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Geneva, 24 – 28 August 2020

**Guidelines for assuring the quality, safety, and efficacy of plasmid DNA
vaccines**

Proposed revision of Annex 1 of WHO Technical Report Series, No. 941

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed revision of *Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines, Annex 1, WHO Technical Report Series No. 941* to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the ECBS. Written comments proposing modifications to this text MUST be received by 13 June 2020 using the Comment Form available separately and should be addressed to:

Department of Health Products Policy and Standards (HPS), World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland. Comments may also be submitted electronically to the Responsible Officer: Dr Hye-Na Kang at kangh@who.int.

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

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Guidelines for assuring the quality, safety, and efficacy of plasmid DNA vaccines

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Appendix 1. Model summary protocol for the manufacturing and control of plasmid DNA vaccines

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

9
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11



1 **Abbreviations**

2

3 DCVMN Developing Countries Vaccine Manufacturers Network

4 DNA deoxyribonucleic acid

5 ECBS Expert Committee on Biological Standardization

6 GCP good clinical practice

7 GMO genetically modified organism

8 GMP good manufacturing practice(s)

9 HPLC high-performance liquid chromatography

10 HPV human papillomavirus

11 IHNV infectious hematopoietic necrosis virus

12 INN International Nonproprietary Name

13 IU International Unit(s)

14 MCB master cell bank

15 MERS Middle East Respiratory Syndrome

16 NRA national regulatory authority

17 PCR polymerase chain reaction

18 RNA ribonucleic acid

19 SARS Severe Acute Respiratory Syndrome

20 WCB working cell bank

21 WNV West Nile virus

22 WHO World Health Organization

23

1 Introduction

2

3 Vaccination involves stimulating the immune system of an individual with an infectious
4 agent or components of an infectious agent but modified in a manner to ensure that the
5 vaccine does not cause undue harm or disease to the vaccinee. Further, efficacious
6 vaccination ensures that when the individual is confronted with that infectious agent, his/her
7 immune system can respond adequately to control the invading organism before it causes
8 overt disease. For over a hundred years, vaccination has been achieved by one of two basic
9 approaches:

10

- 11 • introducing attenuated microorganisms that replicate within the vaccinee without
12 causing disease and synthesize the appropriate immunogens that subsequently
13 stimulate the immune system; or
- 14 • introducing into a vaccinee pathogen-specific antigens against which the immune
15 system will react directly.

16

17 Since the 1990s, a novel approach to vaccination against a broad array of target antigens and
18 diseases has been in development. This technology involves the direct introduction of
19 plasmid deoxyribonucleic acid (DNA) containing the gene encoding the immunogen against
20 which an immune response is sought into the vaccinee for the *in-situ* production of the target
21 immunogen(s). This is referred to as plasmid DNA vaccines or DNA vaccines. This approach
22 offers a combination of potential advantages, including the stimulation of both B and T-cell
23 responses, stability of the vaccine across a broad temperature range, absence of infectivity of
24 the immunogen itself, the speed with which the vaccine can be constructed (for example in
25 the face of an epidemic or pandemic), and the relative ease and generic nature of large-scale
26 manufacture. It may be feasible to produce the same DNA vaccine in different facilities in
27 different geographies to facilitate accessibility and availability of the vaccine during routine
28 immunization or outbreak settings, ensuring a more stable supply of vaccine. Further,
29 plasmid DNA is stable, and as such can be stored and delivered efficiently and effectively to
30 rural settings without requiring a cold chain (depending on the formulation). DNA vaccines
31 do not generate anti-vector immunity nor off-target acquired immunity to DNA in the
32 vaccinee. There is no need for biosafety containment as DNA vaccines are not designed to be
33 infectious and the target infectious pathogen is not used in the construction or production of
34 the vaccine. Integration of the vector into the DNA of the vaccinee is not observed. DNA
35 vaccines can be viewed as a platform technology in which the gene insert can be readily
36 changed without necessarily having to change the manufacture or control of the resulting new
37 product (except for immunogen-specific tests for identity and potency). Many scientific
38 publications address the potential of DNA vaccination (1-10).

39

40 Immune responses in animal models have been obtained using genes from a variety of
41 infectious agents including influenza virus, hepatitis B virus, human immunodeficiency virus,
42 human papillomaviruses, Marburg virus, Middle East Respiratory Syndrome (MERS)
43 coronavirus, rabies virus, Severe Acute Respiratory Syndrome (SARS) virus, West Nile virus

1 (WNV), Zika virus, plasmodia, mycoplasma, and others (10,11). In many cases, protection
2 from disease in animal models has also been demonstrated, and many properties of the
3 immune response generated by the delivery of plasmid DNA vaccines have been
4 demonstrated in animal models. In addition to infectious diseases, plasmid DNAs have been
5 studied in clinical trials for cancer as well as autoimmune and allergic diseases, such as
6 peanut allergy (12-18). Development of plasmid DNA for therapy for HPV infection is
7 subject of clinical investigations in humans and is another example of the application of this
8 technology. The value and advantages of plasmid DNAs need to be assessed on a case-by-
9 case basis; their utility will depend on the nature of the organism being vaccinated against or
10 the targeted disease, the nature of the immunogen or activity of the gene-insert, the type of
11 immune response required for effectiveness, and the delivery system and route of
12 administration.

13
14 The development and application of DNA vaccines continues to evolve. Since the WHO
15 *Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines* were
16 adopted by the Expert Committee for Biological Standardization (ECBS) in 2005 (19), many
17 clinical trials of DNA vaccines have taken place, and much experience in their manufacture
18 and control has accrued. This revision reflects the experience gained, especially in relation to
19 the data derived from nonclinical and clinical safety testing, which address many of the
20 concerns expressed in the prior versions of these guidelines. The control of these vaccines
21 should continue to be approached in a flexible manner to enable further modifications as
22 more experience is gained in their production and use, and as other components or delivery
23 systems are included. The intention is to provide a scientifically sound basis for the consistent
24 manufacture and control of these vaccines for use in humans to ensure their continued safety
25 and efficacy following licensure. Given the potential of DNA vaccination as a platform
26 technology to address priority pathogens of public health emergencies (20-23), the need for
27 international regulatory convergence for DNA vaccines is urged. This document provides up-
28 to-date guiding principles for evaluation of quality, safety and efficacy of DNA vaccines for
29 human use. It is worth noting that while plasmid DNAs are generated through recombinant
30 DNA (rDNA) technology, guidelines specific to rDNA products generally do not apply to
31 DNA vaccines, as those guidelines are intended to cover the manufacture of biotherapeutic
32 proteins generated in cell lines (or cells such as yeast).

34 **Background**

35
36 DNA vaccines are able to generate functional antibodies and both CD4+ and CD8+ T-cell
37 responses. The ability to generate MHC-Class I restricted CD8+ T cells (cytolytic T
38 lymphocytes), which generally are not induced following administration of proteins or
39 inactivated viruses, may be important for key responses against certain pathogens, as well as
40 enabling cross-strain specific responses when many antibody responses are strain-specific.
41 Because the encoded protein is synthesized *in vivo* by the vaccinee following administration,
42 DNA vaccines can encode membrane-bound proteins, such as full-length HIV Env gp160,
43 instead of solely the soluble versions, such as gp120 (24). This can be important because key

1 neutralizing epitopes (including broadly neutralizing epitopes against more than one strain of
2 HIV) are located in protein regions that would be excluded, or not formed in a monomeric
3 truncated soluble version. Unlike certain other vectors (such as viral vectors that may be used
4 in a heterologous prime-boost regimen with DNA vaccines), DNA vaccines do not stimulate
5 adaptive immune responses against the vector (plasmid backbone), although the DNA itself
6 can stimulate certain innate immune responses (25). In other words, they do not generate anti-
7 vector immunity, which could blunt antigen-specific responses following multiple
8 administrations.

9
10 DNA vaccines would seem ideal for use in boosting immune responses, as they could be used
11 repeatedly (and for different purposes) because they do not generate anti-vector immune
12 responses. However, existing data demonstrate that DNA vaccines seem to excel for priming
13 immune responses. These primed immune responses are boosted by delivery of a
14 heterologous vaccine such as a protein antigen or a different gene-based vector, such that the
15 resulting immune responses are often more potent than if either modality is used alone for
16 both prime and boost, or in the reverse order with the DNA given last (26-33). In some cases,
17 the priming immune response from DNA vaccines is only revealed once a heterologous boost
18 is administered (34, 35). The responses to the heterologous boost may be amplified compared
19 with giving a homologous regimen of the booster vaccine (36-39). The DNA prime can also
20 modulate the type of immune response observed following the heterologous boost, in
21 comparison with the booster vaccine alone (32, 35, 39). In other cases, robust responses to
22 the DNA vaccine alone can be observed (40). Clearly, the nature of the immune response
23 depends on the immunogen expressed and immunomodulatory elements in the design or
24 formulation of the DNA vaccine, as well as the method of delivery (41). Evaluation of the
25 contribution of the DNA vaccine to the immunogenicity of any given vaccination regimen
26 may be best assessed by the ultimate immune responses of the regimen as a whole in
27 comparison with a regimen not including DNA vaccination. This is not to suggest that
28 immune responses to the plasmid DNA prime should not also be evaluated, but rather that the
29 priming response may be best assessed in the context of the boosted response.

