WHO Expert Committee on Biological Standardization

Seventy-fourth report
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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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WHO Expert Committee on Biological Standardization

Seventy-fourth report

This report contains the collective 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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Seventy-fourth meeting held via video conferencing on 18 to 22 October 2021

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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.

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Dr J. Shin

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**Abbreviations**

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<td>ACT</td>
<td>WHO Access to COVID-19 Tools (Accelerator)</td>
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<td>AFP</td>
<td>alpha-fetoprotein</td>
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<td>AG-BRA</td>
<td>Advisory Group for Blood Regulation, Availability and Safety</td>
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<td>ATMP</td>
<td>advanced therapy medicinal product</td>
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<tr>
<td>BAU</td>
<td>binding antigen unit</td>
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<td>BCG</td>
<td>bacillus Calmette–Guérin</td>
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<td>CBER</td>
<td>Center for Biologics Evaluation and Research</td>
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<td>CCP</td>
<td>COVID-19 convalescent plasma</td>
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<td>Coalition for Epidemic Preparedness Innovations</td>
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<td>DNA</td>
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<td>GBT Plus Blood</td>
<td>WHO Global Benchmarking Tool Plus Blood</td>
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<td>human immunodeficiency virus</td>
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<td>ICDRA</td>
<td>International Conference of Drug Regulatory Authorities</td>
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<td>interleukin-6</td>
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<td>IU</td>
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<td>IVD</td>
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<td>LMIC</td>
<td>low- and middle-income countries</td>
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<td>mAb</td>
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<td>patient blood management</td>
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<td>PEI</td>
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<td>Ph. Eur. BRP</td>
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<tr>
<td>RDT</td>
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<td>SAGE</td>
<td>Strategic Advisory Group of Experts (on Immunization)</td>
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<td>variant of concern</td>
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<td>VWF</td>
<td>von Willebrand factor</td>
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<td>VZV</td>
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1. Introduction

The seventy-fourth meeting of the WHO Expert Committee on Biological Standardization was held from 18 to 22 October 2021 via video conference due to the travel restrictions imposed during the coronavirus disease 2019 (COVID-19) pandemic. The meeting was opened on behalf of the Director-General of WHO and the Assistant Director-General, Access to Medicines and Health Products, by Dr Clive Ondari, Director, Health Products Policy and Standards. Dr Ondari began by welcoming Committee members, meeting participants and observers, noting that this was one of the most long-standing WHO expert committees. Dr Ondari also noted the particularly large number of new members of the Committee for the current meeting and expressed his gratitude for the commitment and support of all members in these challenging times. Dr Ondari singled out Dr Harvey Klein for particular thanks following his recent retirement from the Committee, and expressed appreciation for his considerable contribution to the WHO Blood Regulators Network and to the blood-related activities of the Committee over the past 15 years.

Dr Ondari noted that the work of WHO had inevitably been impacted by the additional demands arising from the COVID-19 pandemic. Nevertheless, there remained a pressing need for WHO to maintain its wide range of global public health activities. Reflecting on the broad agenda of the current meeting, Dr Ondari looked forward to receiving the expert advice of the Committee in these areas, and thanked the secretariat for their efforts in preparing for the meeting. Dr Ondari acknowledged the hard work of all of the WHO expert committees in addressing both the COVID-19 and non-COVID-19 activities of WHO.

The WHO response to COVID-19 was continuing at all levels of the organization, and was geared towards accelerating the development and availability of diagnostics, therapeutics and vaccines. Dr Ondari noted the crucial ongoing contribution of the Committee to all three of these pillars of the WHO Access to COVID-19 Tools (ACT) Accelerator, and expressed the view that the speed of the Committee in facilitating the development of WHO written and measurement standards for COVID-19 had in part been made possible by its previous experience of other emerging pathogens. In this regard, the Executive Board of the World Health Assembly had placed on record its appreciation for the rapid development of the WHO regulatory considerations document on the evaluation of messenger RNA (mRNA) vaccines. Dr Ondari also highlighted the pivotal role of the National Institute for Biological Standards and Control (NIBSC) in providing measurement standards to support COVID-19 vaccine clinical trials with unprecedented speed. In addition, upcoming WHO guidance documents on the evaluation and use of monoclonal antibodies (mAbs) for the treatment of infectious diseases, including COVID-19, will make an important contribution to the therapeutics pillar of the ACT Accelerator. The advice of
Dr Ondari briefly reviewed the agenda of the meeting noting that several cross-cutting activities with other WHO committees and groups would be discussed, including policy matters arising from the Strategic Advisory Group of Experts on Immunization (SAGE), the emergency use listing (EUL) of COVID-19 vaccines and priorities in vaccine development. Regarding biotherapeutics, an update was to be provided on the upcoming revised WHO Guidelines on evaluation of biosimilars and on the importance of this revision in accelerating universal access to biosimilar medicines by 2030. Dr Ondari concluded by saying that he also looked forward to receiving the Committee’s input on planned WHO activities in the developing area of cell and gene therapies.

Dr Ivana Knezevic, Secretary to the Committee, thanked Dr Ondari for his opening remarks. Dr Knezevic reminded meeting participants that the setting of norms and standards and promoting their implementation is a core function of WHO and a vitally important element in providing WHO leadership in global health matters. For example, since 2020, a wide range of activities related to the COVID-19 pandemic had been initiated by WHO, including the development of WHO written and measurement standards for consideration and endorsement by the Committee. As noted above by Dr Ondari, the important contribution made by the Committee to the prompt adoption and rapid availability of WHO guidance on the evaluation of mRNA vaccines had been recognized by the Executive Board of the World Health Assembly in January 2021.

Noting that this was the fourth meeting of the Committee to be held via video conference, Dr Knezevic reflected on the large attendances at the three previous meetings held in 2020. Going forward, there would now be two meetings per year – in April and October. Although this decision had partly been driven by the demand for standards related to COVID-19 response efforts, it was felt that biannual meetings would more generally facilitate the timely establishment of WHO written and measurement standards, and the endorsement of project proposals. In order to provide the necessary breadth of expertise and ensure all WHO regions were represented, the Expert Advisory Panel on Biological Standardization from which Committee members were selected would continue to be expanded.

Dr Knezevic then outlined the procedures and working arrangements for the present meeting. An open information-sharing session involving all
participants including non-state actors would be held on Monday 18 October 2021. Committee members, regulatory authority representatives and subject matter experts from governmental organizations would then participate in the main meeting from Monday 18 October to Thursday 21 October 2021. On Tuesday and Wednesday, the agenda would be divided into two parallel tracks with vaccines and biotherapeutics discussed in one track and blood products and in vitro diagnostics in the other. All final decisions and recommendations on the adoption of WHO written standards and the establishment of WHO measurement standards would be made in a closed session held on Friday 22 October attended only by Committee members and WHO staff. Dr Knezevic concluded by thanking WHO staff, WHO drafting and working group members, colleagues from WHO collaborating centres and custodian laboratories, and the many individual experts present for their invaluable support.

Following the conclusion of the open information-sharing session, the meeting officials were elected. In the absence of dissent, Mrs Teeranart Jivapaisarnpong was elected as Chair and Professor Klaus Cichutek as Vice-chair for the plenary sessions, with Dr Ian Feavers as Rapporteur. Professor Cichutek was elected as Chair for the vaccines and biotherapeutics track with Dr Feavers elected as Rapporteur. Professor Salwa Hindawi was elected as Chair for the blood products and in vitro diagnostics track with Dr Diana Teo elected as Rapporteur. Dr Knezevic presented the declarations of interests completed by all members of the Committee and by WHO temporary advisers and participants. After evaluation, WHO had concluded that none of the interests declared constituted a significant conflict of interest and that the individuals concerned would be allowed to participate fully in the meeting.

The Committee then adopted the proposed agenda and timetable (WHO/BS/2021.2012).
2. General

2.1 Strategic directions in biological standardization

2.1.1 WHO overview

Dr Knezevic began her overview by noting the link between the work of the Committee and that of other WHO expert committees and groups, and by outlining the WHO and other resources relevant to WHO biological standardization activities. Within WHO, the Norms and Standards for Biologicals (NSB) and the Blood and other Products of Human Origin (BTT) teams were part of the Technical Standards and Specifications Unit which itself was part of the Health Policy and Standards Department. Other resources included WHO collaborating centres, stakeholders, external experts and the Expert Advisory Panel on Biological Standardization. Among recent improvements, BTT had been strengthened and the Expert Advisory Panel expanded to ensure the availability of expertise in new areas of work and to improve geographical representation. Dr Knezevic then outlined the four strategic priorities of the WHO five-year plan to help build effective and efficient regulatory systems, namely: (a) strengthening national and regional regulatory systems; (b) improving regulatory preparedness for public health emergencies; (c) reinforcing and expanding WHO prequalification and product risk assessment; and (d) increasing the impact of WHO regulatory support activities.

Dr Knezevic then highlighted several recent cross-cutting WHO COVID-19 activities in the context of the three pillars of the ACT Accelerator. In the case of the vaccines pillar (COVAX), such activities had included the development of WHO written and measurement standards, and collaboration with a range of working groups set up by WHO and partner organizations. WHO standards were now cited in the criteria for EUL of COVID-19 vaccines and therefore play an important role in WHO prequalification of such vaccines. The holding of prequalification meetings with manufacturers had helped to ensure the correct use of the relevant measurement standards and interpretation of written standards. For the diagnostics pillar, activities had included the development of WHO antigen standards for rapid diagnostic tests (RDTs) as well as an antibody standard and reference panel to support diagnostic assays and seroepidemiological studies. With regard to the therapeutics pillar, two guidance documents on mAbs were now being developed. The first of these focused on manufacturing aspects and was currently the subject of public consultation on the WHO website while the second, on the nonclinical and clinical evaluation of mAbs for use in treating infectious diseases, would shortly be posted on the website for comments. In addition, following the establishment of the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin in 2020 and a recent Cochrane rapid review on the use of CCP, consideration was now being
given to the revision of the current WHO interim guidance on maintaining a safe and adequate blood supply during the pandemic, and on the safe collection of CCP.

Reflecting on the importance of WHO collaborating centres during the pandemic, Dr Knezevic specifically highlighted the rapid development and evaluation of WHO measurement standards undertaken by NIBSC with the support of other collaborating centres and laboratories worldwide, as well as the key contributions made by WHO collaborating centres to the production of WHO written standards. Dr Knezevic noted that NIBSC in the United Kingdom, the National Institutes for Food and Drug Control in China and the Paul-Ehrlich-Institut in Germany would complete their re-designations as WHO collaborating centres in 2021.

Dr Knezevic continued by briefly reviewing the current status of WHO EUL of COVID-19 vaccines (see section 2.1.3 below), reiterating that this clearly emphasized the importance of biological standards in the WHO prequalification process. In the context of the therapeutics pillar of the ACT Accelerator, the WHO Therapeutics and COVID-19 living guideline provides conditional recommendations on the use of the neutralizing mAbs casirivimab and imdevimab, as well as recommendations for or against the use of a range of other proposed therapeutic products. WHO had also facilitated a number of interactions among COVID-19 stakeholders, including product developers, regulatory groups and networks, and national regulators. In several cases, such activities had provided direct opportunities to promote the appropriate use of WHO biological standards.

Dr Knezevic then briefly summarized the current status of regulatory systems in different jurisdictions, noting that 28% of countries had now met the World Health Assembly resolution WHA67.20 goal of having a stable, well-functioning and integrated regulatory system as assessed by the WHO Global Benchmarking Tool. Dr Knezevic concluded by reflecting on the evolving scientific and regulatory challenges now being faced, and on the importance of international cooperation in ensuring the safety, quality and efficacy of locally used medical products, making best use of available resources and expertise, avoiding duplication, and concentrating regulatory efforts and resources where they were most needed.

### 2.1.2 Vaccines, biotherapeutics, and cell and gene therapy products: recent and planned activities in biological standardization

Dr Knezevic reported on recent and planned WHO standardization activities for vaccines, biotherapeutics, and cell and gene therapy products (CGTPs). Currently, a total of 103 WHO written standards set out the key principles for evaluating a broad range of biological products, and thus provide a basis both for the setting of national requirements and for WHO prequalification. These documents are
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monitored and where necessary revised in order to reflect significant advances in scientific knowledge and experience. WHO then supports the implementation of these written standards by regulators and manufacturers through workshops and relevant training. Dr Knezevic reminded the Committee that during its meetings in 2020, four new or revised WHO written standards for vaccines had been recommended for adoption by WHO.

Dr Knezevic informed the Committee that guidance had now been provided on the WHO Biologicals website on the application of existing WHO guiding principles to the evaluation of COVID-19 vaccines. WHO had also conducted a review of the scientific evidence supporting the development of mRNA vaccines and had published a review of the scientific and regulatory aspects of their development and evaluation. These and other efforts were intended to facilitate international convergence of manufacturing and regulatory practices, thereby improving access to this important new class of vaccine. Dr Knezevic presented an overview of COVID-19 mRNA vaccines currently in clinical development and noted that their success was driving the development of mRNA vaccines for other infectious diseases. At the current meeting, the Committee would be asked to consider the new WHO guidance document on evaluating the quality, safety and efficacy of mRNA vaccines for the prevention of infectious diseases (see section 3.4.2 below) along with an amendment to the current WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines (see section 3.4.1 below).

Written standards scheduled for consideration by the Committee in 2022 included revised WHO Guidelines on evaluation of biosimilars, the two-part regulatory considerations document on mAbs for use in treating infectious diseases outlined above in section 2.1.1 and a manual on the development of secondary antibody standards. The Committee was updated on recent progress in the revision of the WHO Guidelines on evaluation of biosimilars which was now scheduled for submission to the Committee in April 2022. In addition, in order to ensure consistency across the range of related documents, WHO guidance on biosimilar mAbs and the WHO Questions and Answers document on biosimilars would also be revised in 2022 for submission to the Committee in 2022–2023. Other new or revised WHO written standards likely to be submitted to the Committee from 2022 onwards included: (a) revised WHO Recommendations for oral poliomyelitis vaccines (OPVs) and other revised WHO guidance on poliomyelitis vaccine production and evaluation; (b) revised WHO Guidelines on dengue vaccines and on rotavirus vaccines; and (c) revised WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards. Requests had also been received from external stakeholders for the revision of the current WHO written standards on post-approval changes to vaccines and on pandemic
influenza preparedness, and of the WHO global model regulatory framework for medical devices including in vitro diagnostics (IVDs). Work had also started on developing WHO guidance on the standardization of enteric vaccines and would continue during 2022.

Dr Knezevic then turned to the WHO measurement standards developed since 2013 – including the three standards established on the advice of the Committee in 2020 to support the development of COVID-19 vaccines, therapeutics and diagnostics. It was anticipated that use of the WHO COVID-19 antibody standards would facilitate the harmonized interpretation of clinical study data by allowing antibody titres to be expressed in International Units (IU) thereby supporting efforts to identify an immune correlate of protection defined in IU/mL. The announcing of the establishment of the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin in the scientific press and its subsequent rapid worldwide distribution highlighted its importance as a key tool for vaccine developers, national reference laboratories and academic groups.

Among the other main outcomes of recent Committee meetings had been the endorsement of a proposal to develop a WHO standard for SARS-CoV-2 antigen to support the development, assessment and comparability of antigen-based RDTs and an update on progress would be provided at the current meeting (see section 8.1.3 below).

Going forward, WHO would continue to provide technical support to users of its COVID-19 standards, for example through dissemination of its Considerations for evaluation of COVID-19 vaccines document which provides advice to manufacturers on both the process and criteria that will be used by WHO to evaluate COVID-19 vaccines submitted either for prequalification or EUL. Technical assistance will also be provided to users of WHO COVID-19 standards through COVAX, the vaccines pillar of the ACT Accelerator, and through WHO participation in the recently established Technical Advisory Group on COVID-19 Vaccine Composition.

Dr Knezevic concluded by outlining recent WHO efforts in the standardization of CGTPs. In February 2020, WHO had initiated the development of a white paper on the fundamental principles and issues related to the regulatory oversight of CGTPs and an update on progress would be provided at the current meeting (see sections 3.3.1 and 3.3.2 below). A survey had also been conducted of CGTP regulation in jurisdictions with relevant marketing authorization experience to better understand the current global regulatory landscape and inform the development of future WHO guidance on these advanced products. The survey results will be analyzed and published in 2022. In addition to these preparatory steps, the Committee was reminded that in 2019 it had recommended the establishment of two WHO measurement standards relating to the integration of lentiviral vectors, and had subsequently endorsed
further measurement standards projects in this area, including on replication-competent lentiviruses, pluripotent stem cell identity for flow cytometry and mesenchymal stromal cells.

2.1.3 **WHO EUL of COVID-19 vaccines: an update**

The Committee was updated on the WHO EUL of COVID-19 vaccines by Dr Carmen Rodriguez. After briefly summarizing the main features of both WHO prequalification and EUL, Dr Rodriguez highlighted the reliance of both processes on the support of WHO-listed authorities, especially through the provision of regulatory oversight of abbreviated processes.

Following the onset of the COVID-19 pandemic, WHO had reviewed the activities that needed to be aligned to expedite the assessment and availability of vaccines in countries. Vaccine development criteria and submission requirements had been identified and an assessment process established and documented. In-country approval and post-approval monitoring of safety, quality and effectiveness would then follow based on national regulatory reliance on WHO prequalification and EUL. Such a “roadmap” had allowed for product-specific regulatory alignment in these crucial areas, with further alignment and support activities now ongoing.

Dr Rodriguez briefly reviewed the current WHO EUL COVID-19 vaccines and the broad range of post-listing monitoring and reporting commitments that manufacturers were required to undertake. Among these ongoing commitments, manufacturers were expected to provide updated vaccine efficacy and effectiveness data, along with monthly safety reports and benefit–risk evaluations every 6 months. Other post-listing commitments undertaken by manufacturers included serious adverse events reporting, quality complaint disclosure and reporting of any post-listing changes that may impact vaccine quality, safety or efficacy, or any constraints in production or quality control that might affect the emergency use condition granted to the product.

