This report presents the recommendations of a WHO Expert Committee commissioned to coordinate activities leading to the adoption of international recommendations for the production and control of vaccines and other biological products used in medicine, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of issues discussed at an exceptional meeting of the Committee held in August 2020 via WebEx video conferencing during the COVID-19 outbreak. Of particular relevance to manufacturers and national regulatory authorities are the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines, and an Amendment document to the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines were adopted on the recommendation of the Committee.

Subsequent sections of the report provide information on the current status, proposed development and establishment of international reference materials in the areas of: blood products and related substances; in vitro diagnostics; standards for use in high-throughput sequencing technologies; standards for use in public health emergencies; and vaccines and related substances.

A series of annexes is then presented which includes an updated list of all WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological products (Annex 1). The above WHO plasmid DNA Guidelines and WHO Amendment document adopted on the advice of the Committee are then presented as part of this report (Annexes 2–3). Finally, all new and replacement WHO international reference standards for biological products established during the exceptional August 2020 meeting are summarized in Annex 4. The updated full catalogue of WHO international reference standards is available at: http://www.who.int/bloodproducts/catalogue/en/.
The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO’s constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

The Organization seeks through its publications to support national health strategies and address the most pressing public health concerns of populations around the world. To respond to the needs of Member States at all levels of development, WHO publishes practical manuals, handbooks and training material for specific categories of health workers; internationally applicable guidelines and standards; reviews and analyses of health policies, programmes and research; and state-of-the-art consensus reports that offer technical advice and recommendations for decision-makers. These books are closely tied to the Organization’s priority activities, encompassing disease prevention and control, the development of equitable health systems based on primary health care, and health promotion for individuals and communities. Progress towards better health for all also demands the global dissemination and exchange of information that draws on the knowledge and experience of all WHO’s Member countries and the collaboration of world leaders in public health and the biomedical sciences.

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The WHO Technical Report Series makes available the findings of various international groups of experts that provide WHO with the latest scientific and technical advice on a broad range of medical and public health subjects. Members of such expert groups serve without remuneration in their personal capacities rather than as representatives of governments or other bodies; their views do not necessarily reflect the decisions or the stated policy of WHO.

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WHO Expert Committee on Biological Standardization

Seventy-first report

This report contains the views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.
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WHO Expert Committee on Biological Standardization
Meeting held via WebEx video conferencing on 24 to 28 August 2020

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1 The decisions of the Committee were taken in closed session with only members of the Committee present. Each Committee member had completed a Declaration of Interests form prior to the meeting. These were assessed by the WHO Secretariat and no declared interests were considered to be in conflict with full meeting participation.

2 Temporary advisers include representatives of the drafting groups of the WHO written standards submitted to the Committee for adoption, other subject matter experts and the Editor of the report of the Committee which is published each year in the WHO Technical Report Series.

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WHO Expert Committee on Biological Standardization  Seventy-first report

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACT</td>
<td>WHO Access to COVID-19 Tools (Accelerator)</td>
</tr>
<tr>
<td>CAG</td>
<td>Containment Advisory Group</td>
</tr>
<tr>
<td>CCP</td>
<td>COVID-19 convalescent plasma</td>
</tr>
<tr>
<td>CEPI</td>
<td>Coalition for Epidemic Preparedness Innovations</td>
</tr>
<tr>
<td>CHIM</td>
<td>controlled human infection model (study)</td>
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<tr>
<td>CLP</td>
<td>centralized laboratory project</td>
</tr>
<tr>
<td>COVID-19</td>
<td>coronavirus disease 2019</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>EDQM</td>
<td>European Directorate for the Quality of Medicines &amp; HealthCare</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EUL</td>
<td>emergency use listing</td>
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<tr>
<td>GACVS</td>
<td>Global Advisory Committee on Vaccine Safety</td>
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<tr>
<td>GAPIII</td>
<td>WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use</td>
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<tr>
<td>IU</td>
<td>International Unit(s)</td>
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<tr>
<td>IVD</td>
<td>in vitro diagnostic</td>
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<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
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<tr>
<td>LVV</td>
<td>lentiviral vector</td>
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<tr>
<td>mRNA</td>
<td>messenger RNA</td>
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<tr>
<td>NAT</td>
<td>nucleic acid amplification technique</td>
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<tr>
<td>NC3R</td>
<td>National Centre for the Replacement, Refinement &amp; Reduction of Animals in Research</td>
</tr>
<tr>
<td>NIBSC</td>
<td>National Institute for Biological Standards and Control</td>
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<tr>
<td>NRA</td>
<td>national regulatory authority</td>
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<tr>
<td>PMSG</td>
<td>pregnant mare serum gonadotrophin</td>
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<tr>
<td>PvLDH</td>
<td><em>Plasmodium vivax</em> lactate dehydrogenase</td>
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<tr>
<td>R&amp;D</td>
<td>WHO Blueprint for Research and Development: Responding to Blueprint Public Health Emergencies of International Concern</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
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<tr>
<td>RDT</td>
<td>rapid diagnostic test</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>SARS-CoV-2</td>
<td>severe acute respiratory syndrome coronavirus 2</td>
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<tr>
<td>VAERD</td>
<td>vaccine-associated enhanced respiratory disease</td>
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<tr>
<td>YFV</td>
<td>yellow fever virus</td>
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1. Introduction

The seventy-first meeting of the WHO Expert Committee on Biological Standardization was held from 24 to 28 August 2020 by WebEx video conferencing due to the restrictions imposed during the coronavirus disease 2019 (COVID-19) pandemic. This exceptional meeting was held prior to the annual October meeting of the Committee with an emphasis placed on addressing a number of urgent biological standardization issues relating to COVID-19. The meeting was opened on behalf of the Director-General of WHO by Dr Mariângela Batista Galvão Simão, Assistant Director-General, Access to Medicines and Health Products. Dr Simão welcomed the Committee, meeting participants and observers. Referring to the protracted nature of the COVID-19 crisis, she expressed the hope that recent technological advances would help to reduce mortality and more generally the societal impact of the pandemic. She highlighted the urgency of the current meeting and outlined the ways in which the work of the Committee related to the WHO Access to COVID-19 Tools (ACT) Accelerator.

The ACT-Accelerator aims to bring together governments, scientists, businesses, civil society, philanthropists and global health organizations to speed up efforts to end the pandemic. It is structured into four pillars of work that are critical to the overall effort – diagnostics, therapeutics, vaccines and health systems strengthening. The rate of progress in developing vaccines, small molecules and biotherapeutics to deal with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has been unprecedented. Cross-cutting all of these efforts is the Access and Allocation workstream, led by WHO, which is crucial for achieving the aims of the ACT-Accelerator and ensuring equitable access to its outputs. In this regard, Dr Simão recognized the crucial importance of the Committee in establishing the standards needed to ensure the development of safe, effective and quality-assured biological products, and expressed her gratitude for its commitment.

Dr Ivana Knezevic, Secretary to the Committee, thanked Dr Simão for her opening remarks and reiterated that the current meeting would focus almost entirely on issues related to COVID-19. She reminded meeting participants that WHO is a specialized agency of the United Nations and serves as the authority on international health matters on behalf of its 194 Member States. Among its broad range of responsibilities, the setting of norms and standards and promoting their implementation is an affirmed core function of WHO with direct relevance to the work of the Committee. In this regard, two resolutions adopted by the World Health Assembly at its Sixty-seventh session in May 2014 were of particular relevance – resolution WHA67.21 on access to biotherapeutic products (BTPs) including similar biotherapeutic products (SBPs), and resolution WHA67.20 on regulatory system strengthening.
Dr Knezevic outlined the working arrangements of the meeting, specifically with regard to the use of WebEx. Discussions would necessarily be shorter than those held during a face-to-face meeting but access to meeting documents had as usual been provided in advance, along with instructions on participation in virtual meetings. Committee members, regulatory authority representatives and subject matter experts from governmental organizations would participate in the meeting from 24 to 27 August 2020. An open information-sharing session involving all participants, including non-state actors, would be held on 24 August. All final recommendations on the adoption of written standards and the establishment of measurement standards would be made by Committee members only during a closed session on 28 August.

Following the conclusion of the open session on 24 August, Dr Knezevic informed the Committee that none of the declarations of interests received from meeting participants constituted a significant conflict of interest, and moved on to the election of meeting officials. In the absence of dissent, Professor Klaus Cichutek was elected as Chair, Dr Harvey Klein as Vice-Chair and Dr Ian Feavers as Rapporteur. Taking the Chair, Professor Cichutek reiterated the importance of biological standardization in the context of the COVID-19 pandemic and thanked Committee members for their availability during this meeting. It was anticipated that further exceptional meetings of the Committee would be required following its main meeting in October 2020.

The Committee then adopted the proposed agenda and timetable (WHO/BS/2020.2386).
2. Strategic directions in biological standardization: impact of COVID-19

2.1 WHO collaborations and partnerships related to COVID-19

2.1.1 Overview of the WHO response to COVID-19

Ms Emer Cooke, Director, Regulation and Prequalification, outlined the ways in which WHO was coordinating the global effort to develop diagnostic tests, therapeutics and vaccines for COVID-19. The WHO Department of Regulation and Prequalification is closely involved with the WHO research and development effort, with full regulator involvement and engagement. Such engagement is ensured by providing a platform for the rapid exchange of information on COVID-19 developments and by promoting regulatory alignment to facilitate quick access to safe and effective products. This work underpins the aims of the four pillars of the ACT-Accelerator.

The diagnostic pillar, co-led by the Foundation for Innovative New Diagnostics (FIND) and the Global Fund, focuses on saving lives and reducing disease transmission by providing equitable access to accurate and affordable diagnostic tests in low- and middle-income countries. The WHO Prequalification unit is currently assessing candidate in vitro diagnostics (IVDs) for the detection of SARS-CoV-2 for emergency use listing (EUL). In July 2020, the scope of EUL was expanded to include antibody-detection enzyme immunoassays. To date, there had been 50 expressions of interest for the listing of nucleic acid amplification technique (NAT)-based assays and 16 for antibody-detection assays. Of these, 15 NAT-based assays had now been listed as eligible for WHO procurement based on their compliance with WHO EUL requirements.

The therapeutics pillar, co-led by Unitaid and the Wellcome Trust, aims to accelerate the development and equitable delivery of treatments for COVID-19 and to ensure their accessibility regardless of geography and level of economic resource. Different phases of the disease require different treatments and the international WHO Solidarity Trial had so far recruited over 5000 hospitalized patients in 39 countries in a concerted effort to identify effective treatments for each phase. Three sets of WHO target product profiles for the treatment of COVID-19 in hospitalized patients had now been published.

The vaccine pillar (COVAX), co-led by the Coalition for Epidemic Preparedness Innovations (CEPI), Gavi and WHO, focuses on the rapid development of safe and effective vaccines, manufactured in sufficient volume and delivered worldwide. COVAX is organized into three workstreams – with the development and manufacturing workstream overseen by a regulatory advisory group. A diverse range of vaccine platforms were being used to develop candidate COVID-19 vaccines (see section 2.1.2 below) with 31 such vaccines currently in clinical development. WHO will facilitate access to COVID-19 vaccines through
the use of the EUL and through collaboration with national regulatory authorities (NRAs). Product-specific roadmaps for promising candidate vaccines were being developed by WHO to help ensure rapid assessment of vaccine quality, safety and efficacy data as they become available. WHO will share the resulting reports with all regulatory authorities to inform national decision-making.

Ms Cooke concluded by summarizing the achievements of the ACT-Accelerator to date in relation to the development of WHO measurement standards, the revision of the WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines, WHO EUL and prequalification activities, and regulatory preparedness. It was acknowledged that numerous challenges remain, including the need to avoid duplication of efforts across the project, manage expectations among developers and regulators, address divergent regulatory approaches and maintain confidence in regulatory processes under accelerated circumstances.

The Committee discussed the regulatory guidance provided to developers by COVAX and the ways in which such guidance related to its own work. It was agreed that the ad hoc guidance provided by COVAX was likely to be informal and product specific, whereas the Committee provided more formal long-term guidance. It was also noted that although much of the general guidance developed by the Committee was already available as formal recommendations and guidelines, the pressing need for guidance specific to COVID-19 would need to be considered in some detail during the current meeting.

2.1.2 Update from the R&D Blueprint

Dr Ana Maria Restrepo presented an update from the R&D Blueprint team on the efforts being made to mobilize the scientific community to accelerate the development and evaluation of COVID-19 candidate vaccines. Four platform technologies were currently being used to develop such vaccines – whole virus vaccines (attenuated or inactivated); protein-based vaccines (subunit or virus-like particle); viral-vectored vaccines (replicating or non-replicating); and nucleic acid vaccines (DNA or RNA). In total, more than 170 candidate vaccines were currently in development, of which 31 were undergoing clinical evaluation. Ten of the candidate vaccines undergoing clinical evaluation are nucleic acid vaccines, a type that is less familiar to both manufacturers and regulators, and for which no previously licensed products exist. Dr Restrepo set out the following four objectives of the COVID-19 vaccine research and development roadmap:

- to develop and standardize animal models
- to develop and standardize assays to support vaccine development
- to develop a multi-country master protocol for Phase IIb/III vaccine evaluation
- to develop potency assays and manufacturing processes.
Dr Restrepo expanded upon a number of issues in each of these areas, including the urgent need for a standardized animal challenge-protection model to enable the preclinical comparison of different candidate vaccines, and to evaluate their potential to cause vaccine-associated enhanced respiratory disease (VAERD). There is also a need to develop and standardize assays, and to share the reagents and protocols used in order to accelerate the development of international standards and reference panels. This would allow for improvements to be made in data interpretation and harmonization, while also enhancing access to assays and related materials for all developers. Examples of progress made included: (a) the reaching of broad expert consensus on the key elements of potential human challenge studies should these be required to accelerate vaccine development; (b) the production of a target product profile to guide vaccine design; and (c) the development of criteria to guide the prioritization of vaccines for WHO Phase IIb/III clinical studies.