30
31 DNA vaccines have progressed as far as Phase IIb pilot efficacy trials. It is anticipated that
32 some candidate vaccines will proceed to Phase III clinical testing. The strong immune
33 responses observed in animal models have generally not been reproduced in humans, with a
34 few exceptions, including a DNA vaccine for WNV that generated neutralizing antibody in
35 humans at titres that are known to be protective in horses, and good titres were also produced
36 in elderly humans, who generally have suboptimal immune responses to vaccines; a WNV
37 DNA vaccine was licensed for horses in 2005 (42-44). Likewise, immune responses were
38 observed in clinical trials of DNA vaccines for Ebola/Marburg, although a different gene-
39 based vector was advanced that required only a single vaccination vs. 3 administrations
40 needed for the DNA vaccines (45-47).

41
42 Many approaches have been tested and are being evaluated to enhance the immune response
43 in humans. These approaches have different intended mechanisms of increasing

1 immunogenicity and improving efficacy, such as by enhancing cellular uptake, strengthening
2 expression, modulating the immune response towards a more favourable profile, or
3 optimizing adjuvant effects. Several examples are provided below:

4
5 Optimization of the vector itself:

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

15
16 Optimization of the formulation/delivery:

- 17 • complexing the DNA with polymers (to enhance uptake, to improve stability after
18 administration and uptake);
- 19 • encapsulating the DNA on or within microparticles (to assist uptake, presentation and
20 stability after administration and uptake);
- 21 • optimizing administration, e.g. by particle-mediated delivery (gene gun), CO₂ or air
22 injector (jet injector), or electroporation (to enhance uptake);
- 23 • changing the route of administration, e.g. mucosal versus parenteral (to modulate the
24 immune response);
- 25 • boosting with viral vectors or protein antigen following an initial priming with
26 plasmid DNA (to boost and/or modulate immune responses); and
- 27 • co-administering DNA encoding an immune stimulatory molecule (molecular
28 adjuvant), e.g. a cytokine (to enhance immune response, to modulate the immune
29 response).

30
31 Other approaches may be under development now or developed in the future. The above
32 approaches to enhancing the efficacy of a DNA vaccine may raise specific safety concerns
33 and these should be addressed in appropriate nonclinical and clinical safety studies. To date,
34 published data from clinical trials indicate that DNA vaccines are safe, with acceptable
35 reactogenicity profiles (11, 22, 29, 45, 48-50). Whether approaches that result in enhanced
36 expression will increase reactogenicity remains an open question.

37
38 DNA vaccines have been developed for veterinary use, and efficacy in animal target species
39 has been observed in some trials. Potentially protective immune responses have been
40 observed against many infectious agents in several target species including fish, companion
41 animals, and farm animals. Although the quality and safety considerations for veterinary
42 vaccines may differ from those for human use, experience with veterinary DNA vaccines can
43 provide valuable information for the control and use of human DNA vaccines. A DNA

1 vaccine against WNV, which generates protective antibody responses, for use in horses was
2 licensed in the USA in 2005. A DNA vaccine against infectious hematopoietic necrosis
3 virus (IHNV), which affects both trout and salmon, was licensed in 2005 in Canada for use in
4 salmon, and more recently a DNA vaccine against pancreas disease for use in salmon was
5 licensed in 2016 in several countries and currently is in use in farmed salmon (51). This
6 vaccine was evaluated for integration or long-term persistence in salmon, and the risk was
7 found to be “orders of magnitude lower than the upper estimated integration rate calculated in
8 the context of the worst-case scenarios” (52, 53).

9
10 The revised guidelines in this document focus on the quality control of vaccines based on
11 biologically manufactured bacterial plasmid DNA intended for use in humans. Nonclinical
12 and clinical aspects are also briefly described, in that general principles that apply to other
13 vaccines also apply to DNA vaccines, so only notable differences or additions are discussed.
14 The purpose of this document is to provide guidance on:

- 15
- 16 • appropriate methods for the control of the manufacture and characterization of
17 plasmid DNA vaccines;
- 18 • appropriate approaches for the nonclinical and clinical testing of plasmid DNA
19 vaccines; and
- 20 • information specific to plasmid DNA vaccines that may be expected to be included in
21 submissions by manufacturers to national regulatory authorities (NRAs) in support of
22 applications for the authorization of clinical trials and for marketing/licensure.
- 23

24 The main changes compared with the Guidelines published in WHO Technical Report Series,
25 No. 941, Annex 1 (19), include the following:

- 26
- 27 • Updating the introduction with additional data including citations for nonclinical and
28 clinical data that collectively address many historical safety concerns;
- 29 • Revising the scope to preventive DNA vaccines against infectious diseases;
- 30 • Updating the quality section (Part A) to be more consistent with current practices and
31 existing WHO guidelines;
- 32 • Extensively revising the nonclinical section (Part B) to reference more general WHO
33 guidelines that have been adopted since the prior version and to focus on a few
34 specific issues;
- 35 • Adding a clinical section (Part C) that also references a recently revised general WHO
36 guideline and to focus on a few specific issues;
- 37 • Adding a Part D for specific guidance to NRAs; and
- 38 • Adding sections on the model summary protocol for the manufacturing and control of
39 plasmid DNA vaccines (Appendix 1) and the model NRA lot release certificate for
40 plasmid DNA vaccines (Appendix 2).
- 41

42 **Purpose and scope**

43

1 This document provides guidance on quality, nonclinical, and clinical aspects of DNA
2 vaccines (including plasmids encoding adjuvant molecules, if present) intended for use in
3 humans to prevent infectious diseases. Plasmid DNA vaccines intended for veterinary use fall
4 outside the scope of these guidelines.

5
6 The active constituent of a DNA vaccine is a DNA plasmid(s) into which gene(s) encoding
7 the desired immunogen(s) is (are) inserted and prepared in purified plasmid preparations to
8 be administered *in vivo*. Typically, these plasmids possess DNA sequences necessary for
9 selection and replication in bacteria. In addition, they contain eukaryotic promoters and
10 enhancers as well as transcription termination/polyadenylation sequences to promote gene
11 expression in vaccine recipients and may contain immunomodulatory elements. In these
12 guidelines, vaccines are defined as biological medicines for the prevention of infectious
13 diseases.

14
15 Although plasmid DNA products developed for therapeutic use against diseases such as
16 cancer (where plasmids may encode a viral or tumour antigen, as well as immunomodulatory
17 proteins), autoimmune, or allergic diseases are not in the scope of these guidelines,
18 manufacture and quality control of plasmid DNA for these indications may be essentially
19 identical. Consequently, the quality section of these guidelines may be applicable to DNA
20 plasmids for therapeutic as well as preventive use. Likewise, use of plasmid DNA to express
21 monoclonal antibodies for preventive, post-exposure prophylaxis, or therapeutic purposes are
22 outside the scope of this guideline, but the quality section (Part A) may be applicable. The
23 detailed design of relevant nonclinical and clinical testing should consider the proposed use
24 of the DNA plasmid(s) and the risk–benefit situation. Plasmid DNA for use in gene therapy,
25 plasmid DNA derived in eukaryotic cells, viral replicons, bacterial cells acting as a carrier for
26 a plasmid DNA encoding a relevant antigen, and nucleic acid vaccines made entirely by
27 chemical means are all outside the scope of these guidelines.

28
29 The revised guidelines are unlikely to be applicable to vaccines based on RNA vaccines
30 because different requirements are likely to apply for quality, nonclinical, and clinical testing
31 for these types of vaccines and immunotherapeutics.

32
33 In general, recommendations in these guidelines are relevant to the DNA vaccine at the time
34 of application for marketing authorization. Nevertheless, some relevant information is
35 provided in these guidelines with respect to candidate vaccine products in development;
36 otherwise, the respective NRA should be consulted prior to clinical development on a case-
37 by-case basis (54-56).

38
39 Acknowledgement is made that products that blur the lines between viral vectors, cell
40 therapy, and nucleic-acid vaccines are likely to emerge, e.g. RNA replicons. Other
41 developments that complicate the regulatory evaluation of nucleic-acid vaccines are also
42 foreseen; e.g. self-amplifying molecules. However, at the present time, these remain outside
43 the scope of these guidelines.

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Terminology

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

Adjuvants: substances that are intended to enhance relevant immune response and subsequent clinical efficacy of the vaccine (55).

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

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

Candidate vaccine: candidate vaccine is a vaccine that is regarded in national regulations as separate and distinct from other candidate and licensed vaccines (56).

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. The following terms are used in these Guidelines: master cell bank (MCB), which is 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. And working cell bank (WCB), which is a cell bank derived by propagation of cells from an MCB under defined conditions and used to initiate production of cell cultures on a lot-by-lot basis; a WCB is also referred to “as manufacturer’s working cell bank” in other documents.

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

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

5
6 **Formulated bulk:** an intermediate in the drug product manufacturing process, consisting of
7 the final formulation of antigens, adjuvants and excipients at the concentration to be filled
8 into primary containers.

9
10 **Good manufacturing practice (GMP):** a system that ensures that products are consistently
11 produced and controlled to the quality standards appropriate to their intended use and as
12 required by the marketing authorization.

