materials

Details of excipients (including adjuvants) or any other component of the container-closure system of the vaccine, in addition to information on the plasmid that constitutes the immunogen, should be provided and should include information on their source, specification and final concentration in the vaccine.

A.4.4 Control of final formulated vaccine

Specifications for the vaccine should be established and justified. Descriptions of analytical methods and acceptance limits for the vaccine, including information on assay validation, should be provided. It is recommended that the specifications include an assessment of the identity, purity, physical state and quantity of the plasmid, any other relevant quality parameters, potency, endotoxin content and sterility. A justification of the specifications should be provided.

Early in development, the specifications may be limited with wide acceptance criteria. A summary of the results of testing on all lots produced at commercial scale should be provided. Early in development, the results of testing on lots made in accordance with GMP and, if available, of engineering

runs performed to establish manufacturing procedures should be summarized and provided.

The appropriateness of performing tests on the bulk purified plasmid versus the formulated vaccine should be considered on a case-by-case basis and justified.

When more than one plasmid is present in the final formulation it may not be straightforward to distinguish the potency of one plasmid from another. In such cases, assessing in vitro expression for each bulk purified plasmid may be performed to establish the potency of the final formulation. In other words, the potency of the final product may be inferred and calculated from the potency of each of the plasmids present in cases where the potency of each plasmid cannot be distinguished from another in the final product. However, if there is an adjuvant or facilitator in the final formulation that may alter the potency of the individual plasmids then this approach may not be reliable.

Several consecutive lots of vaccine, in final dosage form, should be characterized and analysed by employing validated methods to determine manufacturing consistency. Any differences between one lot and another should be noted. The data obtained from such studies, as well as clinical trial outcomes with various lots, should be used as the basis for defining the vaccine specifications and acceptance criteria to be used for routine lot release.

Not all the tests conducted during product development need to be carried out on every lot of vaccine produced at commercial scale. Some tests are required only to establish the validity or acceptability of a procedure, whereas others might be performed on a limited series of lots to establish consistency of production. Thus, a comprehensive analysis of the initial commercial production lots should be undertaken to establish consistency with regard to identity, purity, quality, content/strength/quantity, potency, safety and stability, but thereafter a more limited series of tests may be appropriate.

A.4.4.1 Identity

Each lot of vaccine should be subjected to an appropriate selection of the tests used to confirm the identity of the final product plasmid. Depending on the scope of the identification tests, confirmation of identity by restriction enzyme mapping, sequencing and/or PCR should be considered.

A.4.4.2 Purity

The purity of each lot of vaccine should be determined and shown to be within specified limits. The form of the final product plasmid(s) should be confirmed – for example, by conducting gel electrophoresis or other method to demonstrate that the vaccine has not degraded. Container-closure system compatibility, leachables and extractables should be assessed and discussed.

Where multi-product facilities or contract manufacturing organizations are used for the manufacturing process, freedom from contamination with other products should be demonstrated to established limits or below detection.

A.4.4.3 Content, strength or quantity

DNA vaccines are dosed based on the quantity of the plasmid by weight. Generally, this is established by absorbance at 260 nm (comparison of absorbance at 260 nm and 280 nm may be useful in assessing purity).

A.4.4.4 Other quality parameters

Quality parameters should be established and controlled. Important quality parameters include appearance and pH. Another important quality parameter is the percentage of the overall amount of plasmid that is supercoiled (plasmid may be present in other forms such as nicked circles or linear). Depending on the product characteristics, the control of other parameters such as osmolality and viscosity may be important. Furthermore, quality may be assessed by methods used to evaluate purity or identity, such as restriction mapping, gel or capillary electrophoresis, and/or HPLC – though these may best be performed on the bulk purified plasmid instead. Other tests, such as the test for residual moisture if the vaccine is lyophilized, may be required to confirm the physical characteristics of the product as well as its formulation.

A.4.4.5 Potency

The potency of each lot of the vaccine should be determined using a suitably quantitative and validated assay. Potency relative to an appropriate in-house reference preparation should be established. Ideally, a potency assay would be established that can ensure the consistency of lots with established clinical performance. Often, this takes the form of an in vitro expression system. The immunogen might be expressed in vitro by transfection of a suitable cell line and either the expressed mRNA or the expressed protein identified, for example, by quantitative RT-PCR (in the case of mRNA) or by immunofluorescence or Western blot (in the case of protein). It may be appropriate to establish potency on the basis of an alternative suitably justified laboratory method (that is, a non-bioassay). Early discussion should be held with the NRA to reach consensus regarding the appropriateness of the proposed method. Consensus should also be sought on the use of a composite measure of content (amount of plasmid DNA as used for dosing) and percentage of supercoiled plasmid for control of potency for release of each vaccine lot. An in vitro method demonstrating expression could then be considered for characterization instead of control of potency.

When multiple plasmids are included in the final formulation, the potency of each immunogen encoded should be assessed. However, if this

cannot be determined at the stage of the final formulation, it may be necessary to assess potency at the stage of the individual plasmid prior to its inclusion in the final formulation (see sections A.4.4 and A.4.4.7).

When a cell-based potency assay is used, it is important to control the cells using cell banking to ensure a consistent supply of cells for testing. Furthermore, the cells should be assessed for freedom from adventitious agents, mycoplasmas/spiroplasmas (the latter, only if relevant), bacteria/fungi and mycobacteria (if relevant), and only suitably controlled cells used.

A.4.4.6 Safety, including sterility and endotoxin testing

Each lot of vaccine should be tested for sterility. If the vaccine is to be administered by a non-parenteral route, omission of the sterility test and inclusion of an appropriate alternative bioburden test needs to be appropriately justified. Furthermore, a test for endotoxin should be conducted on each lot and appropriate specifications defined. If required by the NRA, a test for pyrogenicity may be performed – however, animal testing should be avoided whenever alternative satisfactory testing is allowed. For ethical reasons, it is desirable to apply the 3Rs concept of "Replace Reduce Refine" to minimize the use of animals and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation. Pyrogenicity may be determined using the monocyte activation test. The test known as the innocuity, abnormal toxicity or general safety test should not be required or requested (see section A.3.4.4 above).

A.4.4.7 Multi-component vaccines

Additional factors must be considered when more than one plasmid forms the final formulated vaccine. Plasmids in multi-component vaccines may encode additional antigens or cytokines or other biologically active molecules that enhance the efficacy or affect the safety of the vaccine. For each plasmid, the development overview, the control of production and the characterization of the bulk purified plasmid must be described as above. Likewise, for multi-component DNA vaccines that contain components (for example, immunomodulatory molecules or cytokine proteins) in addition to the plasmid(s), the role of the additional components should be addressed. Careful consideration must be given to the control of the final formulated vaccine. For example, potency may depend upon the combination of plasmids and their interaction and not on any single plasmid component of a multi-component vaccine.

In some cases it may not be feasible to measure potency in the context of a mixture of closely related antigens, and the potency of the individual plasmids may have to be measured in terms of expression (of mRNA or protein) in the individual bulk purified plasmids. If agreed to by the NRA, a composite measure

of content (amount of plasmid DNA as used for dosing) and percentage of supercoiled plasmid may be used for control of potency of the lot. In all cases, the approach taken and its justification should be clearly described.

A.4.5 Reference materials

A suitable lot of the final formulated vaccine (or batch of bulk purified plasmid) that has been clinically evaluated should be fully characterized in terms of its chemical composition, purity and biological activity, including full sequencing, and should be retained for use as a chemical and biological reference material. This material should be used as the basis for evaluation of product quality for commercial production lots.