Dr Restrepo went on to explain the rationale and potential benefits of a proposed international randomized controlled trial to evaluate a wide range of COVID-19 candidate vaccines. Given the current number and diversity of such vaccines, this approach would increase the likelihood of identifying more than one effective vaccine, while reducing the danger of a vaccine with only modest efficacy being approved and deployed; which potentially could cause more harm than good. It would also permit the enrolment of participants in locations with high rates of disease, resulting in the rapid accumulation of safety and efficacy data to support licensure, while also eliminating the inefficiency of designing and conducting separate studies. A further key objective of this so-called WHO Solidarity Trial would be the evaluation of overall vaccine safety, including evaluation of the potential for VAERD. Such an approach would also help to foster international collaboration and commitment, and facilitate the equitable deployment of vaccines.

The Committee expressed its broad support for the WHO Solidarity Trial and was assured that more than 250 research sites around the world had been identified as potential participants. Many of the sites that had expressed their willingness to be involved were in locations that continue to experience levels of COVID-19 sufficient to allow for the rigorous evaluation of vaccine efficacy. The Committee emphasized that in the face of intense political pressure for COVID-19 vaccines and therapeutics, such objective and evidence-driven approaches would be crucial in ensuring their efficacy and safety.

2.1.3 Priorities of the Coalition for Epidemic Preparedness Innovations

Dr Paul Kristiansen presented the priorities of CEPI and COVAX in responding to the COVID-19 pandemic. Both CEPI and COVAX had rapidly invested in a portfolio of vaccine research and development projects. The strategic objectives of COVAX in this respect were speed, scale and access, with the aim of supplying
2 billion vaccine doses by the end of 2021. At the same time, COVAX has engaged with the scientific community in order to accelerate progress in a number of cross-cutting enabling activities that support vaccine development and facilitate vaccine use in clinical trials and outbreak response settings. Examples of such enabling activities include the development of biological standards, assays and animal models.

With regard to coronavirus vaccine discovery and development, the Bill & Melinda Gates Foundation have opened up the resources of the Global Health Vaccine Accelerator Platform to holders of Bill & Melinda Gates Foundation and CEPI grants. Through partnership with the biotechnology contract research organization Nexelis, grant holders can access a wide range of high-throughput preclinical and research immunoassays for mouse, hamster, ferret, non-human primate and human studies. Nexelis will also produce and characterize small batches of the full-length spike protein (including receptor-binding domain) of SARS-CoV-2 for research use and assay development.

In collaboration with NIBSC, the development of COVID-19 antibody reference materials had also rapidly progressed. One convalescent serum (available as a research reagent) and a reference sera panel had been made available since the end of April 2020. In addition, a working antibody standard consisting of a characterized serum pool, and with a unitage based on a limited inter-laboratory study, would be made available in November 2020. Subject to endorsement by the Committee, a proposal would be made to conduct an international collaborative study to establish the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin.

In the area of assay development, NIBSC had now developed neutralization assays (for both pseudotyped and live viruses) and an enzyme-linked immunosorbent assay (ELISA) for SARS-CoV-2 to support vaccine development studies. Standard operating procedures for both assay types were now available to vaccine developers. To further facilitate assay harmonization, the centralized laboratory project (CLP) had also been established. Requests for proposals had been solicited from laboratories that could test preclinical and clinical trial samples from Phase I and IIa studies. Securing sufficient testing capacity worldwide will help to avoid bottlenecks and promote global assay harmonization. It was expected that contracts would be in place by the end of August 2020, with CLP services to be made available free of charge to all vaccine developers. Dr Kristiansen explained that to support decisions on vaccine development within the COVAX portfolio, a single laboratory would test samples from the dose-selection studies conducted by all COVAX-funded vaccine developers. In addition, individual vaccine developers, whether COVAX funded or not, would be able to use laboratories within the CLP network to test samples and generate data in support of vaccine licensure. CEPI had also established a network of high-quality institutions for the development of suitable animal
models – all of which were in compliance with the principles of the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3R). Priorities in this area include the development of suitable animal models for challenge studies, provision of sufficient animal testing capacity to CEPI-funded candidate vaccine developers, and the development of animal models for assessing pulmonary immune pathology and vaccine-enhanced disease.

The Committee agreed with the approach taken by COVAX and recognized the important contribution of CEPI to the development of standardized assays and animal models. Noting the speed with which antibody reference materials had been produced, the Committee highlighted that the above-mentioned proposal to develop the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin would be considered during the current meeting (see section 7.1.2 below).

2.2  
WHO COVID-19 vaccine-related activities

2.2.1  
Overview from WHO

Dr Knezevic outlined the impact of the COVID-19 pandemic on the activities of WHO and began by noting that this had included changes to the workings of the Committee. The current exceptional seventy-first meeting of the Committee would focus predominantly on the development of WHO written and measurement standards needed for the response to the pandemic. In April 2020, WHO had set out the guiding principles for COVID-19 vaccine standardization on its website and had listed a number of existing WHO written standards relevant to the development, production and evaluation of candidate COVID-19 vaccines. However, it was clear that additional written standards specific to COVID-19 were also required and this issue would be reviewed in more detail during the current meeting (see section 2.2.3 below).

WHO had also provided information on the status of ongoing measurement standards projects relevant to the development and evaluation of COVID-19 diagnostics, vaccines and other products. The development of measurement standards to facilitate the development, assessment and validation of molecular and antibody assays for COVID-19 was being led by NIBSC with support from numerous laboratories worldwide (see section 7.1 below). Should these standards be established by the Committee at its next exceptional meeting in December 2020, they will have been produced in a timeframe of 9 months compared to the normal timeframe of 2–3 years.

Considerable interest had been expressed in the use of challenge studies based on the controlled human infection model (CHIM) to study COVID-19 prevention and treatment. In May 2020, the WHO Global Health Ethics team had approved a number of key criteria for assessing the ethical acceptability of COVID-19 human challenge studies, and the R&D Blueprint
team had established an advisory group to consider the feasibility of such a study. A roadmap had now been drafted to provide guidance on the selection and manufacture of challenge viruses, and on challenge doses, dose selection, clinical instructions and the measurement of immune responses. Meetings of interested parties had been organized by the Wellcome Trust, with a number of regulators encouraging continued discussion of this issue. Although current levels of COVID-19 were sufficiently high not to require CHIM studies in the immediate term, work had started on the production of a challenge virus stock.

Dr Knezevic went on to highlight the work of several WHO working groups focusing on different aspects of SARS-CoV-2 infection. Two working groups in particular—the working group on assays and standards, and the working group on animal models—had already provided considerable information in relation to the development of COVID-19 vaccines and therapeutics, and on the pathogenesis of the disease. One specific issue raised during discussions in the working group on animal models was the need for standardization in the propagation of virus working stocks. Responses received to date from 20 institutions indicated that 86 such stocks were currently being maintained in nine countries. Once all the data have been received, guidance will be prepared for a broad scientific audience on the key issues in virus propagation.

Dr Knezevic concluded by summarizing a number of cross-cutting issues within the ACT-Accelerator of particular relevance to the work of the Committee. As vaccine development progressed, WHO recognized the importance of collaboration between the Committee, the Strategic Advisory Group of Experts on Immunization (SAGE) and the Global Advisory Committee on Vaccine Safety (GACVS). The requirement for COVID-19 vaccine guidance would therefore be further discussed during the current meeting. In addition, the activities of the diagnostics pillar of the ACT-Accelerator would be supported by the molecular and serological reference standards now being proposed for endorsement (see section 7.1 below) as well as by potential future antigen standards. Furthermore, the development of therapeutic monoclonal antibody products for COVID-19 would need to take into consideration WHO guiding principles for biotherapeutics.

The Committee recognized the additional burden of work placed on WHO as a result of the COVID-19 pandemic and acknowledged the rapid progress made in the development of measurement standards. The Committee also welcomed the expedited revision of the WHO Guidelines on plasmid DNA vaccines (see section 3.2.1) given their relevance to the current crisis. Commenting on the prospective use of CHIM studies for the evaluation of COVID-19 vaccines and therapies, the Committee felt that any guidance developed should focus on the quality of the challenge strain and comprise general principles rather than focus on a specific disease. It was anticipated
that the potential development of such guidance would be discussed at a future meeting. However, the development of guidance on issues such as the ethical considerations for such studies was not within the remit of the Committee.

### 2.2.2 Development of a WHO guidance document on regulatory considerations in the evaluation of mRNA vaccines

Dr Margaret Liu provided an overview of the current status of messenger RNA (mRNA) vaccine development. Although no nucleic acid vaccine has yet been approved for human use, the approach represents a highly promising platform technology for quickly developing vaccines against priority pathogens during public health emergencies. As a result, mRNA-based candidate COVID-19 vaccines had been among the first to enter clinical trials during the current pandemic.

Dr Liu briefed the Committee on the progress made in drafting a proposed WHO guidance document on regulatory considerations in the evaluation of mRNA vaccines. In 2017, revision of the WHO Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines had been initiated. However, based on feedback received during the informal consultations, it had been recognized that the production and clinical evaluation of RNA vaccines differed considerably from that of DNA vaccines. It had therefore been decided not to extend the scope of the revised Guidelines to include RNA vaccines but to produce instead a separate WHO guidance document on regulatory considerations in the evaluation of such vaccines that could be updated as more scientific and clinical data became available.

It is intended that the document will broadly follow the format of more formal WHO vaccine guidelines, with sections on scope, general considerations, quality issues, and nonclinical and clinical evaluation, focusing on aspects of particular relevance to mRNA vaccines. Examples of relevant quality issues in the manufacturing process include the use of a DNA template, the need to carefully determine the nucleotide sequences used and the purification of the resulting mRNA. In addition, as RNA is known to be inflammatory in humans, nonclinical safety studies would first be needed, for example to evaluate the potential for local and systemic inflammation, and induction of VAERD. Such safety studies would also need to incorporate toxicity testing (including mitochondrial toxicity), assess the potential of the candidate vaccine to cause or enhance autoimmune disease and investigate the possibility of reductions in lymphocyte numbers, as had previously been observed with an mRNA vaccine. The theoretical risk of inducing antigen tolerance would also need to be evaluated in relevant animal models. In addition, although RNA cannot itself integrate into the genome of a vaccine recipient, this possibility should also be assessed during preclinical development given the presence of endogenous
human retroviruses. As vaccine potency in vivo is a complex function of the complete vaccine formulation, including the mRNA itself, potency assays could potentially include in vitro assays of the sequence only, or of protein expression in vitro, rather than in vivo immunogenicity. In all cases, both the ability of the vaccine to elicit a protective immune response in the target population and the potential for safety issues should continue to be assessed throughout the clinical development of a candidate mRNA vaccine. Because of the ability of RNA to induce innate immune responses, the impact of mRNA vaccines on other vaccines administered concomitantly or near concomitantly should also be assessed. Dr Liu concluded by summarizing the key issues specifically associated with prospective COVID-19 mRNA vaccines. These included the increased risk of toxicity or inflammation resulting from high doses of mRNA and the potential for immune-response enhanced disease in other organs due to the distribution of the angiotensin-converting enzyme 2 (ACE2) receptor.

The Committee noted that as several candidate COVID-19 mRNA vaccines were already in clinical development there was a need to provide guidance on their development and evaluation as soon as possible. However, the Committee also noted that there was currently insufficient clinical experience with this class of vaccine to support the production of formal WHO guidelines or recommendations. As the presentation given by Dr Liu had raised both generic mRNA vaccine issues and specific COVID-19 mRNA vaccine issues, the Committee proposed that the guidance document be developed as quickly as possible with broad applicability to the mRNA vaccine platform regardless of the target disease. Self-amplifying constructs comprising only mRNA should be included in the scope of the document. If necessary, an appendix could be added to the document covering issues specific to COVID-19 mRNA vaccines. It was envisaged that once sufficient clinical experience with mRNA vaccines had been gained then more formal WHO guidelines could be developed.

2.2.3  A need for standardization

Dr Philip Minor outlined to the Committee the findings of a review of candidate COVID 19 vaccines that had been conducted to identify key areas where standardization was needed. Based on the 18 candidate COVID 19 vaccines in clinical development as of 2 July 2020, the review covered non-replicating viral-vectored vaccines, inactivated vaccines, nucleic acid (DNA and RNA) vaccines, protein subunit vaccines and virus-like particle vaccines. No live attenuated COVID 19 vaccines were in clinical studies at that time. There has been broad experience with inactivated viral vaccines, which are well established with a long history of safety and efficacy. Similarly, subunit vaccines are a well-established platform. However, vectored vaccines and nucleic acid vaccines are less well established with none having been routinely used in humans to date. Almost all of the candidate COVID 19 vaccines were based on the spike protein, which
is roughly analogous to the influenza haemagglutinin and other attachment proteins of lipid-containing viruses.