13
14 **Heterologous prime-boost:** DNA vaccines are often given with another vaccine, such as a
15 viral-vectored vaccine or a protein subunit vaccine, in a combination regimen consisting of
16 heterologous prime-boost. In other words, one vaccine is given in a priming dose series and a
17 different vaccine (or a combination of the two) is administered as a booster.

18
19 **Immunogenicity:** the capacity of a vaccine to elicit a measurable immune response.

20
21 **Marketing authorization:** a formal authorization for a medicine (including vaccines) to be
22 marketed. Once an NRA approves a marketing authorization application for a new medicine,
23 the medicine may be marketed and may be available for physicians to prescribe and/or for
24 public health use (also referred to as product licensing, product authorization, or product
25 registration).

26
27 **Plasmid:** a circular, extrachromosomal bacterial DNA element that undergoes autonomous
28 replication in bacterial cells. It usually carries a few genes, some of which confer resistance
29 to various antibiotics or other selection markers; such resistance or selection marker is used to
30 discriminate between organisms that contain the plasmid and those that do not.

31 32 **General considerations**

33
34 The guidelines cover DNA vaccines noting that the method or specific device to deliver the
35 vaccine (e.g. injector, electroporator) may be integral to achieving efficacy. In the case of
36 requiring a specific device, other delivery methods may not be interchanged, unless justified
37 (57, 58). Product labelling information for the vaccine will need to take this into account.
38 Regulatory pathways for licensure of vaccine in context of delivery device may vary by
39 regulatory jurisdiction, and early discussions with NRA(s) are advised. If the plasmid DNA
40 vaccine is to be marketed along with a novel device (e.g. combination products or Combi -
41 kit), the NRA shall decide the regulatory requirements for seeking approval marketing
42 authorization best suited for the needs of their country. In some jurisdictions, the vaccine and
43 device taken together may be considered to be a combination product, with a defined

1 regulatory pathway for marketing authorization. Whatever the regulatory approach in the
2 jurisdiction in which marketing authorization is being sought, it is important to recognize that
3 the marketing authorization should reflect the device (and device parameters) used to deliver
4 the candidate vaccine during the pivotal efficacy trial(s) and for which there is a sufficiently
5 large safety database. Further, if there is more than one vaccine in the regimen and they are
6 produced by different manufacturers, it will be important to consult with the NRA(s) for
7 guidance on how best to proceed for licensure or marketing authorization.

8
9 Formulation may be crucial to the safety and effectiveness of any vaccine, but for DNA
10 vaccines, when a transfectant, facilitator, adjuvant, or plasmid-encoded adjuvant (e.g.
11 cytokine gene) is included in the formulation, special attention should be given to the
12 formulation that is demonstrated to be safe and efficacious in the pivotal efficacy and/or large
13 safety trials.

14
15 The current generation of DNA vaccines made from bacteria are produced biologically and
16 are considered to be a biological product. While the plasmid is generated by recombinant
17 DNA technology, it should be clarified that a plasmid DNA vaccine is not an organism; thus,
18 it is not a genetically modified organism (GMO) per se, nor is it a gene-transfer or gene-
19 therapy product. There is a wealth of evidence that DNA vaccines to date do not persist or
20 even biodistribute throughout the body of the vaccinee when delivered parenterally into
21 muscle, subcutaneous tissue, or various dermal layers (59-67). What predominantly
22 biodistributes is the immune response generated, following uptake of the plasmid DNA and
23 *in situ* expression of the immunogen(s), along with cross-priming from myocytes to
24 professional antigen-presenting cells (68,69). The local response to plasmid DNA inoculation
25 is that cells either take up the plasmid and then express the immunogen(s) encoded in the
26 DNA vaccine and/or the nucleic acid is degraded by normal molecular mechanisms. As a
27 consequence, the plasmid DNA clears from the injection site over time. It is the immune
28 response that may persist.

29
30 The quality section of these guidelines addresses the control of the bulk purified plasmid,
31 including control of the manufacturing process and the starting materials, characterization
32 and control of the purified plasmid, control of the final formulated vaccine including
33 formulation, control of materials used in formulation, and stability of the bulk purified
34 plasmid and the final formulated vaccine. The appropriate use of reference materials and
35 international standards is also described. Whenever changes to the manufacturing process are
36 implemented, comparability of lots, especially with those used in pivotal studies and the
37 commercial process, should be demonstrated.

38
39 The nonclinical and clinical sections of these guidelines reference existing general WHO
40 guidelines (54-56) and also address some issues that may apply to DNA vaccines more than
41 to other types of vaccines. This revision includes a clinical section for the first time. The
42 nonclinical section has been made more succinct in light of additional data on the concerns
43 that were initially raised before there was such extensive nonclinical and clinical experience

1 with DNA vaccines. The existing nonclinical and clinical databases taken as a whole support
2 the conclusion that prior concerns about integration, autoimmunity, and immunopathology
3 have not been observed (59-67). To date, based on clinical experiences, the reactogenicity
4 observed relate more to the delivery method than to the DNA vaccine itself, notably with
5 electroporation or with particle-mediated bombardment (1, 4, 21, 25-31, 70-72).

6
7 The control, nonclinical testing, and clinical development of each vaccine should be
8 considered individually, and any special features of that candidate vaccine should be taken
9 into account. Consultation with the NRA(s) is useful to assure efficient development of any
10 given candidate vaccine.

11 **Part A. Guidelines on manufacturing and control of plasmid DNA vaccines**

12 **A.1 Definitions**

13 **A.1.1 International name and proper name**

14 The international name should be “plasmid DNA vaccine”. The proper name should
15 be the equivalent to the international name in the language of the country of origin.

16 The use of the international name should be limited to vaccines that meet the specifications
17 elaborated below. Defined recombinant nucleic acids used as active substances in vaccines,
18 whether of biological or synthetic origin, could be assigned an International Nonproprietary
19 Name (INN) upon request (73, 74).

20 **A.1.2 Descriptive definition**

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

27 **A.2 General manufacturing guidelines**

28 Plasmid DNA vaccines are considered to be similar to bacterial and viral vaccines produced
29 by traditional methods, where adequate control of the starting materials and manufacturing
30 process is as important as that of the final product. The guidelines therefore place
31 considerable emphasis on the control strategy of the manufacturing process of the vaccine as
32 well as on comprehensive characterization and batch and lot release of the bulk and the
33 vaccine itself.

34 The general manufacturing requirements contained in good manufacturing practices (GMP)
35 for pharmaceutical products: main principles (75) and biological products (76) should apply
36 to the design, establishment, operation, control and maintenance of manufacturing facilities
37 for DNA vaccines. The requirements should also apply to the vaccine filled in the final form,
38 records, retained samples for future studies and needs, labelling, distribution and transport, as
39

1 well as storage and expiry date for DNA vaccines (75, 76). Quality control during the
2 manufacturing process relies on the implementation of quality systems, such as GMP, to
3 ensure the production of consistent commercial vaccine lots with product characteristics
4 similar to those of lots shown to be safe and effective in clinical trials. Throughout the
5 process, a number of in-process control tests should be established (with acceptable limits) to
6 allow quality to be monitored for each lot from the beginning to the end of production. It is
7 important to note that while most release specifications are product-specific, DNA vaccines,
8 as a product class with shared characteristics, tend to meet product class-specific
9 specifications for many release parameters. Whatever the case, these should be agreed with
10 the NRA(s) as part of the clinical trial or marketing authorization.

11
12 DNA vaccines for use in clinical trials should also be prepared under GMP conditions
13 suitable for the stage of clinical development (i.e. full compliance may not be possible in
14 initial or early development when manufacturing and control procedures remain in
15 development and may not be validated yet; however, it would be expected that validated
16 procedures would be used in early development if they are procedures shared with other
17 DNA vaccines made in that facility that have attained higher phases of development in which
18 the procedures have been already validated). Appropriate attention needs to be given to the
19 quality of all reagents used in production, including the components of fermentation medium.
20 Particular attention to the sourcing of components of animal or human derivation is required.
21 Many of the general requirements for the quality control of biological products, such as tests
22 for endotoxin, stability and sterility, also apply to DNA vaccines.

23
24 Particular attention should be given to DNA vaccines prepared in multi-use facilities, as
25 would be typical in initial or early clinical development. Cleaning validation would be
26 expected even in early development for such multi-use facilities, even though such validation
27 would normally occur later (though prior to commercial production) in a dedicated facility.
28 One novel aspect particular to DNA vaccines is that cleaning procedures need to be verified
29 with an assay sensitive enough to detect not only microorganisms and other biological
30 materials that may be residual from prior manufacture, but also residual full-length plasmid
31 DNA from prior lots of products made using the same equipment and facility. This issue of
32 carry-over or potential for cross-contamination is a specific concern because of the amounts
33 (often, mg quantities) at which DNA vaccines are administered, which may vary from other
34 products made using the same equipment or in the same facility due to formulation and
35 delivery method. Manufacturers also should define the methods they carry out to prevent
36 cross-contamination.