In future, IS expressed in IU may be prepared by a WHO collaborating centre. When such IS become available, it will be important to compare the internal reference material against the IS so that IU may be assigned, and in order to fully validate the quality control tests or assays. By using this approach, comparisons can also be made in a more reliable and less variable way whenever new reference materials need to be prepared.

Likewise, IS may be useful for the interpretation of nonclinical and clinical assays of immune responses or other biomarkers of relevance to the DNA vaccine under development or being evaluated for marketing authorization (see Parts B and C below).

A.5 Records

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (*78*) and WHO good manufacturing practices for biological products (*79*) should be followed, as appropriate to the level of development of the candidate vaccine.

A.6 Retained samples

A sufficient number of samples should be retained for future studies and needs. Vaccine lots that are to be used for clinical trials may serve as a reference material in the future and a sufficient number of vials should be reserved and stored appropriately for that purpose.

A.7 Labelling

The guidance on labelling provided in WHO good manufacturing practices for pharmaceutical products: main principles (*78*) and WHO good manufacturing practices for biological products (*79*) should be followed as appropriate. The label on the carton enclosing one or more final containers, or the leaflet accompanying each container, should include the following information at a minimum:

- the name of the vaccine;
- the names and addresses of the manufacturer and distributer;
- a statement that specifies the nature and content of adjuvant contained in one human dose, if any;
- the immunization schedule, and the recommended route(s) of administration;
- the number of doses, if the product is issued in a multi-dose container;
- the name and concentration of any preservative added;
- a statement on the nature and quantity, or upper limit, of any antibiotics present in the vaccine;
- the temperature recommended during storage and transport;
- the expiry/retest date;
- any special dosing schedules; and
- contraindications, warnings and precautions, and information on concomitant vaccine use and on known adverse reactions.

A.8 Distribution and transport

The guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (*78*) and WHO good manufacturing practices for biological products (*79*) appropriate for a candidate vaccine should be followed. Shipments should be maintained within specified temperature ranges, as applicable, and packages should contain cold-chain monitors if the temperature needs to be controlled (*84*). If it is claimed that a cold-chain is not required then the conditions under which stability has been established (for example, maximum temperature and maximum length of time at that temperature) should be described and data supporting these claims provided.

A.9 Stability testing, storage and expiry date

The relevant guidance provided in WHO good manufacturing practices for biological products (*79*) and in the WHO Guidelines on stability evaluation of vaccines (*82*) appropriate to the respective plasmid DNA vaccine should be followed. Furthermore, the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (*85*) might also be applicable. Any statements concerning storage temperature and expiry date that appear on the primary or secondary packaging should be based on experimental evidence and should be submitted to the NRA for approval.

A.9.1 Stability testing

Adequate stability studies form an essential part of vaccine development. The stability of the final product in the container proposed for use should, therefore, be determined and the results used to establish a shelf-life under appropriate storage conditions. Parameters that might be stability-indicating should be measured. These may include parameters such as appearance, quantity and percentage of supercoiled plasmid. The parameters to be measured should be described and specifications defined. Real-time stability studies should be undertaken for this purpose but accelerated stability studies at elevated temperatures may provide complementary supporting evidence of the stability of the product and confirm the stability-indicating nature of the assays used to determine stability. Container-closure system compatibility with storage stability should be assessed (including in terms of leachables and extractables) and discussed. The stability assessment should comply with the WHO Guidelines on stability evaluation of vaccines (*82*).

A.9.2 Storage conditions

The vaccine should not be stored for a length of time, and/or at a temperature, greater than that shown by the manufacturer to be compatible with a minimal loss of potency before being distributed by the manufacturing establishment or before being issued from a storage site. The maximum duration of storage should be fixed with the approval of the NRA, based on the results of stability studies, and should be such as to ensure that all quality specifications for the final product (including the minimum potency specified on the container or package) are maintained until the end of shelf-life. During clinical trials, this period should ideally be at least equal to the expected duration of vaccine administration in the clinical trial.

A.9.3 Expiry date

The expiry date should be defined on the basis of shelf-life in the final container and should be supported by stability studies approved by the NRA. The expiry date should be based on the date of blending of the final bulk, the date of filling or the date of the first valid potency test on the final lot, as appropriate, and agreed with the NRA.

Part B. Nonclinical evaluation of plasmid DNA vaccines

The nonclinical evaluation of the candidate vaccine should be considered on a product-specific basis taking into account the intended clinical use of the product. The selection of appropriate studies relating to the toxicology and

pharmacology (proof-of-concept) of the product may be determined based on either or both of the following WHO guidelines:

- WHO guidelines on nonclinical evaluation of vaccines (*27*); and
- WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (*28*).

One relevant issue for DNA vaccines would be a situation in which a plasmid component encodes a cytokine or other immunomodulatory protein. In such cases, the choice of animal model selected for nonclinical evaluations may need to take into account the species specificity of any biological activity of the product. It may be necessary to conduct proof-of-concept studies with species-relevant analogues to the human-specific product to be developed. Toxicological evaluations, including of immunotoxicity, may be performed with the human-specific product and/or the analogue, and concurrence from the NRA should be sought in this matter.

Another potential issue for DNA vaccines would be their use in heterologous prime-boost regimens. In cases where there are no pre-existing nonclinical or clinical data on the individual vaccines used in the regimen (or, at least, on the DNA vaccine component of the regimen), the nonclinical programme may be the same or similar to that described in the existing guidelines listed above (*27*, *28*). However, when there is significant clinical experience with each vaccine in the regimen expressing the same or related immunogens – for example, other viral envelope proteins or other influenza haemagglutinins, or in cases where only limited modifications were made to the amino acid sequence of a previously tested candidate vaccine to produce the new candidate vaccine – it is expected that the nonclinical programme could be abbreviated (*86*). The existing clinical experience would be more informative of the safety and performance of the vaccine components in the combined regimen than animal data would be. It may be appropriate to assess certain safety parameters within an immunogenicity (or challenge-protection) study of the new vaccine regimen(s) to determine whether the safety profile in animals appears to be similar to that observed in previous studies of the same DNA vaccine plasmid backbone expressing a related immunogen. This approach is consistent with the principles of the 3Rs to refine, reduce or replace the use of animals in product safety testing whenever suitable alternative methods are available.

Similarly, for new DNA vaccines based on existing plasmid backbones for which there is already significant nonclinical (and possibly clinical) experience, an abbreviated nonclinical programme should be considered (*21*, *22*, *69*, *70*). If the new gene insert is related to other antigens that have already been studied in nonclinical (and possibly clinical) programmes, a case may be made to support a safe starting dose and regimen for the new vaccine based on the existing nonclinical and clinical data without the need for additional toxicology studies.

In the context of the rapid development of a vaccine against a priority pathogen during a public health emergency, consideration may be given to an abbreviated nonclinical programme. In cases where the plasmid is constructed from a backbone that has already been clinically tested using a related antigen (for example, in the case of a pandemic influenza strain when a seasonal or other potential pandemic strain antigen has been tested) then the nonclinical programme might be limited to an immunogenicity study (or studies). However, such a study should collect as many safety data as feasible given that many nonclinical immunogenicity studies are performed without full compliance to good laboratory practices. Depending on the species used, where it is feasible to collect blood not only for immunogenicity assessments but also for haematology and chemistry assessments, these analyses should be performed. In addition, depending on the species used, if the animals are sacrificed at the end of the immunogenicity study then gross pathology and targeted histopathology should be performed. Information obtained from physical examinations or clinical findings should also be captured and reported to the NRA. If the species used is too small to permit individual clinical pathology (for example, mice) or if animals are not sacrificed because they will be used in other research after the immunogenicity study is performed (for example, non-human primates) then whatever safety data can be collected should be reported to the NRA. Where safety information is available on veterinary vaccines expressing related antigens, this information might usefully be provided to the NRA.