A number of issues were found to be common to many of the candidate vaccines. Most notably, the assays currently used to measure humoral antibody responses are diverse and poorly standardized. These include ELISAs, wild-type neutralization assays and pseudotype neutralization assays. The ELISA antigens used vary and are often of uncertain provenance and quality. In addition, although the ability to elicit cell-mediated immunity is generally regarded as a desirable property of vaccines, potential immune-mediated long-term adverse events make this a crucially important issue for vaccine safety, and here too there was a need for improved standardization of the relevant assays. Furthermore, there was a need to clarify the value of the various animal models of SARS-CoV-2 infection used, and to better define their limitations in predicting vaccine efficacy or immune-mediated adverse events. With regard to such events, there was no evidence to date that candidate COVID-19 vaccines cause VAERD. However, an analogous effect had been reported in a SARS-CoV-1 mouse model and this issue would need to be fully investigated. Dr Minor also noted that many candidate COVID-19 vaccines contain adjuvants not yet widely used in established vaccine programmes. The potential risk of VAERD arising from misdirected immune responses would therefore also need to be addressed, highlighting the critical importance of rigorously evaluating any prospective adjuvant.

Dr Minor highlighted that existing WHO written standards and other documents of direct relevance to both current and future COVID-19 vaccine development included:

- Guidelines on the quality, safety and efficacy of plasmid DNA vaccines (see section 3.2.1 below) – relevant for all vaccines using this platform;
- Guidelines on the quality, safety and efficacy of Ebola vaccines – relevant when considering the use of viral-vectored vaccines in responding to an emerging disease;
- Guidelines on the quality, safety and efficacy of respiratory syncytial virus vaccines – which covers many of the above issues (for example, VAERD); and
- Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines.

There are presently no WHO written standards specifically on SARS coronavirus vaccines or on RNA vaccines. Guidance not directly related to vaccine manufacture might also usefully be produced on: (a) the design, validation and standardized comparison of antibody assays; and (b) the quantitation of cellular immune responses and their standardization, specifically in relation to VAERD.
Although WHO guidance on novel vaccine adjuvants was available, it might usefully be revisited if the criteria for avoiding VAERD can be identified.

Dr Minor concluded by outlining a number of measurement standards requirements in this area. A proposal to develop a First WHO International Standard for anti-SARS-CoV-2 immunoglobulin was to be considered for endorsement at the current meeting (see section 7.1.2 below). Although a number of antibody panels were available, better coordination of their distribution would be helpful. In addition, the currently available SARS-CoV-2 antigen targets for ELISA were of variable or unknown quality, and the establishment of antigen reference standards would therefore facilitate the standardization of antibody binding assays. Reference materials for the standardized measurement of Th1 and Th2 immune responses and a positive control for SARS-CoV-2 VAERD would also help in assessing the safety of candidate COVID 19 vaccines.

The Committee discussed at length the need for COVID-19 vaccine guidelines and concluded that at present such a document would be complex to draft in terms of its scope, and would require too much time to be of help in the current pandemic. The Committee was also of the view that there was currently insufficient data on many of the candidate vaccines to inform the development of comprehensive guidelines. Instead, it was proposed that a questions and answers document on COVID-19 vaccines and therapeutics be developed quickly and without formal public consultation, which could be updated as new information emerged.

The Committee considered the suggestion to develop WHO guidelines on the standardization of antibody assays to be interesting. It concluded, however, that experienced manufacturers and regulators already had a good understanding of the issues raised, and that once again formal guidelines would not be available quickly enough to be of help during the current crisis. Instead, it was suggested that this would be an appropriate subject for publication as an article in a scientific journal.

With regard to measurement standards, the Committee noted the work being led by CEPI to obtain quality-assured vaccine antigens for use in antibody binding assays but did not rule out the possibility of recommending the development of WHO SARS-CoV-2 antigen reference standards should they be needed. Given the current lack of scientific consensus on a suitable positive control material for VAERD, the Committee did not feel that a corresponding standardization project could be initiated at this time.

2.2.4 Proposal from the WHO Pharmacovigilance secretariat
Dr Christine Guillard informed the Committee that the number and variety of developmental COVID 19 vaccines presented particular challenges for regulators carrying out risk–benefit assessments – especially for vaccines based on innovative nucleic acid or viral-vectored platforms. Dr Guillard highlighted the
value of using standardized tools to facilitate such assessments and made specific reference to a number of templates established by the Brighton Collaboration. This initiative aims to enhance vaccine research by providing standardized, validated and objective methods for monitoring the safety profiles and risk–benefits of vaccines.

In its role as an independent clinical and scientific advisory body providing WHO with evidence-based advice on vaccine safety issues, GACVS had reviewed the standardized templates, including those for viral-vectored, nucleic acid, protein subunit and inactivated vaccines, with a focus on their value in risk assessment. Dr Guillard requested the Committee to consider the value of these risk–benefit assessment templates in facilitating and harmonizing the regulatory review of new vaccines, and to review the appropriateness of the existing Brighton Collaboration and other tools for its endorsement and broader use.

Following discussion of the issues raised by this proposal, the Committee concluded that the development of risk–benefit assessment templates for vaccine technology platforms was not recommended as each vaccine product would require its own individual risk–benefit assessment. The Committee further noted that it would not be standard procedural practice for it to recommend the adoption of Brighton Collaboration or other external resources, and it had no immediate plans to do so.

2.3 WHO COVID-19 blood-related activities

2.3.1 Overview from WHO

Dr Yuyun Maryuningsih updated the Committee on the blood-related activities of WHO since the declaration in January 2020 that COVID-19 constituted a public health emergency of international concern. Shortly after the declaration, countries began to report declines in blood donation as people became reluctant to leave their homes. In February 2020, the Asia Pacific Blood Network developed a white paper on the anticipated challenges and risks to blood safety posed by COVID-19. In addition, the Asia Pacific Blood Network and the WHO Blood Regulators Network agreed to draft a position paper focusing on blood quality and safety.

In March 2020, WHO published its interim guidance on maintaining a safe and adequate blood supply during the COVID-19 pandemic (see section 2.3.2 below). Following a series of video conferences with stakeholders this guidance was revised to respond to regional requests for more flexibility and to reflect a broader international consensus. Adapted from WHO guidance for national blood services on protecting the blood supply during infectious disease outbreaks, the revised guidance aims to mitigate the risk of transmission of SARS-CoV-2 through transfusion, along with the risks of staff or donor
exposure. The guidance also addresses the impact of the reduced availability of blood donors reported by countries, the need to manage demand for blood and blood products, and the collection of COVID-19 convalescent plasma (CCP).

WHO had also hosted two webinars – the first of which had been held in May 2020 on maintaining the blood supply and blood safety, and the second, in July 2020, on the collection of CCP. Each of these virtual meetings had been well attended with over 200 participants from all six WHO regions taking part. Other WHO activities had included engagement with the press on the issue of the impact of COVID-19 on organ donation and transplantation.

Despite the additional demands placed on WHO and WHO collaborating centres as a result of the pandemic, including the pressing need to develop COVID-19 reference standards, the overall work of WHO in this area has largely continued through video conferencing and it is anticipated that most of the relevant WHO objectives for 2020 will be achieved.

The Committee recognized the additional workload undertaken by WHO during the COVID-19 pandemic and acknowledged its important contribution to maintaining safe blood supplies worldwide during the crisis. The Committee looked forward to hearing more about the interim guidance document during the current meeting.

2.3.2 WHO interim guidance on maintaining a safe and adequate blood supply during the COVID-19 pandemic and on the collection of CCP

Dr Diana Teo reiterated to the Committee that the COVID-19 pandemic had significantly impacted upon the supply of blood and blood components in many countries. In response, WHO had developed an interim guidance document on maintaining a safe and adequate blood supply during the COVID-19 pandemic and on the collection of CCP. This document is intended for use by blood services, national health authorities and others responsible for the provision of blood and blood components. The document is based on, and intended for use in conjunction with, existing WHO guidance on the protection of blood supplies during infectious disease outbreaks and takes into account published risk assessments in relation to COVID-19.

In the absence of evidence of bloodborne transmission, the risk of acquiring SARS-CoV-2 through blood transfusion is currently considered to be minimal. However, reductions in donations and the closure of collection facilities have affected blood supplies worldwide. Blood services need to respond quickly to the changing pandemic and should therefore be represented in the national outbreak response, and in the development and implementation of emergency response plans. In support of this, the interim document provides guidance on: (a) mitigating the potential risk of viral transmission during blood transfusion; (b) mitigating the risk of exposure of staff and donors to the virus;
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(c) managing the impact of reduced numbers of donors during the pandemic; and (d) managing the demand for blood and blood products. The guidance also highlights the importance of ensuring the continuity of supply of critical materials and equipment. It also emphasizes the vital importance of good communication, education of donors and stakeholder engagement.

The document also provides guidance on the collection and preparation of CCP. WHO recognizes CCP as an experimental therapy appropriate for evaluation in clinical studies or for use as a starting material in the manufacture of experimental hyperimmune immunoglobulin. This position is based on the belief that the potential benefits of providing antibodies that may neutralize SARS-CoV-2 outweigh the risks of administration of these plasma products. One commonly agreed criterion for CCP donor acceptance is the meeting of an established minimum neutralizing antibody titre required for plasma to be suitable for use as CCP. Potential donors may also be tested for the presence of antibodies to SARS-CoV-2 using antigen-binding assays (such as ELISA), though the correlation with antibody neutralization titres is uncertain.

The Committee noted that, based on experience with antibody assays against a wide range of pathogens, the expression of antibody assay results as titres can result in high inter-laboratory variation. Such variation can be reduced by several measures, including the expression of results relative to a reference standard. The Committee therefore strongly urged that statements on acceptability criteria for CCP donor acceptance and potency statements for CCP products should be expressed in terms of relative potency. Once a WHO international reference standard for SARS-CoV-2 immunoglobulin had been established (see section 7.1.2 below) then such statements should be expressed in International Units (IU).
3. International Recommendations, Guidelines and other matters related to the manufacture, quality control and evaluation of biological products

3.1 Biotherapeutics other than blood products

3.1.1 Second WHO International Standard for serum gonadotrophin (equine)

The Second WHO International Standard for serum gonadotrophin (equine) (NIBSC code: 62/001) defines the activity of pregnant mare serum gonadotrophin (PMSG) medicinal products previously used to induce superovulation in humans. Since the development of 62/001 in the 1960s the use of PMSG in humans has ceased and it is now exclusively a veterinary product. Since veterinary products do not fall within the remit of the Committee, and in light of upcoming higher-priority standard replacement projects, NIBSC had previously decided that this standard would not be replaced once stocks became exhausted. The Committee was informed that stocks of this reference standard had duly been exhausted in October 2019, with only a small archive of ampoules retained to assist in the future preparation of a replacement reference material by an interested party and to ensure continuity of the IU.

However, the decision not to replace 62/001 had resulted in unforeseen consequences for the European Directorate for the Quality of Medicines & HealthCare (EDQM) as the material is used in two monographs which cannot currently be applied. During meetings between EDQM and the European Commission, concerns were expressed about the large number of manufacturers of the veterinary products, the unavailability of alternative methods, the potential lack of harmonization between products and the need for updated marketing authorization dossiers. These concerns resulted in a proposal to revise the monographs and to consider replacement of the standard.

The Committee acknowledged that discontinuation of the WHO reference standard for equine serum gonadotrophin would be problematic for manufacturers of PMSG products for veterinary use and consideration was given to the following two options:

- evaluation of a candidate replacement international standard by EDQM through a collaborative study with the aim of submitting the results to the Committee for establishment; or
- discontinuation of the international standard and establishment of a European Pharmacopoeia standard instead.

The Committee expressed its preference for the first option and would formally request EDQM to prepare the replacement international standard. NIBSC indicated its willingness to provide samples of the current international
standard to ensure continuity of the assigned IU following the change of custodian laboratory. The Committee noted that many manufacturers would have sufficient stocks of working standards to last until the replacement became available. Nevertheless, it requested that the amount of archived material required for the collaborative study be determined so that any remaining material could be released to manufacturers in critical need.

3.2 Vaccines and related substances

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

DNA vaccine technology involves the direct administration of plasmid DNA containing a gene (or genes) which encodes immunogen(s) against which an immune response is sought. This leads to the in situ production of the target immunogen(s) in the vaccine recipient. Plasmid DNA vaccines (or simply DNA vaccines) are now at an advanced stage of clinical development. Although no DNA vaccine has yet been licensed for use in humans, the approach offers numerous potential advantages. In addition, there has now been over two decades of preclinical and clinical experience with candidate DNA vaccines against a wide array of infectious diseases, and significant experience in their manufacture and control has been gained. Such experience has now allayed many of the early theoretical concerns expressed in previous WHO guidance. As a result, the WHO Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines adopted in 2005 no longer reflected current regulatory expectations and practices.

Given the potential of DNA vaccination as a platform technology for rapidly responding to public health emergencies, and the development of candidate DNA vaccines against SARS-CoV-2, the availability of an up-to-date written standard for such vaccines is a matter of urgency. Although the revision of the document was initiated prior to the emergence of SARS-CoV-2, the guiding principles it provides for evaluating the quality, safety and efficacy of DNA vaccines for human use are applicable to COVID-19 DNA vaccines. As well as providing guidance on the approval and lot release of fully developed products, the revised Guidelines also provide useful guidance to developers of candidate DNA vaccines. It is envisaged that the Guidelines would be used in conjunction with relevant disease-specific WHO written standards. During the revision process, consideration had been given to whether the Guidelines could also incorporate guidance on mRNA vaccines but it had been concluded that a separate document would be required due to the distinct manufacturing processes and safety issues involved (see section 2.2.2 above).