To date, regulatory approval of COVID-19 vaccines has been based on data submitted from clinical trials based on efficacy end-points. Data from these trials and from studies of immunity to natural infection were still being analysed to identify immune correlates of protection, which to date have not been determined and which may vary between vaccine platforms. With the roll-out of effective vaccines, clinical end-point efficacy studies were however becoming increasingly difficult and were no longer feasible in many regions. As an alternative approach, comparative immunogenicity (immunobridging) study designs were being considered on the assumption that neutralizing antibodies were the vaccine-induced immune marker most likely to imply protection against SARS-CoV-2 infection – an assumption that explains why some regulators were willing to accept post-approval changes to current vaccines. The evaluation of new
COVID-19 vaccines would however present a particular challenge and although innovative approaches have been proposed there was currently no consensus among regulators on the best approach to take. With regard to additional and booster doses, some regulators had selectively approved the use of an additional vaccine dose to complete the primary immunization schedule for certain immunocompromised groups. Currently, there was no regulatory consensus on the use of booster doses for fully vaccinated individuals in the general population, though a small number of regulators have amended their emergency authorizations to approve a booster dose of specific vaccines for subsets of the general population. Dr Rodriguez concluded by listing several issues on which the Committee may be able to offer specific advice, including the need for further WHO guidance in a number of key areas.

Responding to questions from the Committee, Dr Rodriguez confirmed that WHO EUL was valid for 1 year before reassessment based on the nature and status of the public health emergency. A number of COVID-19 vaccine manufacturers were currently seeking marketing authorization and would go through the prequalification process. Clarification was also given that although prequalification required the continued oversight of a mature regulatory authority, the authority of record on an EUL was the regulator in the country of origin of the product.

Acknowledging the challenge of approving innovative vaccines when Phase III trials based on clinical end-points were not feasible, the Committee noted that regulators were not prepared to commit to immunobridging studies and to date no product had been approved based on data from such studies. WHO was monitoring this issue and if regulatory consensus emerged on the use of immunobridging data then WHO guidance would be needed. Reflecting on the considerable complexity of immunobridging criteria and the ongoing discussions among international regulators, the Committee felt that immunobridging studies would be less likely to be needed once an immune correlate of protection had been established, and that regulatory guidance should only be developed once the situation became clear. Noting the importance of the First WHO International Standard for anti-SARS-Cov-2 immunoglobulin in efforts to establish an immune correlate of protection, the Committee applauded the considerable efforts of WHO in supporting users of its measurement standards and promoting the use of IU for serological assays. It was suggested that a similar approach might be taken to promoting the use of IU in the diagnostics area.

The Committee reviewed the request made for additional guidance and noted that WHO advocated the application of its existing guiding principles to COVID-19 vaccines as far as possible. WHO should therefore prioritize the development of guidance that was either unavailable or which could not be inferred from guidance on other vaccines or infectious diseases. It was noted,
for example, that guiding principles for the evaluation of viral vectored vaccines were already provided in the existing WHO Guidelines on the quality, safety and efficacy of Ebola vaccines.

### 2.1.4 Update from the WHO Product Development for Vaccines Advisory Committee

The Committee was updated on the activities of the WHO Product Development for Vaccines Advisory Committee (PDVAC), which has continued to convene virtually throughout the pandemic. With regard to tuberculosis vaccines, the majority of candidate vaccines in the current pipeline were intended to boost waning immunity to bacillus Calmette–Guérin (BCG) in adolescents and adults. PDVAC had advised WHO to develop a roadmap of the steps required for the prospective introduction of vaccines already in or approaching Phase III trials. As both next-generation BCG and non-BCG tuberculosis vaccines were under development, PDVAC had also suggested that WHO consider reviewing, and where necessary revising, its existing written standards relevant to tuberculosis, while also noting the current lack of measurement standards for non-BCG tuberculosis vaccines.

The Committee was then reminded that WHO guidelines on the quality, safety and efficacy of group B streptococcus vaccines were being developed that, among other aims, would set out the standards required for WHO prequalification. A workshop had been held and a series of meetings with regulators and policy-makers planned for 2022 to discuss potential licensure strategies based on the identification of an immune correlate of protection. Work was also continuing on the harmonization of assays for measuring the immunological response to group B streptococcus infection and vaccination, including the development of a WHO measurement standard based on pooled serum from individuals immunized with a hexavalent candidate vaccine that was expected to proceed to Phase III trials in 2023.

PDVAC has also continued to monitor the development of RSV immunization products, with several candidate vaccines now in Phase III trials, including maternal vaccines and mAbs intended to provide passive immunization to young infants. WHO Preferred Product Characteristics for RSV vaccines and mAbs were published in 2017 and 2021 respectively. Regulatory submissions for the most advanced candidate mAbs are expected towards the end of 2022, and an RSV-specific supplement to the upcoming WHO regulatory considerations document on the nonclinical and clinical evaluation of mAbs for infectious diseases (see section 2.1.1 above) is planned. To accelerate access to these products in LMIC, WHO is raising awareness of the burden of RSV and of prospective products for disease prevention, and is developing tools to facilitate in-country decision-making.
PDVAC has also continued to monitor the development of mAbs against other infectious diseases. For example, several mAbs against human immunodeficiency virus (HIV) were being developed as longer-acting alternatives to daily oral pre-exposure prophylaxis. In collaboration with global HIV, hepatitis and sexually transmitted infections programmes, WHO Preferred Product Characteristics for broadly neutralizing mAbs for HIV prevention had been drafted and subjected to public consultation, with publication scheduled for early 2022. In addition, two rabies mAb products were now on the market with others in clinical development. In 2018, the WHO position paper on rabies was updated to include a recommendation to use rabies mAbs as an alternative to blood-derived rabies immunoglobulin. In 2021, rabies mAbs were added to the WHO Model List of Essential Medicines and a rabies-specific supplement to the WHO regulatory considerations document on the nonclinical and clinical evaluation of mAbs for infectious diseases is planned.

The update concluded with a review of the enteric vaccine development pipeline, noting in particular the prospects for Salmonella combination vaccines in the future. With regard to the ongoing development of a WHO Shigella reference serum to support assay standardization, the utility of controlled human infection models in vaccine development was raised. Although WHO regulatory considerations for human challenge trials have been published, there may be a need for specific guidance on trials involving enteric pathogens.

The Committee welcomed the recent developments outlined which had generated a lot of interest, particularly with regard to the development of novel vaccines and other products. Nevertheless, despite the availability of new platforms, many challenges remain, including the need to elicit the most appropriate immune responses, the lack of a correlate of protection for tuberculosis vaccines and the need to identify protective epitopes for HIV. Commenting specifically on the development of mAbs against HIV, the Committee was assured that several longer-acting alternatives to daily prophylaxis were in the pipeline. The Committee then asked about the prospect of further mRNA vaccine developments following the success of this approach for COVID-19 vaccines but was informed that PDVAC had not yet specifically discussed this issue.

With regard to written standards, the Committee noted the proposal to incorporate disease-specific supplements into the upcoming WHO guidance on nonclinical and clinical evaluation of mAbs for infectious diseases, while also emphasizing the current need to focus on COVID-19. The Committee also acknowledged that novel tuberculosis and recombinant BCG vaccine developments would probably require the revision or amendment of existing WHO guidance and noted the absence of WHO measurement standards for prospective non-BCG vaccines. The Committee urged WHO to continue to regularly review the need for both written and measurement standards in this area.
2.1.5 Report of the October meeting of the Strategic Advisory Group of Experts on Immunization

Dr Joachim Hombach updated the Committee on matters discussed at the October meeting of the Strategic Advisory Group of Experts on Immunization (SAGE). With regard to polio vaccination it had been noted that during the first 9 months of 2021 there had only been two paralytic cases of wild poliovirus type 1 and 326 cases of circulating vaccine-derived poliovirus, predominantly serotype 2 (cVDPV2). Given the desire of countries to use only inactivated poliomyelitis vaccine (IPV), SAGE recommended a primary three-dose IPV series for low-risk areas with an optional two-dose series for the lowest risk areas. It was further agreed that the same early schedules currently recommended for the whole cell pertussis pentavalent vaccine would also be applicable to the hexavalent vaccine containing IPV. SAGE also endorsed the wider use under WHO EUL of novel OPVs against type 2 polioviruses based on genetically stabilized viruses to allow countries to promptly respond to outbreaks caused by cVDPV2.

Dr Hombach then drew the attention of the Committee to the interim SAGE recommendation applicable to all WHO EUL COVID-19 vaccines that moderately or severely immunocompromised people should receive an additional dose of vaccine as part of an extended primary series. Interim recommendations had also been issued on the use of an extended primary immunization series for inactivated COVID-19 vaccines produced by certain manufacturers to address their lower efficacy in older people. Dr Hombach also informed the Committee that an inactivated COVID-19 vaccine (Covaxin) formulated with a novel adjuvant had been authorized for use in adults by the regulatory authority of India and subsequently in 14 other jurisdictions. Preliminary results indicated that the vaccine was safe and highly effective against hospitalization and death while the delta variant was predominant in the population. However, data on the use of the vaccine during pregnancy were limited, with developmental and reproductive toxicology data still anticipated and there was no WHO recommendation to use this vaccine in pregnant women. No policy recommendations would be issued until the vaccine had been granted WHO EUL.

In a joint session, SAGE and the Malaria Policy Advisory Group had recommended a four-dose schedule of the RTS,S/AS01 malaria vaccine for children from the age of 5 months in regions with moderate to high malaria transmission, with the possibility of a five-dose schedule in areas with highly seasonal malaria. These recommendations were based on the results of a pilot implementation programme conducted in Ghana, Kenya and Malawi involving more than 800,000 children. Malaria vaccine delivery through routine childhood immunization programmes has the ability to reach vulnerable children not protected by other interventions and in the pilot programme led to statistically significant reductions in both severe hospitalized malaria cases and hospitalization
with malaria infection. There was also no evidence that safety signals noted during Phase III trials had been caused by the vaccine.

SAGE also reinforced the importance of seasonal influenza vaccination, particularly among the most vulnerable groups, with vaccination in both prior and current seasons producing higher levels of protection than no or partial vaccination. In addition, co-administration of inactivated seasonal influenza vaccine with any WHO EUL COVID-19 vaccine would be acceptable and expected to maximize the uptake of both vaccines. SAGE also stressed the need to improve access to hepatitis E vaccine. Although a subunit vaccine has been available since 2015, it is not widely used and manufacturers were urged to apply for WHO prequalification. To help address the current lack of data to support its use, WHO had been urged to work with Gavi, the Vaccine Alliance, on the inclusion of hepatitis E vaccination for outbreak response, and on hepatitis E diagnosis and surveillance in Gavi-eligible countries.

During discussion, it was clarified that the data on the impact of prior vaccination with seasonal influenza vaccine on subsequent levels of protection had been derived from a systematic review and were based on vaccinations in consecutive years. Reflecting on the low incidence of poliovirus infections, the Committee also asked whether bivalent vaccination campaigns were continuing and was informed that the majority of countries still used bivalent OPV in their routine schedule, with IPV only recommended for very low risk countries.

The Committee then enquired about the issues to be discussed at upcoming SAGE sessions and was informed that a decision-making framework for the implementation of booster doses of COVID-19 vaccines would shortly be proposed. This was a complex issue as decisions are ultimately made in-country and are vaccine specific. As it would be premature to issue vaccine-by-vaccine recommendations at present, the decision-making framework was being put forward. Discussion then moved on to the possibility of SAGE recommendations being issued on heterologous boosting and childhood immunization in the context of COVID-19 vaccine supply issues. The Committee was informed that SAGE was undertaking a systematic review of the available data and it now looked likely that it would recommend a more flexible approach to the use of different booster vaccines in due course. Although children were at low risk of severe disease, SAGE was considering a recommendation to immunize adolescents based on the burden of disease and on the potential to reduce transmission as vaccine supplies increased.

2.1.6 Blood products and in vitro diagnostics: recent and planned activities in biological standardization

Dr Yuyun Maryuningsih began by informing the Committee of the establishment of the Advisory Group for Blood Regulation, Availability and Safety (AG-BRAS)
in July 2021. This advisory group had been established to provide technical advice and recommendations to WHO in the fields of blood regulation and transfusion medicine. As highlighted by the adoption of several World Health Assembly resolutions, there is a global need to ensure the safety and availability of blood products. Furthermore, the development of the WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023 now provides a global strategic direction. In January 2020, the merging of the WHO blood products and blood safety and transplantation teams resulted in the combined and strengthened WHO Blood and other Products of Human Origin (BTT) team, and an associated need for wide-ranging expert guidance from experts in the field. The new advisory group would reflect the need for a suitable diversity of expertise and experience, while also ensuring representation from all six WHO regions. Currently comprising 25 members serving in their individual capacities, the functions of AG-BRAS were: (a) to advise on the development of WHO norms, standards and guidelines for ensuring the safety, quality and availability of blood products; (b) to advise on scaling up the implementation of WHO policies and strategies, and strengthening national systems for blood supply and regulation; and (c) to provide scientific assessments of current and emerging threats to the safety and availability of blood and blood products. To date, two virtual meetings of the advisory group had been conducted, with a third scheduled in late 2021 to develop its workplan.

Dr Maryuningsih then summarized the outcomes of a recent workshop on blood and blood products held as part of a virtual meeting of the International Conference of Drug Regulatory Authorities (ICDRA). Meeting recommendations to countries included a call for ministries of health to provide effective leadership and governance in the development of national blood regulation systems. Countries were also urged to use the WHO Global Benchmarking Tool Plus Blood (GBT Plus Blood) to identify gaps and needs in national blood regulation, with training on the use of this tool having been conducted in nine countries. ICDRA also recommended that national governance mechanisms are established and implemented to support collaboration among regulators, blood establishments and hospitals in developing and improving well-functioning blood systems. ICDRA recommendations for WHO included: (a) ensuring the provision of support to countries in developing and strengthening their national blood regulatory systems as a WHO Action framework priority; (b) providing periodical reports on the progress achieved in implementing the framework; and (c) supporting the implementation of WHO guidance and blood policies at country level, particularly in LMIC.

Dr Maryuningsih then provided an update on the current status of several WHO documents developed in 2021 that were intended to facilitate achievement of the high-level strategic objectives set out in the WHO Action framework. A
policy brief on the urgent need to implement patient blood management (see section 3.2.2 below) and educational modules on clinical use of blood (see section 3.2.1 below) had both been completed with publication expected by the end of 2021. In addition, planning clearance had been obtained for a WHO guidance document on screening donated blood for transfusion-transmissible infectious agents, and for tools for the stepwise implementation of a haemovigilance system, with both resources now under development. WHO had also organized or contributed to a series of webinars to promote the use of recently published WHO guidance in this area, and had held a training workshop on the use of the WHO GBT Plus Blood tool. Dr Maryuningsih noted that consideration would also need to be given to updating the WHO interim guidance on maintaining a safe and adequate blood supply and collecting convalescent plasma in the context of the COVID-19 pandemic. Dr Maryuningsih concluded by reporting on the eighth meeting of the WHO network of collaborating centres for blood products and in vitro diagnostics held in September 2021 at which the relevant WHO measurement standards to be considered by the Committee at the current meeting had been discussed.

The Committee sought clarification of the envisaged role of AG-BRAS, particularly with regard to the existing WHO collaborating centre process for proposing and developing WHO measurement standards for blood products for review by the Committee. Clarification was provided that AG-BRAS would focus on WHO written standards rather than measurement standards, and that WHO would continue to rely on its network of collaborating centres for the latter. Further clarification was given that in addition to the need for technical advice and recommendations on both blood regulation and transfusion medicine following the formation of the BTT team, WHO norms and regulations governing WHO-managed networks and engagement with expert bodies also required balanced geographical and other representation in its advisory mechanisms. It was noted that several members of the long-established WHO Blood Regulators Network were also now members of AG-BRAS, and it was expected that the establishment of AG-BRAS would help rationalize all of the various advisory mechanisms in this field that WHO had relied on in the past.

The Committee acknowledged the many past contributions made by the WHO Blood Regulators Network in strengthening harmonization and standardization efforts in blood product safety and quality, expressed its support for the new advisory group going forward and looked forward to its contributions in furthering progress in this area.

2.1.7 Update on the use of convalescent plasma for COVID-19

Dr Cynthia So-Osman updated the Committee on the use of CCP for the treatment of COVID-19. Both CCP and hyperimmune serum containing polyclonal
immunoglobulin G (hyper IgG) had been used early in the COVID-19 pandemic based on the reported efficacy of convalescent plasma used for diseases such as severe acute respiratory syndrome in 2003, A(H1N1) influenza in 2009, and Middle East respiratory syndrome in 2012. However, definitive evidence of the effectiveness of this approach for COVID-19 was not available. In addition, drugs shown to be effective in initiating recovery from COVID-19 (such as tocilizumab and dexamethasone) were not specifically directed towards clearing the virus.

Dr So-Osman outlined the three product types currently available – namely CCP, hyper IgG and mAbs. CCP can be prepared very quickly once convalescent donors are available, while hyper IgG and mAbs both take significantly longer to be produced. In addition, the relatively high costs of producing hyper IgG and mAbs would likely constrain their availability. The Committee was informed that Cochrane living systematic reviews were currently in progress for all three products with the aim of searching the literature for definitive evidence of their effectiveness.

The first Cochrane living systematic review on the use of CCP or hyper IgG to treat COVID-19 had been published in May 2020, with a subsequent review published in July 2020. Both reviews found no difference in primary outcome (effectiveness) among patients who received or did not receive CCP. A more recent review had been published in May 2021 based on 13 studies involving a total of 48 509 participants (including the large RECOVERY study with more than 11 000 patients). These studies had mostly been carried out in hospitals, primarily among patients with moderate to severe COVID-19. It was firmly concluded that CCP conferred no benefit in treating such patients. It was however unclear whether CCP was of any benefit in treating those with mild or asymptomatic disease. With 130 studies still ongoing, unpublished or recently published, these living systematic reviews would continue.

Dr So-Osman reflected on why CCP appeared not to work in these studies and highlighted the Dutch CONCOVID study which had shown no difference in viral clearance between hospitalized COVID-19 patients provided with standard care and those treated with CCP. However, many of the patients had developed antibodies by day 7 of infection and it was hypothesized that CCP might be effective if transfused earlier and at a high titre. The results of studies involving the use of CCP within 7 days of disease onset, and its use in immunocompromised seronegative patients, had now been published or were being finalized and would be included in the next Cochrane living systematic review update. In February 2021, the US Food and Drug Administration (FDA) issued a Letter of Authorization which stipulated that only early and high-titre CCP treatments could be used.