In the context of the rapid development of a vaccine against a priority pathogen during a public health emergency where the plasmid backbone has previously been clinically tested but the antigen is novel (that is, not related to any other antigen that has been clinically tested) then the approach outlined above might not be sufficient. Decisions about the type of nonclinical safety/toxicology information that will be required could be guided by what and how much is known about the natural disease in terms of its pathology, particularly its immunotoxicity. If the natural disease is associated with immunopathology due to cross-reactivity, autoimmunity or immunity-associated disease enhancement then toxicology studies would likely be needed to ensure that the novel antigen was not associated with these effects. In cases where the natural disease is not associated with immunopathology or where little is known about the natural disease, discussion with the NRA should be undertaken. Finally, in cases where the plasmid backbone or both the plasmid backbone and the antigen are novel, discussion with the NRA should again be undertaken.

Although biodistribution studies were previously suggested for DNA vaccines, the data acquired to date have not shown reason to continue with such evaluations. Plasmid DNA remains largely at the injection site and does not biodistribute at clinically relevant levels or widely throughout the body. Furthermore, it does not target the ovaries or testes and clears from the body

by degradation (*70*, *71*, *87*, *88*). However, most of these data were collected in adult animals. A limited amount of information is available from developmental toxicology and biodistribution studies in maternal or fetal animals (*88*). The publication of any developmental toxicology studies already performed on DNA vaccines is encouraged.

For DNA vaccines against priority pathogens for use during public health emergencies identified by the WHO Blueprint for Research and Development: Responding to Public Health Emergencies of International Concern (R&D Blueprint), the following documents may be of relevance and should be consulted:

- WHO guidelines on nonclinical evaluation of vaccines (*27*);
- WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (*28*);
- An R&D Blueprint for action to prevent epidemics. Plan of action. May 2016 (*89*); and
- An R&D Blueprint for action to prevent epidemics. Accelerating R&D and saving lives. Update 2017 (*90*).

WHO has published more than 60 guidelines and recommendations documents for vaccines against specific diseases, and those that cover the disease of relevance for a given DNA vaccine should be consulted. It is anticipated that further such documents on specific DNA vaccines will be considered for development at the appropriate time once a disease-specific DNA vaccine nears submission for marketing authorization.

Part C. Clinical evaluation of plasmid DNA vaccines

The clinical evaluation expectations for clinical trial authorization or marketing authorization will depend upon the disease against which the DNA vaccine is being or has been developed, and the vaccine mode of action (or mechanism of action) for preventing that disease. Clinical studies should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (*91*) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*29*). It should be noted that the issue of post-licensure pharmacovigilance is discussed in the latter guidelines.

One issue of relevance to DNA vaccines is their use in heterologous prime-boost regimens and some guidance on this issue is provided in the above guidelines. A challenge for marketing authorization will be the labelling of each of the vaccines in the regimen that ultimately demonstrates efficacy, as this type of heterologous prime-boost regimen remains novel at this time, and health care workers and public health systems are not necessarily ready for this approach.

Clear labelling to prevent mix ups and mis-dosing will be crucial to a successful public health campaign or during routine use. Another issue that may require attention is the attribution of safety events observed following immunization, and how to clearly establish whether an event was due to the prime or the boost vaccine, even if the event occurred late (for example, after boosting).

One potential advantage of DNA vaccines may be their suitability for use during pregnancy. This issue is discussed in section 5.6.4 and succeeding subsections of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*29*). Further potentially useful information may be obtained from regional or NRA-specific guidelines. Such guidelines are not specific to DNA vaccines and may apply to a variety of product types, but they do provide guidance on clinical trial designs and labelling issues relevant to immunization during pregnancy. As with any vaccine, careful safety and efficacy evaluation in this vulnerable population is crucially important.

When a specific delivery device has been used to demonstrate the efficacy of a DNA vaccine, the labelling should reflect the device parameters used in the pivotal trial(s), as should the protocols for those trials. The labelling should make it clear to the user that only the authorized device must be used. It will be important to consider how pharmacovigilance plans will capture any off-label use of an alternative device, including needle and syringe delivery. The impact of using alternative devices in terms of vaccine safety and potential reduction or loss of vaccine efficacy should be evaluated. The clinical trial design for the pivotal efficacy trial(s) will be important in terms of whether the control group(s) will have the same device used to deliver the placebo or other type of control (for example, another vaccine). It is important to maintain a double-blinded randomized placebo-controlled trial design to obtain pivotal efficacy data, whenever feasible. However, the appropriateness of using the delivery device with a substance other than the intended candidate vaccine has to be considered, in terms of ethics and risk–benefit considerations. Labelling should be consistent with the requirements of the NRA.

For DNA vaccines against priority pathogens for use during public health emergencies identified by the WHO R&D Blueprint, the following documents may be of relevance and should be consulted:

- An R&D Blueprint for action to prevent epidemics. Plan of action. May 2016 (*89*);
- An R&D Blueprint for action to prevent epidemics. Accelerating R&D and saving lives. Update 2017 (*90*).
- List of Blueprint Priority Diseases;[10] and

[10] See: http://www.who.int/blueprint/priority-diseases/en/

- WHO Target Product Profiles.[11]

As stated above, WHO has now published more than 60 guidelines and recommendations documents for vaccines against specific diseases, and further such documents for DNA vaccines will be considered for development at the appropriate time once disease-specific DNA vaccines near submission for marketing authorization.

Part D. Guidelines for NRAs

D.1 General

The guidance for NRAs and national control laboratories (NCLs) given in the WHO Guidelines for national authorities on quality assurance for biological products (*92*) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (*93*) should be followed. These guidelines specify that no new biological product should be released until consistency of lot manufacturing and quality has been established and demonstrated by the manufacturer. The guidelines 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 or efficacy of DNA vaccines, should be discussed with and approved by the NRA. When marketing authorization for a DNA vaccine against a specific disease is imminent, WHO guidelines specifically for such vaccines may be prepared through the consultative development and adoption process of the WHO Expert Committee on Biological Standardization. For DNA vaccines that target diseases for which other types of vaccines and corresponding guidelines are available, it may be appropriate to consider Part A from these Guidelines and Parts B and C from the disease-specific guidelines in tandem.

For control purposes, the relevant international standards available at the time should be obtained for the purpose of calibration of the national/regional/working standards. The updated full catalogue of WHO International Reference Preparations is available at: http://www.who.int/bloodproducts/catalogue/en/. Until the international/national standard preparation is established, the NRA may obtain the product-specific/working reference to be used for lot release from the manufacturer.

As with any vaccine, consistency of production has been recognized as an essential component in the quality assurance of DNA vaccines. The NRA should carefully monitor production records and quality control test

[11] See: https://www.who.int/research-observatory/analyses/rd_blueprint/en/index3.html

results for clinical lots, as well as for a series of consecutive lots of the vaccine produced using the procedures and control methods that will be used for the marketed vaccine.

D.2 Official release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Guidelines or disease-specific WHO guidelines, as relevant to the product.

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

A lot release certificate signed by the appropriate NRA/NCL official should then be provided if requested by the manufacturing establishment, and should certify that the lot of vaccine in question meets all national requirements and/or Part A of these WHO Guidelines. The certificate should provide sufficient information on the vaccine lot. The purpose of this official national lot release certificate is to facilitate the exchange of vaccines between countries and should be provided to importers of the vaccines. A model NRA/NCL Lot Release Certificate for plasmid DNA vaccines is provided below in Appendix 2.