Issues particularly associated with DNA vaccines include their typical administration via specific devices or delivery systems that are likely to affect their efficacy. In many regulatory jurisdictions, the vaccine and the device will
be considered together as a combination product, thus determining the ways in which such products will be evaluated and approved. In addition, DNA vaccines are often envisaged as the first component of a prime-boost immunization strategy in which the booster dose is another type of vaccine, possibly produced by a different manufacturer.

The Committee agreed that such issues would require clear regulatory pathways. However, NRAs would also need to make decisions on a product-by-product basis and in all cases early discussion between manufacturers and NRAs would be essential in reaching consensus. The Committee discussed at length the question of how the potency of plasmid DNA vaccines should be assessed. Traditionally, vaccine potency is assessed based on an in vivo or cell-based bioassay. In practice, this would be challenging for plasmid DNA vaccines, which are generally transfected into a cell line and the expression of the antigen gene assessed by an immunochemical method such as Western blot. This approach is semi-quantitative at best, challenging to validate and requires a continued supply of antibody reagents. A plasmid DNA vaccine might therefore be best considered as a specified and well-characterized biological substance, with its chemical structure defining the product better than its biological activity. Dosing would then be based on DNA concentration rather than a potency unit, with the percentage of supercoiled plasmid and the nucleotide sequence considered to be critical quality attributes. The Committee supported the use of in vitro approaches to potency assessment for such vaccines as outlined in the revised guidelines, emphasizing that the expression of the antigen-encoding sequence must be demonstrated during vaccine development. In addition, where a specific delivery device was used to deliver a DNA vaccine, the final labelling should reflect the device parameters used in the pivotal trial(s) and must make it very clear to end users that only the authorized device should be used.

The Committee then discussed whether the title of the revised Guidelines should reflect their specific applicability to plasmid DNA vaccines used in the prevention of infectious disease. The Committee felt that this was clearly set out in the scope of the document, which explicitly excluded therapeutic DNA vaccines. It also agreed that although the word “plasmid” was not essential in the title it was more precise and should be retained.

The Committee reviewed a number of further comments received during public consultation and, after making a number of changes to the text, recommended that the document WHO/BS/2020.2380 be adopted and annexed to its report (Annex 2). Recognizing the pressing importance of promoting regulatory preparedness for public health emergencies, the Committee urged WHO to expedite the dissemination of the revised Guidelines.
Amendment to the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines

At its sixty-ninth meeting in 2018, the Committee had recommended the adoption of the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines. These Guidelines incorporated a number of biosafety measures consistent with the WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) with the aim of minimizing the risk of the accidental reintroduction of poliovirus from a vaccine manufacturing facility into the community. In order to fully align with GAPIII the Guidelines included several requirements related to the physical design of the facility and to quality control testing which were added after the final round of public consultation in 2018. Following publication of the Guidelines, poliomyelitis vaccine manufacturers had requested WHO to reconsider these particular requirements, taking into consideration the use of facility-specific risk-based approaches.

In consultation with the Containment Advisory Group (CAG), the Committee at its seventieth meeting recommended the amendment of the Guidelines, specifically with regard to: (a) the requirement for showering when exiting the containment facility; (b) allowing the use of non-dedicated quality control laboratories; and (c) permitting the removal of certain samples for testing outside the containment facility. The guidance on showering had now been amended to incorporate risk assessment on condition that such assessment was consistent with GAPIII and CAG policies. The proposed amendment to the Guidelines also explicitly sets out the conditions that have to be met when non-dedicated quality control laboratories are used, and clarifies the circumstances under which certain types of samples can be removed for testing outside the containment facility. The Committee was informed that only these issues had been addressed in the proposed amendment and that no objection to the changes had been raised by CAG.

The Committee was in general agreement with the proposed amendments. However, slight concern was expressed that the wording used in two of the amended sections would mean that the Guidelines would effectively be dependent upon the most recent GAPIII policies in place at the time. This would potentially alter the interpretation of the guidance over time as GAPIII policies evolved. The WHO Polio Operations and Research department indicated that although the revision of GAPIII was under way this would take time to complete. Meanwhile, CAG had agreed to the proposed amendments. The Committee indicated its support for the much needed flexibility outlined in the amendment and highlighted that flexibility in relation to the “shower-out” requirements would still be needed once the global eradication of wild poliovirus was declared.
Following further consideration of these issues, the Committee urged that the planned revision process for GAPIII should involve poliomyelitis vaccine manufacturers and the Guidelines amendment drafting group. In addition, the final decision on “shower-out” requirements should be based on the results of ongoing studies of its effectiveness and of the level of risk of skin exposure within containment facilities during poliomyelitis vaccine manufacturing and testing. After making a number of minor changes to the text, the Committee recommended that the document WHO/BS/2020.2381 be adopted and annexed to its report (Annex 3).

3.2.3 Revision of the WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines

The WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines were adopted in 2010. The Committee was informed that WHO had now received a request from Sanofi Pasteur to consider the revision of Appendix 2 of the recommendations, which specifically addresses the testing of new virus master and working seeds in non-human primates. Appendix 2 sets out the ways in which virus master and working seed lots should be tested for viscerotropism, immunogenicity and neurotropism, both in terms of clinical evidence and histological lesions, based on comparison against a reference virus approved by the NRA. Clinical scores are based on daily examination of the test animals over a period of 30 days by personnel familiar with the clinical signs of encephalitis in primates. Each animal is given a numerical score each day based on a four-point scale, where a score of 4 represents the most severe clinical signs.

During recent tests of yellow fever virus (YFV) working seed lots, which included Sanofi Pasteur’s new Vero-YFV, discrepancies were observed in the clinical scoring of two lots, even though the overall test package results were similar. The viscerotropism and immunogenicity results indicated that the animals had been correctly inoculated, and histological scoring met the acceptance criteria. It was therefore concluded that histological scoring was more reliable than clinical scoring. This conclusion was further supported by observed differences in the clinical scores obtained for the same reference PV26 virus in tests carried out in 2011 and 2018.

As such data and the published literature both suggest that clinical evaluation is less reliable than histological evaluation in the non-human primate model, Sanofi Pasteur had requested the revision of both Appendix 2 and the European Pharmacopoeia 0537 monograph in order to align assessment of the neurotropism of yellow fever vaccines in monkeys with the corresponding assessment of neurovirulence in oral poliomyelitis vaccines. In the case of the latter, the clinical signs are recorded but do not form part of the assessment
or pass/fail criteria. Prior to proceeding with the establishment of a drafting group of subject matter experts to prepare a draft amendment text, WHO was seeking the advice of the Committee. The Committee agreed that this was a good proposal and that a drafting group should be established to consult with as many manufacturers and other stakeholders as possible.
4. International reference materials – blood products and related substances

All reference materials established at the meeting are listed in Annex 4.

4.1 WHO international reference standards for blood products and related substances

4.1.1 Third WHO International Standard for thrombin

The Second WHO International Standard for thrombin has primarily been used by manufacturers of diagnostic and therapeutic thrombin products for the purpose of potency assignment. Such products increasingly include fibrin sealant or “glue” kits for use as topical haemostats, sealants or adhesives in surgical procedures. The principal constituents of such products are thrombin and fibrinogen. Following its establishment in 2003, the international standard had been in high demand and was now in need of replacement.

An international collaborative study involving 19 laboratories in 13 countries was conducted to evaluate the suitability of two candidate materials (NIBSC codes 01/578 and 19/188) for use as the Third WHO International Standard for thrombin. The potencies of the two candidate materials were estimated relative to the current international standard using plasma or fibrinogen clotting assays and/or chromogenic/fluorogenic assays. Data were received for a total of 111 assays, including 91 clotting assays, 16 chromogenic assays and four fluorogenic assays. Inter- and intra-laboratory variation was low, with geometric coefficients of variation of < 5% for all assay methods and substrates. In line with the collaborative study carried out in 2002 to establish the current international standard, candidate material 01/578 produced clotting assay potency estimates that were significantly lower than those obtained using chromogenic assays. However, the mean potency estimates obtained for coded duplicates of candidate material 19/188 were almost identical, with combined overall mean potencies of 90.4 IU/ampoule by clotting assay and 88.1 IU/ampoule by chromogenic assay. The closer accordance of potency estimates obtained for 19/188 using the different assays indicated a higher α-thrombin content compared to 01/578 and equivalence with the current international standard. Accelerated degradation studies indicated that the candidate material 19/188 was stable, with predicted potency losses at −20 °C of 0.036% and 0.041% per year by clotting and chromogenic assay respectively. The material also proved to be stable following reconstitution, with little loss in activity over the assay period.
The Committee considered the report of the study (WHO/BS/2020.2384) and noted that the proposal appeared to be clear and without issue. It therefore recommended that the candidate material 19/188 be established as the Third WHO International Standard for thrombin with an assigned unitage of 90 IU/ampoule based on the results of clotting assays.
5. International reference materials – in vitro diagnostics

All reference materials established at the meeting are listed in Annex 4.

5.1 WHO international reference standards for in vitro diagnostics

5.1.1 First WHO International Standard for *Plasmodium vivax* antigen

*Plasmodium vivax* is the second most abundant human malaria parasite after *P. falciparum* and the accurate diagnosis of vivax malaria is essential for determining appropriate treatment. It is recommended that suspected cases of malaria are confirmed by parasitological diagnosis using microscopy or rapid diagnostic tests (RDTs) prior to the initiation of anti-malaria chemotherapy. Malaria RDTs detect *Plasmodium*-specific antigens in patient blood samples and have a range of different antigen targets depending on their specificity. The principal *P. vivax* antigen targeted by RDTs is lactate dehydrogenase (PvLDH).

To date, the development of WHO international reference standards for malaria has focused on *P. falciparum* and a range of molecular and serological reference materials for the standardization and quality control of assays for its detection has been established. Efforts were now under way to develop corresponding reference materials specifically for *P. vivax*, prompted in part by the reported variability of RDTs used for its detection in endemic settings. At its meeting in 2017, the Committee had endorsed a project to develop a *P. vivax* antigen standard to support the development and quality control of malaria RDTs.

An international collaborative study had been conducted to assess the suitability of a candidate PvLDH material (NIBSC code 19/116) comprising red blood cell lysates obtained from *P. vivax*-infected donors, alongside clinical isolates and recombinant protein. Sixteen laboratories in 11 countries participated in the study, which involved ELISA and RDT manufacturers, research institutes and universities, and end users in the field of malaria diagnostics. Antigen detection was evaluated separately for ELISAs and RDTs, with laboratories participating in either or both parts of the study. The overall aims of the study were: (a) to determine whether the candidate material 19/116 was detectable in a wide range of antigen detection assays; (b) to harmonize measurements across different assays and laboratories; and (c) to assess whether the candidate material 19/116 behaved similarly in RDTs to PvLDH found in *P. vivax* clinical isolates.

PvLDH was consistently detected by the majority of laboratories, and reporting both potency and limit-of-detection values relative to the candidate material 19/116 reduced inter-laboratory variability. The candidate material was detectable using all of the RDTs analysed. In each case it reacted with the appropriate target band and was not detected by any *P. falciparum*-specific bands serving as negative controls. The geographically diverse clinical isolates exhibited
the same qualitative behaviour as the candidate material across all devices, indicating a high degree of similarity of performance. Currently available data indicated that the candidate material was stable under the recommended storage conditions of −70 °C or below; however, accelerated and real-time thermal degradation studies were ongoing and long-term storage recommendations would be made once more data became available.

The Committee enquired whether the proposed material might exhibit limitations if used with innovative RDTs based on other *P. vivax* antigens. Assurance was given that, although the principal target in the collaborative study had been PvLDH, as a fixed parasite preparation the proposed material should be suitable for future RDT developments. The Committee considered the report of the study (WHO/BS/2020.2385) and recommended that the candidate material 19/116 be established as the First WHO International Standard for *Plasmodium vivax* antigen, with an assigned unitage of 1000 IU/ampoule.

All reference materials established at the meeting are listed in Annex 4.

6.1 Proposed new projects and updates – standards for use in high-throughput sequencing technologies

6.1.1 Proposed First WHO International Reference Panel A for adventitious virus detection by high-throughput sequencing

The development of metagenomic approaches based on high-throughput sequencing offers the possibility of significantly improving the detection of adventitious agents in biological products. To realize this possibility, appropriate reference materials are required for the optimization and comparison of different methods. Since 2016, a multiplex reference material (NIBSC code 11/242-001) has been available but feedback received from the initial study participants and potential end users had highlighted a number of required improvements.

The development of a new WHO international reference panel was therefore being proposed based on reduced complexity, improved purity and characterization relative to 11/242-001. The new panel would be used as a parallel or spiked-in run control to facilitate the optimization and comparison of adventitious virus detection methods. Anticipated users of the panel include biological product manufacturers, contract research/testing laboratories, regulatory agencies and academic laboratories. The panel would consist of seven individual virus stocks – human herpesvirus 1, porcine circovirus 1, SV40 polyomavirus, canine parvovirus 2b, feline leukaemia virus, simian rotavirus SA11 and influenza (A)H1N1 virus. Each vial would contain approximately $10^6$ infectious particles with information provided on genome titres, particle counts and nucleotide sequences. The proposed collaborative study would involve up to 20 laboratories incorporating the panel into their adventitious agent detection testing. The use of the panel would permit meaningful comparisons to be drawn between different methods and laboratories. It would also allow for the determination of limits of detection based on the physical properties of different viruses, and for the validation of existing infectivity methods. It was anticipated that the collaborative study would be completed in 2022 with its report submitted to the Committee in 2023.