Future Cochrane initiatives would evaluate the use of CCP and hyper IgG to prevent SARS-CoV-2 infection and the use of hyper IgG in treating
COVID-19. The first review of mAb use in COVID-19 patients had been published in September 2021 and found insufficient evidence to support the use of this approach in the overall patient population – though it may have a role to play in specific patient subgroups.

The Committee observed that there were some indications that the very early use of high-titre CCP in people at high risk of severe disease may lead to a less severe disease course. Dr So-Osman noted that of four reported studies on the use of CCP during the pre-hospitalization stage and during early symptomatic infection, only one had shown any benefit, with the remaining studies reporting no difference in outcome. Dr So-Osman further clarified that the Cochrane reviews had primarily involved randomized control trials which allowed for specific patient subgroups (for example, immunocompromised patients) to be evaluated and the results aggregated to improve the available data. A Phase III randomized control study of CCP had recently been concluded that could potentially also contribute to the subgroup data. The Committee queried whether it was possible to identify the specific level of antibody required for treatment efficacy and was referred to the CONCOR-1 Study in which patients had been divided into groups receiving either high, medium or low antibody titre CCP, and which had shown no evidence of benefit. The Committee concluded that the use of CCP was a developing field and with many studies still ongoing, firm conclusions on its efficacy in different patient subgroups were yet to be reached.

2.1.8 Snakebite envenoming

Dr David Williams updated the Committee on the progress and priorities of the snakebite envenoming programme at WHO. As part of efforts to improve access to safe and effective treatments, a technical and scientific advisory group had been established to develop target product profiles (TPPs) to guide the development of conventional plasma-derived antivenoms, next-generation products and small molecule inhibitors. Four such TPPs, for animal-plasma-derived antivenoms for Africa, would shortly go out for public consultation and TPPs for other products would be developed throughout 2022. Key considerations in the preparation of TPPs include the need to ensure: (a) adequate amounts of active pharmaceutical ingredient; (b) appropriate protein content; (c) initial doses sufficient to neutralize the average adult venom yield for each intended species; and (d) acceptable product stability in countries where the products will be used. Dr Williams then summarized the outcome of laboratory evaluations of the composition and activity of six products assessed as part of WHO benefit–risk assessment of sub-Saharan antivenoms. A high degree of variability had been found in terms of both protein content and active pharmaceutical ingredient content, with four of the products (based on F(ab’)2 fragments) failing to meet their own specifications in this regard, and three
such products containing significant amounts of low molecular weight proteins, indicating poor process and quality control.

Dr Williams informed the Committee that following advances in the field there was now a need to review and revise the current WHO Guidelines for the production, control and regulation of snake antivenom immunoglobulins. One of the outcomes of the WHO benefit–risk assessments of sub-Saharan antivenoms had been the recognition of the need for a fundamental reassessment of how antivenoms were designed and how venoms were selected as immunogens. Given the substantial benefits offered by intact IgG antivenoms in terms of safety, production efficiency, viral safety, quality, efficacy and cost, a critical review of the use of F(ab′)2 and other fragments was now warranted. The assessments had also indicated that the current WHO guidance on the quality control, stability and storage of antivenoms would benefit from revision, along with improved guidance on the preclinical and clinical evaluation of antivenoms to reflect new technologies and methods, including potential methods for replacing in vivo testing in the context of the ethical use of animals. The revision process would begin in 2022 with the aim of presenting the updated document to the Committee in 2023.

A key barrier to the development of a WHO prequalification procedure for antivenoms is the lack of reference materials for all regions, with wide species and geographical variations presenting a particular challenge. Although reference venoms are available in eastern Asian and some Latin American countries, they are not available in sub-Saharan Africa or South Asia, where the incidence of snakebites is highest. WHO is exploring the development of venom reference standards for at least six medically important African snake species representing the full spectrum of envenoming syndromes. A network of national control laboratories with relevant experience will be needed to work with laboratories at the forefront of snake venom and antivenom characterization to expedite the development of such reference standards. A WHO prequalification process could then be implemented that incorporated the current bespoke antivenom benefit–risk assessment procedure along with elements of existing WHO prequalification procedures. A protocol for the development of reference materials in this area will be presented at a future Committee meeting and discussion of this and related WHO activities with the Committee in 2022 would be timely.

Dr Williams concluded by informing the Committee of a substantial upgrade to the WHO snakebite information and data platform. Information and data had now been better integrated and a range of tools now under development would shortly be incorporated. These include a DHIS2 data-reporting module on snakebite burden and antivenom use, a tool to improve access to health care, antivenoms and other health commodities, and an antivenom cost-effectiveness tool.

Reflecting on the challenges and complexity of developing antivenom reference materials, the Committee speculated as to whether the proposed
Regional standards would have to take into account differences in snake species, but agreed that the approach taken should focus on the venom rather than the snake itself. The use of a well-characterized pool of venom as the immunogen would help to address the inherent variations.

2.2 Feedback from custodian laboratories

2.2.1 Developments and scientific issues identified by custodians of WHO international reference standards

*Center for Biologics Evaluation and Research (CBER), Silver Springs, MD USA*

Dr Celia Witten reviewed the standardization activities of CBER during the last year. CBER COVID-19-related activities had included participation in the collaborative studies to establish WHO international standards both for nucleic acid amplification technique (NAT)-based assays and serological assays. In the context of COVID-19 vaccine development, CBER had participated in WHO technical working groups on viruses, reagents and immunoassays, and animal models, with support also provided to WHO workshops on the standardization of clinical trials and their methodologies. Furthermore, CBER would also be participating in the forthcoming collaborative studies to establish the First WHO International Reference Panel for antibodies to SARS-CoV-2 variants of concern and the Second WHO International Standard for anti-SARS-CoV-2 immunoglobulin (see sections 8.1.1 and 8.1.2 below).

In other areas, CBER had seen an increase in requests for the reference viruses used to validate high-throughput sequencing as an alternative to in vivo adventitious virus detection. Replacements stocks were now being developed and two more viruses (coronavirus and parvovirus) will be included in an expanded panel, with the results of a collaborative bridging study to be presented to the Committee for its consideration in due course. Dr Witten also updated the Committee on a project it had endorsed in 2018 to develop universal reagents for the potency testing of IPV based on human and mouse mAbs specific for the D-antigen. These mAbs and other reagents had now been assessed in preliminary studies and sent to NIBSC for filling so that a collaborative study could start in late 2021.

With regard to the current meeting, CBER had participated in the collaborative studies on the First WHO International Standard for anti-Lassa virus immunoglobulin G (see section 6.1.3 below) and the Third WHO International Standard for von Willebrand factor (concentrate) (see section 5.1.1 below) and had been involved in the drafting of the WHO regulatory considerations document on mRNA vaccines (see section 3.4.2 below). In addition, CBER was participating in the ongoing revision of the WHO Recommendations for
OPVs and in the collaborative study to replace animal neurovirulence testing with whole-genome high-throughput sequencing for routine OPV lot release and monitoring of IPV production consistency – the outcomes of both of which were expected to be presented to the Committee in 2022. CBER staff had also developed the first draft of the white paper on CGTPs scheduled for discussion at the current meeting (see section 3.3.2 below). Upcoming standardization activities include participation in a collaborative study to support establishment of a First WHO International Standard for activated blood coagulation factor X (human), and in projects to replace the current WHO international standards for blood coagulation factor VIII (concentrate) and factor IX (concentrate).

European Directorate for the Quality of Medicines & HealthCare (EDQM), Strasbourg, France

Dr Laurent Mallet updated the Committee on recent standardization activities at EDQM. Dr Mallet began by reminding the Committee that EDQM is the custodian laboratory for international standards for antibiotics. Twenty three international standards for old antibiotics were currently available, eight of which were on the WHO Model List of Essential Medicines. No issues had been identified in relation to these standards since the previous meetings of the Committee in 2020. Since EDQM took over the responsibility for these standards from NIBSC in 2006, 10 replacement standards had been established.

Dr Mallet then provided an overview of the goals and selected ongoing activities of the EDQM biological standardisation programme. Among the projects with implications for the application of the 3Rs principles (Replacement, Reduction, Refinement) regarding the use of animals in research was an international collaborative study to validate a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) as an in vitro alternative to the in vivo potency test for rabies vaccines. A set of samples consisting of 11 vaccines and five virus strains, along with a standard operating procedure and reagents, had been distributed to 31 participants worldwide to evaluate the transferability and robustness of the method. The results of the study were expected to be reported to the Committee in 2022. A second project with direct implications for the 3Rs principles was an initiative to eliminate animal-based pyrogen testing from the European Pharmacopoeia (Ph. Eur.). Despite changes to relevant texts aimed at encouraging users to perform in vitro tests (such as the monocyte activation test) the rabbit pyrogen test continued to be widely used. Dr Mallet set out a timeline for the complete removal of the rabbit pyrogen test from the Ph. Eur. by 2026 as an essential step towards the exclusive use of in vitro tests for the control of pyrogens. The current animal-based tests would be replaced by risk-based analysis of the potential presence of non-endotoxin pyrogens in the product or intermediate being tested. If the presence of non-endotoxin pyrogens could be
ruled out in this way, then the revised Ph. Eur. will recommend the use of the bacterial endotoxin test alone; otherwise, the monocyte activation test will be recommended, either on its own or in addition to the bacterial endotoxin test.

The Committee acknowledged the progress made towards the removal of the rabbit pyrogen test from the Ph. Eur. and strongly supported moves to establish the monocyte activation test as an alternative to animal testing for pyrogens worldwide. Noting that the use of fresh human blood in the test remained a challenge, it highlighted the need for research into the development of alternatives based on the use of suitable cell lines and the application of reporter gene technology. Regarding the development of a quantitative sandwich ELISA as an alternative to the in vivo potency test for rabies vaccines, the Committee noted that essential reagents for the assay were available commercially and their supply was assured. The Committee further noted that ensuring the ability of the replacement assay to detect subpotent vaccine batches would be critical.

**National Institute for Biological Standards and Control (NIBSC), Potters bar, the United Kingdom**

Before beginning his review of recent standardization activities at NIBSC, Dr Marc Bailey first informed the Committee of an ongoing transformation programme at the Medicines and Healthcare products Regulatory Agency (MHRA) of which NIBSC was part.

Dr Bailey then updated the Committee on a specific issue that had arisen with regard to the First WHO International Reference Panel for lentiviral vector copy number, established on the recommendation of the Committee in 2019. Significant differences had been reported between data obtained by droplet digital polymerase chain reaction (PCR) and quantitative PCR when using the standard, which would potentially impact on all PCR-based methods. Independent laboratory analysis had confirmed the presence of method bias. The proposed solution was to change the unitage originally used for the standard (lentiviral copies/cell) to IU/ampoule. This would overcome the problem of the standard being method-dependent, make it consistent with similar WHO standards and reinforce the IU concept in the advanced therapy medicinal product (ATMP) community. A correspondingly revised supplement or new report would be submitted to the Committee for its consideration in 2022.

Dr Bailey went on to update the Committee on NIBSC activities in relation to COVID-19 reference materials, noting in particular the rapid depletion of stocks of the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin and its proposed replacement scheduled for discussion at the current meeting (see section 8.1.2 below). For now, NIBSC was distributing secondary standards calibrated against the current standard. Dr Bailey concluded by reviewing the list of reference materials currently scheduled for submission.
by NIBSC for discussion by the Committee in early 2022, highlighting the challenging deadline for such submissions and noting that more projects would likely be submitted were the meeting to be rescheduled for slightly later in the year.

The Committee began by expressing its profound concern with regard to the potential upcoming changes to the current financing, structure and operating environment of NIBSC. As the foremost WHO collaborating centre in the development of international reference materials, the activities of NIBSC were pivotal not only to the work of the Committee but to the advancement of regulatory science worldwide. The role played by NIBSC in developing and distributing WHO international reference materials was vital in ensuring the national licensing and release of a huge range of much-needed diagnostics, vaccines, biotherapeutics and other biological products. In addition, as one of only four designated essential regulatory laboratories for influenza, NIBSC plays a central role in both seasonal and pandemic influenza surveillance and response activities each year. NIBSC also provides crucial support to the Global Polio Eradication Initiative through its central role in the development of new poliomyelitis vaccines and as the world’s main custodian of poliovirus strains. Without the contribution made by NIBSC the hard-won gains in improving access to vital medicines in some of the poorest countries in the world will be jeopardized and the prospect of achieving many of the goals and targets of internationally agreed initiatives such as the sustainable development goals will be materially damaged. The Committee strongly supported a proposal that WHO send as a matter of urgency a letter to the chief executive of the MHRA and chair of its board, setting out the importance of the work of NIBSC in achieving the global health aspirations of the international community.

_Paul-Ehrlich-Institut (PEI), Langen, Germany_

Dr Heidi Meyer updated the meeting on the recent standardization activities of PEI. These had included participation in the development or revision of a number of WHO written standards, including the new WHO guidance document on evaluating the quality, safety and efficacy of mRNA vaccines for the prevention of infectious diseases (see section 3.4.2 below) and the amendment to the current WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines (see section 3.4.1 below) both of which would be discussed at the current meeting. PEI was also contributing to the ongoing revision of the WHO Guidelines on evaluation of biosimilars.

Dr Meyer then explained how PEI staff had supported the WHO prequalification programme by carrying out product summary file reviews of vaccines and performing lot-release testing of several WHO prequalified vaccines. PEI had also provided support to the WHO prequalification programme for IVDs by assessing various SARS-CoV-2 nucleic acid amplification tests for WHO EUL.
Dr Meyer concluded by reporting on the results of a comparative evaluation of the sensitivity of SARS-CoV-2 antigen RDTs. As of September 2021, 226 different SARS-CoV-2 antigen RDTs had been evaluated using a panel of 50 positive samples covering a wide range of viral load (cycle threshold (Ct) = 17–36). The results revealed a wide range of sensitivity (0/50–43/50 detected) while also highlighting the criticality of the pre-analytical swabbing steps. Of the 226 RDTs evaluated, 20% failed to meet a sensitivity target of detecting more than 75% of samples with a Ct less than 25. It was also noted that the batch-to-batch consistency of some antigen RDTs was poor and that the use of different names for the same original products hampered traceability. The comparative evaluation of antigen RDTs would continue.

Noting the wide range of sensitivity found across the SARS-CoV-2 antigen RDTs evaluated so far, the Committee emphasized the importance of ensuring RDT sensitivity and specificity, and highlighted the potential need for further WHO guidance on this issue.
3. International Recommendations, Guidelines and other matters related to the manufacture, quality control and evaluation of biological products

3.1 General

3.1.1 Assessment of stability in international collaborative studies

The Committee was presented with an overview of the key issues in assessing the stability of WHO international reference standards during their development and evaluation in international collaborative studies. The biological activity of most such standards was designated in arbitrary “International Units” (IU) and their degradation cannot therefore be measured directly as by definition they are the primary standard for the respective biological activity. Although biological standards were often highly stable lyophilized preparations, it was nevertheless important to evaluate their predicted degradation, typically using data from accelerated thermal degradation studies. In this approach, the potency of test samples stored at various temperatures between 4–56 °C is assayed at several time points, usually over a period of between 6 months and 2 years. The potency of these samples relative to that of baseline samples stored at −20 °C is then used to predict the loss in activity expected at −20 °C using the Arrhenius equation. To date, a predicted potency loss of less than 0.1% per year has been considered acceptable.

There are, however, a number of known shortcomings in the use of the Arrhenius equation to predict the rate of loss of biological activity. Although many reference materials appear to exhibit Arrhenius-like behaviour over a limited range of temperatures, the relationship is approximate and may not be sustained over a wider temperature range. Estimates obtained by this method can also have poor precision, while highly stable materials may not lose potency even when stored at elevated temperatures. In addition, assumptions made about the course of the degradation process cannot be tested statistically.

The WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards sets out the stability data and related information required by WHO to support establishment of its international reference standards. This includes information on participating laboratories, assays used, details of the study design and statistical considerations. In addition to the stability of the material during storage, it is also recommended that the stability of the reconstituted standard is determined.

Using the example of the measurement standards to be discussed at the current meeting, the Committee was provided with an overview of potential stability study outcomes. Ideally, the predicted degradation rate will demonstrate acceptable stability but in some cases this rate is either too high to assure stability
or there was no loss of activity at elevated temperatures. In other cases there may be no appropriate model for stability prediction or insufficient time to complete an accelerated thermal degradation study, especially when a standard is urgently required. For real-time stability monitoring, options include monitoring the potency of the standard over time relative to another appropriate preparation and/or monitoring other parameters of the standard (such as physicochemical characterization data). As the precise situation will vary for different standards, a clear vision is required of the stability monitoring goals, potential outcomes, and the assay and statistical tools to be used.

Noting that the vast majority of reference materials were stable and relatively few turned out to be unsatisfactory, the Committee discussed the key issues raised and the stability data expected in collaborative study reports. Although monitoring the stability of reference materials in real time was considered ideal, the comprehensive post-establishment stability monitoring of WHO international standards was considered too resource intensive to be achieved in practice. Furthermore, as the acceptable annual loss of potency varied among standard preparations this should be considered on a case-by-case basis rather than regarding 0.1% per year as a requirement for all reference materials. Observing that the acceptable level of stability typically relates to the intended use of the standard and to the precision of the assays involved, it was agreed that a decision tree to inform the design of stability studies rather than simply following the Arrhenius model should be included when the current WHO Recommendations were revised. This would help project leaders to know what the Committee expected in cases where accelerated thermal degradation studies failed to predict a degradation rate or where the predicted degradation rate was too high to assure stability. The Committee commented on the wealth of experience now available on how the characteristics and formulation of different reference materials affect their stability, suggesting the possibility of more-flexible approaches to stability studies conducted as part of collaborative studies. It was noted in this regard that the stability of reconstituted reference materials was increasingly being assessed in collaborative studies. The Committee concluded by highlighting the importance of feedback from users of reference materials in identifying any emerging stability issues.

3.2 Blood products and related substances
3.2.1 Educational modules on clinical use of blood – first tranche

The WHO Educational modules on clinical use of blood have been developed as an update to the WHO Clinical use of blood in medicine, obstetrics, paediatrics, surgery and anaesthesia, trauma and burns which has been widely used by a diverse community of health professionals since its publication in 2001. The Educational modules will provide a new practical resource for a similarly broad
global audience to help guide decisions and improve training and knowledge in the field of transfusion medicine. It is intended that the modules will thus support implementation of the WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023, and complement the WHO Aide-mémoire for national health programmes on clinical use of blood and other resources.