37
38 It is recognized that the level of detail required by a regulatory authority increases as product
39 development proceeds. During the initial phases of clinical development, the information
40 contained in a clinical trial application should be adequate to allow an assessment of the
41 safety risks derived from the manufacturing process. This would include, for example, testing
42 of the cell banks for identity, identification and specifications for all materials used in the
43 process, assessment of risks from biologically-sourced materials, certification or phase-

1 appropriate GMP compliance of the manufacturing facility, a brief description of the process
2 and tests, results of testing of the clinical trial material and preliminary stability of the final
3 product. As with all vaccines, for late-stage clinical trials, the level of detail on the Quality
4 (manufacturing and controls) expected would increase.

5
6 Changes made to the product composition (e.g. addition of adjuvant or preservatives) or
7 manufacture (process, site or scale) during the development of clinical lots should be
8 adequately described. Depending on how the final product composition is changed, e.g.
9 addition of novel excipients, new preclinical studies might be warranted. For changes to the
10 manufacturing process such as scale-up or change to the purification process, comparability
11 of the clinical trial material with the one from previous processes should be evaluated. The
12 comparability studies might include immunogenicity data from animal models, results from
13 physico-chemical analyses, process and product-related impurity studies, and stability data
14 (77).

15 16 **A.3 Manufacture and control of bulk purified plasmid (bulk)**

17 **A.3.1 General information and description of manufacturing process and process** 18 **controls**

19 A brief overview of the development and manufacture of the plasmid(s) should include a
20 justification for the selection of the gene(s) of interest, other gene(s) encoded in the plasmid
21 (e.g. selection markers or antibiotic resistance gene), and regulatory elements used. Any gene
22 expression optimization modifications should be described. The sequence of the complete
23 plasmid should be provided.

24 25 **A.3.2 Manufacture**

26 **A.3.2.1 Control of materials**

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

31
32 Reference to internationally accepted pharmacopoeias or details on the specifications should
33 be provided.

34 35 ***A.3.2.1.1 Control of source and starting materials of biological or animal origin***

36 Information regarding the source, manufacture and characterization of all biologically-
37 sourced materials or materials that may have used biological materials during manufacture
38 should be provided. Risk assessment for bovine spongiform encephalopathy agents should be
39 provided if bovine materials were used at any stage.

40 41 ***A.3.2.1.2 Source, history and generation of the host cell and plasmid***

1 Information should be provided on the bacterial host cell including its source, phenotype and
2 genotype. Particular attention should be given when using a host cell that is a novel strain or
3 species of bacterium, including to the types of toxins they may express.
4

5 The complete nucleotide sequence of the plasmid DNA vaccine should be provided together
6 with appropriate annotation indicating the important elements, such as the
7 promoters/enhancers, termination sequences, drug-resistance or other marker for selection in
8 bacteria, and bacterial origin of replication. In most cases, it is likely that the gene for the
9 immunogen will be optimized for expression and synthesized chemically before being
10 recombined into the plasmid DNA. As such, the gene will have a novel sequence and not be
11 present in any database. In contrast, if the gene is obtained from other sources such as
12 amplification from a natural element by PCR, the source of that material should be provided.
13

14 As part of characterization, a DNA sequence homology check of the plasmid with the
15 international databases (e.g. the National Center for Biotechnology Information, National
16 Institutes of Health, USA, and/or other international nucleotide databases) should be
17 performed to investigate the presence of unintended sequences of biological significance,
18 such as those encoding cellular growth factors, other known immunogens, or viral sequences.
19

20 The identity of the plasmid after transformation into the bacterial cell to be used for
21 production should be confirmed. While sequencing is preferred, representative restriction
22 enzyme maps may also be useful. The candidate vaccine selected to advance in clinical
23 development and for marketing authorization should be demonstrated to be genetically stable.
24

25 ***A.3.2.1.3 Cell banking system, characterization and testing***

26 The production of a plasmid DNA vaccine should be based ideally on a cell bank system
27 involving an MCB and a WCB.
28

29 For early stage clinical trials, it may be appropriate to use the MCB to initiate production,
30 although manufacturers are expected to prepare a WCB for later clinical studies. Ideally,
31 initiating production from a well-characterized WCB is expected for commercial
32 manufacturing.
33

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

1
2 The DNA sequence of the entire plasmid should be confirmed at the stage of the MCB and/or
3 WCB.

4
5 The genetic stability of the plasmid should be confirmed by characterizing the size and
6 complete sequence.

7 8 **A.3.2.2 Process development and in-process control**

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

12
13 Validation of the manufacturing process should demonstrate reproducible and consistent
14 clearance of process and product-related contaminants to levels acceptable for the intended
15 use in humans.

16
17 Process validation is not generally required for a product used in early-stage clinical trials
18 although critical steps such as aseptic processing, sterility of final product and cleaning
19 validation - particularly when multi-product facilities or contract manufacturing organizations
20 are used for the manufacturing - should be validated or carefully and convincingly controlled
21 prior to initiation of clinical development.

22 23 **A.3.3 Characterization**

24 **A.3.3.1 Characterization of bulk purified plasmid**

25 A summary of the characterization of the bulk purified plasmid(s) should be provided in
26 addition to in-process and lot-release testing. Rigorous characterization by chemical, physical
27 and biological methods will be essential paying particular attention to the use of a range of
28 analytical techniques based on different principles.

29
30 During development, the sequence of the entire plasmid should be determined, as discussed
31 in sections A.2, A.3.2.1.2, and A.3.2.1.3 above.

32
33 The immunogenicity elicited by the DNA vaccine should be characterized. Whenever other
34 immunomodulatory elements or genes are included, their contribution to the mode-of-action
35 (immunogenicity) of the DNA vaccine should also be determined in order to justify their
36 inclusion.

37
38 Potential impurities in the purified product should be described and investigated. These
39 impurities include residual host cell proteins, endotoxins, residual host cell RNA and
40 chromosomal DNA, materials used in the manufacturing process and medium components.
41 Data should be provided on the contaminants present in the purified plasmid, with estimates
42 of their maximum acceptable or lowest achievable levels. For contaminants and residuals
43 with known or potential toxic effects, a toxicological risk assessment is expected. Degraded

1 plasmid DNA may be assessed as part of analytical procedures such as polyacrylamide gel
2 electrophoresis, high performance liquid chromatography and/or capillary electrophoresis. An
3 important characteristic of the purified bulk plasmid to determine is the degree to which the
4 plasmid remains supercoiled or has been partially converted to relaxed circles or linear forms.
5

6 **A.3.3.2 Consistency of manufacturing**

7 Prior to seeking marketing authorization, a number of consecutive batches should be
8 characterized and analysed by employing validated methods to determine consistency of
9 manufacture. Any differences between one batch and another outside the accepted range for
10 the parameters tested should be noted. The data obtained from such studies, as well as
11 clinical-trial outcomes with various lots, should be used as the basis for justification of the
12 chosen specifications.
13

14 During early-stage development, few lots will have been made, and demonstration of
15 consistency may be limited. Demonstration of consistency will occur as manufacturing
16 experience is gained during product development. Characterization of consistency of lots is
17 generally done either during advanced development, when the manufacturing process has
18 been scaled up for commercial manufacture but prior to submission of a licence or marketing
19 application. Whenever changes to the manufacturing process are implemented, comparability
20 of lots, especially to those used in pivotal studies and the intended commercial process,
21 should be demonstrated. Comparability protocols and strategies for demonstrating
22 comparability are discussed in the WHO *Guidelines on procedures and data requirements for*
23 *changes to approved vaccines (77)*.
24

25 **A.3.4 Control of bulk purified plasmid**

26 Specifications for critical quality attributes for the identity, purity, quality, and safety of the
27 bulk purified plasmid should be established and justified. Descriptions of analytical methods
28 used and acceptance limits defined, including assay validation information, should be
29 provided. A summary of the results of testing of all batches produced at commercial scale
30 should be provided.
31

32 Early in development, results from testing batches made in accordance with GMP and, if
33 available, engineering runs performed to establish manufacturing procedures should be
34 summarized and provided.
35

36 It is recommended that the specifications for the bulk purified plasmid include, at a
37 minimum, an assessment of the identity, purity, physical state and quantity of the plasmid,
38 endotoxin content and sterility or bioburden. A justification of the specifications should be
39 provided.
40

41 Early in development, the specifications may be limited and have somewhat wide acceptance
42 criteria. Not all the tests conducted during product characterization need to be carried out on
43 each batch of vaccine. Some tests are required only to establish the validity or acceptability of

1 a procedure, whereas others might be performed on a limited series of batches to establish
2 consistency of production. Thus, a comprehensive analysis of the initial commercial
3 production batches should be undertaken to establish consistency with regard to identity,
4 purity, quality, safety, and stability; thereafter, a limited series of tests may be appropriate.
5

6 **A.3.4.1 Identity**

7 The identity of each batch should be confirmed by an appropriate means; such as PCR
8 analysis, sequencing, restriction enzyme analysis, *in vitro* expression (mRNA or protein) of
9 the gene insert of the plasmid accompanied by confirmation of the identity of the expressed
10 antigen.
11

12 **A.3.4.2 Purity**

13 Limits based on process capability and regulatory guidance should be established for all
14 impurities detected, and these should be identified and characterized as appropriate. The
15 degree of contamination with chromosomal DNA, RNA and proteins should be evaluated and
16 limits established, and the acceptance criteria should be established and specified.