Following a brief discussion of the composition of the panel, the Committee endorsed the proposal (WHO/BS/2020.2383) to develop a First WHO International Reference Panel A for adventitious virus detection by high-throughput sequencing.
6.1.2 Proposed WHO international reference reagents for adventitious virus detection by high-throughput sequencing

The importance of high-throughput sequencing technologies as an alternative or supplementary approach to the currently recommended adventitious virus detection assays was again highlighted. Such assays are not standardized and are time consuming to perform, typically requiring > 30 days to produce results. In addition, the replacement of in vivo adventitious virus testing assays is in line with the 3Rs concept of “Replace Reduce Refine” for minimizing the use of animals in research. The proposed international reference reagents would be used as reference materials for standardizing and validating the detection of adventitious viruses in biological products using high-throughput sequencing methods. Five virus families with distinct physical and chemical resistance properties had now been selected to test various steps in the workflow of such methods. The viruses and cell lines for their propagation had been obtained from the American Type Culture Collection (ATCC) where large-scale virus stocks are prepared that meet the criteria for infectious titre and genome copy number. The five proposed international reference reagents each consisted of one of the following viruses – porcine circovirus type 1, mammalian orthoreovirus type 1, feline leukaemia virus, human respiratory syncytial virus and Epstein-Barr virus. It was envisaged that these reference materials would be used to demonstrate the sensitivity, specificity and reproducibility of the key steps of sample processing, cDNA synthesis, library preparation, sequencing and bioinformatics analysis.

During an ongoing international collaborative study involving vaccine manufacturers, contract research organizations and regulatory agencies, eight laboratories had carried out spiking studies to evaluate the detection of the five reference viruses using independent protocols, sequencing platforms and bioinformatics pipelines. All study participants used the five reference reagents to spike at different concentrations into a background of a high-titre virus to mimic the testing of a viral vaccine seed for adventitious virus contamination. The resulting data were currently being analysed and it was anticipated that the study report would be submitted to the Committee in October 2020.

The Committee noted that manufacturers had been well represented during the development of the virus strain collection. In addition, the strains were not being presented as a single panel, thus allowing for flexibility in their use. It was clarified that although the reference materials had not been inactivated (as they were intended to evaluate all of the steps involved) they were all BSL-2 viruses and there would therefore be no implications for their distribution. The Committee endorsed the proposal (WHO/BS/2020.2383) to develop WHO international reference reagents for adventitious virus detection by high-throughput sequencing.
The Committee then discussed the increasing importance of high-throughput sequencing technologies as a regulatory tool for ensuring the safety of biological products. Taken together, this and the previous proposal (see section 6.1.1 above) highlighted the need for virus reference standards in this area. It was noted that although each of the proposed reference materials comprised a collection of different viruses, their differential presentation (either as a set of individual viruses or single combined panel) would be complementary, with each having its own merits, thereby justifying the endorsement of both proposals. To ensure that these materials would be clearly distinguishable, the Committee noted that much of the detail on each one would need to be covered in the instructions for use.

The consideration of these two similar proposals then led to a more general discussion on how best to coordinate the activities of WHO collaborating centres to avoid the potential duplication of effort – an issue that had previously arisen in Committee discussions. Given the burden of work on custodian laboratories, the Committee considered that this issue might usefully be discussed by the WHO network of collaborating centres on standardization and regulatory evaluation of vaccines, and possibly other networks. However, it also reiterated its view that the above proposals were complementary and did not in this case represent a duplication of effort.

All reference materials established at the meeting are listed in Annex 4.

7.1 Proposed new projects and updates – standards for use in public health emergencies

7.1.1 Proposed First WHO International Standard for SARS-CoV-2 RNA for NAT-based assays

SARS-CoV-2 is the aetiological agent of COVID-19 with an estimated 10% of cases requiring medical intervention, of which a small percentage progress to severe pneumonia and death. On 30 January 2020, WHO declared COVID-19 to be a public health emergency of international concern, with the pandemic subsequently declared on 11 March 2020. The accurate diagnosis of infection with SARS-CoV-2 is essential both for clinical treatment of the individual and for informing national policies and approaches for reducing the spread of infection. The WHO Prequalification unit was currently assessing candidate IVDs for the detection of SARS-CoV-2 for EUL. To date, 50 expressions of interest had been received for the listing of NAT-based assays and 15 such assays had now been listed as eligible based on their compliance with WHO EUL requirements. There was now an urgent need for a common international reference material for the assessment and standardization of such diagnostic assays.

It was proposed that two types of material would be explored: (a) a high-titre SARS-CoV-2 preparation grown at NIBSC and inactivated by a validated treatment; and (b) SARS-CoV-2 RNA packaged inside lentiviral vector (LVV) particles to produce safe, non-infectious and non-replicating chimeric constructs. The proposed international collaborative study would involve around 27 laboratories worldwide performing NAT-based assays for SARS CoV 2, including national control laboratories, manufacturer laboratories, and clinical and academic laboratories. Using a panel of inactivated SARS-CoV-2 preparations and LVV constructs, the study would assess the suitability of each candidate material to serve as the proposed WHO international standard for use in the harmonization of SARS-CoV-2 NAT-based diagnostic assays. The candidate materials would be characterized in terms of their reactivity and specificity in different assay systems, and through measurement of their potency in a range of typical assays. The LVV constructs would also be assessed for commutability with the SARS-CoV-2 preparation as part of the study. Given the urgent need for the proposed standard it was intended that a progress report on the collaborative study would be submitted to the Committee in October 2020 followed by the final report in December 2020 for a decision on the establishment of the proposed standard.
The Committee discussed a number of issues raised by the proposal, most notably the quality of existing assays and the choice of target viral strains. Although current in-house NAT-based assays generally work, it was felt that they were poorly standardized and the development of the proposed standard was therefore important. The Committee enquired whether there was a need for sub-genomic standards for replicating virus but recognized that none of the current diagnostic assays were based on sub-genomic RNA and that it was too late to make changes to the proposed collaborative study. Instead, the Committee suggested that a literature review be carried out to evaluate whether the candidate materials would be suitable for assays based on sub-genomic RNA. The Committee further suggested that the variable performance of some in-house assays may be a consequence of the choice of primers and considered whether the target strains to be used in the collaborative study were likely to work with any primer. The Committee requested that the match between primer sequence and the target strains be checked.

Overall, the Committee felt that this was an excellent proposal and agreed with the choice of target strains. The Committee therefore endorsed the proposal (WHO/BS/2020.2383) to develop a First WHO International Standard for SARS-CoV-2 RNA for NAT-based assays.

7.1.2 Proposed First WHO International Standard for anti-SARS-CoV-2 immunoglobulin

Serological assays are essential for assessing the epidemiological impact of COVID-19. Such assays can be used to determine the number of people who have been infected, and to better understand the transmission of SARS-CoV-2, particularly as most cases with mild symptoms go undetected. In addition, serological assays are required to measure immune responses to vaccines in clinical development and to support the use of therapeutic antibody preparations. COVID-19 vaccines were now being developed at an unprecedented rate, with around 170 candidate vaccines currently in the pipeline – 31 of which were in clinical development.

Plasma or serum taken from convalescent patients would be used for the proposed standard as these are likely to be commutable due to their similarity to the clinical samples analysed in the assay. The project was being supported by CEPI which had helped to identify donors of candidate material in several affected countries. All of the clinical and other materials obtained had been screened for bloodborne viruses and rendered non-infectious following viral inactivation using a validated solvent-detergent method.

The proposed international collaborative study would involve more than 50 laboratories worldwide performing a range of serological assays for anti-SARS-CoV-2 immunoglobulin, including ELISAs and neutralization assays.
Study participants would include national control laboratories, vaccine and diagnostic kit manufacturer laboratories, and clinical and academic laboratories. Each laboratory would receive a panel of samples including: (a) the candidate material comprising a pool of high-titre plasma from 11 donors; (b) NIBSC research reagent 20/130; and (c) various samples covering a range of anti-SARS-CoV-2 immunoglobulin titres. The suitability of the candidate material to serve as a WHO international standard for the harmonization of SARS-CoV-2 serological assays would then be evaluated. This would be achieved by determining the reactivity and specificity of the various antibody preparations in different assay systems, measuring their potencies and assessing their commutability. Based on the proposed timeline and the data returned so far, it was anticipated that the final report of the collaborative study would be available for submission to the Committee in December 2020.

The Committee unanimously recognized the urgent need for a WHO international reference material to standardize the assays used in COVID-19 vaccine evaluation and in the diagnosis of SARS-CoV-2 infection. The Committee further recognized that the preparation of the candidate material and the collaborative study will have been completed in the shortest possible timeframe, and noted the crucial importance of the advance availability of the NIBSC research reagent, which had facilitated assay development earlier in the year. The Committee also discussed the prospective use of the reference material in standardizing antibody levels in therapeutic CCP and strongly urged that potency statements are made in IU as soon as the standard is established. The Committee endorsed the proposal (WHO/BS/2020.2383) to develop a First WHO International Standard for anti-SARS-CoV-2 immunoglobulin.
8. International reference materials – vaccines and related substances

All reference materials established at the meeting are listed in Annex 4.

8.1 WHO international reference standards for vaccines and related substances

8.1.1 WHO International Reference Reagent for anti-malaria (*Plasmodium vivax*) plasma (human)

To date, the development of WHO international reference standards for malaria has focused on disease caused by *P. falciparum*. However, as the next most common cause of malaria, work was now under way to develop corresponding standards for *P. vivax*. In addition to the *P. vivax* antigen standard proposed for establishment at the current meeting (see section 5.1.1 above) there was also a need for a serology standard to support vaccine development and immunoepidemiological studies. Although vaccines for the prevention of *P. vivax* malaria are not yet as advanced as those for *P. falciparum*, clinical trials are ongoing and an appropriate serological reference reagent would be valuable in harmonizing the assays used to evaluate immune responses.

An international collaborative study involving 15 laboratories in seven countries had been conducted to assess a candidate material (NIBSC code 19/198) for its suitability to serve as a WHO international reference reagent for use in ELISAs and potentially in other serological methods. The candidate material comprised lyophilized human plasma derived from patients with acute *P. vivax* infection. Selective pooling had been used to maximize the titres of antibodies against two candidate vaccine antigens currently in clinical trials – PvCSP and PvDBP-RII. Study results indicated that the use of candidate material 19/198 as a reference material reduced the variability of ELISA results both within and between laboratories. Accelerated thermal degradation studies indicated that long-term storage at −80 °C would ensure sufficient stability across the breadth of antibodies in the material and further stability monitoring was ongoing.

The Committee acknowledged that many blood services worldwide use antibody tests to screen donors and felt that a *P. vivax* antibody reference reagent would be useful. As there were at present only relatively few tests for screening individuals in *P. vivax* endemic areas, the proposed reference reagent would help to ensure accurate and harmonized results. The Committee welcomed the analysis that had been conducted of the immunoglobulin isotypes present in the material. Noting that the unit assignment was based only on ELISA data, the Committee queried the likely utility of the proposed reference reagent for other assay types. Although immunofluorescent methods could be ruled out
on the basis of their limited use and at best semi-quantitative results, it was considered that Luminex® and other multiplex-based methods would likely be of importance in the future. The Committee considered the report of the study (WHO/BS/2020.2382) and recommended that the candidate material 19/198 be established as the First WHO International Reference Reagent for anti-malaria (Plasmodium vivax) plasma (human) with an assigned unitage of 100 U/ampoule.
Annex 1

WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological products

WHO Recommendations, Guidelines and other documents are intended to provide guidance to those responsible for the development and manufacture of biological products as well as to others who may have to decide upon appropriate methods of assay and control to ensure that such products are safe, reliable and potent. WHO Recommendations (previously called Requirements) and Guidelines are scientific and advisory in nature but may be adopted by an NRA as national requirements or used as the basis of such requirements.

Recommendations and guidance on biological products are formulated by international groups of experts and published in the WHO Technical Report Series7 as listed below. A historical list of Requirements and other sets of Recommendations is available on request from the World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland.