The Committee was informed that the module development process had been a collaborative effort between WHO and the International Society of Blood Transfusion (ISBT). Materials were developed by an international panel of authors with broad expertise in transfusion medicine, with feedback provided by a panel of critical readers drawn from countries worldwide and by the WHO/ISBT steering committee. This process had ensured that critical inputs could be invited on the modules to ensure their relevance across the diverse range of settings in which they might be used.

A brief outline of the nine modules comprising the first tranche of materials was provided and clarification given that these educational materials were intended to provide simple and clear information that could be readily grasped by a general audience, including students. As the aim was to provide an introduction to the topic rather than an encyclopaedic text, the materials focus on important principles, key points and practical application, and were illustrated with clear diagrams and examples of clinical scenarios.

Organized as a series of modules that could be individually updated as needed, the materials would be made available in both electronic and hard copy formats, and would be accessible online. Publication of the first tranche of modules was planned for the end of 2021 and would be followed by a series of webinars hosted by ISBT in collaboration with WHO. The modules would be promoted and disseminated by WHO and through ISBT and other policy and professional networks. Based on the extensive use of the 2001 WHO document and the positive feedback received, a similarly wide uptake was anticipated for the modules and their translation into multiple languages was anticipated. In addition, a second tranche of modules was now in preparation and would likely be finalized shortly. Topics in the second tranche would complement those in the first tranche and would include the principles of appropriate use of blood and blood products and patient blood management, major haemorrhage and massive transfusion, trauma resuscitation, platelet transfusion, plasma transfusion, adverse events and transfusion in under-resourced countries.

The Committee recognized the timeliness and importance of the Educational modules as an update of the 2001 document. Given the global reach of the earlier document, the Committee welcomed the steps taken to ensure adequate representation of all regions in the authorship and review processes. This included Latin America where the previous document, translated into
Portuguese and Spanish, had been widely used. The Committee was assured that representation from all regions would be ensured in both the finalization of the first tranche of modules and the development and review of the second tranche.

A number of additional topics for inclusion were then suggested by the Committee, including a separate module on the blood donor. In response, it was clarified that references to blood donors were generally restricted to stewardship and responsibility for the blood as the focus of the materials was on clinical decision-making given the target audience. However, it was recognized that the topic of walking donors overlaps both the donor and clinical settings and could be considered in future updates. Other topics of current interest were also highlighted including the use of convalescent plasma and advanced therapies, but in such fast-moving fields requiring regular updating only the broader principles and aspects could realistically be included in what was intended to become a reference resource of established information and practices.

3.2.2 WHO policy brief: the urgent need to implement patient blood management

The Committee was informed of the development of a WHO policy brief intended to support implementation of the WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023. This policy brief specifically relates to Strategic Objective 4 of the Action framework on implementation of patient blood management (PBM) to optimize the clinical practice of transfusion.

Anaemia and blood loss taken together represent one of the biggest public health and health economic burdens at the global level, with an estimated 2.9 billion individuals with anaemia and/or micronutrient deficiencies, and more than 600 million individuals with chronic or acute blood loss and/or bleeding disorders. There is therefore an urgent need for countries to implement PBM to help address this largely preventable and greatly underestimated threat to population health. By addressing the problems of anaemia, blood loss and coagulopathy through a patient-centred, systematic and evidence-based approach, PBM has the potential to significantly improve global population health and the clinical outcomes of hundreds of millions of surgical, medical and obstetric patients, while significantly reducing health resource utilization and health care costs.

The Committee was provided with an overview of the three pillars of PBM and clarification was given of the importance of distinguishing PBM from the concept of optimal blood use. Optimal blood use programmes are based on ensuring clinically indicated transfusions at the minimal effective dose and have a narrower focus compared to the broader clinical approach of PBM. The goal of PBM was not to reduce or restrict the use of blood transfusions or any other therapy but rather to place emphasis on the patient’s own blood as a valuable
resource even before transfusion is considered. A thorough understanding of such differences will help to shift the focus from the blood product to the patient.

There is now a significant body of scientific evidence of the clinical benefits of therapeutic strategies based on PBM, including improved clinical outcomes. A strong economic argument can also be made in terms of improved cost-effectiveness, alleviated cost constraints and almost immediate returns on investment. Additionally, there is an ethical obligation not to ignore and withhold any medical model that is beneficial not only for society at large but also for highly vulnerable populations, individual patients and blood donors. The WHO policy brief therefore calls on countries to adopt a national PBM policy, introduce the necessary governance, and reallocate resources to improve population health status and individual patient outcomes, thus reducing overall health care expenditures. As a next step in this initiative, the development of WHO implementation guidelines for PBM was planned for 2022–2023 involving a large multidisciplinary and multi-profession team drawn from all relevant sectors.

The Committee acknowledged the importance of PBM and commended the next step to develop WHO guidelines on its implementation. Strong concern was expressed that, if accurate, the estimated total number of people worldwide with anaemia and/or micronutrient deficiency would represent approximately one third of the global population. If so, there was a clear and urgent need to strengthen PBM and its implementation in countries.

3.2.3 WHO guidance on screening donated blood for transfusion-transmissible infectious agents

The Committee was informed of progress made in the updating of the 2010 WHO document Screening donated blood for transfusion-transmissible infections: recommendations. The update process had been initiated and guided by the outcomes of a 2018 WHO review meeting involving independent experts in the field. The updated document, scheduled for publication by the end of 2021, is intended to assist countries that require support in developing a national screening programme to maximize the microbial safety of blood donations. Guidance will be provided on the key infectious agents for which all donations should be screened, the most appropriate methodology and screening targets, and the systems required to support effective and reliable screening. The updated guidance is also intended to assist national blood systems in advocating for the political support and resources needed.

The Committee was provided with an overview of the key elements and general guidance provided in the updated document. Key elements include critical and screening-specific elements of infectious disease screening programmes, while the general guidance covers important broader aspects of blood services and their activities which help to support effective screening programmes. The
Committee was informed that the overall aims of the update process had been to ensure that the core guidance remained appropriate while also bringing up to date the supporting evidence, background information and messages. In addition, a number of core messages had been strengthened, and the terminology made clearer and more precise. Developments in understanding and technology had also been incorporated, along with guidance on the setting of acceptable risk levels. Specific guidance was now provided on molecular screening and on a number of “additional” infectious agents for which screening may be required.

During discussion, the Committee enquired whether mention had been made of the work of the Committee and WHO collaborating centres in developing assay calibrants and reference materials, and whether these were of relevance. Confirmation was given that these were indeed relevant to the updated guidance now provided on quality assurance and quality control. Although the work of the Committee was not specifically mentioned in the text, reference had been made to WHO programmes on ensuring quality assurance and quality control, and to the provision of reference materials. A suggestion to add information, including website links, on the relevant WHO reference materials now available to support evaluation of assay performance and assay validation was accepted and would be included in the final version of the document.

In light of the concerns raised about RDTs and their promotion and use in many countries, the Committee enquired whether mention of these had been made in the updated document. Clarification was given that although RDTs were not directly advocated in the document, they can have a role to play in some situations. The issue was not the RDTs per se but ensuring their proper evaluation and validation for their intended use. The Committee emphasized the vital importance of this issue and the generally poor understanding of the variability in quality of such tests, citing the SARS-CoV-2 and HIV RDTs as examples. It was acknowledged that the issues around RDT evaluation and validation were considerable, and that a process of global evaluation and rapid dissemination of the outcomes would significantly benefit blood services worldwide.

The Committee noted that the updated document focused primarily on the screening of donations, and asked if pathogen inactivation might not be a better approach, considering the threat of future emerging viruses. Clarification was given that although pathogen inactivation had a role and was mentioned as part of the overall structure in countries that use the approach, it would not be of value to many countries until it could be applied effectively across all products and the associated issues of reduced yield and high cost addressed. The review group had felt that this evolving situation could not properly be addressed in the updated document. In addition, advocating for its use might be unhelpful and could detract from the key messages to be conveyed to the envisaged target audience of the document.
3.2.4  WHO tools for the stepwise implementation of a haemovigilance system

The Committee was informed of the development of a WHO document intended to support implementation of the WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023. The document relates to one of the outcomes of Strategic Objective 4 of the Action framework, which includes the putting in place of haemovigilance systems at both national and organizational levels. Specifically, the document identifies and enumerates the haemovigilance tools and other resources currently used in countries worldwide. These resources will be made available to all countries through a freely accessible online repository to support the stepwise implementation of a national haemovigilance system. It is envisaged that countries at different stages of developing a haemovigilance system, including countries with already well-established systems, will benefit from free access to such resources.

The document builds upon two earlier WHO documents – the WHO Aide-mémoire for ministries of health: national haemovigilance system and the WHO guide to establishing a national haemovigilance system. Document development had started in late 2020 with the establishment of a working group comprising more than 20 experts from the International Haemovigilance Network, ISBT, national haemovigilance systems and WHO. Working group members collected and contributed relevant tools and resources from across the global haemovigilance community. Both document development and the collection of related resources were based on a highly inclusive approach involving broad stakeholder engagement and feedback. In addition, as haemovigilance can be initiated and enhanced even by committed individuals within a variety of organizations, the target audience for the document was very broad and included national level and other policy-makers, blood establishment staff, blood donor organizations, public health institutions, patient groups, scientific and professional bodies, and developmental partners and international organizations.

In 2020, a survey had been conducted to identify the priority needs in this area, with responses received from 87 countries across all six WHO regions. The provision of educational and training tools and modules in the context of WHO technical support was identified as the top priority. As this involves a number of technical and other challenges, including the need to avoid tools becoming outdated or inaccessible, it had been decided that the ISBT Haemovigilance website would act as the primary repository, with a smaller set of resources placed in the WHO Notify Library. The Committee was presented with an overview of the document structure and contents, which included a directory of the tools and resources available on the ISBT website. A number of the templates and example materials were available in English and French, with plans in place to include other languages. A brief demonstration was then given of how users could navigate the directory when looking for specific resources. In 2021, the document had been
widely shared, with positive feedback and suggestions received. Any identified

gaps in the tools would now be addressed and a final draft document produced

and submitted for WHO clearance by the end of 2021.

The Committee commended the contribution the document would make
to the establishment and strengthening of haemovigilance systems, especially
in developing countries. One important area where guidance was needed was
the analysis of haemovigilance data and follow-up actions to be taken. It was a
common misperception in some countries that the simple provision of data to a
medical information system was equivalent to haemovigilance. The Committee
was informed that this issue had been observed among some survey respondents
at all levels of the haemovigilance system. As a result, this issue had been addressed
in the document which emphasizes that comprehensive haemovigilance was not
just about collecting data but also included data analysis and feedback to hospitals
and blood centres so that improvements could be made. With regard to the need
for ongoing maintenance of the online tools and resources, the Committee was
informed that discussions were taking place to identify the best approach.

The Committee enquired as to the reasons for such a broad target

audience, noting for example that patient groups are very different from blood
establishments and ministries of health. The Committee wondered if it might be
more effective to develop specific tools and resources for each group. In response,
it was highlighted that many countries lack strong national health authorities
and that even though the document was primarily intended for national systems
staff, stakeholders at all system levels had been included. In addition, it was
important to recognize that small initial steps could be taken in some countries
by individuals, some of whom may be involved in efforts to improve patient and
donor safety. The Committee noted that in some cases the Ministry of Health will
even delegate responsibility for the implementing of a haemovigilance system,
and expressed its agreement with the overall approach that had been taken.

3.3 Cell and gene therapy products

3.3.1 Standardization of cell and gene therapy products

The Committee was provided with an overview of developments in cell and gene
therapy products (CGTPs) worldwide. Following outstanding efficacy results for
many of the first-authorized ATMPs the number of such products was increasing
globally with manufacturers increasingly approaching multiple regulatory
authorities. This was now a rapidly evolving area, with 53 clinical trials of gene-
editing approaches primarily based on CRISPR/Cas9 technology either active or
completed – though the evaluation of long-term safety issues remained ongoing. In
addition, the first CD19 CAR T-cell products were now in commercial use, leading
the way for evaluation of other such products, including CAR T-cells for solid
tumours. Current issues in relation to this product type include ensuring specificity
for the target antigen, challenges in manufacturing and potential toxicity. Similarly, the number of approved ex vivo retrovirus and lentivirus gene therapy products was also increasing – with critical challenges here including the risk of integrational mutagenesis or oncogenesis, and the need to determine optimal treatment timing. In addition, adeno-associated viruses were now being widely used for the treatment of monogenic diseases, with ongoing issues including the need to ensure persistence of the desired effect while avoiding immune responses directed against the virus vector.

By 2020, more than 1200 ATMP clinical trials were ongoing, with rapid progress being made through the clinical trial phases. As a result, there would likely be numerous such products submitted for regulatory approval in the near future. The Committee was reminded of the inherent and unique risks associated with ATMPs due to their biological complexity and the current lack of long-term safety data for the majority of approaches. Early risk identification and mitigation approaches would therefore be key requirements going forward. In addition, numerous manufacturing, quality control, testing and legislative issues continue to impact on the clinical development of ATMPs. Among these, the generation of clinical evidence to support licensure is particularly challenging with regard to the choice of comparators, dose and end-points. In the case of severe conditions, many therapies are potentially curative or ameliorating but no relevant standard of care exists for comparison, while studies often involve “last-line” patients for whom best supportive care is not attractive. In the case of orphan treatments, the patient populations eligible for clinical trials were frequently small and heterogeneous.

After being provided with an overview of existing regulatory frameworks and guidance documents for CGTPs in different jurisdictions, the Committee discussed a number of specific regulatory aspects in this area. Among these, the challenge of developing suitable animal-based and in vitro approaches to ensure the safety of ATMPs prior to their use in humans was highlighted. In addition, with regard to the evaluation of point-of-care products, the Committee noted that it was difficult to control products in hospitals and this issue had yet to be adequately resolved by the major regulators. It was suggested that this and other issues raised in this presentation, including the need for early risk identification and mitigation, should be addressed in the WHO white paper now under development (see section 3.3.2 below).

### 3.3.2 WHO white paper on regulatory convergence for CGTPs

The Committee was updated on the development of a WHO white paper on regulatory convergence for CGTPs. During a WHO working group meeting on CGTPs held in February 2020, consensus had been reached that CGTPs could be divided into two categories. The first category consists of products that are well established and relatively uncomplicated (such as skin or bone grafts) and where safety and efficacy are assumed. The primary issue with such products
was preventing the transmission of infectious diseases. Although significant issues remain to be addressed in cases where multiple components are mixed or where stem cells are used, there is now a high degree of commonality among different regulatory authorities in the ways in which this category of minimally manipulated cells is regulated. The second category involves a far higher level of complexity that raises a number of complex regulatory considerations with regard to the manufacturing and quality control of the associated ATMPs. Such considerations include whether manufacturing is centralized or decentralized, ensuring appropriate good manufacturing practice for ATMPs, and aligning different standards and practices across different regulatory jurisdictions.

Furthermore, establishing the critical quality attributes of such products will be crucial and will require the establishment of well-defined TPPs. Other manufacturing issues warranting regulatory consideration include how ATMPs are transported and the delivery systems used to administer them. In addition to manufacturing issues, the nonclinical development of ATMPs and the clinical evidence needed to support their approval will be crucially important elements of any prospective regulatory framework. For some ATMPs with potentially long-lasting impact, post-marketing pharmacovigilance will be especially important. Finally, a range of educational, ethical, scientific, political and economic factors would also need to be considered in the context of ATMP regulation.

The Committee was informed that the principal purpose of the white paper was to develop a common language and risk-based classification for these highly complex products, thus facilitating regulatory convergence and dialogue between countries on the measures needed to improve their global accessibility. The white paper was broadly divided into the two categories of product outlined above. Clarification was given that RNA products such as prophylactic vaccines that result in transient protein expression were considered to be outside the scope of the white paper. A first round of public consultation in late 2021 will provide an opportunity for all stakeholders to comment on the proposed document.

The Committee expressed its strong support for the development of the WHO white paper, noting that this once again highlighted the proliferation of these increasingly complex and challenging products, several of which had already come before the Committee for its consideration. Noting as an example the interchangeable use of “tissue products” and “tissue engineering products” in different regulatory jurisdictions, the Committee welcomed the proposed harmonization of terminology. However, without additional specialized expertise, providing WHO with fully informed guidance in this field will present a significant challenge. WHO was therefore urged to consider expanding the expertise both of the Committee and of its Biologicals team to provide the specialist knowledge required to address the formidable regulatory and other challenges associated with this complex class of biological product.
3.4 Vaccines and related substances

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

Yellow fever is a viral haemorrhagic fever endemic to countries in Africa, and Central and South America. It is transmitted from human to human via mosquitoes and can result in high case-fatality rates among non-vaccinated young children and the elderly. Highly effective live attenuated yellow fever vaccines derived from strain 17D have been in use since the late 1930s and a wealth of data is available on their safety. However, rare serious adverse reactions associated with their use include neurological and viscerotropic disease. It is therefore crucially important to assess the levels of neurotropism and viscerotropism exhibited by new virus master or working seeds compared to those exhibited by vaccines shown to be clinically safe. Appendix 2 of the current WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines sets out how virus master and working seed lots should be tested for viscerotropism, immunogenicity and neurotropism in non-human primates, both in terms of clinical evidence and histological lesions, based on comparison against a reference virus approved by the NRA.

In 2018, one yellow fever vaccine manufacturer reported that three working seed lots had been tested in monkeys using a single reference and discrepancies observed between the clinical scoring results of two lots. Further investigation had concluded that histological scoring was more reliable than clinical scoring. Differences in the clinical scores for the same reference virus (PV26) obtained in 2011 and 2018 provided further evidence of the inconsistency of clinical scoring even when using the same material. Following these observed discrepancies, WHO was requested to amend the current guidance in such a way as to be consistent with the approach used to test the neurovirulence of OPV seed lots in which clinical signs are recorded but do not contribute to the pass/fail criteria.

At its meeting in August 2020, the Committee had supported a proposal to set up a working group comprising regulators, manufacturers and academics to review the WHO guidance on the clinical evaluation of non-human primates provided in Appendix 2. While acknowledging that the neurotropism test was subjective and analysis could be challenging, the working group agreed that clinical evaluation nonetheless provided important information and should be retained as part of the test. The resulting amendments to Appendix 2 were: (a) improved guidance on non-human primate testing with respect to observation and assessment of clinical signs; (b) provision of recommendations regarding failure outcomes for the clinical signs; and (c) a recommendation to monitor reference virus performance.