17 Comparison of the absorbance at 260 nm and 280 nm may be useful for purity assessment,
18 such as the extent of contamination introduced by RNA and cellular proteins. Residual levels
19 of medium components (including antibiotics, if applicable) and other materials from process
20 steps should also be controlled. The analysis should include sensitive and reliable assays for
21 process- and product-related contaminants and strict upper limits should be specified for their
22 content in the bulk purified plasmid. A maximum allowable limit should be established and
23 justified. It is important that the techniques used to demonstrate purity be based on as wide a
24 range of physicochemical properties as possible. Measuring residual levels of process- or
25 product-related impurities as part of quality control may be discontinued after processes have
26 been adequately validated for their suitable removal. Container-closure system compatibility,
27 leachables and extractables should be assessed and discussed in the application.
28

29 Where multiproduct facilities or contract manufacturing organizations are used for the
30 manufacturing process, freedom from contamination with other products, especially other
31 DNA plasmids made in the same facility, should be demonstrated to be below defined limits.
32

33 **A.3.4.3 Physical state, quantification of plasmid**

34 The proportion of supercoiled plasmid should be determined and specifications set.
35 Quantification of the plasmid amount is usually by absorbance at 260 nm. Any additional
36 quality parameters relevant to the bulk purified plasmid should also be determined and
37 specifications set, e.g. pH or viscosity might be important for certain products, to ensure
38 stability and quality at the bulk purified plasmid stage.
39

40 **A.3.4.4 Safety**

41 Relevant safety tests should be described. These may include: tests for endotoxins and/or
42 pyrogens, a test for bacterial and fungal sterility including demonstration of lack of

1 bactericidal or fungicidal activity of the test article or a test for bioburden including quantity,
2 identification, and freedom from objectionable organisms.

4 **A.3.5 Reference materials**

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

12
13 In early development, use of an engineering run batch or a batch used to produce the lot of
14 DNA vaccine used in the pivotal nonclinical studies may be used until a suitable clinical trial
15 lot has been identified and characterized for use in advanced development and commercial
16 manufacture.

18 **A.3.6 Stability**

19 The stability assessment should be in compliance with the WHO *Guidelines for Stability*
20 *Evaluation of Vaccines* (78). The types of studies conducted, the protocols used, and the
21 results of the studies should be summarized in an appropriate format such as tables, graphs or
22 a narrative document. The summary should include results as well as conclusions with
23 respect to appropriate storage conditions or shelf-life. Data on stability to support the shelf-
24 life of the bulk and any future extension of it should be based on long-term, real-time stability
25 studies under actual conditions.

26
27 Limited stability information would be expected during initial clinical development. For
28 example, some regulators accept three months of real-time stability at the time of application
29 for clinical trial authorization, but this should be agreed with the NRA. Lots should be
30 labelled with a re-test or re-pass date, if required by the NRA.

32 **A.4 Manufacture and control of final formulated vaccine (vaccine)**

33 **A.4.1 Composition**

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

38 **A.4.2 Manufacture**

39 A flow chart should be provided that illustrates the manufacturing steps from the bulk
40 purified plasmid to the final formulated vaccine. The chart should include all steps (i.e. unit
41 operations), identification of materials and in-process and quality control tests. In some cases,
42 this may involve simple dilution of the purified bulk; in other cases, a more complex
43 formulation may be envisaged including combining purified bulks of more than one plasmid.

1 A narrative description of each process step depicted in the flow chart should be provided.
2 Information should be included on, for example, scale, buffers and other additives, major
3 equipment, and process controls, including in-process tests and critical process operational
4 parameters with acceptance criteria.
5

6 **A.4.3 Control of materials**

7 Details of excipients, including adjuvants, or any other component of the container-closure
8 system of the vaccine in addition to the plasmid constituting the immunogen should be
9 provided, including their source, specification, method of conjugation, if appropriate, and
10 final concentration in the vaccine.
11

12 **A.4.4 Control of final formulated vaccine**

13 Specifications for the vaccine should be established and justified. Descriptions of analytical
14 methods and acceptance limits for the vaccine, including information on assay validation
15 should be provided. It is recommended that the specifications include an assessment of the
16 identity, purity, physical state and quantity of the plasmid, any other relevant quality
17 parameters, potency, endotoxin content and sterility. A justification of the specifications
18 should be provided.
19

20 Early in development, the specifications may be limited with wide acceptance criteria.

21 A summary of the results of the testing on all lots produced at commercial scale should be
22 provided. Early in development, results from testing lots made in accordance with GMP, and
23 if available, engineering runs performed to establish manufacturing procedures should be
24 summarized and provided.
25

26 The appropriateness of performing tests on the bulk purified plasmid versus the formulated
27 vaccine should be considered on a case-by-case basis and justified.
28

29 When more than one plasmid is contained in the final formulation, it may not be readily
30 achieved to distinguish the potency of one plasmid from another. In such cases, assessing in
31 vitro expression on each bulk purified plasmid may be performed to establish the potency of
32 the final formulation. In other words, the potency of the final product may be inferred and
33 calculated from the potency of each of the plasmids contained, if the potency of each plasmid
34 cannot be distinguished from another in the final product. However, if there is an adjuvant or
35 facilitator in the final formulation that may alter the potency of the individual plasmids; this
36 approach may not be reliable.
37

38 Several consecutive lots of vaccine, in final dosage form, should be characterized and
39 analysed by employing validated methods to determine manufacturing consistency. Any
40 differences between one lot and another should be noted. The data obtained from such
41 studies, as well as clinical trial outcomes with various lots, should be used as the basis for
42 defining the vaccine specifications and acceptance criteria to be used for routine lot release.
43

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

8 9 **A.4.4.1 Identity**

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

14 15 **A.4.4.2 Purity**

16 The purity of each lot of vaccine should be determined and be shown to be within specified
17 limits. The form of the final product plasmid(s) should be confirmed; e.g. gel electrophoresis
18 or other method to demonstrate that the vaccine has not degraded should be conducted.
19 Container-closure system compatibility, leachables and extractables should be assessed and
20 discussed.

21
22 Where multiproduct facilities or contract manufacturing organizations are used for the
23 manufacturing process, freedom from contamination with other products should be
24 demonstrated to established limits or below detection.

25 26 **A.4.4.3 Content, Strength, or Quantity**

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

30 31 **A.4.4.4 Other quality parameters**

32 Quality parameters should be established and controlled. Important quality parameters
33 include appearance and pH. Another important quality parameter is the percentage of
34 supercoiled plasmid to overall amount of plasmid (which may be present in other forms such
35 as nicked circles or linear). Depending on the product characteristics, control of other
36 parameters such as osmolality or viscosity may be important. Further, quality may be
37 assessed by methods used to evaluate purity or identity, such as restriction mapping, gel or
38 capillary electrophoresis, and/or high-performance liquid chromatography (HPLC) though
39 these may best be performed on the purified bulk plasmid instead. Other tests, such as the test
40 for residual moisture if the vaccine is lyophilized, may be required to confirm the physical
41 characteristics of the product as well as the formulation.

42 43 **A.4.4.5 Potency**

1 The potency of each lot of the vaccine should be determined using a suitably quantitative and
2 validated assay. It is preferable that potency relative to an appropriate in-house reference
3 preparation be established. It is preferable that a potency assay be established that can be
4 correlated to functional activity. Often, this takes the form of a quantitative *in vitro*
5 expression system. The immunogen might be expressed *in vitro* by transfection of a suitable
6 cell line and either the expressed mRNA or the expressed protein identified, for example, by
7 quantitative RT-PCR (in the case of mRNA) or by immunofluorescence or Western blot (in
8 the case of protein). It may be appropriate to establish potency on the basis of an alternative
9 suitably justified laboratory method (i.e. a non-bioassay). Early discussion with the NRA(s)
10 to gain consensus regarding appropriateness of a proposed method should be undertaken.

11
12 When multiple plasmids are included in the final formulation, the potency of each
13 immunogen encoded should be assessed. However, if this cannot be determined at the stage
14 of the final formulation, it may be necessary to assess potency at the stage of the individual
15 plasmid prior to inclusion in the final formulation (see discussion in Sections A.4.4 and
16 A.4.4.7).

17
18 When a cell-based potency assay is used, it is important to control the cells used by banking
19 to ensure a consistent supply of cells for testing. Furthermore, the cells should be assessed for
20 freedom from adventitious agents, mycoplasma/spiroplasma (the latter, if relevant),
21 bacteria/fungi, and mycobacteria (if relevant) and only suitably controlled cells used.

22 23 **A.4.4.6 Safety, including sterility and endotoxin testing**

24 Each lot of vaccine should be tested for sterility. If the vaccine is to be administered by a
25 non-parenteral route, omission of the sterility test and inclusion of an appropriate alternative
26 bioburden test needs to be appropriately justified. Further, a test for endotoxin should be
27 conducted on each lot, and appropriate specifications should be defined. This may be
28 determined by use of the monocyte activation test. If required by an NRA, a test for
29 pyrogenicity may be performed; however, animal testing should be avoided whenever
30 alternative satisfactory testing is allowed. For ethical reasons, it is desirable to apply the 3Rs
31 concept of “Replace Reduce Refine” to minimize the use of animals in research, and
32 consideration should be given to the use of appropriate *in vitro* alternative methods for safety
33 evaluation. Particularly, the test known as the innocuity, abnormal toxicity, or general safety
34 test should not be required nor requested.