Authors and acknowledgements

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The resulting draft document was then posted on the WHO Biologicals website for a first round of public consultation from 30 July to 20 September 2019. Comments were received from: Ms T. Cervinkova (*provided the*

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The second draft was prepared by the same drafting group as above, taking into consideration the public comments received, and the discussions and consensus reached during a second WHO informal consultation on the guidelines for evaluation of the quality, safety and efficacy of DNA vaccines (*26*), held in Geneva, Switzerland, 9–10 December 2019 and attended by: Dr E. Abwao, Pharmacy and Poisons Board, Kenya; Dr C. Bae, Ministry of Food and Drug Safety, Republic of Korea; Dr C. Blades, Agência Nacional de Vigilância Sanitária, Brazil; Dr J. Boyer and Dr K.E. Broderick, Inovio Pharmaceuticals, the USA; Dr P. Duffy and Dr J. Ledgerwood, National Institutes of Health, the USA; Dr A. Farnsworth, Health Canada, Canada; Dr J. Gangakhedkar, Central Drugs Standard Control Organisation, India; Dr D. Gutsch and Dr A. Khan, Merck & Co., Inc., the USA (*IFPMA representatives*); Dr R. Hafiz, Saudi Food & Drug Authority, Saudi Arabia; Dr N. Jackson, Coalition for Epidemic Preparedness Innovations, Norway; Dr D. Kaslow, PATH Vaccine Development Global Program, the USA; Dr M.A. Liu, Karolinska Institutet, Sweden; Dr J. Maslow, GeneOne Life Science Inc., the USA; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr E. Nkansah, Food and Drugs Authority, Ghana; Mr Y. Park, GeneOne Life Science Inc., Republic of Korea; Dr A. Patel, the Wistar Institute, the USA; Dr K. Peden, United States Food and Drug Administration, the USA; Dr T. Racine, Université Laval, Canada; Dr N. Rose, National Institute for Biological Standards and Control, the United Kingdom; Dr P. Roy, London School of Hygiene & Tropical Medicine, the United Kingdom; Dr R. Sheets, consultant, the USA; Dr M. Song, International Vaccine Institute, Republic of Korea; Dr W. Wei, Center for Drug Evaluation, China; and Dr H-N. Kang, Dr I. Knezevic and Dr M. Alali, World Health Organization, Switzerland. The resulting document WHO/BS/2020.2380 was then posted on the WHO Biologicals website for a second round of public consultation from 13 May to 13 June 2020.

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References

1. Suschak JJ, Williams JA, Schmaljohn CS. Advancements in DNA vaccine vectors, non-mechanical delivery methods, and molecular adjuvants to increase immunogenicity. Hum Vaccin Immunother. 2017;13(12):2837–48 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5718814/, accessed 18 August 2020).
2. Manickan E, Karem KL, Rouse BT. DNA vaccines – a modern gimmick or a boon to vaccinology? Crit Rev Immunol. 2017;37(2–6):483–98 (abstract: https://pubmed.ncbi.nlm.nih.gov/29773031/, accessed 18 August 2020).

3. Porter KR, Raviprakash K. DNA vaccine delivery and improved immunogenicity. Curr Issues Mol Biol. 2017;22:129–38 (abstract: https://pubmed.ncbi.nlm.nih.gov/27831541/, accessed 18 August 2020).
4. Li L, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. Expert Rev Vaccines. 2016;15(3):312–29 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4955855/, accessed 18 August 2020).
5. Marć MA, Domínguez-Álvarez E, Gamazo C. Nucleic acid vaccination strategies against infectious diseases. Expert Opin Drug Deliv. 2015;12(12):1851–65 (https://www.tandfonline.com/doi/full/10.1517/17425247.2015.1077559, accessed 18 August 2020).
6. Grunwald T, Ulbert S. Improvement of DNA vaccination by adjuvants and sophisticated delivery devices: vaccine-platforms for the battle against infectious diseases. Clin Exp Vaccine Res. 2015;4(1):1–10 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4313101/, accessed 18 August 2020).
7. Tregoning JS, Kinnear E. Using plasmids as DNA vaccines for infectious diseases. Microbiol Spectr. 2014;2(6) (https://www.asmscience.org/content/journal/microbiolspec/10.1128/microbiolspec.PLAS-0028-2014, accessed 18 August 2020).
8. Williams JA. Improving DNA vaccine performance through vector design. Curr Gene Ther. 2014;14(3):170–89 (abstract: https://pubmed.ncbi.nlm.nih.gov/25142448/, accessed 18 August 2020).
9. Wahren B, Liu MA. DNA vaccines: recent developments and the future. Vaccines (Basel). 2014;2(4):785–96 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4494247/, accessed 18 August 2020).
10. Liu MA. DNA vaccines: an historical perspective and view to the future. Immunol Rev. 2011;239(1):62–84 (abstract: https://pubmed.ncbi.nlm.nih.gov/21198665/, accessed 18 August 2020).
11. Ledgerwood JE, Graham BS. DNA vaccines: a safe and efficient platform technology for responding to emerging infectious diseases. Hum Vaccin. 2009;5(9):623–6 (https://www.tandfonline.com/doi/pdf/10.4161/hv.8627, accessed 19 August 2020).
12. Smith TRF, Patel A, Ramos S, Elwood D, Zhu X, Yan J et al. Immunogenicity of a DNA vaccine candidate for COVID-19. Nat Commun. 2020;11(1):2601 (https://www.nature.com/articles/s41467-020-16505-0, accessed 19 August 2020).
13. Pierini S, Perales-Linares R, Uribe-Herranz M, Pol JG, Zitvogel L, Kroemer G et al. Trial watch: DNA-based vaccines for oncological indications. Oncoimmunology. 2017;6(12):e1398878 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5706602/, accessed 19 August 2020).
14. Gottlieb P, Utz PJ, Robinson W, Steinman L. Clinical optimization of antigen specific modulation of type 1 diabetes with the plasmid DNA platform. Clin Immunol. 2013;149(3):297–306 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4800754/, accessed 19 August 2020).
15. Garren H, Robinson WH, Krasulová E, Havrdová E, Nadj C, Selmaj K et al. Phase 2 trial of a DNA vaccine encoding myelin basic protein for multiple sclerosis. Ann Neurol. 2008;63(5):611–20 (abstract: https://onlinelibrary.wiley.com/doi/abs/10.1002/ana.21370, accessed 19 August 2020).
16. Zhu Z, Yu J, Niu Y, Sun S, Liu Y, Saxon A et al. Enhanced prophylactic and therapeutic effects of polylysine-modified Ara h 2 DNA vaccine in a mouse model of peanut allergy. Int Arch Allergy Immunol. 2016;171(3–4):241–50 (https://www.karger.com/Article/FullText/453264, accessed 19 August 2020).

17. Trimble CL, Morrow MP, Kraynyak KA, Shen X, Dallas M, Yan J et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. Lancet. 2015;386(10008):2078–88 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4888059/, accessed 19 August 2020).

18. Maldonado L, Teague JE, Morrow MP, Jotova I, Wu TC, Wang C et al. Intramuscular therapeutic vaccination targeting HPV16 induces T cell responses that localize in mucosal lesions. Sci Transl Med. 2014;6(221):221ra13 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4086631/, accessed 19 August 2020).

19. Tebas P, Kraynyak KA, Patel A, Maslow JN, Morrow MP, Sylvester AJ et al. Intradermal SynCon® Ebola GP DNA vaccine is temperature stable and safely demonstrates cellular and humoral immunogenicity advantages in healthy volunteers. J Infect Dis. 2019;220(3):400–10 (https://academic.oup.com/jid/article/220/3/400/5395966, accessed 19 August 2020).

20. Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines. In: WHO Expert Committee on Biological Standardization: fifty-sixth report. Geneva: World Health Organization; 2007: Annex 1 (WHO Technical Report Series, No. 941; https://www.who.int/biologicals/publications/trs/areas/vaccines/dna/Annex%201_DNA%20vaccines.pdf?ua=1, accessed 18 August 2020).

21. International Health Regulations (2005). Second edition. Geneva: World Health Organization; 2008 (https://www.who.int/ihr/9789241596664/en/, accessed 18 August 2020).

22. Tebas P, Roberts CC, Muthumani K, Reuschel EL, Kudchodkar SB, Zaidi FI et al. Safety and immunogenicity of an anti-Zika virus DNA vaccine – preliminary report. N Engl J Med. 2017 (https://www.nejm.org/doi/pdf/10.1056/NEJMoa1708120, accessed 19 August 2020).

23. Gaudinski MR, Houser KV, Morabito KM, Hu Z, Yamshchikov G, Rothwell RS et al. Safety, tolerability, and immunogenicity of two Zika virus DNA vaccine candidates in healthy adults: randomised, open-label, phase 1 clinical trials. Lancet. 2018;391(10120):552–62 (https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(17)33105-7/fulltext, accessed 19 August 2020).

24. Paules CI, Marston HD, Fauci AS. Coronavirus infections – more than just the common cold. JAMA. 2020;323(8):707–8 (https://jamanetwork.com/journals/jama/fullarticle/2759815, accessed 19 August 2020).

25. Beasley DWC. New international guidance on quality, safety and efficacy of DNA vaccines. NPJ Vaccines. 2020;5:53 (https://www.nature.com/articles/s41541-020-0199-0, accessed 19 August 2020).

26. Sheets R, Kang H, Meyer H, Knezevic I. WHO informal consultation on the guidelines for evaluation of the quality, safety, and efficacy of DNA vaccines. Geneva, Switzerland, December 2019. NPJ Vaccines. 2020;5:52 (https://www.nature.com/articles/s41541-020-0197-2, accessed 19 August 2020).

27. WHO guidelines on nonclinical evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-fourth report. Geneva: World Health Organization; 2005: Annex 1 (WHO Technical Report Series, No. 927; http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/ANNEX%201Nonclinical.P31-63.pdf?ua=1, accessed 18 August 2020).

28. Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines. In: WHO Expert Committee on Biological Standardization: sixty-fourth report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 987; https://www.who.int/biologicals/areas/vaccines/TRS_987_Annex2.pdf?ua=1, accessed 25 November 2019).

29. Guidelines on clinical evaluation of vaccines: regulatory expectations. In: WHO Expert Committee on Biological Standardization: sixty-seventh report. Geneva: World Health Organization; 2017: Annex 9 (WHO Technical Report Series, No. 1004; http://www.who.int/entity/biologicals/expert_committee/WHO_TRS_1004_web_Annex_9.pdf?ua=1, accessed 18 August 2020).

30. Keefer MC, Graham BS, Belshe RB, Schwartz D, Corey L, Bolognesi DP et al. Studies of high doses of a human immunodeficiency virus type 1 recombinant glycoprotein 160 candidate vaccine in HIV type 1-seronegative humans. The AIDS Vaccine Clinical Trials Network. AIDS Res Hum Retroviruses. 1994;10(12):1713–23 (abstract: https://pubmed.ncbi.nlm.nih.gov/7888231/, accessed 19 August 2020).

31. Martin JE, Sullivan NJ, Enama ME, Gordon IJ, Roederer M, Koup RA et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol. 2006;13(11):1267–77 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1656552/, accessed 19 August 2020).

32. Liu MA. Immunologic basis of vaccine vectors. Immunity. 2010;33(4):504–15 (https://www.cell.com/immunity/fulltext/S1074-7613(10)00364-X?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS107476131000364X%3Fshowall%3Dtrue, accessed 19 August 2020).

33. Nabel GJ, Kaslow DC, Ulmer JB, Liu MA. DNA vaccines. In: New generation vaccines. New York (NY): Marcel Dekker; 2010:386–95.

34. Liu MA. Gene-based vaccines: recent developments. Curr Opin Mol Ther. 2010;12(1):86–93 (abstract: https://pubmed.ncbi.nlm.nih.gov/20140820/, accessed 20 August 2020).

35. Enama ME, Ledgerwood JE, Novik L, Nason MC, Gordon IJ, Holman L et al. Phase I randomized clinical trial of VRC DNA and rAd5 HIV-1 vaccine delivery by intramuscular (IM), subcutaneous (SC) and intradermal (ID) administration (VRC 011). PLoS One. 2014;9(3):e91366 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3951381/, accessed 20 August 2020).

36. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med. 2013;369(22):2083–92 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4030634/, accessed 20 August 2020).

37. Churchyard GJ, Morgan C, Adams E, Hural J, Graham BS, Moodie Z et al. A phase IIA randomized clinical trial of a multiclade HIV-1 DNA prime followed by a multiclade rAd5 HIV-1 vaccine boost in healthy adults (HVTN204). PLoS One. 2011;6(8):e21225 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3152265/, accessed 18 August 2020).

38. Leifert JA, Whitton JL. Immune responses to DNA vaccines: induction of CD8 T cells. Madame Curie Biosciences Database [website]. Austin (TX): Landes Bioscience 2000–2013 (https://www.ncbi.nlm.nih.gov/books/NBK6523/, accessed 20 August 2020).

39. Kardani K, Bolhassani A, Shahbazi S. Prime-boost vaccine strategy against viral infections: mechanisms and benefits. Vaccine. 2016;34(4):413–23 (abstract: https://www.sciencedirect.com/science/article/pii/S0264410X15017430?via%3Dihub, accessed 20 August 2020).

40. De Rosa SC, Thomas EP, Bui J, Huang Y, deCamp A, Morgan C et al. HIV-DNA priming alters T-cell responses to HIV-adenovirus vaccine even when responses to DNA are undetectable. J Immunol. 2011;187(6):3391–401 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3180898/, accessed 20 August 2020).

41. Mulligan MJ, Russell ND, Celum C, Kahn J, Noonan E, Montefiori DC et al. Excellent safety and tolerability of the human immunodeficiency virus type 1 pGA2/JS2 plasmid DNA priming vector vaccine in HIV type 1 uninfected adults. AIDS Res Hum Retroviruses. 2006;22(7):678–83 (abstract: https://pubmed.ncbi.nlm.nih.gov/16831092/, accessed 20 August 2020).