The Committee sought clarification regarding the extent of agreement between the histological and clinical signs, and wondered if vaccine assessment
might simply rely on histological evaluation. The Committee was informed that this possibility had been discussed by the working group but manufacturers still felt that clinical signs provided useful information and there was insufficient data to support the discarding of clinical scoring at present. Having encouraged the development of high-throughput sequencing technologies to assess the safety of OPV at its recent meetings, the Committee was also interested in the potential application of this approach to yellow fever vaccine testing. Clarification was given that although the implementation of this technology was considered highly likely in future, there was currently insufficient data on the critical mutations or lot-to-lot consistency of viral sequences to introduce this as a release test at present.

The Committee was then provided with an overview of a number of issues addressed by the drafting group following feedback received during public consultation. These included: (a) the need to be clear on whether testing was for “neurotropism” or “neurovirulence”; (b) the feasibility of conducting randomized, double-blind controlled tests in non-human primates; and (c) the potential benefit of telemetry to make testing more objective. Acknowledging that “neurotropism” was the term used throughout the current main text of the Recommendations, and that only the appendix was to be amended, the Committee accepted the proposal to add a definition of neurotropism to the beginning of the amendment. The Committee did not feel that randomized, double-blind controlled tests were feasible with non-human primates and this requirement should therefore not be added. Accepting a manufacturer’s argument that the benefits of telemetry remain to be demonstrated, the Committee agreed that the text referring to telemetry should be deleted but a sentence should be inserted in small print encouraging manufacturers to explore the possible use of telemetry to render assessment more objective.

The Committee noted that new reference preparations of yellow fever vaccine virus strains were also now needed for use in non-human primate testing. However, the development of such standards would be challenging and should first be discussed with manufacturers and other stakeholders. The Committee further noted that other yellow fever vaccine platforms were being developed and that although no attempt had been made at the current time to review the 2010 WHO Recommendations in their entirety, an open stakeholder meeting might usefully be organized to determine the need for such a revision.

The Committee then recommended that the document WHO/BS/2021.2401 be adopted and annexed to its report (Annex 2).

3.4.2 Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations

Recent advances in the manufacturing and stabilization of mRNA have established the approach as an important vaccine technology and the speed with which candidate mRNA vaccines can be developed makes them eminently suitable for
use during public health emergencies. The unprecedented pace of development and clinical evaluation of candidate COVID-19 mRNA vaccines has highlighted the urgent need for WHO guidance on evaluating the quality, safety and efficacy of such vaccines. During discussions of the recently revised WHO Guidelines on the quality, safety and efficacy of plasmid DNA vaccines, the Committee had expressed its support for the development of a separate WHO guidance document on regulatory considerations for mRNA vaccines that could be updated as more scientific and clinical data became available. It was proposed that the document would cover the manufacture, quality control, and nonclinical and clinical evaluation of mRNA vaccines intended for the prevention of infectious diseases in humans.

The development of the resulting document had presented several challenges. Although the methods of mRNA vaccine production are generally understood, the precise details of their manufacture and control remain confidential, and the technologies employed can differ significantly. Moreover, compared to other vaccines there is only limited experience of their nonclinical and clinical evaluation. Another challenge had been the need to develop the document within a highly compressed timeline in order to support vaccine development and regulatory approval during the ongoing pandemic. Although its drafting had been expedited to promptly offer regulatory guidance on COVID-19 mRNA vaccine development and evaluation, the document would be broadly applicable to mRNA vaccines against other infectious diseases. The scope of the document was limited to mRNA and self-amplifying mRNA packaged in lipid nanoparticles for the in vivo expression of antigen to elicit an active immune response. Replicating agents, viral vectors, other RNA replicons, mRNA vaccines intended for therapeutic purposes and mRNA products expressing mAbs were outside the scope of the current document.

The Committee was provided with a detailed overview of the structure and content of the proposed document, and of the document development process. Given the considerable level of interest in developing COVID-19 mRNA vaccines, two rounds of public consultation had generated numerous comments. Specific issues addressed included the need for a more comprehensive definition of the relatively new concept of “platform technology”. While acknowledging that the definition used was more stringent than was generally understood in the scientific community, the Committee agreed that from a regulatory perspective the wording used was appropriate. Other terminology issues addressed included reaching agreement on the use of “drug substance” and “drug product” instead of “antigen” and “final vaccine” to reflect the fact that although the mRNA encodes the antigen it is not itself the antigen. The Committee agreed with the consensus that had been reached by the drafting group on these and several other aspects of mRNA vaccine manufacturing. Noting that manufacturers may
regard some aspects of mRNA production as being similar to a pharmaceutical, the Committee recommended that the document clearly indicate that good manufacturing practices for biologicals would be the expected standard. The Committee further noted that in vivo potency tests for mRNA vaccines were unreliable and should not be encouraged. Potency measurements should reflect the clinical performance of a vaccine, with potency assays used for any strain changes validated. The Committee went on to review the overall document and made several general suggestions. Among these, it was suggested that the more usual regulatory term “benefit–risk” (rather than “risk–benefit”) should be used in this and future WHO written standards.

After receiving reassurances that several relevant aspects not explicitly covered in the proposed document were already sufficiently addressed in existing and more general WHO guidelines on nonclinical and clinical evaluation, the Committee recommended that the document WHO/BS/2021.2402 be adopted and annexed to its report (Annex 3).
4. International reference materials – biotherapeutics other than blood products

4.1 WHO international reference standards for biotherapeutics other than blood products

4.1.1 Third WHO International Standard for follicle-stimulating hormone (human, recombinant) for bioassay

Follicle-stimulating hormone (FSH) is a glycoprotein produced in the anterior pituitary gland. It is involved in the regulation of follicular growth, pubertal maturation and reproductive processes, working in synergy with luteinizing hormone to regulate ovulation. FSH is used in fertility treatments such as in vitro fertilization. Early FSH products, which were extracted from urine, were of variable purity and have largely been replaced by recombinant products. Today, a number of such human recombinant products are on the market including a number of biosimilars. The potency of therapeutic FSH is typically determined using an in vivo bioassay with the dose expressed in IU traceable to the WHO international standard. Stocks of the Second WHO International Standard for follicle-stimulating hormone (human, recombinant) for bioassay (NIBSC code 08/282) were now critically low, prompting the production of a candidate replacement material to ensure the continuity of the IU for FSH products.

A quantity of manufacturer-donated purified human recombinant FSH was formulated and filled in 0.5mL aliquots into glass ampoules for evaluation of its suitability as a replacement standard material. The candidate material (NIBSC code 20/218) was evaluated in an international collaborative study involving six laboratories in six different countries. Using a method provided in the study protocol, each laboratory carried out independent in vivo bioassays to estimate the content of the candidate material 20/218 relative to the current WHO international standard 08/282. Results from 16 valid assays indicated an overall geometric mean value of 137 IU/ampoule FSH for the candidate material. Low intra- and inter-laboratory variability (geometric coefficient of variation = 1–8%) showed that estimates were in good agreement. No significant loss of activity was observed in samples stored at elevated temperatures for 6 months. Although this prevented estimation of the rate of loss of activity per annum, the lack of observed degradation, together with the proven stability of the previous FSH standards with a similar formulation, suggested that the material would likely exhibit good long-term stability when stored at −20°C. Further bioassays would be performed in 12 months in order to allow prediction of the annual rate of loss of activity.

The Committee asked for more information on the precise assays performed by each of the collaborative study participants and was assured that there was only one bioassay available at present. Therefore, all laboratories had
performed the Steelman-Pohley assay as described in Ph. Eur., with the exception of one laboratory which made a minor adjustment to the sample diluent. The Committee considered the report of the study (WHO/BS/2021.2410) and recommended that the candidate material 20/218 be established as the Third WHO International Standard for follicle-stimulating hormone (human, recombinant) for bioassay, with an assigned unitage of 137 IU/ampoule. The Committee requested that the report of the study be revised to reflect the minor assay adjustment made by one of the laboratories and this was duly done (WHO/BS/2021.2410 rev.1).

4.2 Proposed new projects and updates – biotherapeutics other than blood products

4.2.1 Proposed Second WHO International Standard for interleukin-6 (human, recombinant)

Interleukin-6 (IL-6) is a pleiotropic cytokine associated with regulation of inflammation, haematopoiesis, cancer progression and immune responses. As IL-6 stimulates the inflammatory and autoimmune processes in many diseases, therapeutic anti-IL-6 products continue to be developed for a diverse range of treatments. The current First WHO International Standard for interleukin-6 (human, recombinant) was established in 1992 and is used in a broad range of applications by manufacturers of both biotherapeutics and immunoassays, as well as by researchers. It is a critical reagent in cell-based assays used for the potency testing of therapeutic mAbs and for the potency testing of IL-6 preparations used as cell culture supplements for CGTPs. It is also used to calibrate immunoassays used to measure serum IL-6 levels, including during the nonclinical and clinical evaluation of immunotherapies. The Committee was informed that the increased use of the current international standard in recent years, which had been exacerbated by the COVID-19 pandemic, had led to the rapid depletion of stocks which, at the current rate of use, will be exhausted by the end of 2022. There was therefore an urgent need for a replacement international standard.

It was proposed that recombinant human IL-6, sourced from a commercial supplier, would be lyophilized in ampoules and evaluated in a collaborative study that would include both bioassays and immunoassays. Although the current international standard was expressed in Chinese hamster ovary cells, it is proposed that IL-6 expressed in *E. coli* be used for the replacement standard. The Committee was informed that material had now been sourced and a trial fill completed. Upon completion of the definitive fill, an international collaborative study involving 17 laboratories representing industry, contract research organizations and control laboratories would assess the suitability of the candidate material as a replacement international standard for bioassays and immunoassays, and
would assign a unitage relative to the current WHO international standard. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2022.

The Committee recognized the urgent need for this international standard and after due consideration endorsed the proposal (WHO/BS/2021.2411) to develop a Second WHO International Standard for interleukin-6 (human, recombinant).
Von Willebrand disease is the most frequent inherited bleeding disorder and is caused by a deficiency and/or abnormality of von Willebrand factor (VWF). VWF is essential for platelet subendothelial adhesion and platelet-to-platelet interactions, and is a specific carrier of blood coagulation factor VIII in plasma. Where transfusion therapy is necessary, disease treatment relies upon the use of purified VWF concentrates. The WHO international standard for VWF is used for the potency estimation and labelling of these therapeutic products. The current Second WHO International Standard for von Willebrand factor (concentrate) (NIBSC code 09/182) was in high demand and it was estimated that stocks would be exhausted by mid-2022.

Candidate replacement materials had therefore been prepared using clinical grade VWF purified from two plasma-derived VWF concentrates (NIBSC codes 18/248 and 08/296) and one recombinant VWF product (NIBSC code 20/156). An international collaborative study involving 48 laboratories in 22 countries had been conducted to assign potency values for VWF:antigen (VWF:Ag), VWF:ristocetin cofactor (VWF:RCo) and VWF:collagen binding (VWF:CB) to the three candidate materials. Values were calculated relative to the current WHO international standard and to the Sixth WHO International Standard for factor VIII/VWF (plasma) to ensure continuity of the IU and confirm the equivalence of the IU values applied to each type of standard (concentrate and plasma). Study participants also investigated the possibility of assigning values to the replacement standard for VWF:glycoprotein IbR (VWF:GPIbR) and VWF:glycoprotein IbM (VWF:GPIbM) to reflect the development of newer immunological assays based on VWF binding to immobilized recombinant GPIb receptors. Due to the rapid uptake of these newer assays and the resulting urgent need for international standardization, the Sixth WHO International Standard for factor VIII/VWF (plasma) had also been established in 2018 as the WHO International Reference Reagent for GPIbR and GPIbM methods.

The study results showed no significant variability in overall mean potency estimates for VWF:Ag and VWF:RCo for all three candidate materials when measured relative to either the current WHO international standard for concentrate or the current WHO international standard for plasma. Greater variability was observed for VWF:CB and was associated with the different methods and different
collagen types used. Only candidate material 18/248 showed no significant method differences for estimates relative to the current international standard, highlighting the importance of the “like versus like” principle of biological standardization. Estimates calculated relative to the WHO international standard for concentrate were observed to be less variable than estimates calculated relative to the WHO international standard for plasma – supporting the use of a separate international standard for each material. Candidate material 18/248 was also associated with the lowest inter-laboratory variability for all three analytes. Mean estimates for GPIbR and GPIbM were also calculated for the candidate materials relative to the current WHO international standards. Candidate material 18/248 was again associated with low inter-laboratory variability and provided the greatest equivalence between the various estimates made.

An accelerated thermal degradation study carried out to evaluate the stability of the candidate material 198/248 showed little loss of potency after 3 months. Real-time stability studies of the current WHO international standard for VWF, concentrate has demonstrated no measurable loss of activity even after 12 years of storage at 20 °C. No stability issues were therefore anticipated and ongoing monitoring would be conducted. Stability testing of the reconstituted material indicated that it remained stable when stored on ice for 4 hours.

The Committee queried the short duration of the stability studies and the resulting limited data and was informed that this had been due to an oversight in placing the candidate materials into storage for studies after filling. However, early indications from stability testing at 56 °C storage for 3 months were that this would not be an issue – a conclusion supported by the extreme stability of the current international standard during its lifetime. Nonetheless, the stability of the materials would be checked again in 6 and 12 months.

The Committee questioned the inclusion of a recombinant product in the study and were informed that licensed recombinant products were not available when the current international standard had been established. A recombinant material had therefore been included in the study to provide an understanding of the relationship between the concentrate standard and the recombinant material. Discussion then turned to the recent transition to the newer GPIbR and GPIbM assays and the need for specific guidance on this. The Committee was informed that this was unlikely to pose a problem for manufacturers establishing a new product as potency could be defined using VWF:RCo, VWF:GPIbR or VWF:GPIbM methods without the need to compare the different analyte values. However, the potency values obtained using these different methods could not be reconciled as they were independent of each other. As a result, manufacturers of existing products planning to switch to a newer method would have to understand and demonstrate the relationship between the clinically proven RCo potency values originally used for such products and those of the new analyte to be assayed.
The Committee also discussed the clinical and industry implications of having different patients and products assessed using different types of activity, each dependant on different assays or references. It was observed that some clinical laboratories were using GPIbR and GPIbM assays to monitor patients and that in cases where a manufacturer moved toward the use of these new methods and standards then these should also be used for patient monitoring – however, it was unclear at present if this would happen in practice.

The Committee considered the report of the study (WHO/BS/2021.2408) and recommended that the candidate material 18/248 be established as the Third WHO International Standard for von Willebrand factor (concentrate) with the following assigned values: VWF:Ag = 12.0 IU/ampoule; VWF:RCo = 8.7 IU/ampoule; VWF:CB = 9.8 IU/ampoule; VWF:GPIbR = 8.6 IU/ampoule; and VWF:GPIbM = 7.3 IU/ampoule.

Ferritin is the main storage protein for iron in many tissues, with the highest concentrations found in liver, spleen and bone marrow. The protein consists of a 24-subunit heterogeneous shell (composed of varying ratios of both heavy and light chain subunits) surrounding an iron core that can contain up to 4500 iron atoms. With its predominantly light subunit form, circulating ferritin is not itself iron-bearing but the level of serum ferritin directly reflects the level of stored iron, with levels being low during iron deficiency and high during iron overload. Serum ferritin levels are normally quantitated using an antibody test that detects the ferritin protein. The Third WHO International Standard for ferritin (human, recombinant) (NIBSC code 94/572) has been distributed to more than 200 laboratories in 38 countries since 1997 and is used to calibrate immunoassays and standardize the results of different assay methodologies. The Committee was informed that stocks of this standard were now low and a replacement standard was required.

WHO international standards for ferritin were originally prepared using ferritin derived from human liver and spleen but due to difficulties in acquiring suitable tissue the current WHO international standard had been prepared using recombinant light chains. Two candidate materials for the replacement standard were therefore prepared using lyophilized preparations of recombinant ferritin light chains (human) expressed either in *E. coli* BL21(DE3) (NIBSC code 19/118) or ExpiCHO-S™ (NIBSC code 19/162). The two candidate materials were shown to be immunologically similar to the current international standard.

An international collaborative study involving 12 laboratories in nine countries using 11 different immunoassay platforms was conducted to assign potency values to the two candidate materials relative to the current WHO international standard. Three lyophilized clinical serum samples with high,
normal and low levels of ferritin were also obtained from the Welsh External Quality Assurance Scheme for use in commutability studies. The sample potencies obtained were reported by the laboratories according to the assay platform calibrant, with these potencies then being expressed relative to the current WHO international standard by NIBSC. It was observed that some laboratories still claimed traceability of their results to the first and second WHO international standards despite these not been available for almost 25 years.

Results indicated that inter-laboratory variability was reduced when the current WHO international standard was used as a reference material. Variability was also reduced when the results from two laboratories were excluded due to invalid assays resulting from non-linearity and non-parallelism. All laboratories correctly identified the commutability samples, though wider variation was observed for results obtained for the sample with the low ferritin level as this was below the limit of quantitation for most of the laboratory systems. Inter-laboratory variability was lower when using either candidate reference material compared to in-house standards.

Combined study results indicated overall mean potencies of 10.5 µg/ampoule for candidate material 19/118 and 8.0 µg/ampoule for candidate material 19/162 relative to the current international standard. Better overall agreement was observed between all laboratories and between assay methods for the potency of candidate material 19/118 compared to candidate material 19/162. Accelerated degradation studies indicated no significant loss of activity in either candidate material after a period of 2 years and it was anticipated that they would remain stable under long-term storage at −20 °C.

The Committee considered the report of the study (WHO/BS/2021.2409) and recommended that candidate material 19/118 be established as the Fourth WHO International Standard for ferritin (human, recombinant) with an assigned content of 10.5 µg/ampoule and expanded uncertainty limits of 10.2–10.8 µg/ampoule (95% confidence; k = 2.23).