35 36 **A.4.4.7 Multi-component vaccines**

37 Additional factors must be considered when more than one plasmid forms the final
38 formulated vaccine. Plasmids in multi-component vaccines may encode additional antigens
39 or cytokines or other biologically active molecules that enhance the efficacy or affect the
40 safety of the vaccine. For each plasmid, the development overview, the control of production
41 and the characterization of the bulk purified plasmid must be described as above. Likewise,
42 for multi-component DNA vaccines that contain components (e.g. immunomodulatory
43 molecules or cytokine proteins) in addition to the plasmid(s), the role of the additional

1 components should be addressed. Careful consideration has to be given to the control of the
2 final formulated vaccine. For example, potency may depend on the combination of plasmids
3 and their interaction and not on any single plasmid component of a multicomponent vaccine.
4

5 On the other hand, it may not be feasible to measure potency in the context of a mixture of
6 closely related antigens and potency of the individual plasmids may have to be measured as
7 expression in the individual purified bulk plasmids. Clarity on the approach taken and its
8 justification should be described.
9

10 **A.4.5 Reference materials**

11 A suitable lot of the final formulated vaccine, or batch of bulk purified plasmid, that has been
12 clinically evaluated, should be fully characterized in terms of its chemical composition, purity
13 and biological activity, including full sequencing, and retained for use as a chemical and
14 biological reference material. This material should be used as the basis for evaluation of
15 product quality for commercial production lots.
16

17 In future, international standards (IS), expressed in International Units (IU), may be prepared
18 by a WHO collaborating centre. When such IS become available, it will be important to
19 compare the internal reference material with the IS, so that IU may be assigned and in order
20 to fully validate quality control tests or assays. In this manner, comparisons can be made in a
21 more reliable and less variable way whenever new reference materials are needed to be
22 prepared.
23

24 Likewise, IS may be useful for interpretation of nonclinical and clinical assays of immune
25 responses or other biomarkers of relevance to the DNA vaccine under development or being
26 evaluated for marketing authorization (also see the WHO guidelines listed in Parts B and C
27 for further guidance on this issue).
28

29 **A.5 Records**

30 The recommendations given in the WHO *Good manufacturing practices for pharmaceutical*
31 *products: main principles (75)* should apply, as appropriate to the level of development of the
32 candidate vaccine.
33

34 **A.6 Retained samples**

35 A sufficient number of samples should be retained for future studies and needs. Vaccine lots
36 that are to be used for clinical trials may serve as a reference material in the future, and a
37 sufficient number of vials should be reserved and stored appropriately for that purpose.
38

39 **A.7 Labelling**

40 The labelling recommendations provided in the WHO *Good manufacturing practices for*
41 *biological products (76)* should be followed as appropriate. The label of the carton enclosing
42 one or more final containers, or the leaflet accompanying the container, should include, at a
43 minimum, as agreed with the NRA:

- 1 • the name of the vaccine;
- 2 • a statement that specifies the nature and content of adjuvant contained in one human
- 3 dose, if any;
- 4 • the immunization schedule, and the recommended route(s) of administration;
- 5 • the number of doses, if the product is issued in a multiple-dose container;
- 6 • the name and concentration of any preservative added;
- 7 • a statement on the nature and quantity, or upper limit, of any antibiotics present in the
- 8 vaccine;
- 9 • the temperature recommended during storage and transport;
- 10 • the expiry/retest date;
- 11 • any special dosing schedules;
- 12 • contraindications, warnings and precautions, and information on concomitant vaccine
- 13 use and on adverse events.

14

15 **A.8 Distribution and transport**

16 The recommendations provided in the WHO *Good manufacturing practices for biological*
17 *products (76)* appropriate for a candidate vaccine should apply. Shipments should be
18 maintained within specified temperature ranges, as applicable, and packages should contain
19 cold-chain monitors, if temperature requirements need to be controlled (79). If claiming that
20 the cold-chain is not required, then the conditions under which stability has been established
21 (e.g. temperature highs and maximum length of time) should be described and data
22 supporting these claims provided.

23

24 **A.9 Stability testing, storage and expiry date**

25 The recommendations given in *Good manufacturing practices for biological products (76)*
26 and in the *Guidelines on stability evaluation of vaccines (78)* appropriate for the respective
27 plasmid DNA vaccine should apply. Furthermore, the *Guideline on the stability evaluation of*
28 *vaccines for use under extended controlled temperature conditions* might apply (80). The
29 statements concerning storage temperature and expiry date that appear on the primary and
30 secondary packaging should be based on experimental evidence and should be submitted to
31 the NRA for approval.

32

33 **A.9.1 Stability**

34 Adequate stability studies form an essential part of vaccine development. The stability of the
35 final product in the container proposed for use should, therefore, be determined and the
36 results used to set a shelf life under appropriate storage conditions. Parameters that might be
37 stability-indicating should be measured. These may include parameters such as appearance,
38 quantity, and percentage super-coiled. The parameters to be measured should be described
39 and specifications defined. Real-time stability studies should be undertaken for this purpose,
40 but accelerated stability studies at elevated temperatures may provide complementary
41 supporting evidence for the stability of the product and confirm the stability-indicating nature
42 of the assays used to determine stability. Container-closure system compatibility for storage
43 stability, including leachables and extractables should be assessed and discussed. The

1 stability assessment should comply with WHO *Guideline for Stability Evaluation of Vaccines*
2 (78).

4 **A.9.2 Storage conditions**

5 The vaccine should not be stored for a length of time and at a temperature greater than that
6 shown by the manufacturer to be compatible with a minimal loss of potency before being
7 distributed by the manufacturing establishment or before being issued from a storage site.

8 The maximum duration of storage should be fixed with the approval of the NRA, based on
9 results of stability studies, and should be such as to ensure that all quality specifications for
10 the final product, including the minimum potency specified on the container or package, are
11 maintained until the end of shelf life. During clinical trials, this period should ideally be at
12 least equal to the expected duration of vaccine administration in the clinical trial.

14 **A.9.3 Expiry date**

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

20 **Part B. Nonclinical evaluation of plasmid DNA vaccines**

21
22 The nonclinical evaluation of the candidate vaccine should be considered on a product-
23 specific basis taking into account the intended clinical use of the product. The selection of
24 appropriate studies relating to the toxicology and pharmacology (proof-of-concept) of the
25 product may be determined from either or both of the following WHO guidelines:

- 27 • WHO *Guidelines on the Nonclinical Evaluation of Vaccines* (54)
- 28 • WHO *Guidelines on the Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted*
29 *Vaccines* (55)

30
31 One issue of relevance for DNA vaccines would be the case when a plasmid component
32 encodes a cytokine or other immunomodulatory protein. In such cases, the choice of animal
33 model selected for nonclinical evaluations may need to consider the species specificity of any
34 biological activity of the product. It may be necessary to conduct proof-of-concept studies
35 with species-relevant analogues to the human-specific product to be developed. Toxicological
36 evaluations, including immunotoxicity, may be performed with the human-specific product
37 and/or the analogue, and concurrence from the NRA(s) should be sought in this matter.

38
39 Another issue that may be relevant to DNA vaccines would be their use in heterologous
40 prime-boost regimens. When there are no pre-existing nonclinical or clinical data on the
41 individual vaccines in the regimen (or, at least, the DNA vaccine component of the regimen),
42 the nonclinical program may be the same or similar to the existing guidelines. However,
43 when there is significant clinical experience with each vaccine in the regimen expressing the

1 same or related immunogens (e.g. other viral envelope proteins, other influenza
2 hemagglutinins, limited modifications in amino acid sequences between new candidate and
3 previously tested candidate), it is expected that the nonclinical program could be abbreviated
4 (81). The existing clinical experience would be more informative to the safety and
5 performance of the vaccine components in a combined regimen than would be animal data. It
6 might be suitable to assess certain safety parameters within an immunogenicity (or challenge-
7 protection) study of the new vaccine regimen(s) to determine whether the safety profile
8 appears similar in animals as previous studies performed on the DNA vaccine plasmid
9 backbone expressing a related immunogen. This approach is consistent with the principles of
10 the 3Rs to refine, reduce, or replace the use of animals in product safety testing, whenever
11 suitable alternative methods are available.

12

13 Likewise, for new DNA vaccines based on existing plasmid backbones for which there is
14 already significant nonclinical (and possibly clinical) experience, an abbreviated nonclinical
15 program should be considered (21, 22, 66, 67). If the new gene insert is related to other
16 antigens that have already been studied nonclinically or clinically, a case may be made to
17 support a safe starting dose and regimen for the new vaccine based on the existing nonclinical
18 and clinical data without need for additional toxicology studies.