42. Jaoko W, Karita E, Kayitenkore K, Omosa-Manyonyi G, Allen S, Than S et al. Safety and immunogenicity study of multiclade HIV-1 adenoviral vector vaccine alone or as boost following a multiclade HIV-1 DNA vaccine in Africa. PLoS One. 2010;5(9):e12873 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2943475/, accessed 20 August 2020).
43. Koup RA, Roederer M, Lamoreaux L, Fischer J, Novik L, Nason MC et al. Priming immunization with DNA augments immunogenicity of recombinant adenoviral vectors for both HIV-1 specific antibody and T-cell responses. PLoS One. 2010;5(2):e9015 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2814848/, accessed 21 August 2020).
44. Liu J, Hellerstein M, McDonnel M, Amara RR, Wyatt LS, Moss B et al. Dose-response studies for the elicitation of CD8 T cells by a DNA vaccine, used alone or as the prime for a modified vaccinia Ankara boost. Vaccine. 2007;25(15):2951–8 (abstract: https://pubmed.ncbi.nlm.nih.gov/17360078/, accessed 21 August 2020).
45. Viegas EO, Kroidl A, Munseri PJ, Missanga M, Nilsson C, Tembe N et al. Optimizing the immunogenicity of HIV prime-boost DNA-MVA-rgp140/GLA vaccines in a phase II randomized factorial trial design. PLoS One. 2018;13(11):e0206838 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6264478/, accessed 21 August 2020).
46. Flatz L, Roychoudhuri R, Honda M, Filali-Mouhim A, Goulet JP, Kettaf N et al. Single-cell gene-expression profiling reveals qualitatively distinct CD8 T cells elicited by different gene-based vaccines. Proc Natl Acad Sci U S A. 2011;108(14):5724–9 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3078363/, accessed 21 August 2020).
47. Honda M, Wang R, Kong WP, Kanekiyo M, Akahata W, Xu L et al. Different vaccine vectors delivering the same antigen elicit CD8+ T cell responses with distinct clonotype and epitope specificity. J Immunol. 2009;183(4):2425–34 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2858449/, accessed 21 August 2020).
48. Barouch DH, McKay PF, Sumida SM, Santra S, Jackson SS, Gorgone DA et al. Plasmid chemokines and colony-stimulating factors enhance the immunogenicity of DNA priming-viral vector boosting human immunodeficiency virus type 1 vaccines. J Virol. 2003;77(16):8729–35 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC167238/, accessed 21 August 2020).
49. Martin JE, Pierson TC, Hubka S, Rucker S, Gordon IJ, Enama ME et al. A West Nile virus DNA vaccine induces neutralizing antibody in healthy adults during a phase 1 clinical trial. J Infect Dis. 2007;196(12):1732–40 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2714735/, accessed 21 August 2020).
50. Ledgerwood JE, Pierson TC, Hubka SA, Desai N, Rucker S, Gordon IJ et al. A West Nile virus DNA vaccine utilizing a modified promoter induces neutralizing antibody in younger and older healthy adults in a phase I clinical trial. J Infect Dis. 2011;203(10):1396–404 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3080891/, accessed 21 August 2020).
51. West Nile shot for horses is first licensed DNA vaccine. Minneapolis (MN): Center for Infectious Disease Research and Policy; 2005 (http://www.cidrap.umn.edu/news-perspective/2005/07/west-nile-shot-horses-first-licensed-dna-vaccine, accessed 22 August 2020).
52. Martin JE, Sullivan NJ, Enama ME, Gordon IJ, Roederer M, Koup RA et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol. 2006;13(11):1267–77 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1656552/, accessed 22 August 2020).
53. Sarwar UN, Costner P, Enama ME, Berkowitz N, Hu Z, Hendel CS et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. J Infect Dis. 2015;211(4):549–57 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4318920/, accessed 22 August 2020).

54. Kibuuka H, Berkowitz NM, Millard M, Enama ME, Tindikahwa A, Sekiziyivu AB et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial. Lancet. 2015;385(9977):1545–54 (abstract: https://pubmed.ncbi.nlm.nih.gov/25540891/, accessed 22 August 2020).

55. Buchbinder SP, Grunenberg NA, Sanchez BJ, Seaton KE, Ferrari G, Moody MA et al. Immunogenicity of a novel Clade B HIV-1 vaccine combination: results of phase 1 randomized placebo controlled trial of an HIV-1 GM-CSF-expressing DNA prime with a modified vaccinia Ankara vaccine boost in healthy HIV-1 uninfected adults. PLoS One. 2017;12(7):e0179597 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5519050/, accessed 22 August 2020).

56. Mori T, Kanda Y, Takenaka K, Okamoto S, Kato J, Kanda J et al. Safety of ASP0113, a cytomegalovirus DNA vaccine, in recipients undergoing allogeneic hematopoietic cell transplantation: an open-label phase 2 trial. Int J Hematol. 2017;105(2):206–12 (abstract: https://pubmed.ncbi.nlm.nih.gov/27796740/, accessed 22 August 2020).

57. Stenler S, Blomberg P, Smith CE. Safety and efficacy of DNA vaccines. Plasmids vs. minicircles. Hum Vaccin Immunother. 2014;10(5):1306–8 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4896608/, accessed 22 August 2020).

58. Dalmo RA. DNA vaccines for fish: review and perspectives on correlates of protection. J Fish Dis. 2018;41(1):1–9 (abstract: https://pubmed.ncbi.nlm.nih.gov/29064091/, accessed 22 August 2020).

59. Houston R, Moxon S, Nogué F, Papadopoulou N, Ramon M, Waigmann E. Assessment of the potential integration of the DNA plasmid vaccine CLYNAV into the salmon genome. EFSA J. 2017;15(1):e04689 (https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4689, accessed 22 August 2020).

60. Weniger BG, Anglin IE, Tong T, Pensiero M, Pullen JK, Nucleic Acid Delivery Devices for HIV Vaccines Workshop Group. Workshop report: nucleic acid delivery devices for HIV vaccines: workshop proceedings, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA, May 21, 2015. Vaccine. 2018;36(4):427–37 (abstract: https://pubmed.ncbi.nlm.nih.gov/29174315/, accessed 22 August 2020).

61. Ake JA, Schuetz A, Pegu P, Wieczorek L, Eller MA, Kibuuka H et al. Safety and immunogenicity of PENNVAX-G DNA prime administered by Biojector 2000 or CELLECTRA electroporation device with modified vaccinia Ankara-CMDR boost. J Infect Dis. 2017;216(9):1080–90 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5853809/, accessed 22 August 2020).

62. Ledwith BJ, Manam S, Troilo PJ, Barnum AB, Pauley CJ, Griffiths TG 2nd et al. Plasmid DNA vaccines: assay for integration into host genomic DNA. Dev Biol (Basel). 2000;104:33–43 (abstract: https://pubmed.ncbi.nlm.nih.gov/11713822/, accessed 22 August 2020).

63. Ledwith BJ, Manam S, Troilo PJ, Barnum AB, Pauley CJ, Griffiths TG 2nd et al. Plasmid DNA vaccines: investigation of integration into host cellular DNA following intramuscular injection in mice. Intervirology. 2000;43(4–6):258–72 (abstract: https://pubmed.ncbi.nlm.nih.gov/11251381/, accessed 22 August 2020).

64. Kim BM, Lee DS, Choi JH, Kim CY, Son M, Suh YS et al. In vivo kinetics and biodistribution of a HIV-1 DNA vaccine after administration in mice. Arch Pharm Res. 2003;26(6):493–8 (abstract: https://pubmed.ncbi.nlm.nih.gov/12877561/, accessed 22 August 2020).

65. Pilling AM, Harman RM, Jones SA, McCormack NAM, Lavender D, Haworth R. The assessment of local tolerance, acute toxicity, and DNA biodistribution following particle-mediated delivery of a DNA vaccine to minipigs. Toxicol Pathol. 2002;30(3):298–305 (abstract: https://pubmed.ncbi.nlm.nih.gov/12051546/, accessed 22 August 2020).

66. Bureau MF, Naimi S, Torero Ibad R, Seguin J, Georger C, Arnould E et al. Intramuscular plasmid DNA electrotransfer: biodistribution and degradation. Biochim Biophys Acta. 2004;1676(2):138–48 (abstract: https://pubmed.ncbi.nlm.nih.gov/14746908/, accessed 22 August 2020).

67. Wang Z, Troilo PJ, Wang X, Griffiths TG II, Pacchione SJ, Barnum AB et al. Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation. Gene Ther. 2004;11:711–21 (https://www.nature.com/articles/3302213, accessed 22 August 2020).

68. Manam S, Ledwith BJ, Barnum AB, Troilo PJ, Pauley CJ, Harper LB et al. Plasmid DNA vaccines: tissue distribution and effects of DNA sequence, adjuvants and delivery method on integration into host DNA. Intervirology. 2000;43(4–6):273–81 (abstract: https://pubmed.ncbi.nlm.nih.gov/11251382/, accessed 22 August 2020).