5.2 Proposed new projects and updates – blood products and related substances

5.2.1 Proposed Sixth WHO International Standard for blood coagulation factor IX (concentrate)

Blood coagulation factor IX (FIX) therapeutic products are used as a replacement therapy for the bleeding disorder haemophilia B, which is caused by an FIX deficiency. The current WHO international standard is used for the potency labelling of all FIX (plasma-derived, recombinant and extended half-life) and prothrombin complex concentrates used in the treatment of congenital and acquired FIX deficiency. There are currently more than 20 FIX therapeutic
products licensed worldwide, with more under development and approximately 600 ampoules of the international standard are distributed every year, mostly to FIX product manufacturers, IVD manufacturers, regulatory agencies and national control laboratories.

Although the successive WHO international standards have functioned well in the potency labelling of both plasma-derived and recombinant FIX products, substantial discrepancies occur when assaying extended half-life FIX products using different reagents and kits. As all licensed extended half-life FIX products were originally potency assigned relative to the Fourth WHO International Standard for blood coagulation factor IX (concentrate), with the labelled IU supported by clinical trial data, the current fifth WHO international standard had been prepared using the same plasma-derived factor concentrate to ensure continuity of the IU – an approach that has worked well for all licensed FIX products. In addition, the Ph. Eur. Human coagulation factor IX concentrate Biological Reference Preparation (BRP) batch 3 also originated from the same source as the current WHO international standard, which has supported traceability of the IU. The Committee was informed that stocks of both reference materials were now running low and replacements were needed.

It is proposed that the same plasma-derived FIX concentrate will once again be used to prepare the candidate material to ensure continuity and consistency of the IU for all FIX products. As EDQM has expressed a wish to maintain the current harmonized approach, the same batch of material would also be used to replace the Ph. Eur. BRP. The Committee was informed that approximately 20 000 ampoules of the current WHO international standard and BRP had been produced in 2015 and that in order to increase the current replacement period of 6–7 years a larger batch would be required. In addition, the plasma-derived FIX concentrate used as the bulk source material for both the fourth and fifth WHO international standards was being phased out by the manufacturer and may not be available for replacement standards in future. Sufficient candidate material had therefore been donated to the NIBSC for three fills of 25 000 ampoules each, which would ensure sufficient material for successive standards over the next 18 years. The proposed collaborative studies would be run simultaneously in two phases, with submission of the results for consideration by the Committee anticipated in 2022.

During discussion, clarification was given that the proposed two-phase approach was due to known assay and other discrepancies, and that the aim would be to ensure that the same pattern of discrepancies would be maintained in all successive WHO international standards. Discussion then turned to the continued availability of the source material, with concerns expressed regarding the rate of uptake of the international standard and the time between replacement standards. A suggestion was made to assign the full batch of candidate material to
the development of the successive WHO international standards while allocating another batch of material for EDQM use. It was explained that this had been considered but it was thought preferable to use the same batch of candidate material for both reference materials to maintain harmonization and reduce discrepancies. EDQM expressed its appreciation for the approach proposed by NIBSC, reiterated its desire to maintain full harmonization and highlighted that this would also avoid the risk of separate batches expiring at different times. EDQM undertook to work closely with NIBSC to ensure the best use of the materials. Discussion then took place on the optimal filling strategy and clarification given that the current intention was to perform all three fills in 2021 to ensure the stability of the material. Candidate materials derived from all three fills would then be evaluated in the collaborative study to minimize the work that would be needed to establish the future seventh and eighth WHO international standards.

After due consideration, the Committee endorsed the proposal (WHO/BS/2021.2411) to develop a Sixth WHO International Standard for blood coagulation factor IX (concentrate).

6.1 WHO international reference standards for in vitro diagnostics

6.1.1 First WHO International Standard for Mycobacterium tuberculosis (H37Rv) DNA for NAT-based assays

Tuberculosis (TB) remains a major cause of death worldwide and is a particular public health concern in LMIC with inadequate diagnostic facilities. TB is a respiratory disease caused by the bacterium Mycobacterium tuberculosis and timely and accurate diagnosis is crucial for effective treatment and the prevention of transmission. The field testing of TB diagnostics is an important element in achieving the goal set by the international health community to end global TB epidemics by 2030. Sputum-smear microscopy and culture techniques are effective in diagnosing highly infectious TB but less so for the early diagnosis of infection in people with less-pronounced symptoms, with culture methods in particular having long turnaround times. More-sensitive RDTs based on nucleic acid amplification techniques (NATs) are now available with other new tests in development.

The Committee was reminded that experts at a 2018 WHO workshop had discussed advances in TB diagnostics and had identified the need for a WHO international standard for M. tuberculosis DNA to serve as the primary calibrator for NAT-based assays. The use of the international standard would improve the harmonization of such assays and allow different laboratories to compare analytical data using different assay formats. At its meeting in October 2018, the Committee had endorsed a proposal to develop a WHO international standard based on the commonly used laboratory strain H37Rv.

A single batch of 2992 vials of lyophilized, heat-inactivated H37Rv with a concentration of approximately 106 genome copies per mL had therefore been prepared. Preliminary studies confirmed the consistency of filling and effectiveness of the heat inactivation process. This candidate material (NIBSC code 20/152) was then evaluated for its suitability to serve as a WHO international standard for both quantitative PCR assays and RDTs in an international collaborative study involving eight laboratories in seven countries. It was concluded that the use of candidate material 20/152 in quantitative PCR assays reduced inter-laboratory variability. In addition, serial dilution of the material allowed for estimation of the end-point titres/limit of detection of various RDT kits. There was no observable loss of genome copies when the vials were stored at elevated temperatures up to 56 °C for 12 months, while the reconstituted material was stable for up to 4 weeks at −20 °C and up to 1 week at 4 °C.

The Committee commended the study of this necessary standard and reflected on the difficulty of recruiting diagnostic laboratories that were currently heavily committed to the COVID-19 response – an issue for recent collaborative studies in other areas. The Committee did not support a suggestion that “DNA” be
omitted from the name of the standard as this would be inconsistent with similar diagnostic standards. Instead, any potential clarification required of the precise nature of the reference material should be addressed in the instructions for use (IFU). However, it did agree that the strain designation should be included in the name of the standard. Clarification was then given that one of the main intended uses of the standard would be to evaluate and monitor the fitness for purpose of RDTs and the Committee noted the importance of such standards in the WHO prequalification of IVDs.

The Committee considered the report of the study (WHO/BS/2021.2403) and recommended that the candidate material 20/152 be established as the First WHO International Standard for *Mycobacterium tuberculosis* (H37Rv) DNA for NAT-based assays with an assigned unitage of 6.3 log_{10} IU/vial.

6.1.2 First WHO International Standard for varicella zoster virus DNA for NAT-based assays

Varicella zoster virus (VZV) is a member of the Herpesviridae family of DNA viruses and is a highly contagious and widely distributed human pathogen causing a significant public health burden. VZV is the aetiological agent of chickenpox, which primarily occurs in childhood. However, the virus persists in the body asymptomatically after primary infection, establishing latency in the trigeminal and dorsal root ganglia from where it can reactivate from dormancy to cause herpes zoster in adults. Immunocompromised patients, including transplant patients, are at increased risk of developing herpes zoster with disseminated VZV infection potentially life-threatening in such patients. In addition, congenital varicella syndrome can result in a variety of problems among affected infants, including severe damage to the nervous system. Five VZV clades distinguishable by single nucleotide polymorphisms and exhibiting a clear geographical distribution have now been identified.

VZV infection is primarily diagnosed using PCR-based methods that allow for the rapid and sensitive detection of the virus in a range of clinical samples, including serum, plasma, whole blood, vesicle fluid, vesicle swab and cerebrospinal fluid. Quantitative viral load assays and antiviral resistance testing for transplantation patients, as well as vaccine/wild-type differentiation tests, are also performed in clinical settings. In 2015, the Committee had noted the need for standardization in this area and had endorsed a proposal to develop a WHO international standard for use with NAT-based assays.

A candidate freeze-dried VZV preparation was propagated in cell culture from a primary paediatric clinical isolate and evaluated in an international collaborative study involving 12 laboratories in nine countries. Using their routine VZV NAT-based assays, each laboratory evaluated the candidate material (NIBSC code 19/164) alongside two proprietary VZV comparator materials (Ellen and
v-Oka strains), as well as four clinical samples and two spiked samples to permit a preliminary assessment of commutability. Agreement on the mean potency estimates reported across laboratories for both qualitative and quantitative assays significantly improved when results were expressed relative to the candidate material. In addition, accelerated thermal degradation study data obtained at 12 months and 18 months post-production indicated that the candidate material was stable and suitable for long-term storage. Nucleotide sequencing data revealed a pattern of single nucleotide polymorphisms consistent with Clade 3, with no major genome rearrangements.

Noting the reported higher variability of results relative to the v-Oka comparator strain, the Committee enquired whether the other samples in the collaborative study panel had been sequenced. It was informed that work was ongoing and a wider commutability study would allow the effect of clade differences to be assessed. However, the higher variability observed might also have been due to the small number of study participants. The Committee noted in particular the small number of laboratories that had contributed qualitative assay data and, despite assurance that the results indicated that the candidate material had harmonized results, it was suggested that further studies be carried out post-establishment.

The Committee considered the report of the study (WHO/BS/2021.2405) and recommended that the candidate material 19/164 be established as the First WHO International Standard for varicella zoster virus DNA for NAT-based assays with an assigned unitage of $7.0 \log_{10} \text{IU/vial}$.

6.1.3 **First WHO International Standard for anti-Lassa virus immunoglobulin G; and First WHO International Reference Panel for anti-Lassa virus immunoglobulin G**

Lassa fever is a zoonotic disease that occurs as seasonal outbreaks in several West African countries. The causative Lassa virus (LASV) is transmitted to humans from infected rats or person-to-person through contact with contaminated bodily fluids. Although approximately 80% of infected people are asymptomatic, around 20% of infections result in severe disease, including viral haemorrhagic fever. Overall mortality among hospitalized cases is around 20% but higher rates have been observed in some outbreaks. LASV has been identified by the WHO Blueprint for Research and Development: Responding to Public Health Emergencies of International Concern (R&D Blueprint) as a top ten priority pathogen with outbreak potential. While the antiviral medication ribavirin has been used as a treatment, a number of vaccines and other treatments are under development and reliable assays are now needed for their evaluation. The establishment of WHO international reference materials for LASV antibodies would allow for the standardization of serological assays thus facilitating the development of
vaccines and therapeutics, and support epidemiological studies of the disease. In collaboration with the Coalition for Epidemic Preparedness Innovations (CEPI), NIBSC had now carried out a study to develop a WHO international standard and a WHO international reference panel for anti-LASV immunoglobulin G.

Pooled convalescent plasmas had been evaluated in an international collaborative study involving 17 laboratories in seven countries. A total of 12 samples had been evaluated, including two anti-LASV mAb mixtures, to determine their suitability to serve as WHO international reference materials. A wide range of neutralization assays (using live LASV or pseudotyped virus systems) and binding assays (directed against the glycoprotein (GP), the nucleoprotein (NP) or a combination of the two) were used. In addition to the candidate international standard material (NIBSC code 20/202), a reference panel was also assembled consisting of: (a) two high-titre antibody preparations from Nigeria (NIBSC code 20/228) and Sierra Leone (NIBSC code 20/244); (b) a mid-titre pool from Nigeria (NIBSC code 20/226) and convalescent plasma from one individual with mid-titre neutralizing, but high-titre binding antibodies (NIBSC code 20/204); and (c) three low-titre pools from Nigeria (NIBSC code 20/226) and Sierra Leone (NIBSC codes 20/246 and 20/248). The candidate material 20/202 was assessed as part of a blinded sample panel that also included the reference panel, a working standard for LASV antibody and the two mAb mixtures.

Expressing the LASV antibody titres of the study samples relative to the candidate material 20/202 reduced inter-laboratory variability and improved comparability of the results obtained both by neutralization assays and by binding assays (for both viral GP and NP). The rankings assigned by study participants to the samples in the reference panel were similar but not identical, with the low-titre preparations challenging the sensitivity of some assays. Thus, the reference panel proved to be a useful tool in the assessment of serological assay sensitivity for LASV antibodies. The mAb mixture also harmonized the results of both the neutralizing and binding assays, though slightly less effectively than the candidate material 20/202. The stability of the lyophilized plasma pool 20/202 was assessed in an accelerated thermal degradation study. Relative to a baseline sample stored at −20 °C, there was minimal loss of potency for up to 1 month at 37 °C and up to 6 months at ambient temperature (20 °C). Using the Arrhenius equation, the predicted loss of potency for candidate material 20/202 was estimated at 0.17% per year when stored at −20 °C.

The Committee queried whether the serum panel samples obtained from Nigeria and Sierra Leone were sufficiently representative of the broad range of LASV lineages. It was agreed that other samples from different sources might usefully be added to the panel in future to improve its representativeness. Reflecting on the challenge of producing reference materials for emerging pathogens, it was suggested that further studies into the generation of suitable reference materials
using mAbs might be worthwhile. A key issue to be addressed would the current focus placed on mAb development in the context of mechanisms of protection against infectious disease, which would not necessarily reflect the requirements of diagnostic assays.

The Committee considered the report of the study (WHO/BS/2021.2406) and recommended that the candidate material 20/202 be established as the First WHO International Standard for anti-Lassa virus immunoglobulin G with a unitage of 25 IU/ampoule for neutralizing antibody, 250 IU/ampoule for anti-GP binding IgG and 250 IU/ampoule for anti-NP binding IgG. The Committee also recommended that the proposed panel consisting of candidate materials 20/204, 20/222, 20/226, 20/228, 20/244, 20/246 and 20/248 be established as the First WHO International Reference Panel for anti-Lassa virus immunoglobulin G without an assigned unitage.

6.1.4 First WHO International Standard for anti-thyroid peroxidase antibodies

Autoimmune thyroid disease is the most common autoimmune disease and is caused by anti-thyroid microsome autoimmunity. The target of such autoimmunity is thyroid peroxidase (TPO), with antibodies to TPO present in thyroid diseases such as Hashimoto’s thyroiditis and hyperthyroidism. Immunoassays that measure anti-TPO antibodies are important for the diagnosis of thyroid autoimmunity and disease. An NIBSC reference reagent (NIBSC code 66/387) developed in the 1960s had been widely used to calibrate these immunoassays but stocks were now heavily depleted and a replacement reference material urgently needed. At its meeting in 2018, the Committee had endorsed a proposal to develop a First WHO International Standard for anti-thyroid peroxidase antibodies.

A pool of human serum exhibiting high anti-TPO antibody titres had been obtained from three donors, filled into ampoules and freeze dried. The resulting candidate material (NIBSC code 19/260) was then evaluated in an international collaborative study involving seven laboratories in six countries to assess its suitability to serve as a WHO international standard for anti-TPO antibody immunoassays. Data from a total of 13 different immunoassays was analysed. In addition, human serum and plasma samples containing a range of anti-TPO concentrations had also been included in the study to assess the commutability of the candidate material in native samples.

Study results indicated that the reference reagent, 66/387 and the candidate material 19/260 behaved in a similar manner in the various immunoassays used and were in good agreement with one another, indicating that the establishment of candidate material 19/260 as a replacement standard would permit the continued calibration of such assays. Relative to reference reagent 66/387, the overall geometric mean potency for candidate material 19/260 was estimated to be 571 IU/ml (95% confidence interval = 493–662 IU/ml), with a median
value of 533 IU/ml and a robust mean value of 555 IU/ml. Using a difference-in-bias approach, the commutability of both materials was demonstrated for all laboratory methods used in the study. The stability of candidate material 19/260 was assessed in a thermal degradation study over a period of 7 months. Based on the Arrhenius equation, the annual loss of activity when stored at −20 °C was predicted to be approximately 0.013%.

It was noted that as autoantibodies were inevitably donor specific, any replacement standard obtained from different donors would differ from the previous reference reagent. Accepting that this was an insurmountable issue, the Committee was content that candidate material 19/260 behaved similarly to the current reference reagent 66/387 and would harmonize the results of immunoassays. The Committee considered the report of the study (WHO/BS/2021.2404) and recommended that the candidate material 19/260 be established as the First WHO International Standard for anti-thyroid peroxidase antibodies with an assigned unitage of 555 IU/ampoule. The Committee further recommended that the IFU should make clear that the replacement material represented a different population of autoantibodies than the previous reference reagent 66/387.

6.2 Proposed new projects and updates – in vitro diagnostics

6.2.1 Proposed Second WHO International Standard for alpha-fetoprotein (human cord serum)

Alpha-fetoprotein (AFP) is a 70 kDa glycoprotein in the serum albumin family that is generally present at low levels in adults and at slightly increased levels in pregnant women. Significantly elevated AFP levels can be seen in disease processes such as chronic active hepatitis and hepatocellular carcinoma, and in the amniotic fluid in the presence of congenital abnormalities such as anencephaly, omphalocele and spina bifida. AFP levels in serum, plasma or amniotic fluid are measured via immunoassay as part of disease diagnosis and monitoring, or for the detection of fetal congenital abnormalities during pregnancy. The majority of such immunoassays are calibrated against the current First WHO International Standard for alpha-fetoprotein (human cord serum) (NIBSC code 72/225). The Committee was informed that stocks of this international standard, which had been established in 1975, would be exhausted in 2022 and a replacement reference material was urgently required. The replacement reference material was expected to be used by manufacturer, clinical and academic laboratories to calibrate their in-house immunoassays. Based on current level of usage, the predicted demand was 60–70 ampoules per annum.

The current WHO international standard had been prepared using pooled human cord serum obtained from a large number of donors, and had an assigned unitage of 100 000 IU/ampoule (0.121 mg/ampoule). The Committee was informed that this level was very high in comparison with the dynamic
International reference materials – in vitro diagnostics

The Committee expressed concern that the replacement international standard would only be submitted for proposed establishment in 2023 despite the expected depletion of the current international standard in 2022, and enquired as to the implications of this. Assurance was given that steps had been taken by NIBSC to minimize the period during which an international standard would not be available, with laboratories currently restricted to one ampoule per year. In addition, source materials had now been secured and initial preparations were under way. The Committee endorsed the proposal (WHO/BS/2021.2411) to develop a Second WHO International Standard for alpha-fetoprotein (human cord serum).