19

20 In the situation of rapid development of a vaccine against priority pathogens for public health
21 emergencies, consideration may be given to an abbreviated nonclinical program as follows:
22 In the case where the plasmid is constructed from a backbone that has already been tested
23 clinically with a related antigen (e.g. in the case of a pandemic influenza strain when a
24 seasonal or other potential pandemic strain antigen has been tested), then the nonclinical
25 program might be limited to an immunogenicity study(ies). However, that/those studies
26 should collect as much safety data as feasible given that many nonclinical immunogenicity
27 studies are performed without full compliance to Good Laboratory Practices. Depending on
28 the species used, if it is feasible to collect blood not only for immunogenicity, but also for
29 hematology and chemistry assessments, these analyses should be performed. In addition,
30 depending on the species used, if the animals are sacrificed at the end of the immunogenicity
31 study, then gross pathology and targeted histopathology should be performed. In addition,
32 information about physical exams or clinical findings should be captured and reported to the
33 NRA. If the species used are too small (e.g. mice) to permit individual clinical pathology or a
34 species that is not sacrificed because the animals will be used in other research after the
35 immunogenicity study is performed (e.g. non-human primates), then whatever safety data can
36 be collected should be reported to the NRA. If there is safety information about veterinary
37 vaccines expressing related antigens, this information might be useful to provide to the NRA.

38

39 In the case of rapid development of a vaccine against priority pathogens for public health
40 emergencies where the plasmid backbone has been tested clinically, but the antigen is novel
41 (not related to another antigen that has been tested clinically), then this approach might not be
42 sufficient. The decision about what type of nonclinical safety/toxicology information required
43 might be guided by what and how much is known about the natural disease in terms of the

1 pathology, particularly immunotoxicity. If the natural disease is associated with
2 immunopathology due to cross-reactivity, autoimmunity, or immunity-associated disease
3 enhancement, then toxicology studies would likely be needed to assure that the novel antigen
4 is not associated with these effects. In the cases where natural disease is not associated with
5 immunopathology or where little is known about the natural disease, discussion with the
6 NRA should be undertaken. Finally, in the cases where both the plasmid backbone and the
7 antigen are novel, discussion with the NRA also should be undertaken.

8
9 Although biodistribution studies were previously suggested for DNA vaccines, data acquired
10 to date have not shown reason to continue such evaluations. Plasmid DNA remains largely at
11 the injection site, does not biodistribute at clinically relevant levels nor widely throughout the
12 body. Further, it does not target the ovaries nor testes and clears from the body by
13 degradation (67, 68, 83). However, most of these data were collected in adult animals. There
14 is a limited amount of information available from developmental toxicology or
15 biodistribution studies in maternal or fetal animals (83). This leaves a slight evidence gap
16 regarding the safe use of plasmid DNA vaccines in maternal immunization. Publication of
17 any developmental toxicology studies already performed on DNA vaccines is encouraged.

18
19 In the case of DNA vaccines against priority pathogens for public health emergencies, as
20 identified by the WHO R&D Blueprint, the following documents may be of relevance and
21 should be consulted:

- 22
- 23 • WHO *Guidelines on Nonclinical Evaluation of Vaccines* (54)
- 24 • WHO *Guidelines on the Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted*
25 *Vaccines* (55)
- 26 • An R&D Blueprint for Action to Prevent Epidemics [May 2016] (84)
- 27 • An R&D Blueprint for Action to Prevent Epidemics [Update 2017] (85)

28
29 Finally, the WHO has more than sixty guidelines and recommendations for vaccines against
30 specific diseases, which may be consulted if they cover the disease of relevance for the given
31 DNA vaccine. It should be anticipated that further disease/product-specific guidelines would
32 be considered at the appropriate time when any given DNA vaccine is near to submission for
33 marketing authorization.

34 35 **Part C. Clinical evaluation of plasmid DNA vaccines**

36
37 The clinical evaluation expectations for clinical trial authorization or marketing authorization
38 will be driven by the disease against which the DNA vaccine is being or has been developed
39 and the mode-of-action (or mechanism-of-action) of the vaccine to prevent that disease.
40 Clinical studies should adhere to the principles described in the WHO *Guidelines for good*
41 *clinical practice (GCP) for trials on pharmaceutical products* (86) and the WHO *Guidelines*
42 *on clinical evaluation of vaccines: regulatory expectations* (56). Likewise, post-marketing
43 pharmacovigilance is discussed in this latter guideline.

1
2 One issue that is of relevance to DNA vaccines is their use in heterologous prime-boost
3 regimens. Some guidance on this issue is provided in the Guidelines listed above. One
4 challenge for marketing authorization will be labelling of each of the vaccines in the regimen
5 that ultimately demonstrates efficacy, as this type of heterologous prime-boost regimen
6 remains novel at this time, and healthcare workers and public health systems are not
7 necessarily ready for this approach. Labelling to prevent mix-ups and mis-dosing will be
8 crucial to a successful public health campaign or routine use. Another matter that may require
9 attention is attribution of safety events seen following immunization and how it will be
10 clearly established whether the event is due to the prime or the boost vaccine, even if the
11 event occurs late (e.g. after boosting).

12
13 A potential advantage of DNA vaccines may be their use during pregnancy. This issue is
14 discussed in the above guidelines (56) in Section 5.6.4 and succeeding subsections.
15 Additional information that may be useful may be obtained from regional or NRA-specific
16 guidelines. These additional guidelines are not specific to DNA vaccines and may apply to a
17 variety of product types, but they do provide guidance on clinical trial designs and labelling
18 issues relevant to maternal immunization.

19
20 When a specific delivery device has been used to demonstrate efficacy of a DNA vaccine, it
21 will be important to consider pharmacovigilance plans to capture any off-label use of an
22 alternative device, including needle and syringe delivery. Clinical trial design for the pivotal
23 efficacy trial(s) will be important in terms of whether the control group(s) will have the same
24 device used to deliver the placebo or other type of control (e.g. another vaccine). It is
25 important to maintain a double-blinded, randomized, controlled trial design to gain pivotal
26 efficacy data, whenever feasible. However, appropriateness of use of the delivery device with
27 a substance other than the intended candidate vaccine has to be considered, in terms of ethics
28 and risk/benefit considerations. Finally, the labelling should reflect the device parameters
29 (e.g. pulse rate) used in the pivotal trial(s), as should the protocols for those trials.

30
31 In the case of DNA vaccines against priority pathogens for public health emergencies, as
32 identified by the WHO R&D Blueprint, the following documents may be of relevance and
33 should be consulted:

- 34
35
- 36 • The WHO R&D Blueprint¹;
 - 37 • List of Blueprint Priority Diseases²;
 - 38 • R&D Blueprint Plan of Action³;
 - WHO Target Product Profiles⁴

¹ See <http://www.who.int/blueprint/about/en/>

² See <http://www.who.int/blueprint/priority-diseases/en/>

³ See http://www.who.int/research-observatory/analyses/rd_blueprint/en/index5.html

⁴ See http://www.who.int/research-observatory/analyses/rd_blueprint/en/index5.html

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Finally, as stated above, the WHO has more than sixty guidelines and recommendations for vaccines against specific diseases⁵ and further disease/product-specific guidelines would be considered at the appropriate time when any given DNA vaccine is near to submission for marketing authorization.

Part D. Guidelines for NRAs

D.1 General guidelines

The general recommendations for control laboratories given in the WHO *Guidelines for national authorities on quality assurance for biological products* (87) and WHO *Guidelines for independent lot release of vaccines by regulatory authorities* (88) should apply after the vaccine product has been granted a marketing authorization. These recommendations specify that no new biological substance should be released until consistency of lot manufacturing and quality has been established and demonstrated. The recommendations do not apply to material for clinical trials.

The detailed production and control procedures as well as any significant changes in them that may affect the quality, safety and efficacy of DNA vaccines should be discussed with and approved by the NRA. Close to marketing authorization of each DNA vaccine, specific guidelines for the vaccine and the disease it targets may be prepared by WHO through their consultative process for adoption by the WHO ECBS. For DNA vaccines that target diseases for which there are existing vaccines and corresponding guidelines, it may be appropriate to consider in tandem Part A from these guidelines and Parts B and C from the disease-specific guidelines.

For control purposes, the international standards currently in force should be obtained for the purpose of calibration of the national/regional/working standards (89). The NRA may obtain the product-specific/working reference from the manufacturer to be used for lot release until the international/national standard preparation is established.

Consistency of production has been recognized as an essential component in the quality assurance of DNA vaccines, as with any vaccine. In particular, the NRA should carefully monitor production records and quality control test results for clinical lots, as well as a series of consecutive lots of the vaccine, produced using the procedures and control methods that will be used for the marketed vaccine.

D.2 Official release and certification

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

⁵ See <https://www.who.int/biologicals/vaccines/en/>

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

5
6 A lot release certificate signed by the appropriate official of the NRA should be provided, if
7 requested by a manufacturing establishment, and should certify whether or not the lot of
8 vaccine in question meets all national requirements, as well as Part A of these guidelines. The
9 certificate should provide sufficient information on the vaccine lot. The official national
10 release certificate should be provided to importers of the vaccines. The purpose of the
11 certificate is to facilitate the exchange of vaccines between countries. A model NRA Lot
12 Release Certificate is provided below in Appendix 2.