69. Sheets RL, Stein J, Manetz TS, Duffy C, Nason M, Andrews C et al. Biodistribution of DNA plasmid vaccines against HIV-1, Ebola, Severe Acute Respiratory Syndrome, or West Nile virus is similar, without integration, despite differing plasmid backbones or gene inserts. Toxicol Sci. 2006;91(2):610–9 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2377020/, accessed 22 August 2020).

70. Sheets RL, Stein J, Manetz TS, Andrews C, Bailer R, Rathmann J et al. Toxicological safety evaluation of DNA plasmid vaccines against HIV-1, Ebola, Severe Acute Respiratory Syndrome, or West Nile virus is similar despite differing plasmid backbones or gene inserts. Toxicol Sci. 2006;91(2):620–30 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2366098/, accessed 22 August 2020).

71. Ulmer JB, Deck RR, DeWitt CM, Donnelly JJ, Liu MA. Generation of MHC class I-restricted cytotoxic T lymphocytes by expression of a viral protein in muscle cells: antigen presentation by non-muscle cells. Immunology. 1996;89(1):59–67 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1456656/, accessed 22 August 2020).

72. Fu TM, Ulmer JB, Caulfield MJ, Deck RR, Friedman A, Wang S et al. Priming of cytotoxic T lymphocytes by DNA vaccines: requirement for professional antigen presenting cells and evidence for antigen transfer from myocytes. Mol Med. 1997;3(6):362–71 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2230213/, accessed 22 August 2020).

73. Morrow MP, Tebas P, Yan J, Ramirez L, Slager A, Kraynyak K et al. Synthetic consensus HIV-1 DNA induces potent cellular immune responses and synthesis of granzyme B, perforin in HIV infected individuals. Mol Ther. 2015;23(3):591–601 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4351468/, accessed 22 August 2020).

74. Roy MJ, Wu MS, Barr LJ, Fuller JT, Tussey LG, Speller S et al. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. Vaccine. 2000;19(7–8):764–78 (abstract: https://pubmed.ncbi.nlm.nih.gov/11115698/, accessed 22 August 2020).

75. Gorse GJ, Newman MJ, deCamp A, Hay CM, De Rosa SC, Noonan E et al. DNA and modified vaccinia virus Ankara vaccines encoding multiple cytotoxic and helper T-lymphocyte epitopes of human immunodeficiency virus type 1 (HIV-1) are safe but weakly immunogenic in HIV-1-uninfected, vaccinia virus-naive adults. Clin Vaccine Immunol. 2012;19(5):649–58 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3346329/, accessed 22 August 2020).

76. Guidance on the use of international nonproprietary names (INNs) for pharmaceutical substances. Geneva: World Health Organization; 2017 (https://www.who.int/medicines/services/inn/FINAL_WHO_PHARM_S_NOM_1570_web.pdf?ua=1, accessed 22 August 2020).

77. Robertson JS, Chui WK, Genazzani AA, Malan SF, de la Rica Manjavacas AL, Mignot G et al. The INN global nomenclature of biological medicines: a continuous challenge. Biologicals. 2019;60:15–23 (https://www.sciencedirect.com/science/article/pii/S104510561930048X?via%3Dihub, accessed 22 August 2020).

78. WHO good manufacturing practices for pharmaceutical products: main principles. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-eighth report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 986; http://www.who.int/medicines/areas/quality_safety/quality_assurance/TRS986annex2.pdf?ua=1, accessed 18 August 2020).

79. WHO good manufacturing practices for biological products. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 2 (WHO Technical Report Series, No. 999; http://www.who.int/biologicals/areas/vaccines/Annex_2_WHO_Good_manufacturing_practices_for_biological_products.pdf?ua=1, accessed 18 August 2020).

80. Guidelines on procedures and data requirements for changes to approved vaccines. In: WHO Expert Committee on Biological Standardization: sixty-fifth report. Geneva: World Health Organization; 2015: Annex 4 (WHO Technical Report Series, 993; http://www.who.int/biologicals/vaccines/Annex4_Guidelines_changes_to_approved_vaccines_eng.pdf?ua=1, accessed 18 August 2020).

81. WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products. Geneva: World Health Organization; 2003 (http://www.who.int/biologicals/publications/en/whotse2003.pdf, accessed 18 August 2020).

82. Deletion of the innocuity/abnormal toxicity test for biological products. In: WHO Expert Committee on Biological Standardization: sixty-ninth report. Geneva: World Health Organization; 2019 (WHO Technical Report Series, No. 1016, section 3.1.3, pp. 32–33; https://www.who.int/biologicals/expert_committee/WHO_TRS_1016_web.pdf?ua=1, accessed 23 June 2020).

83. Guidelines on stability evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-seventh report. Geneva: World Health Organization; 2011: Annex 3 (WHO Technical Report Series, No. 962; http://www.who.int/biologicals/vaccines/Annex_3_WHO_TRS_962-3.pdf?ua=1, accessed 18 August 2020).

84. Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-fifth report. Geneva: World Health Organization; 2011: Annex 9 (WHO Technical Report Series, No. 961; http://www.who.int/medicines/areas/quality_safety/quality_assurance/ModelGuidanceForStorageTransportTRS961Annex9.pdf, accessed 18 August 2020).

85. Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 5 (WHO Technical Report Series, No. 999; http://www.who.int/biologicals/areas/vaccines/Annex_5_Guidelines_on_Stability_evaluation_vaccines_ECTC.pdf?ua=1, accessed 18 August 2020).

86. Sheets RL, Stein J, Bailer RT, Koup RA, Andrews C, Nason M et al. Biodistribution and toxicological safety of adenovirus type 5 and type 35 vectored vaccines against human immunodeficiency virus-1 (HIV-1), Ebola, or Marburg are similar despite differing adenovirus serotype vector, manufacturer's construct, or gene inserts. J Immunotoxicol. 2008;5(3):315–35 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2777703/, accessed 20 August 2020).

87. Tuomela M, Malm M, Wallen M, Stanescu I, Krohn K, Peterson P. Biodistribution and general safety of a naked DNA plasmid, GTU-MultiHIV, in a rat, using a quantitative PCR method. Vaccine. 2005;23(7):890–6 (abstract: https://pubmed.ncbi.nlm.nih.gov/15603889/, accessed 20 August 2020).

88. Sheng-Fowler L, Tu W, Fu H, Murata H, Lanning L, Foseh G et al. A mouse strain defective in both T cells and NK cells has enhanced sensitivity to tumor induction by plasmid DNA expressing both activated H-Ras and c-Myc. PLoS One. 2014;9(10):e108926 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4193875/, accessed 20 August 2020).

89. An R&D Blueprint for action to prevent epidemics. Plan of action. May 2016. Geneva: World Health Organization; 2016 (http://www.who.int/blueprint/about/r_d_blueprint_plan_of_action.pdf?ua=1, accessed 20 August 2020).

90. An R&D Blueprint for action to prevent epidemics. Accelerating R&D and saving lives. Update 2017. Geneva: World Health Organization; 2017 (Document WHO/HIS/HIA/17.5; http://www.who.int/blueprint/about/brochure-2017.pdf?ua=1, accessed 20 August 2020).

91. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. In: WHO Expert Committee on the Use of Essential Drugs: sixth report. Geneva: World Health Organization; 1995: Annex 3 (WHO Technical Report Series, No. 850; https://www.who.int/publications/i/item/92-4-120850-3, accessed 18 August 2020).

92. Guidelines for national authorities on quality assurance for biological products. In: WHO Expert Committee on Biological Standardization: forty-second report. Geneva: World Health Organization; 1992: Annex 2 (WHO Technical Report Series, No. 822; http://www.who.int/biologicals/publications/trs/areas/biological_products/WHO_TRS_822_A2.pdf, accessed 18 August 2020).