6.2.2 Proposed First WHO International Standard for anti-thyroglobulin antibodies

Thyroglobulin plays a major role in the synthesis, storage and release of thyroid hormone. The presence of thyroglobulin antibodies (TgAbs) is diagnostic of thyroid autoimmune diseases such as Hashimoto’s thyroiditis, Graves’ disease and other hyperthyroid and hypothyroid disorders. Measurement of TgAb level is also used to diagnose and monitor differentiated thyroid cancers. The majority of immunoassays used to detect TgAb in serum and plasma are calibrated using the current International Reference Preparation of anti-thyroglobulin serum (NIBSC code 65/093) established in 1978. Since its establishment, this reference material had been widely used and stocks were now expected to be exhausted in 2023. It was therefore proposed that a replacement WHO international standard be developed for use as a calibrant for TgAb immunoassays. Based on current level of usage, the predicted demand was 85–95 ampoules per annum, with an expected fill of 1000–1500 ampoules ensuring sufficient stocks for 10–15 years.

The current international reference preparation had been produced from a large number of plasma donors with high titres of TgAb. It was proposed that serum, source plasma or recovered plasma from a similar group of donors be obtained and pooled to produce a candidate replacement material. The candidate material would be characterized and value assigned against the current international reference preparation 65/093 in an international collaborative study.
involving manufacturer, clinical and academic laboratories. It was anticipated that the collaborative study outcomes would be submitted for consideration by the Committee in 2023.

During discussion, it was ascertained that the source material to be purchased would likely be plasma collected by plasmapheresis and tested for several markers by the supplier. The Committee noted that there would be a shift from the current international reference preparation to a WHO international standard and enquired about the implications of this both for the source material and its characterization. In response, it was surmised that the initial status of the long-established current reference material may simply have been due to the small number of laboratories (n = 3–4) involved in its characterization. The characterization and unitage assignment of the WHO international standard would involve a greater number of laboratories. It was further noted that reference preparations had commonly been established in situations where likely uptake levels could not be firmly determined, or where there had been uncertainty about the need for a WHO international standard. However, it was clear in this instance that there was both wide uptake and a recognized need for an international standard.

The Committee endorsed the proposal (WHO/BS/2021.2411) to develop a First WHO International Standard for anti-thyroglobulin antibodies.

6.2.3 Proposed Fifth WHO International Standard for hepatitis B virus DNA for NAT-based assays

Hepatitis B virus (HBV) remains a major global health problem with an estimated 257 million people living with chronic HBV infection in 2015. NAT-based assays for HBV are routinely used for the diagnosis of hepatitis B and for monitoring antiviral therapies. Such assays are also used to screen blood donations, as well as cells, tissues and organs, to ensure blood and transplantation safety. The WHO international standard is used in the calibration of secondary reference materials and in the validation of NAT-based HBV assays by blood centres, clinical laboratories, control authorities and IVD manufacturers. The Committee was informed that stocks of the current Fourth WHO International Standard for hepatitis B virus DNA for NAT-based assays (NIBSC code 10/266) would be exhausted in 2 years, and that this would also be the time period required to replace the standard.

All previous WHO international standards for HBV DNA had been derived from the same Eurohep R1 reference material (genotype A2, HBsAg subtype adw2) diluted in pooled human plasma. In each case, filling had taken place off-site in 0.5 mL volumes with a limited fill of around 2000 ampoules resulting in a replacement period of approximately 5 years for each WHO international standard, starting in 1999. The Committee was informed that
sufficient residual Eurohep R1 reference material remained to prepare a batch of 3000–3500 ampoules based on a fill volume of 0.5 mL. An international collaborative study involving 10–20 laboratories would be conducted to evaluate the candidate material against the current WHO international standard.

The Committee was informed that although current laboratory analysers require larger sample volumes, this would not necessarily require the standard volume to be increased as its current concentration allows for dilution to obtain a larger sample volume. Producing a batch that would last for at least 10 years would require a minimum of 5000 ampoules. In order to achieve this, new HBV source material would be needed which would potentially result in sequence variations compared to the current reference material. This would necessitate sequencing of the whole genome to ensure that any replacement material matched, as closely as possible, the existing WHO international standard. Variations in the new source material could also lead to a potential drift in the IU value which would need to be minimized by: (a) selecting collaborative study participants similar to those involved in assessing the current WHO international standard; (b) including the First WHO International Standard for hepatitis B virus DNA for NAT-based assays (80 ampoules remaining) in the calibration studies; and (c) using digital PCR in the evaluation. Efforts were currently under way to source new stocks of HBV-positive plasma. Subject to the prompt sourcing and characterization of the new stocks, it was envisaged that the collaborative study outcomes would be submitted for consideration by the Committee in 2023.

The Committee acknowledged the need to minimize any risks associated with changes in source material and noted that the sourcing and analysis of any new material would take time. After considering the various options available, the Committee recommended that replacement of the current international standard should proceed using the residual stocks of Eurohep R1 reference material as this would allow sufficient time for new material to be sourced for future WHO international standards for HBV DNA.

The Committee noted that the current WHO international standard was based on the HBV genotype A which is highly prevalent in Europe and Africa but less common in the Far East, where genotypes B and C were more prevalent. Such genotype differences might affect the interpretation of assay performance in different regions – an issue that had previously been raised for other NAT-based assays such as those for hepatitis C virus RNA. It was suggested that calibrating panels containing different subtypes against the proposed WHO international standard might help to resolve this issue. In any case, the possibility of including other genotypes in future WHO international standards of this type should be investigated.

The Committee endorsed the proposal (WHO/BS/2021.2411) to develop a Fifth WHO International Standard for hepatitis B virus DNA for NAT-based assays.
6.2.4 Update on replacement challenges for the First WHO International Standard for anti-rubella immunoglobulin

Rubella virus, the causative agent of rubella disease, was first identified in 1962. Although the disease is typically mild, infection early in pregnancy can result in miscarriage or congenital rubella syndrome in the infant. Although effective vaccines are available and have been widely used, sensitive and accurate diagnostic methods remain important in detecting infection during pregnancy and in monitoring progress towards local and global eradication of the disease. The Committee was reminded that, since 1966, a series of three WHO anti-rubella measurement standards have been used. The latest of these (RUBI-1-94) consists of normal human immunoglobulin obtained from healthy donors and has been in use since its establishment in 1996 as the First WHO International Standard for anti-rubella immunoglobulin.

Today, a wide and increasing range of assays is used to assess anti-rubella antibody levels, including neutralization, haemagglutination inhibition, single radial haemolysis, ELISAs and more recently other forms of antibody-binding assays. It has become evident that the use of the current WHO international standard leads to different results across laboratories using different methods. Over time there has been a shift away from the evaluation of functional antibody activity towards the measurement of antibody in high-throughput binding assays that are quicker and require less skill to perform. Other issues include the previous lowering of the cut-off point for establishing immune protection from 15 IU/mL to 10 IU/mL, with one recent study finding that different test kits use different cut-offs and grey zone ranges – highlighting the importance of using the same test method when comparing results or performing follow-up testing.

In 2017, the Committee reviewed the findings of a WHO consultation on the use of the First WHO International Standard for anti-rubella immunoglobulin and made the following recommendations: (a) the standard should continue to be made available; (b) the IFU should clearly highlight to users the potential lack of commutability; (c) stakeholders should reconsider the appropriateness of quantitative anti-rubella measurement and the use of the 10 IU/mL value as a cut-off when assessing immune protection; and (d) the use of high-specificity qualitative assays should be considered as an alternative to antibody quantitation.

The Committee was reminded that stocks of the current WHO international standard were now limited and likely to be exhausted within 2 years. As the use of this measurement standard had changed over time, the Committee was asked for its advice on the best approach to its replacement and whether a single material could realistically harmonize both functional and binding assays, as well as meet the needs of both vaccine manufacturers and diagnostic kit manufacturers. A proposal was made to use a panel of anti-rubella immunoglobulin samples covering a range of antibody concentrations...
and calibrated against the current WHO international standard, and a potential collaborative study design was outlined that would assess usage in different assays and possibly allow for differentiation between vaccine development and diagnostic applications.

The Committee agreed that the failure of the current international standard to harmonize modern diagnostic tests resulted from the lack of a clearly defined measurand or measuring system. Consistent with its previous discussions on this issue, the Committee discouraged the quantitative use of a standard to assign units as this fails to harmonize assays and therefore serves no purpose. It concluded that only qualitative assays should be used for the diagnosis of previous infection or vaccination, and that a panel without assigned unitage would facilitate assay development. Commenting that the rubella field was very active, the Committee encouraged the involvement of stakeholders in decisions regarding the development and use of the proposed reference panel.

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

7.1.1 Proposed test protocol and WHO international reference reagents for whole-genome sequencing in the routine lot release of OPV and control of sIPV

Polio eradication remains a top priority for WHO and will depend upon a continuous supply of safe and effective vaccines. A number of live attenuated OPV and Sabin IPV (sIPV) products have now been approved for use in many countries. In addition, following polio eradication, a significant number of additional manufacturers are expected to begin sIPV production to meet an increased demand for such vaccines in the near future, with demand for novel OPVs based on genetically stabilized viruses also anticipated.

Genetic stability is a crucial consideration in ensuring the safety of vaccines. The Sabin strains currently used to manufacture OPV accumulate reversions during replication in humans and cell cultures that potentially increase their virulence. Traditionally, the genetic stability of OPV was assessed using in vivo neurovirulence tests in monkeys or in transgenic mice expressing the human poliovirus receptor. Since 2002, the genotypic stability of such vaccines has also been assessed using the in vitro mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) test, which measures the proportion of revertant mutations present in the 5′-UTR of the viral genome. However, MAPREC is technically challenging, requires radioisotopes and only measures one mutation in the entire genome with implications for the evaluation of other mutations affecting viral properties.

In 2019, the Committee had been updated on the first phase of an international collaborative study, jointly coordinated by NIBSC and the FDA, on the use of high-throughput sequencing as an alternative to MAPREC quantification of mutations in live viral vaccines. It was found that high-throughput sequencing was sufficiently sensitive to detect low frequency variants in the linear range of raw MAPREC data, with good correlation between the two methods in assessing single point mutations. Following the success of this initial study, the Committee was presented with an overview of a potential future approach to routine OPV lot release based on the use of whole-genome high-throughput sequencing and a series of proposed next steps was set out.

The first of these steps would be to conduct a second-phase international collaborative study to explore the suitability of whole-genome high-throughput sequencing as a replacement for neurovirulence testing by assessing test
procedures and reference reagents for the lot release of OPV or sIPV. A series of consistency lots of monovalent OPV will be analyzed by whole-genome high-throughput sequencing and the results compared to those obtained in neurovirulence testing using non-human primates or transgenic mice. The study, sponsored by PATH, will again be jointly coordinated by NIBSC and FDA. Several OPV manufacturers had agreed to provide vaccine lots for use in the study and as prospective reference materials, and a statistical method was being developed for data analysis.

The Committee discussed the relative sensitivities of the MAPREC and high-throughput sequencing methods, noting that both were sufficiently sensitive to detect < 1% mutations in the 5’-UTR – a level of mutation considered to be safe. Reflecting on the limited sequence variation observed between vaccine lots, and noting that many mutations arising in vaccine strains are well characterized, the Committee envisaged that neurovirulence testing may eventually not be required. Enquiring about the affordability of high-throughput sequencing for vaccine developers and control laboratories in LMIC, the Committee was assured that today the approach was technically straightforward, inexpensive and accessible. Part of this proposed project would be to support manufacturers adopting this approach through workshops and training sessions. The Committee commended the progress that had been made and, acknowledging the importance of implementing modern molecular methods for lot release and the considerable benefits that would result from the elimination of in vivo neurovirulence testing, it endorsed the proposal (WHO/BS/2021.2411) to develop a test protocol and WHO international reference reagents for whole-genome sequencing in the routine lot release of OPV and control of sIPV.

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

8.1.1 Proposed First WHO International Reference Panel for antibodies to SARS-CoV-2 variants of concern

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of the ongoing COVID-19 pandemic. At its meeting in December 2020, the Committee recommended the establishment of the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin and the First WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulin. These reference materials are intended to facilitate COVID-19 vaccines and therapeutics development through the harmonization of serological assay data worldwide. However, in late 2020, SARS-CoV-2 “variants of concern” (VOCs) began to emerge with mutations that rendered them more transmissible. The impact of such variants on the effectiveness of vaccines and therapeutics now requires continual evaluation.

It was proposed that a WHO international reference panel consisting of CCP or serum from patients infected with VOCs be developed to facilitate the development of serological assays for VOCs in order to support the continuing development of vaccines and therapeutic strategies. Following collaboration between WHO, CEPI and NIBSC, three donations of CCP or serum from individuals each infected with a VOC had now been offered by a number of sources. The sourcing and receipt of all required donations was now ongoing. All candidate materials would be treated to inactivate any contaminating viruses, sequenced and filled to produce the proposed five-member panel, though flexibility may be required in terms of panel composition should a new VOC emerge. The suitability of the panel to serve as a reference material for assessing serological assays for SARS-CoV-2 VOCs will then be determined as part of a proposed collaborative study to evaluate a replacement reference material for the First WHO International Standard anti-SARS-CoV-2 immunoglobulin (see section 8.1.2 below). To date, three of the five panel sera have been obtained, representing the original Wuhan-like strain and the Alpha and Delta VOCs. Once complete, the panel would be promptly evaluated, with the collaborative study outcomes expected to be submitted for consideration by the Committee in early 2022.

The Committee enquired about the intended primary use of the proposed reference panel and clarification was given that it would principally support the development of live-virus neutralization assays and was not intended to be used
in diagnostic testing. There was also no plan to assign unitages to the panel as this was a known complex issue and would probably cause considerable confusion among end users. Instead, data from the collaborative study would be included in the IFU to guide the interpretation of results.

Noting the importance of the reference panel, the Committee acknowledged the challenge of adding sera corresponding to newly emerging VOCs, especially as more people became vaccinated. However, the Committee also recognized the need for the timely availability of the panel, which should not be delayed due to difficulties sourcing sera for any specific VOC. It agreed with the proposal to develop a flexible panel with a clearly defined process for adding further sera as new VOCs emerged. Reflecting on the challenge of obtaining serum samples for the current panel, the Committee made a number of suggestions regarding the sourcing of Gamma variant serum. It would also be important to make clear how the WHO reference panel differed from similar national reference panels now in development. It was noted that the First WHO Repository of red blood cell transfusion relevant bacterial reference strains might provide a useful precedent for such WHO reference materials.

The Committee endorsed the proposal (WHO/BS/2021.2411) to develop a First WHO International Reference Panel for antibodies to SARS-CoV-2 variants of concern.

8.1.2 Proposed Second WHO International Standard for anti-SARS-CoV-2 immunoglobulin

The First WHO International Standard for anti-SARS-CoV-2 immunoglobulin was established on the recommendation of the Committee in December 2020. Despite imposing a limit of five ampoules per user, more than 2400 ampoules had been distributed by August 2021 to 581 individual customers globally and the standard stock had been exhausted. A replacement standard is therefore urgently needed to ensure both continuity of supply and traceability of the assigned unitage.

The development of a replacement material presents a number of challenges. As the IU is an arbitrary unit that does not correspond to a physical measurement, it cannot be calculated for each variant. Users are therefore advised to report the potency of antisera specifically for each variant used and not to make direct comparisons between VOCs. In addition, diagnostic kit manufacturers have requested the use of higher titre sera as vaccinees tend to exhibit higher antibody titres than those convalescing from infection. The proposed replacement standard would therefore consist of a pool of high-titre serum or plasma obtained from individuals convalescing from infection with a VOC. The pool will be prepared from selected sera sourced for the development of the WHO international reference panel for VOCs (see section 8.1.1 above). The ideal candidate material
would exhibit high antibody titres against all current VOCs, and be sufficient to fill at least 5000 ampoules. It had been observed that serum from recovered and subsequently vaccinated individuals exhibited higher antibody titres and a broader response to the different VOCs than convalescent sera.

Another significant challenge was the need to avoid the inappropriate use of the reference material in antibody-binding assays. The current WHO international standard had been assigned an IU based on neutralizing antibodies and concern had been raised by the Committee during its establishment that assigning the same unitage for use in antibody-binding assays based on different antigens could result in the incorrect use of the standard. To allow for comparison of relative antibody titres to different antigens, the material would be assigned an arbitrary binding antigen unit (BAU). Comparison of the current WHO international standard with clinical samples across a range of antibody-binding assay platforms had demonstrated its commutability with clinical material but had also highlighted the potential unsuitability of IU assignment for such assays. As many commercial assays based on different target antigens were calibrated in BAU and several publications had reported results accordingly, the continued use of the unit would be useful.

Data from the collaborative study would be used to assign an IU unitage relative to the current WHO international standard based on neutralization assays. A value would be calculated for each VOC with arbitrary values to be assigned should new VOCs emerge. Regarding the timeline for development of the replacement standard, it was clarified that if the Committee recommended the use of high-titre sera from recovered and subsequently vaccinated individuals then such material would need to be sourced, potentially shifting the establishment of the replacement standard from early to late 2022. Mindful that any such change in the source material might also affect its assigned unitage, the Committee advised that the replacement material should be as similar as possible to the current WHO international standard and should be assessed using the same neutralization measurement system. It was noted that the development of future standards using suitable convalescent serum would be challenging because of changes in the predominant VOC, the rapidly changing COVID-19 vaccination status of donors and the declining interest in collecting CCP. Given the interest in using high-titre sera from infected and subsequently vaccinated individuals, the Committee suggested that such material could, if sourced in time, be included in the collaborative study and the data subsequently reviewed.

Acknowledging the ongoing high level of demand for the current WHO international standard and recognizing its importance in the harmonization of serological assays globally, the Committee endorsed the proposal (WHO/BS/2021.2411) to develop a Second WHO International Standard for anti-SARS-CoV-2 immunoglobulin.
8.1.3 Update on the development of the First WHO International Standard for SARS-CoV-2 antigen

The Committee was updated on the development of the First WHO International Standard for SARS-CoV-2 antigen. The antigen assay landscape for SARS-CoV-2 was now highly complex, with several different antigens targeted either alone or in combination, and various types of test sample and numerous formats used. Worldwide, more than 200 SARS-CoV-2 antigen tests have received regulatory approval or are in development. A common reference reagent was urgently required to support their development, evaluation and monitoring. The current project had therefore been endorsed by the Committee at its meetings in late 2020 and the aim remained of presenting the outcomes of a full collaborative study for consideration by the Committee in early 2022. Several candidate materials had now been sourced, including whole inactivated virus, N protein expressed in E. coli and trimeric S protein expressed in HEK293 cells, and their concentrations determined using mass spectrometry.