Reports of the WHO Expert Committee on Biological Standardization published in the WHO Technical Report Series can be purchased from:

WHO Press
World Health Organization
20 avenue Appia, 1211 Geneva 27
Switzerland
Telephone: + 41 22 791 3246
Fax: +41 22 791 4857
Email: bookorders@who.int
Website: www.who.int/bookorders

Individual Recommendations, Guidelines and other documents may be obtained free of charge as offprints by writing to:

Technical Standards and Specifications unit
Department of Health Product Policy and Standards
Access to Medicines and Health Products
World Health Organization
20 avenue Appia, 1211 Geneva 27
Switzerland

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7 Abbreviated in the following pages to “TRS”. 
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8 Available online at: [https://www.who.int/biologicals/expert_committee/QA_for_SBPs_ECBS_2018.pdf?ua=1](https://www.who.int/biologicals/expert_committee/QA_for_SBPs_ECBS_2018.pdf?ua=1)
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<td>Regulation and licensing of biological products in countries with newly developing regulatory authorities</td>
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<td>Yellow fever vaccines, laboratories approved by WHO for the production of</td>
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<td>Yellow fever virus, production and testing of WHO primary seed lot 213-77 and reference batch 168-736</td>
<td>Adopted 1985, TRS 745 (1987)</td>
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Annex 2

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

Replacement of Annex 1 of WHO Technical Report Series, No. 941

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

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

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

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

Since the 1990s, a novel third approach to vaccination against a broad array of target antigens and diseases has been in development. This technology involves the direct administration of plasmid deoxyribonucleic acid (DNA) containing the gene encoding the immunogen against which an immune response is sought, leading to the in-situ production of the target immunogen(s) in the vaccine recipient. Such vaccines are referred to as “plasmid DNA vaccines” or simply “DNA vaccines” (with both terms used interchangeably throughout these Guidelines). This approach offers a combination of potential advantages, including the stimulation of both B-cell and T-cell responses, stability of the vaccine across a broad temperature range, absence of infectivity of the immunogen itself, the speed with which the vaccine can be constructed (for example, in the face of an epidemic or pandemic) and the relative ease and generic nature of large-scale manufacture. It may be feasible to produce the same DNA vaccine in different facilities in different countries to facilitate accessibility and availability of the vaccine during routine immunization or in outbreak settings, thus ensuring a more stable supply of vaccine. Furthermore, DNA vaccines can be more stable than other more common vaccine types and may therefore, depending on their formulation, be stored and efficiently delivered in the absence of a cold chain. DNA vaccines do not generate anti-vector immunity or off-target acquired immunity to DNA in the vaccine recipient. DNA vaccines are not designed to be infectious and the target infectious pathogen is not used in their construction or production. However, the manufacturing of such vaccines in bacteria may require appropriate biosafety containment in accordance with local regulations. Although chromosomal integration of the plasmid DNA was initially a major theoretical concern, the data obtained to date have not
borne out this concern. In summary, DNA vaccines can be viewed as a platform technology in which the gene insert can be readily changed without necessarily having to change the manufacturing or control of the resulting new product (with the exception of the immunogen-specific tests for identity and potency). Numerous scientific publications have addressed the potential benefits of DNA vaccination (1–10).

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

The development and application of DNA vaccines continues to progress. Since the WHO Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines were adopted by the Expert Committee for Biological Standardization in 2005 (20), many clinical trials of DNA vaccines have taken place and considerable experience in their manufacture and control has accrued. The current revision reflects this experience, especially in relation to data derived from nonclinical and clinical safety testing, which address many of the concerns expressed in previous versions of these Guidelines. The control of DNA vaccines should continue to be approached in a flexible manner to enable further modifications as more experience is gained in their production and use, and as other components or delivery systems are included. The intention of the current document is to provide a scientifically sound basis for the consistent manufacture and control of DNA vaccines for human use to ensure their continued safety and efficacy following licensure. Given the potential of DNA vaccination as a platform technology for addressing priority pathogens during public health emergencies (21–26), international regulatory convergence for DNA vaccines is needed. This document provides up-to-date guiding principles.
for evaluating the quality, safety and efficacy of DNA vaccines for human use. It is worth noting that while plasmid DNAs are generated using recombinant DNA (rDNA) technology, existing guidelines specific to rDNA products generally do not apply to DNA vaccines, as such guidelines are intended to cover the manufacture of biotherapeutic proteins generated in cell lines.

**Purpose and scope**

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

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

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

- updating the Introduction with additional data, including citations for nonclinical and clinical data that collectively address many historical safety concerns;
- restricting the scope to preventive DNA vaccines against infectious diseases;
- updating the quality section (Part A) to make it more consistent with current practices and with other WHO guidelines;
- extensively revising the nonclinical section (Part B) to include references to general WHO guidelines adopted since the previous version and to better focus on a number of specific issues;
- adding a clinical section (Part C) that also includes references to recently revised general WHO guidelines and that also focuses on a number of specific issues;
- adding a section on specific guidance to NRAs (Part D); and
- adding a model summary protocol for the manufacturing and control of plasmid DNA vaccines (Appendix 1) and a model NRA/NCL Lot Release Certificate for plasmid DNA vaccines (Appendix 2).

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

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

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

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

The guidance provided in these Guidelines will be relevant to the DNA vaccine at the time of application for marketing authorization. Nevertheless,
some relevant information is provided regarding candidate vaccine products in development; in any case, the respective NRA should be consulted prior to clinical development on a case-by-case basis (27–29).

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

**Terminology**

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

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

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

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

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

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

**Final lot**: a collection of sealed final containers that is homogeneous with respect to the composition of the product and the avoidance of contamination during filling. A final lot must therefore have been filled from a formulated bulk in one continuous working session.
**Final product:** a finished dosage form (for example, suspension or lyophilized cake) that contains an active ingredient, generally but not necessarily in association with inactive ingredients (excipients) or adjuvants. Also referred to as “finished product” or “drug product” in other documents.

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

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

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

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

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

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

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

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

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

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

Background

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

Theoretically, DNA vaccines would be ideal for use in boosting immune responses as they could be used repeatedly (and for different purposes) because they do not generate anti-vector immune responses. However, existing data demonstrate that DNA vaccines excel at priming immune responses. These primed immune responses are boosted by the subsequent delivery of a heterologous vaccine (for example, a protein antigen or a different gene-based vector) such that the resulting immune responses are often more potent than if either modality is used alone for both prime and boost or if the DNA vaccine is given second (32–39). In some cases, the priming immune response to a DNA vaccine is only revealed once a heterologous boost has been administered (40, 41). The responses to the heterologous boost may be amplified compared to the responses to a homologous regimen of the booster vaccine (42–45). The DNA prime can also modulate the type of immune response observed following the heterologous boost; an effect not seen with the booster vaccine alone (38, 41, 45). In other cases, robust responses to the DNA vaccine alone can be observed (46). Clearly, the nature of the immune response will depend upon the immunogen expressed and the immunomodulatory elements in the design
or formulation of the DNA vaccine, as well as on the method of delivery (47).
Evaluation of the contribution of the DNA vaccine to the immunogenicity of any given vaccination regimen may best be assessed by the ultimate immune responses of the regimen as a whole in comparison with a regimen that does not include DNA vaccination. This is not to suggest that immune responses to the plasmid DNA prime should not also be evaluated, but rather that the priming response may best be assessed in the context of the boosted response.

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

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

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

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

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

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

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

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

The current generation of DNA vaccines made from bacteria are produced biologically and are considered to be a biological product. In addition, even though the plasmid is generated by recombinant DNA technology, it should be clarified that a plasmid DNA vaccine is not an organism; thus, it is not a genetically modified organism (GMO) per se, nor is it a gene-transfer or gene-therapy product. There is a wealth of evidence that DNA vaccines to date do not persist or even biodistribute throughout the body of the vaccine recipient when delivered parenterally into muscle, subcutaneous tissue or various dermal layers (62–70). What does predominantly biodistribute is the immune response generated following uptake of the plasmid DNA and in situ expression of the immunogen(s), along with cross-priming from myocytes to professional
antigen-presenting cells (71, 72). The local response to plasmid DNA inoculation is that cells take up the plasmid and then express the immunogen(s) encoded in the DNA vaccine and/or the nucleic acid is degraded by normal molecular mechanisms. As a consequence, the plasmid DNA clears from the injection site over time, while it is the immune response that may persist.

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

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

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

A.1 Definitions

A.1.1 International name and proper name

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

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

A.1.2 Descriptive definition

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

A.2 General manufacturing guidelines

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

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

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

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

It is recognized that the level of detail required by a regulatory authority increases as product development proceeds. During the initial phases of clinical development, the information contained in a clinical trial application should be adequate to allow for assessment of the safety risks derived from the manufacturing process. This would include, for example, the methods and results of testing of the bacterial cell banks for identity, identification and specifications
for all materials used in the process, assessment of risks from biologically sourced materials, certification or phase-appropriate GMP compliance of the manufacturing facility, a brief description of the process and tests, results of testing of the clinical trial material, and preliminary stability of the final product. As with all vaccines, the level of detail expected on the Quality (manufacturing and control) would increase for late-stage clinical trials.

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

A.3  **Control of bulk purified plasmid (bulk)**

A.3.1  **General information**

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

A.3.2  **Manufacture**

A.3.2.1  **Control of materials**

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

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

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

Information, including proper certification, regarding the source, manufacture and characterization of all biologically sourced materials or materials produced using biologically sourced materials should be provided. Risk assessments for
bovine spongiform encephalopathy agents should be provided if bovine materials were used at any stage. Compliance is expected with the WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (81).

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

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

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

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

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

A.3.2.1.3 Cell bank system, characterization and testing

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

For early-stage clinical trials, it may be appropriate to use the MCB to initiate production – though manufacturers are expected to prepare a WCB for later clinical studies. Initiating production from a well-characterized WCB is expected for commercial manufacturing.
A well-characterized bacterial cell containing the plasmid should be cloned and used to establish the MCB. The preparation of the MCB and WCB should be conducted according to GMP with appropriate precautions taken to prevent contamination. Information should be provided on the origin and storage conditions of the cell banks. Evidence for the viability of the MCB and WCB under storage and recovery conditions should also be provided by the time of application for marketing authorization. New WCBs should be fully characterized and meet established acceptance criteria. Specific phenotypic features that can form a basis for identification of the transformed cell should be described. Prior to their use, either a protocol for establishing and releasing new WCBs or information on each new WCB should be provided for regulatory review and concurrence.

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

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

A.3.2.2 Process development and in-process control

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

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

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

A.3.3 Characterization

A.3.3.1 Characterization of bulk purified plasmid

A summary of the characterization of the bulk purified plasmid(s) should be provided in addition to in-process and lot-release testing. Rigorous characterization using a range of orthogonal chemical, physical and biological methods will be essential.
During development, the nucleotide sequence of the entire plasmid should be determined, as discussed in sections A.3.2.1.2 and A.3.2.1.3 above. Demonstration of expression of the full-length protein(s) without truncated or alternative forms should also be provided.

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

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

A.3.3.2 Consistency of manufacturing

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

During early-stage development, few lots will have been made, and demonstration of consistency may be limited. Demonstration of consistency will occur as manufacturing experience is gained during product development. Demonstration of the consistency of lots is generally performed during advanced development (when the manufacturing process has been scaled-up for commercial manufacture) but prior to submission of a licence or marketing application. Whenever changes to the manufacturing process are implemented, the comparability of lots, especially to those used in pivotal studies and the intended commercial process, should be demonstrated. Comparability protocols and strategies for demonstrating comparability are discussed in the WHO Guidelines on procedures and data requirements for changes to approved vaccines (80).
A.3.4  **Control of bulk purified plasmid**

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

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

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

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

A.3.4.1  **Identity**

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

A.3.4.2  **Purity**

Limits based on process capability and regulatory guidance should be established for all impurities detected, and these should be identified and characterized as appropriate. The degree of contamination with host cell chromosomal DNA, RNA and proteins should be evaluated and limits established, and acceptance criteria established and specified. Comparison of the absorbance at 260 nm and 280 nm may be useful for purity assessment, for example of the extent of contamination introduced by RNA and cellular proteins. However, other suitable methods may be appropriate for purity assessment. Residual levels of medium components (including antibiotics, if applicable) and other materials from process steps should also be controlled. The analysis should include sensitive
and reliable assays for process- and product-related contaminants and strict upper limits should be specified for their content in the bulk purified plasmid. A maximum allowable limit should be established and justified. It is important that the techniques used to demonstrate purity be based on as wide a range of physicochemical properties as possible. Measuring residual levels of process- or product-related impurities as part of quality control may be discontinued after suitable processes for their removal have been adequately validated. Plans and specifications for the periodic revalidation of processes should be described. Until such processes have been validated, impurities should continue to be measured for a number of lots, as acceptable to the NRA. In the case of major changes to manufacturing, process revalidation or continued measurement for the number of lots agreed to by the NRA would be expected. Container-closure system compatibility, leachables and extractables should be assessed and discussed in the marketing authorization application.

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

A.3.4.3 Physical state and quantification of plasmid

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

A.3.4.4 Safety

Relevant safety tests should be described and may include tests for: (a) endotoxins; (b) bacterial and fungal sterility (including demonstration of lack of bactericidal or fungicidal activity of the test article); or (c) bioburden (including quantity, identification and freedom from specified unwanted organisms). Although a test for pyrogenicity may be performed if required by the NRA, animal testing should be avoided whenever alternative satisfactory testing is accepted. For ethical reasons, it is desirable to apply the 3Rs concept of “Replace Reduce Refine” to minimize the use of animals, and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation. In particular, manufacturers and regulators should take note of the decision of the WHO Expert Committee on Biological Standardization in 2018 to discontinue the inclusion of the general safety (innocuity) test in routine lot release testing.
requirements for all vaccines in WHO Recommendations, Guidelines and other guidance documents for biological products (82). This test should therefore not be required or requested.

A.3.5  **Reference materials**
An in-house reference preparation should be established for use in assay standardization. Information on the reference standards or reference materials used for testing of the bulk purified plasmid should be provided by the time of application for marketing authorization. A suitable batch (that is, one that has been clinically evaluated) should be fully characterized in terms of its chemical composition, purity, biological activity and complete sequence, and retained for use as a chemical and biological reference material. A plan for replacing the initial reference material upon exhaustion should be agreed with the NRA.