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14

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17

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1 **Appendix 1**

2

3 **Model summary protocol for the manufacturing and control of plasmid** 4 **DNA vaccines**

5

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

10

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

19

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

26

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

28

- 29 • International name:
- 30 • INN (if applicable):
- 31 • Trade name/commercial name:
- 32 • Product licence (marketing authorization) number:
- 33 • Country:
- 34 • Name and address of manufacturer:
- 35 • Name and address of product licence-holder, if different:
- 36 • Plasmid designation (if applicable):
- 37 • Gene Insert(s) (if applicable):
- 38 • Lot number(s):
- 39 • Type of container:
- 40 • Number of filled containers in this final lot:
- 41 • Number of doses per container:

- 1 • Preservative and nominal concentration (if applicable):
- 2 • Summary of the composition (summary of qualitative and quantitative composition of
- 3 the vaccine, including any adjuvant and other excipients):
- 4 • Target group:
- 5 • Shelf life approved (months):
- 6 • Expiry date:
- 7 • Storage conditions:

8

9 **2. Control of source material**

10

11 **2.1 Plasmid seeds (where applicable)**

12 **2.1.1 Seed banking system**

- 13 ▪ Name and identification of plasmid(s):
- 14 ▪ Origin of all genetic components (if applicable):
- 15 ▪ Construction of plasmid DNA vaccine:
- 16 ▪ Nucleotide sequence of the transgene and plasmid backbone:
- 17 ▪ Antigenic analysis, copy number, yield (in vitro/in vivo):
- 18 ▪ Seed bank genealogy with dates of preparation, passage number and date of coming
- 19 into operation:
- 20 ▪ Tests for contaminating bacteria, fungi, (for plasmid seeds):
- 21 ▪ Details of animal or human components of any reagents used in the manufacture of
- 22 seed banks, including culture medium:
- 23 ▪ Genetic stability at the level of a plasmid pre-master seed or plasmid master seed to its
- 24 sequence at, or preferably beyond, the anticipated maximum passage level:
- 25 ▪ Confirmation of approval for use by manufacturer, and the basis for that approval.

26

27 **2.1.2 Tests on working seed lot production (if applicable)**

- 28 ▪ Antibiotic resistance (if applicable):
- 29 ▪ Marker genes or selection genes (if applicable and different from antibiotic resistance
- 30 gene):
- 31 ▪ Identity:
- 32 ▪ Bacterial and fungal contamination:

33

34 **2.2 Cultures and culture media (where applicable)**

35 **2.2.1 Cell banking system**

- 36 ▪ Name and identification of cell strain and bank:
- 37 ▪ Details of any manipulations (including genetic manipulations) performed on the
- 38 parental cell strain in the preparation of the production cell strain:
- 39 ▪ Culture medium:
- 40 ▪ Cell bank genealogy with dates of preparation, passage number and date of coming into
- 41 operation:

- 1 ▪ Confirmation of approval for use by manufacturer, and the basis for that approval:
- 2 ▪ Test for absence of bacterial and fungal contamination:
- 3 ▪ Details of animal or human components of any reagents used in manufacture of cell
- 4 banks, including culture medium:
- 5 ▪ Genetic stability (if genetically manipulated).

7 **2.2.2 Tests on working cell bank production (if applicable)**

- 8 ▪ Identification of cell bank
- 9 ▪ Culture medium:
- 10 ▪ Cell bank genealogy with dates of preparation, passage number and date of coming into
- 11 operation:
- 12 ▪ Confirmation of approval for use by manufacturer, and the basis for that approval:
- 13 ▪ Test for absence of bacterial and fungal contamination:
- 14 ▪ Details of animal or human components of any reagents used in manufacture of cell
- 15 banks, including culture medium:
- 16 ▪ Genetic stability (if genetically manipulated).

18 **3. Control of vaccine production**

20 **3.1 Control of purified plasmid bulk (for each monovalent plasmid, if applicable)**

21 **3.1.1 Information on manufacture:**

- 22 ▪ Batch number(s):
- 23 ▪ Date of manufacture of each batch:
- 24 ▪ Identification of reagents used during production or other phases of manufacture,
- 25 including media components and antibiotics, if applicable):
- 26 ▪ Total volume of purified plasmid bulk:
- 27 ▪ Volume(s), storage temperature, storage time and approved storage period:

29 **3.1.2 Tests on purified plasmid bulk(s):**

- 30 ▪ Identity:
- 31 ▪ Purity:
- 32 ▪ Antigen content (quantity):
- 33 ▪ Physical state (i.e., % supercoiled):
- 34 ▪ Sterility (bacteria and fungi):
- 35 ▪ Residual levels of reagents used during production or other phases of manufacture,
- 36 including media components and antibiotics, if applicable):
- 37 ▪ Residual protein content:
- 38 ▪ Residual DNA derived from the expression system:
- 39 ▪ Residual RNA:
- 40 ▪ Endotoxins:
- 41 ▪ Only if not feasible on final vaccine due to multi-component formulation, potency
- 42 (expression of mRNA or protein):

1

2 **3.2 Control of final bulk (where applicable)**

3 **3.2.1 Information on manufacture**

- 4 ▪ Lot number(s):
- 5 ▪ Date of formulation:
- 6 ▪ Total volume of final bulk formulated:
- 7 ▪ Monovalent bulk plasmid(s) used for formulation:
- 8 ▪ Volume(s), storage temperature, storage time and approved storage period:
- 9 ▪ Lot number/volume added:
- 10 ▪ Name and concentration of added substances (for example, adjuvants, facilitators, etc.,
11 if applicable).

12

13 **3.3.2 Tests on final bulk or final containers, as applicable**

- 14 ▪ Identity:
- 15 ▪ Purity:
- 16 ▪ Antigen content (quantity):
- 17 ▪ Physical state (i.e., % supercoiled):
- 18 ▪ Sterility (bacteria and fungi):
- 19 ▪ Endotoxins:
- 20 ▪ Potency (expression of mRNA or protein)

21

22 **4. Filling and containers**

23

- 24 ▪ Lot number:
- 25 ▪ Date of filling:
- 26 ▪ Type of container:
- 27 ▪ Volume of final bulk filled:
- 28 ▪ Filling volume per container:
- 29 ▪ Number of doses, if the product is presented in a multiple-dose container:
- 30 ▪ Number of containers filled (gross):
- 31 ▪ Number of containers rejected during inspection:
- 32 ▪ Number of containers sampled:
- 33 ▪ Total number of containers (net):
- 34 ▪ Maximum period of storage approved (expiry dating):
- 35 ▪ Storage temperature:

36

37 **5. Control tests on final vaccine lot**

38

- 39 ▪ Inspection of final containers:
- 40 ▪ Identity:
- 41 ▪ Appearance:
- 42 ▪ pH (if applicable):

- 1 ▪ Osmolality (if applicable):
- 2 ▪ Sterility (bacterial and fungal):
- 3 ▪ Preservative (if applicable):
- 4 ▪ Residual moisture content (for freeze-dried product):
- 5 ▪ Endotoxin:
- 6 ▪ Adjuvant content (if applicable):
- 7 ▪ Potency:
- 8 ○ Expression of heterologous antigen in vitro (mRNA or protein):
- 9 ▪ Purity:
- 10 ▪ Extractable volume (if applicable):
- 11 ▪ Residual antibiotics (if applicable):
- 12

13 **6. Certification by the manufacturer**

14
15 Name of Head of Production or Quality Assurance (typed) _____

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

18 I certify that lot no. _____ of [name of] plasmid DNA vaccine, whose
19 number appears on the label of the final containers, meets all national requirements and satisfies
20 Part A¹ of the WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines²
21 and Part A of any disease-specific relevant WHO guidelines.

22
23 Name (typed) _____

24 Signature _____

25 Date _____

26

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

29 ² WHO Technical Report Series, No. XXXX, Annex X.

30

31 **7. Certification by the NRA**

32

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

35

1 **Appendix 2**

2
3 **Model NRA Lot Release Certificate for plasmid DNA vaccines**

4
5
6
7 Certificate no. _____

8
9
10 This certificate is to be provided by the NRA of the country where the vaccine has been
11 manufactured, on request by the manufacturer.

12
13
14 The following lot(s) of [name of] plasmid DNA vaccine produced
15 by _____¹
16 in _____² whose lot numbers appear on the labels of the final containers,
17 complies with the relevant specification in the marketing authorization and provisions for the
18 release of biological products³ and Part A⁴ of the WHO Guidelines on the quality, safety and
19 efficacy of plasmid DNA vaccines⁵ and Part A of the relevant disease-specific WHO guidelines
20 and complies with WHO good manufacturing practices for pharmaceutical products: main
21 principles,⁶ WHO good manufacturing practices for biological products,⁷ and Guidelines for
22 independent lot release of vaccines by regulatory authorities.⁸

23
24 The release decision is based on _____⁹

25
26
27 The certificate may include the following information:

- 28
- 29 ▪ name and address of manufacturer;
 - 30 ▪ site(s) of manufacturing;
 - 31 ▪ trade name and common name of product;
 - 32 ▪ marketing authorization number;
 - 33 ▪ lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
 - 34 ▪ type of container used;
 - 35 ▪ number of doses per container;
 - 36 ▪ number of containers or lot size;
 - 37 ▪ date of start of period of validity (for example, manufacturing date) and expiry date;
 - 38 ▪ storage conditions;
 - 39 ▪ signature and function of the person authorized to issue the certificate;
 - 40 ▪ date of issue of certificate;
 - 41 ▪ certificate number.

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The Director of the NRA (or other appropriate authority)

Name (typed) _____

Signature _____

Date _____

¹ Name of manufacturer.

² Country of origin.

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

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

⁵ WHO Technical Report Series, No. XXXX, Annex X.

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

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

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

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