93. Guidelines for independent lot release of vaccines by regulatory authorities. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 2 (WHO Technical Report Series, No. 978; http://www.who.int/biologicals/TRS_978_Annex_2.pdf?ua=1, accessed 18 August 2020).

Appendix 1

Model summary protocol for the manufacturing and control of plasmid DNA vaccines

The following provisional protocol is intended for guidance and indicates the minimum information that should be provided by the manufacturer to the NRA or NCL after the vaccine product has been granted a marketing authorization. The protocol is not intended to apply to material intended for clinical trials. Information and tests may be added or omitted as necessary with the approval of the NRA or NCL.

Since the development of plasmid DNA vaccines was incomplete at the time of publication of the current document, their detailed requirements had not yet been finalized. Consequently, only the essential requirements are provided in this appendix. Information and tests may be added or omitted as necessary (if adequate justification is provided) to ensure alignment with the marketing authorization approved by the NRA or NCL. It is therefore possible that a protocol for a specific product will differ in detail from the model provided here. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO guidance on a particular product should be provided 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 (package insert) 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 (see Appendix 2) from the NRA or NCL of the country in which the vaccine was produced and/or released stating that the product meets all national requirements as well as Part A of these WHO Guidelines.

1. Summary information on final lot

International name of product: _____

INN (if applicable): _____

Commercial/trade name: _____

Product licence (marketing authorization) number: _____

Country: _____

Name and address of manufacturer: _____

Name and address of licence holder, if different: _____

Plasmid designation (if applicable): _____

Gene insert(s) (if applicable): _____

Final packaging lot number: _____
Type of container: _____
Number of containers in this final lot: _____
Number of doses per final container: _____
Preservative and nominal concentration (if applicable): _____

Summary of the composition (*summary of the qualitative and quantitative composition of the vaccine, including any adjuvant used and other excipients*):

_____

_____

Shelf-life approved (months): _____
Date of manufacture: _____
Expiry date: _____
Storage conditions: _____

2. Control of source material

2.1 Plasmid seeds (where applicable)

2.1.1 Seed banking system

Name and identification of plasmid(s): _____
Origin of all genetic components (if applicable): _____
Construction of plasmid DNA vaccine: _____
Nucleotide sequence of the transgene and plasmid backbone: _____
Antigenic analysis, copy number, yield (in vitro/in vivo): _____

_____

_____

Seed bank genealogy with dates of preparation, passage number and date of coming into operation: _____

_____

Tests for contaminating bacteria, fungi (for plasmid seeds): _____
Details of animal (including human) components of any reagents used in the manufacture of seed banks, including culture medium: _____

_____

Genetic stability at the level of a plasmid pre-master seed or plasmid master seed to its sequence at, or preferably beyond, the anticipated maximum passage level: _____

_____

Confirmation of approval for use by manufacturer, and the basis for that approval: _____

_____

2.1.2 Tests on working seed lot production (if applicable)

Antibiotic resistance (if applicable): _____

Marker genes or selection genes (if applicable and different from antibiotic resistance gene): _____

Identity: _____

Bacterial and fungal contamination: _____

2.2 Cultures and culture media (where applicable)

2.2.1 Cell bank system

Name and identification of cell strain and bank: _____

Culture medium: _____

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: _____

Test for absence of bacterial and fungal contamination: _____

Details of animal (including human) components of any reagents used in manufacture of cell banks, including culture medium: _____

2.2.2 Tests on working cell bank production (if applicable)

Identification of cell bank: _____

Culture medium: _____

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: _____

Test for absence of bacterial and fungal contamination: _____

Details of animal (including human) components of any reagents used in manufacture of cell banks, including culture medium: _____

Genetic stability (if genetically manipulated): _____

3. Control of vaccine production

3.1 Control of purified plasmid bulk (for each monovalent plasmid, if applicable)

3.1.1 Information on manufacture

Batch number(s): _____

Date of manufacture of each batch: _____

Identification of reagents used during production or other phases of manufacture, including media components and antibiotics, if applicable): _____

_____

Total volume of purified plasmid bulk: _____

Volume(s), storage temperature, storage time and approved storage period: _____

_____

3.1.2 Tests on purified plasmid bulk(s)

Identity: _____

Purity: _____

Antigen content (quantity): _____

Physical state (that is, % supercoiled): _____

Sterility (bacterial and fungal): _____

_____

Residual levels of reagents used during production or other phases of manufacture, including media components and antibiotics, if applicable): _____

Residual protein content: _____

Residual DNA derived from the expression system: _____

Residual RNA: _____

Endotoxins: _____

Only if not feasible on final vaccine due to its multi-component formulation, potency (expression of mRNA or protein): _____

3.2 Control of final bulk (where applicable)

3.2.1 Information on manufacture

Lot number(s): _____

Date of formulation: _____

Total volume of final bulk formulated: _____

Monovalent bulk plasmid(s) used for formulation: _____

Volume(s), storage temperature, storage time and approved storage period: _____
Lot number/volume added: _____
Name and concentration of added substances (for example, adjuvants, facilitators, etc., if applicable): _____

_____

3.2.2 **Tests on final bulk or final containers, as applicable**

Identity: _____
Purity: _____
Antigen content (quantity): _____
Physical state (that is, % supercoiled): _____
Sterility (bacterial and fungal): _____
Endotoxins: _____
Potency (expression of mRNA or protein): _____

4. Filling and containers

Lot number: _____
Date of filling: _____
Type of container: _____
Volume of final bulk filled: _____
Filling volume per container: _____
Number of doses, if the product is presented in a multi-dose 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 (expiry dating): _____
Storage temperature: _____

5. Control tests on final vaccine lot

Inspection of final containers: _____
Identity: _____
Appearance: _____
pH (if applicable): _____
Osmolality (if applicable): _____
Sterility (bacterial and fungal): _____

Preservative (if applicable): _____
Residual moisture content (for freeze-dried product): _____
Endotoxin: _____
Adjuvant content (if applicable): _____
Potency: _____
Expression of heterologous antigen in vitro (mRNA or protein): _____
Purity: _____
Extractable volume (if applicable): _____
Residual antibiotics (if applicable): _____

6. Certification by the manufacturer

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

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

I certify that lot no. _____ of [name of] plasmid DNA vaccine, whose number appears on the label of the final containers, meets all national requirements and satisfies Part A[12] of the WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines[13] and Part A of any relevant disease-specific WHO guidance.

Signature _____
Name (typed) _____
Date _____

7. Certification by the NRA/NCL

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

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

[13] WHO Technical Report Series, No. 1028, Annex 2.

Appendix 2

Model NRA/NCL Lot Release Certificate for plasmid DNA vaccines

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

Certificate no. _____

The following lot(s) of [name of] plasmid DNA vaccine produced by _____[14] in _____,[15] whose numbers appear on the labels of the final containers, meet all national requirements[16] and Part A[17] of the WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines[18] and Part A of the relevant disease-specific WHO guidelines and comply with WHO good manufacturing practices for pharmaceutical products: main principles,[19] WHO good manufacturing practices for biological products,[20] and the WHO Guidelines for independent lot release of vaccines by regulatory authorities.[21]

The release decision is based on _____[22]

Final lot number _____
Number of human doses released in this final lot _____
Expiry date _____

The certificate may also include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;

[14] Name of manufacturer.

[15] Country of origin.

[16] 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 or NCL.

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

[18] WHO Technical Report Series, No. 1028, Annex 2.

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

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

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

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

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

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

Signature _____
Name (typed) _____
Date _____