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

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

A.3.6  **Stability**
The stability assessment should be in compliance with the WHO Guidelines on stability evaluation of vaccines (83). The types of studies conducted, the protocols used and the results of the studies should be summarized in an appropriate format such as tables, graphs or a narrative document. The summary should include results and conclusions regarding appropriate storage conditions and shelf-life. Stability data to support the shelf-life of the bulk and any future extension of it should be derived from long-term, real-time stability studies under actual conditions.

Limited stability information would be expected during initial clinical development. For example, some regulators accept three months of real-time stability at the time of application for clinical trial authorization, but this should be agreed with the NRA. Lots should be labelled with a retest or re-pass date, if required by the NRA.
A.4 **Control of final formulated vaccine (vaccine)**

A.4.1 **Composition**

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

A.4.2 **Manufacture**

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

A.4.3 **Control of materials**

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

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

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

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

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

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

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

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

A.4.4.1 Identity

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

A.4.4.2 Purity

The purity of each lot of vaccine should be determined and shown to be within specified limits. The form of the final product plasmid(s) should be confirmed – for example, by conducting gel electrophoresis or other method to demonstrate that the vaccine has not degraded. Container-closure system compatibility, leachables and extractables should be assessed and discussed.
Where multi-product facilities or contract manufacturing organizations are used for the manufacturing process, freedom from contamination with other products should be demonstrated to established limits or below detection.

A.4.4.3 Content, strength or quantity
DNA vaccines are dosed based on the quantity of the plasmid by weight. Generally, this is established by absorbance at 260 nm (comparison of absorbance at 260 nm and 280 nm may be useful in assessing purity).

A.4.4.4 Other quality parameters
Quality parameters should be established and controlled. Important quality parameters include appearance and pH. Another important quality parameter is the percentage of the overall amount of plasmid that is supercoiled (plasmid may be present in other forms such as nicked circles or linear). Depending on the product characteristics, the control of other parameters such as osmolality and viscosity may be important. Furthermore, quality may be assessed by methods used to evaluate purity or identity, such as restriction mapping, gel or capillary electrophoresis, and/or HPLC – though these may best be performed on the bulk purified plasmid instead. Other tests, such as the test for residual moisture if the vaccine is lyophilized, may be required to confirm the physical characteristics of the product as well as its formulation.

A.4.4.5 Potency
The potency of each lot of the vaccine should be determined using a suitably quantitative and validated assay. Potency relative to an appropriate in-house reference preparation should be established. Ideally, a potency assay would be established that can ensure the consistency of lots with established clinical performance. Often, this takes the form of an in vitro expression system. The immunogen might be expressed in vitro by transfection of a suitable cell line and either the expressed mRNA or the expressed protein identified, for example, by quantitative RT-PCR (in the case of mRNA) or by immunofluorescence or Western blot (in the case of protein). It may be appropriate to establish potency on the basis of an alternative suitably justified laboratory method (that is, a non-bioassay). Early discussion should be held with the NRA to reach consensus regarding the appropriateness of the proposed method. Consensus should also be sought on the use of a composite measure of content (amount of plasmid DNA as used for dosing) and percentage of supercoiled plasmid for control of potency for release of each vaccine lot. An in vitro method demonstrating expression could then be considered for characterization instead of control of potency.

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

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

A.4.4.6 Safety, including sterility and endotoxin testing

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

A.4.4.7 Multi-component vaccines

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

In some cases it may not be feasible to measure potency in the context of a mixture of closely related antigens, and the potency of the individual plasmids may have to be measured in terms of expression (of mRNA or protein) in the individual bulk purified plasmids. If agreed to by the NRA, a composite measure
of content (amount of plasmid DNA as used for dosing) and percentage of supercoiled plasmid may be used for control of potency of the lot. In all cases, the approach taken and its justification should be clearly described.

A.4.5 Reference materials

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

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

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

A.5 Records

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

A.6 Retained samples

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

A.7 Labelling

The guidance on labelling provided in WHO good manufacturing practices for pharmaceutical products: main principles (78) and WHO good manufacturing practices for biological products (79) should be followed as appropriate. The label on the carton enclosing one or more final containers, or the leaflet accompanying each container, should include the following information at a minimum:
- the name of the vaccine;
- the names and addresses of the manufacturer and distributor;
- a statement that specifies the nature and content of adjuvant contained in one human dose, if any;
- the immunization schedule, and the recommended route(s) of administration;
- the number of doses, if the product is issued in a multi-dose container;
- the name and concentration of any preservative added;
- a statement on the nature and quantity, or upper limit, of any antibiotics present in the vaccine;
- the temperature recommended during storage and transport;
- the expiry/retest date;
- any special dosing schedules; and
- contraindications, warnings and precautions, and information on concomitant vaccine use and on known adverse reactions.

A.8 Distribution and transport

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

A.9 Stability testing, storage and expiry date

The relevant guidance provided in WHO good manufacturing practices for biological products (79) and in the WHO Guidelines on stability evaluation of vaccines (82) appropriate to the respective plasmid DNA vaccine should be followed. Furthermore, the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (85) might also be applicable. Any statements concerning storage temperature and expiry date that appear on the primary or secondary packaging should be based on experimental evidence and should be submitted to the NRA for approval.
A.9.1 **Stability testing**

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

A.9.2 **Storage conditions**

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

A.9.3 **Expiry date**

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

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

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

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

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

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

Similarly, for new DNA vaccines based on existing plasmid backbones for which there is already significant nonclinical (and possibly clinical) experience, an abbreviated nonclinical programme should be considered (21, 22, 69, 70). If the new gene insert is related to other antigens that have already been studied in nonclinical (and possibly clinical) programmes, a case may be made to support a safe starting dose and regimen for the new vaccine based on the existing nonclinical and clinical data without the need for additional toxicology studies.
In the context of the rapid development of a vaccine against a priority pathogen during a public health emergency, consideration may be given to an abbreviated nonclinical programme. In cases where the plasmid is constructed from a backbone that has already been clinically tested using a related antigen (for example, in the case of a pandemic influenza strain when a seasonal or other potential pandemic strain antigen has been tested) then the nonclinical programme might be limited to an immunogenicity study (or studies). However, such a study should collect as many safety data as feasible given that many nonclinical immunogenicity studies are performed without full compliance to good laboratory practices. Depending on the species used, where it is feasible to collect blood not only for immunogenicity assessments but also for haematology and chemistry assessments, these analyses should be performed. In addition, depending on the species used, if the animals are sacrificed at the end of the immunogenicity study then gross pathology and targeted histopathology should be performed. Information obtained from physical examinations or clinical findings should also be captured and reported to the NRA. If the species used is too small to permit individual clinical pathology (for example, mice) or if animals are not sacrificed because they will be used in other research after the immunogenicity study is performed (for example, non-human primates) then whatever safety data can be collected should be reported to the NRA. Where safety information is available on veterinary vaccines expressing related antigens, this information might usefully be provided to the NRA.

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

Although biodistribution studies were previously suggested for DNA vaccines, the data acquired to date have not shown reason to continue with such evaluations. Plasmid DNA remains largely at the injection site and does not biodistribute at clinically relevant levels or widely throughout the body. Furthermore, it does not target the ovaries or testes and clears from the body
by degradation (70, 71, 87, 88). However, most of these data were collected in adult animals. A limited amount of information is available from developmental toxicology and biodistribution studies in maternal or fetal animals (88). The publication of any developmental toxicology studies already performed on DNA vaccines is encouraged.

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

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

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

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

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

One issue of relevance to DNA vaccines is their use in heterologous prime-boost regimens and some guidance on this issue is provided in the above guidelines. A challenge for marketing authorization will be the labelling of each of the vaccines in the regimen that ultimately demonstrates efficacy, as this type of heterologous prime-boost regimen remains novel at this time, and health care workers and public health systems are not necessarily ready for this approach.
Clear labelling to prevent mix ups and mis-dosing will be crucial to a successful public health campaign or during routine use. Another issue that may require attention is the attribution of safety events observed following immunization, and how to clearly establish whether an event was due to the prime or the boost vaccine, even if the event occurred late (for example, after boosting).

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

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

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

- An R&D Blueprint for action to prevent epidemics. Plan of action. May 2016 (89);
- List of Blueprint Priority Diseases;\(^{10}\) and

\(^{10}\) See: [http://www.who.int/blueprint/priority-diseases/en/]
WHO Target Product Profiles.\textsuperscript{11}

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

\section*{Part D. Guidelines for NRAs}

\subsection*{D.1 General}

The guidance for NRAs and national control laboratories (NCLs) given in the WHO Guidelines for national authorities on quality assurance for biological products (92) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (93) should be followed. These guidelines specify that no new biological product should be released until consistency of lot manufacturing and quality has been established and demonstrated by the manufacturer. The guidelines do not apply to material for clinical trials.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety or efficacy of DNA vaccines, should be discussed with and approved by the NRA. When marketing authorization for a DNA vaccine against a specific disease is imminent, WHO guidelines specifically for such vaccines may be prepared through the consultative development and adoption process of the WHO Expert Committee on Biological Standardization. For DNA vaccines that target diseases for which other types of vaccines and corresponding guidelines are available, it may be appropriate to consider Part A from these Guidelines and Parts B and C from the disease-specific guidelines in tandem.

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

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

\textsuperscript{11} See: \url{https://www.who.int/research-observatory/analyses/rd_blueprint/en/index3.html}
results for clinical lots, as well as for a series of consecutive lots of the vaccine produced using the procedures and control methods that will be used for the marketed vaccine.

D.2 **Official release and certification**

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

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

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

**Authors and acknowledgements**

The preliminary draft of these WHO Guidelines was prepared by Dr R. Sheets, consultant, the USA; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr E. Nkansah, Food and Drugs Authority, Ghana; Dr M.A. Liu, Karolinska Institutet, Sweden; Dr K. Peden, United States Food and Drug Administration, the USA; and Dr H-N. Kang and Dr I. Knezevic, World Health Organization, Switzerland based on the discussions of a WHO informal consultation on regulatory expectations for the evaluation of nucleic acid vaccines, held in Geneva, Switzerland, 21–23 February 2018 and attended by: Dr P. Aprea, Administración Nacional de Medicamentos, Alimentos y Tecnología Médica, Argentina; Dr F. Bähner, CureVac AG, Germany; Dr J. Boyer, Dr L. Gibbs and Dr K.E. Broderick, Inovio Pharmaceuticals, the USA; Dr S. Darbooy, Food and Drug Administration, the Islamic Republic of Iran; Dr P. Duffy and Dr B. Graham, National Institutes of Health, the USA; Dr A. Farnsworth, Health Canada, Canada; Dr U. Gompels, London School of Hygiene & Tropical Medicine, the United Kingdom; Dr E. Grabski, Dr D. Loos and Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr D. Gutsch, Merck Research Laboratories, the USA (International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) representative); Dr W. Huang, National
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The first draft of these WHO Guidelines was then prepared by a WHO drafting group comprising Dr R. Sheets, consultant, the USA; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr M.A. Liu, Karolinska Institutet, Sweden; Dr K. Peden, United States Food and Drug Administration, the USA; and Dr H-N. Kang, World Health Organization, Switzerland, taking into consideration the comments received from: Dr P. Aprea, Administración Nacional de Medicamentos, Alimentos y Tecnología Medica, Argentina; Dr J. Boyer, Inovio Pharmaceuticals, the USA; Dr D. Gutsch, Merck Research Laboratories, the USA; Dr W. Huang, National Institutes for Food and Drug Control, China; Dr D. Kaslow, PATH Vaccine Development Global Program, the USA; Dr M. Page, National Institute for Biological Standards and Control, the United Kingdom; Dr M. Saville, Coalition for Epidemic Preparedness Innovations, Norway; Dr M. Savkina, Federal State Budgetary Institution Scientific Centre for Expert Evaluation of Medicinal Products, Russian Federation; and Dr R. Vogels, Janssen Vaccines & Prevention B.V., Netherlands.

The resulting draft document was then posted on the WHO Biologicals website for a first round of public consultation from 30 July to 20 September 2019. Comments were received from: Ms T. Cervinkova (provided the
consolidated comments of IFPMA), Switzerland; Mr J. Charbonneau (provided the consolidated comments of Health Canada), Canada; and Dr M. Savkina, Federal State Budgetary Institution Scientific Centre for Expert Evaluation of Medicinal Products, Russian Federation.

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

Further changes were made to document WHO/BS/2020.2380 by the WHO Expert Committee on Biological Standardization.

References


Appendix 1

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

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

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

The section concerning the final product should be accompanied by a sample of the label and a copy of the leaflet (package insert) that accompanies the vaccine container. If the protocol is submitted in support of a request to permit importation, it should also be accompanied by a lot release certificate (see Appendix 2) from the NRA or NCL of the country in which the vaccine was produced and/or released stating that the product meets all national requirements as well as Part A of these WHO Guidelines.

1. Summary information on final lot

International name of product: 

INN (if applicable): 

Commercial/trade name: 

Product licence (marketing authorization) number: 

Country: 

Name and address of manufacturer: 

Name and address of licence holder, if different: 

Plasmid designation (if applicable): 

Gene insert(s) (if applicable): 


Final packaging lot number: ________________________________
Type of container: ______________________________________
Number of containers in this final lot: _______________________
Number of doses per final container: ________________________
Preservative and nominal concentration (if applicable): ________

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

___________________________________________________________________________

Shelf-life approved (months): ________________________________
Date of manufacture: _______________________________________
Expiry date: _______________________________________________
Storage conditions: __________________________________________

2. Control of source material

2.1 Plasmid seeds (where applicable)

2.1.1 Seed banking system

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

___________________________________________________________________________

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

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

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

Confirmation of approval for use by manufacturer, and the basis
for that approval: ____________________________________________
2.1.2 Tests on working seed lot production (if applicable)
Antibiotic resistance (if applicable): 
Marker genes or selection genes (if applicable and different from antibiotic resistance gene): 
Identity: 
Bacterial and fungal contamination: 

2.2 Cultures and culture media (where applicable)

2.2.1 Cell bank system
Name and identification of cell strain and bank: 
Culture medium: 
Cell bank genealogy with dates of preparation, passage number and date of coming into operation: 

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

Test for absence of bacterial and fungal contamination: 

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

2.2.2 Tests on working cell bank production (if applicable)
Identification of cell bank: 
Culture medium: 
Cell bank genealogy with dates of preparation, passage number and date of coming into operation: 

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

Test for absence of bacterial and fungal contamination: 

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

Genetic stability (if genetically manipulated): 


3. Control of vaccine production

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

3.1.1 Information on manufacture

Batch number(s): 
Date of manufacture of each batch: 
Identification of reagents used during production or other phases of manufacture, including media components and antibiotics, if applicable:

Total volume of purified plasmid bulk: 
Volume(s), storage temperature, storage time and approved storage period:

3.1.2 Tests on purified plasmid bulk(s)

Identity: 
Purity: 
Antigen content (quantity): 
Physical state (that is, % supercoiled): 
Sterility (bacterial and fungal): 

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

Residual protein content: 
Residual DNA derived from the expression system: 
Residual RNA: 
Endotoxins: 

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

3.2 Control of final bulk (where applicable)

3.2.1 Information on manufacture

Lot number(s): 
Date of formulation: 
Total volume of final bulk formulated: 
Monovalent bulk plasmid(s) used for formulation: 
Volume(s), storage temperature, storage time and approved storage period: ___________________________
Lot number/volume added: ___________________________
Name and concentration of added substances (for example, adjuvants, facilitators, etc., if applicable): ___________________________

3.2.2 Tests on final bulk or final containers, as applicable
Identity: ___________________________
Purity: ___________________________
Antigen content (quantity): ___________________________
Physical state (that is, % supercoiled): ___________________________
Sterility (bacterial and fungal): ___________________________
Endotoxins: ___________________________
Potency (expression of mRNA or protein): ___________________________

4. Filling and containers
Lot number: ___________________________
Date of filling: ___________________________
Type of container: ___________________________
Volume of final bulk filled: ___________________________
Filling volume per container: ___________________________
Number of doses, if the product is presented in a multi-dose container: ___________________________
Number of containers filled (gross): ___________________________
Number of containers rejected during inspection: ___________________________
Number of containers sampled: ___________________________
Total number of containers (net): ___________________________
Maximum period of storage approved (expiry dating): ___________________________
Storage temperature: ___________________________

5. Control tests on final vaccine lot
Inspection of final containers: ___________________________
Identity: ___________________________
Appearance: ___________________________
pH (if applicable): ___________________________
Osmolality (if applicable): ___________________________
Sterility (bacterial and fungal): ___________________________
Preservative (if applicable): ________________________________
Residual moisture content (for freeze-dried product): ________________
Endotoxin: ________________________________
Adjuvant content (if applicable): ________________________________
Potency: ________________________________
Expression of heterologous antigen in vitro
  (mRNA or protein): ________________________________
Purity: ________________________________
Extractable volume (if applicable): ________________________________
Residual antibiotics (if applicable): ________________________________

6. Certification by the manufacturer

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

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

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

Signature ________________________________
Name (typed) ________________________________
Date ________________________________

7. Certification by the NRA/NCL

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

\(^{12}\) With the exception of provisions on distribution and transport, which the NRA may not be in a position to assess.

Appendix 2

Model NRA/NCL Lot Release Certificate for plasmid DNA vaccines

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

Certificate no. ______________________

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

The release decision is based on ______________________.

Final lot number ______________________

Number of human doses released in this final lot ______________________

Expiry date ______________________

The certificate may also include the following information:

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

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14 Name of manufacturer.
15 Country of origin.
16 If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the NRA or NCL.
17 With the exception of provisions on distribution and shipping, which the NRA or NCL may not be in a position to assess.
22 Evaluation of the product-specific summary protocol, independent laboratory testing and/or specific procedures laid down in a defined document, and so on as appropriate.
- trade name and/or common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date);
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate.

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

Signature  ____________________________________________________________
Name (typed)  _______________________________________________________
Date  _______________________________________________________________
Annex 3

Guidelines for the safe production and quality control of poliomyelitis vaccines

Amendment to Annex 4 of WHO Technical Report Series, No. 1016

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Amendments 97
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Introduction

At its sixty-ninth meeting in 2018, the WHO Expert Committee on Biological Standardization recommended the adoption of the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines (1). These Guidelines outline the biosafety measures required for poliomyelitis vaccine production and quality control during the final poliovirus containment stage (Phase III) as defined in the third edition of the WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) (2). The biosafety-related steps outlined in the Guidelines are to be implemented to minimize the risk of accidentally reintroducing poliovirus from a vaccine manufacturing facility into the community after global certification of poliomyelitis eradication. In order to align with GAPIII requirements, the current Guidelines included several requirements related to the physical design of the facility and to quality control testing which were added after the final round of public consultation in 2018. Following the publication of the Guidelines, poliomyelitis vaccine manufacturers requested WHO to reconsider these requirements, taking into consideration the use of facility-specific risk-based approaches. Given the need to balance global vaccine supply (production) and demand, as well as other technical issues associated with the implementation of GAPIII, the Fourth Meeting of the Containment Advisory Group (CAG) was convened on 15–16 July 2019. At this meeting the issues and challenges faced by poliomyelitis vaccine manufacturers were presented for the deliberation of CAG. A consensus was reached that the relevant sections in the Guidelines should be amended to allow for facility-specific risk assessments to be performed in relation to the specific areas highlighted (3). Subsequently, the Committee at its meeting in 2019 recommended amending the Guidelines in accordance with the recommendations made by CAG (4). The amendments provided in the current document comprise:

- a modified requirement for showering when exiting the containment facility;
- permitting the use of non-dedicated quality control laboratories; and
- permitting the testing of certain samples taken from the containment facility outside of containment laboratories.

No attempt was made at this time to review the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines in their entirety and only the above issues have been addressed.
Amendments

Replace section 7.5.6 with the following text:

7.5.6 A full-body shower facility should be available within the personnel exit airlock from the containment facility. The use of a shower upon exit should follow the established procedure supported by the risk assessment and be consistent with the policies established by the latest version of GAPIII23 and with the most recent CAG recommendations.24

Replace section 11.2 with the following text:

11.2 The use of non-dedicated quality control laboratories may be permissible when all of the following conditions are met:

- The non-dedicated quality control laboratories are located within the containment facility.
- All non-poliovirus-related activities performed within the non-dedicated containment laboratories and all personnel admitted into the non-dedicated containment laboratories adhere to all applicable containment procedures.
- A thorough risk assessment compliant with the requirements set out in the latest version of GAPIII23 and with the most recent CAG recommendations24 is performed to identify any additional controls necessary to mitigate the risks introduced by operating non-dedicated laboratories.

Replace section 11.5 with the following text:

11.5 All samples received from the containment production facility should be handled using established procedures to prevent the release of live poliovirus. Procedures used to decontaminate sample containers or packaging materials should be validated and shown to have no impact on sample integrity. The packaging materials should be decontaminated prior to disposal. All samples received from the containment production facilities – with the exceptions described below in sections 11.5.1 and 11.6 – should be tested in containment laboratories. All test procedures using reagents containing live poliovirus should also be performed within the containment laboratories.

11.5.1 On the issue of handling samples outside the containment facility, certain samples (that is, those for water or environment monitoring) taken from within the containment perimeter may be tested outside the containment laboratories if a risk assessment concludes that they are unlikely to contain live poliovirus, based on facility design, equipment used (especially closed systems) and sampling locations (3) provided all sample-handling, transportation and disposal processes adhere to the policies established by the latest version of GAPIII and to the most recent CAG recommendations.

Authors and acknowledgements

The first draft of this document was prepared by Ms A. Bonhomme, Public Health Agency of Canada, Canada; Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr H-N. Kang, World Health Organization, Switzerland.

The document was then posted on the WHO Biologicals website for a first round of public consultation from 25 February to 3 April 2020 and comments were received from: Dr N. Holvast, Bilthoven Biologicals B.V., Netherlands; Dr P. Huntly, Riskren PTE Ltd, Singapore; Dr M. Janssen, Vaccine PQ Team, World Health Organization, Switzerland; Dr K. Mahmood, PATH, the USA; Dr A.E. Malkin, Russian Academy of Sciences, Russian Federation; Dr D. Moffett and Dr H. Singh, Department of Polio Operations and Research, World Health Organization, Switzerland; Dr V. Pithon, Agence nationale de sécurité du médicament et des produits de santé, France; Dr J. Rosenstand Jørgensen, Statens Serum Institut, Denmark; Dr J. Southern, Advisor to the South African Health Products Regulatory Authority, South Africa; Dr W. Wulandari, Indonesian Food and Drug Authority, Indonesia; and the International Federation of Pharmaceutical Manufacturers & Associations (provided by Dr C. Bardone, Sanofi Pasteur; and Dr M. Duchene, Johnson & Johnson).

Taking into consideration the comments received, a second draft document was prepared by Ms A. Bonhomme, Public Health Agency of Canada, Canada; Dr K. Chumakov, United States Food and Drug Administration, the

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USA; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, World Health Organization, Switzerland, with critical inputs provided by Dr D. Moffett and Dr H. Singh, Department of Polio Operations and Research, World Health Organization, Switzerland.

During the posting of the resulting document on the WHO Biologicals website for a second round of public consultation from 17 June to 3 August 2020, written comments were received from: Dr I. Feavers, Consultant, Nacton, the United Kingdom; Dr M. Janssen, Vaccine PQ Team, World Health Organization, Switzerland; Dr V. Pithon, Agence nationale de sécurité du médicament et des produits de santé, France; and Dr W. Wulandari, Indonesian Food and Drug Authority, Indonesia. The document WHO/BS/2020.2381 was then prepared by Ms A. Bonhomme, Public Health Agency of Canada, Canada; Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, World Health Organization, Switzerland.

Further changes were subsequently made to document WHO/BS/2020.2381 by the WHO Expert Committee on Biological Standardization.

References


Annex 4

New and replacement WHO international reference standards for biological products

The provision of global measurement standards is a core normative WHO activity. WHO international reference standards are widely used by manufacturers, regulatory authorities and academic researchers in the development and evaluation of biological products. The timely development of new reference standards is crucial in harnessing the benefits of scientific advances in new biologicals and in vitro diagnostics. At the same time, management of the existing inventory of WHO international reference standards requires an active and carefully planned programme of work to replace established materials before existing stocks are exhausted.

The considerations and guiding principles used to assign priorities and develop the programme of work in this area have previously been set out as WHO Recommendations.27 In order to facilitate and improve transparency in the priority-setting process, a simple tool was developed as Appendix 1 of these WHO Recommendations. This tool describes the key considerations taken into account when assigning priorities, and allows stakeholders to review and comment on any new proposals being considered for endorsement by the WHO Expert Committee on Biological Standardization.

A list of current WHO international reference standards for biological products is available at: http://www.who.int/biologicals.

At its exceptional meeting held on 24–28 August 2020, the WHO Expert Committee on Biological Standardization made the changes shown below to the previous list. Each of the WHO international reference standards shown in this table should be used in accordance with their instructions for use (IFU).

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Additions}\(^{28}\)

<table>
<thead>
<tr>
<th>Material</th>
<th>Unitage</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood products and related substances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombin</td>
<td>90 IU/ampoule</td>
<td>Third WHO International Standard</td>
</tr>
<tr>
<td><strong>In vitro diagnostics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Plasmodium vivax</em> antigen</td>
<td>1000 IU/ampoule</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td><strong>Vaccines and related substances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-malaria (<em>Plasmodium vivax</em>) plasma (human)</td>
<td>100 U/ampoule</td>
<td>WHO International Reference Reagent</td>
</tr>
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\(^{28}\) Unless otherwise indicated, all materials are held and distributed by the National Institute for Biological Standards and Control, Potters Bar, Herts, EN6 3QG, the United Kingdom.
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This report presents the recommendations of a WHO Expert Committee commissioned to coordinate activities leading to the adoption of international recommendations for the production and control of vaccines and other biological products used in medicine, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of issues discussed at an exceptional meeting of the Committee held in August 2020 via WebEx video conferencing during the COVID-19 outbreak. Of particular relevance to manufacturers and national regulatory authorities are the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines, and an Amendment document to the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines were adopted on the recommendation of the Committee.

Subsequent sections of the report provide information on the current status, proposed development and establishment of international reference materials in the areas of: blood products and related substances; in vitro diagnostics; standards for use in high-throughput sequencing technologies; standards for use in public health emergencies; and vaccines and related substances.

A series of annexes is then presented which includes an updated list of all WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological products (Annex 1). The above WHO plasmid DNA Guidelines and WHO Amendment document adopted on the advice of the Committee are then presented as part of this report (Annexes 2–3). Finally, all new and replacement WHO international reference standards for biological products established during the exceptional August 2020 meeting are summarized in Annex 4. The updated full catalogue of WHO international reference standards is available at: http://www.who.int/bloodproducts/catalogue/en/.