A pilot study had been conducted to evaluate the source materials and determine the most suitable antigen preparation for use as the candidate material in the full study. Pilot study participants (n = 17) had determined the end-point titration for each of six samples using a range of different tests. The sample set included inactivated virus preparations, recombinant N protein and recombinant spike protein. In most cases, tests had been provided by the manufacturer, with some study participants also evaluating other tests routinely used in their laboratory. Although ED$_{50}$ values were estimated using the Spearman-Karber method due to time pressures, alternative probit models and other methods are available, and a different method would likely be used in the definitive collaborative study. Pilot study results showed that the virus sample inactivated with 0.01% formaldehyde was detected with the greatest sensitivity and produced the lowest standard deviation between laboratories. Following the production of an interim NIBSC working standard, a candidate WHO international standard was developed based on the Delta variant inactivated with 0.01% formaldehyde. The candidate material will be evaluated in the definitive collaborative study by approximately 30 laboratories using a range of assay technologies.

The Committee enquired if the proposed IU of the candidate material was to be linked to its content, as determined by mass spectroscopy. Clarification was given that no decision had been taken in this regard and the views of the Committee would be welcome. It was felt that as most of the tests depended upon an antibody-antigen interaction it would be more appropriate to use IU rather than SI units for the proposed standard. However, the use of mass spectrometry had been very useful in determining that inactivation did not change the amount of protein in the whole-virus samples and had highlighted potential issues with the use of recombinant proteins in this situation, where detection by lateral flow
tests relied on correct folding of the antigen. Noting that the ED$_{50}$ data from the pilot study could be used to rank the sensitivity of the different tests and that numerous publications were now available on test sensitivity using clinical materials, it was suggested that it might be worthwhile to compare the results of the full study with the published scientific literature to allow for a more detailed comparative analysis of test sensitivity.

9.1 WHO international reference standards for vaccines and related substances

9.1.1 Second WHO International Standard for diphtheria antitoxin (equine)

Diphtheria antitoxin (DAT) products produced from equine serum are essential medicines used for diphtheria therapy and outbreak management, and for prophylaxis against suspected cases of diphtheria in countries where the disease is endemic. In countries with good vaccination coverage, DAT is stockpiled for emergency use. The use of an equine DAT standard calibrated in IU is essential for ensuring that products meet minimum potency requirements. The First WHO International Standard for diphtheria antitoxin (equine) was prepared in Copenhagen in 1934 and consists of a preparation of dried hyperimmune horse serum in ampoules. To conserve stocks, a batch of liquid standard has been produced at NIBSC approximately every 2 years, with a diphtheria antitoxin concentration of 10 IU/mL. In 2016, the Committee had been informed that the stock of dried serum was running low and had endorsed the preparation of a lyophilized replacement standard that would provide a single homogenous batch sufficient for 15–20 years.

The procured candidate material (NIBSC code 18/180) consisted of refined diphtheria immunoglobulin prepared from horse serum and was calibrated using both in vivo and in vitro (Vero cell) toxin neutralization assays, with potency expressed relative to the current WHO international standard. Several formulations were evaluated in a trial fill and were freeze-dried successfully with no loss of biological activity. An international collaborative study was conducted involving 14 laboratories in nine countries, with 10 participants providing data from in vivo assays and eight from in vitro methods. Although sample protocols were provided, participants were encouraged to use their existing in-house assays. Potency was determined by comparing the dose of DAT necessary to protect against the effects of diphtheria toxin with the quantity of a reference preparation necessary to give the same protection. Potency estimates obtained using either the in vivo or in vitro toxin neutralization tests were comparable, with low inter-assay variability. Although use of the Vero cell assay is increasing, in vivo assays continue to be used in many countries and so the potency value for candidate material 18/180 was estimated by combining all data. This gave a value of 57 IU/ampoule, which was similar to the geometric mean potency obtained using the in vivo assays (54 IU/ampoule). The results of accelerated thermal degradation studies predicted no significant loss in activity when stored at −20 °C indicating long-term stability of the material. Real-time stability data indicated that the candidate material would be suitable for use for up to 1 year after reconstitution when stored at 2–8 °C.
The Committee commented on the slow progress towards the use of the Vero cell assay and was informed that many producers of diphtheria antitoxin still opted to use the in vivo assay. Reflecting on whether the small difference (3 IU or 5%) between the potency estimated from all available data and that using only the in vivo assays would be problematic for borderline potency determinations, the Committee was satisfied that this difference would be negligible in the overall context of assay variability. The Committee considered the report of the study (WHO/BS/2021.2407) and recommended that the candidate material 18/180 be established as the Second WHO International Standard for diphtheria antitoxin (equine) with an assigned unitage of 57 IU/ampoule based on calibration by in vivo and in vitro toxin neutralization tests.

9.2 Proposed new projects and updates – vaccines and related substances

9.2.1 Proposed Fifth WHO International Standard for pertussis vaccine (whole cell)

The strict human pathogen Bordetella pertussis is the etiological agent of whooping cough and is transmitted in respiratory droplets. Despite high rates of vaccination in the young, whooping cough (pertussis) remains an important cause of morbidity and mortality globally. There are two types of pertussis vaccine – killed whole cell and acellular (protein antigen). Although acellular pertussis vaccines are widely used in developed countries, more doses of whole cell vaccine are used in routine immunization programmes worldwide each year and an international standard is therefore still required. The potency of whole cell pertussis vaccines is measured in IU based on the current Fourth WHO International Standard for pertussis vaccine (whole cell) established in 2006. Despite restricted sales, stocks of this standard are now becoming heavily depleted and it is estimated that a replacement standard will be required within the next 2 years.

A commercial vaccine producer will donate sufficient material to produce 4000–5000 ampoules of the candidate replacement material, which at the current restricted rate of use should last for approximately 20 years. An international collaborative study will be conducted to assess the suitability of the candidate material, and to assign a unitage calibrated to the current WHO international standard based on data from the Kendrick test. It is anticipated that the study will involve 10–20 laboratories, representing national control laboratories and manufacturers worldwide. It was envisaged that the collaborative study outcomes would be submitted for consideration by the Committee in 2023.

The Committee discussed the significant ethical issues raised by performing stability studies using the Kendrick test, which uses a large number of animals, and for which there is currently no in vitro alternative. The Committee
expressed its support for the ongoing development of alternative in vitro methods and suggested that the use of orthogonal methods be explored further. In order to reduce the use of the Kendrick test, and concluding that accelerated degradation studies of the proposed standard would be part of the collaborative study, the Committee agreed that the stability of reconstituted material only needed to be determined by the custodian laboratory. The Committee endorsed the proposal (WHO/BS/2021.2411) to develop a Fifth WHO International Standard for pertussis vaccine (whole cell).

9.2.2 Proposed WHO International Reference Reagent for diphtheria CRM$_{197}$ antigen

Cross-reacting material 197 (CRM$_{197}$) is a genetically detoxified form of diphtheria toxin in which a single mutation results in the loss of ADP-ribosyltransferase activity. CRM$_{197}$ is widely used as a carrier protein in a number of different polysaccharide conjugate vaccines, including meningococcal, pneumococcal and typhoid glycoconjugates. Although CRM$_{197}$ can be produced using a Corynebacterium diphtheriae expression system, this approach produces relatively low yields and a number of companies are now producing recombinant CRM$_{197}$ using heterologous expression systems. These alternative sources of CRM$_{197}$ carrier protein are likely to become important in helping to meet an increasing global demand for conjugate vaccines against encapsulated bacterial pathogens.

When used for vaccine manufacture, CRM$_{197}$ is characterized using immunochemical and physicochemical methods. It is proposed that a WHO international reference reagent be developed to support the stable and well-characterized control of these analytical methods. The Committee was informed that sufficient recombinant CRM$_{197}$ produced in *E. coli* had been donated as a potential material for this purpose, and will be filled and lyophilized. A collaborative study will be conducted to assess the suitability of the proposed candidate material but units of activity or content will not be assigned. Although a number of different recombinant CRM$_{197}$ materials are available, it is currently not clear whether one material would be suitable for use as a control in analytical techniques applied to other recombinant CRM$_{197}$ products. However, published comparisons of various CRM$_{197}$ materials suggest only subtle differences in some physicochemical characteristics. The carrier protein content of conjugate vaccines is typically quoted in SI units – normally µg/mL in bulk conjugates and µg/dose (or range of µg/dose) in the final product – with UV spectrophotometry being the primary method.

The Committee briefly discussed the potential differences between CRM$_{197}$ produced in different expression systems. Acknowledging that the purpose of the collaborative study would be to identify such differences, it was clear from the published literature that the physicochemical differences were
subtle, with the main differences likely to be in yield, purity and possibly post-translational modification. The Committee recognized the considerable level of interest in manufacturing polysaccharide-conjugate vaccines in LMIC, especially in the WHO African Region. Any reduction in the level of containment required during vaccine production and the increased yield associated with the novel CRM$_{197}$ expression systems would potentially reduce the cost of this important carrier protein.

Noting the importance of demonstrating that the proposed material could be used as a control for both native CRM$_{197}$ and CRM$_{197}$ produced in other expression systems, the Committee endorsed the proposal (WHO/BS/2021.2411) to develop a WHO International Reference Reagent for diphtheria CRM$_{197}$ antigen.
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 Series 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
Email: bookorders@who.int
Website: http://apps.who.int/bookorders

Individual Recommendations and 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

Abbreviated in the following pages to “TRS”.

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<sup>5</sup> 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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Annex 2

Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines
Amendment to Annex 5 of WHO Technical Report Series, No. 978

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Introduction

The WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines were adopted in 2010 (1). Appendix 2 of these Recommendations addresses the testing of new virus master and working seed lots in non-human primates. Specifically, the appendix sets out the ways in which such 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. Following reported discrepancies in the clinical scoring of monkeys during the assessment of working seed lots, WHO received a request from one yellow fever vaccine manufacturer to align the neurotropism assessment outlined in the 2010 Recommendations with that used for the neurovirulence testing of oral poliomyelitis vaccine seed lots in which clinical signs are recorded but do not form part of the assessment or pass/fail criteria (2).

At its seventy-first meeting in August 2020, the WHO Expert Committee on Biological Standardization recommended that a drafting group be established to consult with as many yellow fever vaccine manufacturers and other stakeholders as possible on a proposed revision of Appendix 2 of the 2010 Recommendations (3). At its seventy-third meeting in December 2020, the Committee was updated on the progress that had been made (4). The currently specified approach had now been associated with several technical challenges including: (a) a paucity of data on the performance of the test; (b) the difficulties inherent in conducting a collaborative study involving non-human primates; (c) the lack of an international reference standard for vaccines of the 17D-204 and 17DD lineages and consequent use of different reference materials; (d) reported discrepancies between clinical and histopathological assessments; (e) inconsistencies between staff in the scoring of clinical and histopathological observations; and (f) the sourcing of animals from different locations.

Work on revising Appendix 2 of the 2010 WHO Recommendations commenced in early 2021. On 18–19 March 2021, a virtual WHO working group meeting was held to discuss a proposed draft of the revised text. Overall, there was a consensus among manufacturers and NRAs that clinical evaluation provides important information and should be retained as part of the neurotropism test. However, there was also agreement that the test is somewhat subjective and that analysis can be difficult. It was recognized that there was potential for improvement in both test execution and analysis to increase harmonization between organizations. Based on these working group discussions, the appendix was revised by the WHO drafting group. Following public consultation and further revision, the amendment to the 2010 WHO Recommendations presented below was reviewed by the Committee at its meeting in October 2021.
No attempt was made at this time to review the 2010 WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines in their entirety and only the issues outlined above have been addressed

**Amendment**

Replace Appendix 2 with the following text:

**Appendix 2**

**Tests in non-human primates of new virus master and working seeds**

Neurotropism is defined as the tendency or capacity of a microorganism to cause disease of the nervous system and the yellow fever test in monkeys is a tropism test by this definition. However, it also involves examination of the tendency of the virus to cause viraemia after intracranial inoculation, which could be interpreted as a surrogate of both viscerotropism and the ability to induce an immune response in the same way. The test can differentiate between strains of yellow fever vaccine viruses using all three of these criteria.

Each virus master and working seed lot should be tested for viscerotropism, immunogenicity and neurotropism in a group of 10 test monkeys compared against a second similar group of 10 monkeys injected with a reference virus. The same test and reference groups will be used for all of the viscerotropism, immunogenicity and neurotropism tests. The allocation of animals to the two groups should be blinded to the operators throughout the experiment. For the neurotropism test, the test monkeys inoculated intracranially with the virus seed lot should be compared against the 10 monkeys injected with the reference virus. Existing manufacturers should use a homologous reference – for example, where their working seed is to be replaced by another derived from the same master seed, the existing seed can be used as the reference material, provided it has been shown to produce a vaccine with satisfactory properties. It is recommended that sufficient stocks of such a reference are kept for all future anticipated replacements of the working seed. New manufacturers using a new seed should use a homologous preparation known to produce a satisfactory product as a reference material. The reference virus should be approved by the NRA.

A WHO reference virus, 168-73, is available from the National Institute for Biological Standards and Control, Potters Bar, England. This virus is of the same lineage as the WHO primary seed 213-77 (see Appendix 1, Figure 1), but available published data show that it behaves differently to vaccines of at least one other lineage in the monkey test, being much less neurovirulent and producing a higher viraemia. It is likely, though unproven, that 168-73 will be a satisfactory reference for seeds of the 213-77 lineage. While 168-73 is not suitable as a
A comparator for vaccines of other lineages, its inclusion in the neurotropism test as a common material would make it possible to compare different tests, and one lineage with another, for information.

The monkeys should be *Macaca mulatta* (rhesus monkeys) or *Macaca fascicularis* (cynomolgus monkeys) and should have been demonstrated to be non-immune to yellow fever virus and other flaviviruses using a relevant test (such as the haemagglutination inhibition test, ELISA or seroneutralization assay) immediately prior to injection of the seed virus. Tests should be performed using healthy macaques of both sexes (weighing at least 2 kg and at least 18 months old). The monkeys should not have been previously subjected to any experimentation. The test dose should be injected into one frontal lobe of each monkey, under anaesthetic, and the monkeys should be observed for a minimum of 30 days.

The test dose should consist of 0.25 mL containing not less than 5000 (3.7 log$_{10}$) IU and not more than 50 000 (4.7 log$_{10}$) IU, as shown by titration in cell culture. In addition, the virus titres of the test virus seed lot and the reference virus should be as close as possible.

Historically, the test dose has consisted of 0.25 mL containing the equivalent of not less than 5000 and not more than 50 000 mouse LD$_{50}$, as shown by titration in cell culture.

1. **Viscerotropism test**

The criterion of viscerotropism (indicated by the amount of circulating virus) should be fulfilled as follows: sera obtained from each of the test monkeys on the second, fourth and sixth days after injection of the test dose should be inoculated at dilutions of 1:10, 1:100 and 1:1000 into at least four cell culture vessels per dilution. In no case should 0.03 mL of serum contain more than 500 (2.7 log$_{10}$) IU and in no more than one case should 0.03 mL of serum contain more than 100 (2.0 log$_{10}$) IU.

2. **Immunogenicity test**

The criterion of sufficient virus-neutralizing antibody in the sera (immunogenicity) should be fulfilled as follows: at least 90% of the test monkeys should be shown to have become immune within 30 days following injection of the test dose, as determined by examining their sera in the yellow fever virus neutralization test described below. In some countries it has been shown that, at low dilutions, some sera contain nonspecific inhibitors that interfere with this test. The NRA may therefore require sera to be treated to remove such substances.

Dilutions of 1:10, 1:40 and 1:160 of serum from each test monkey should be mixed with an equal volume of strain 17D vaccine virus at a dilution that has been shown to yield an optimum number of plaques when assayed according to one of the cell culture methods given in Appendix 4. These serum–virus mixtures should be incubated in a water bath at 37 °C for 1 hour and then chilled in an
ice-water bath before inoculation of 0.2 mL aliquots of each mixture into each of four separate cell culture vessels. The vessels should be handled in accordance with one of the cell culture techniques described in Appendix 4. In addition, 10 vessels should be similarly inoculated with a pre-incubated mixture of the same virus with an equal volume of a 1:10 dilution of monkey serum known to contain no neutralizing antibodies to yellow fever virus. At the end of the observation period, the mean number of plaques in the vessels containing virus and non-immune serum should be compared with the mean number of plaques in the vessels containing virus and serum from test monkeys. For the immunogenicity test to be satisfied, serum at the 1:10 dilution from no more than 10% of the test monkeys should fail to reduce the mean number of plaques by 50% as compared with the vessels containing non-immune serum.

3. Neurotropism test

The monkeys in the test group should be compared with 10 monkeys injected with the reference virus with respect to both clinical evidence of encephalitis and the severity of histological lesions of the nervous system (5, 6).

The onset and duration of the febrile reaction should not differ between monkeys injected with the test virus or with the reference virus.

3.1 Clinical evaluation

The monkeys should be examined daily for 30 days by personnel familiar with the clinical signs of encephalitis in primates. All such signs should be recorded individually on a daily basis. Evaluation may include observation from a distance using closed circuit television to gather information. The use of implantable telemetry devices (for example to produce electroencephalograms or to monitor temperature and motor activity) may also be considered.

If necessary, the monkeys may be removed from their cages and examined for signs of motor weakness or spasticity, as described elsewhere (6).

Signs of encephalitis – such as paresis, incoordination, lethargy, tremors or spasticity – should be assigned numerical values for severity by the following grading method. Each day each monkey should be given a numerical score based on this scale:

0: no general signs or signs of CNS involvement;
1: rough coat, not eating;
2: high-pitched voice, inactive, slow moving;
3: shaky movements, tremors, incoordination, limb weakness;
4: inability to stand, limb paralysis or death.
Any animal unexpectedly found to be moribund, cachectic or unable to obtain food or water must be euthanized. A monkey that dies receives the score “4” from the day of death until day 30.

The clinical score for each monkey is the average of its daily scores; the clinical score for a group is the arithmetic mean of the individual scores. The timing of the development of clinical signs and their disappearance, as well as their severity, provides evidence of the phenotypic identity of the test vaccine virus and the reference virus. For the test material to be considered sufficiently comparable to the reference material, as required, it should produce no statistically different clinical signs, including in terms of the timescale of their appearance and resolution. It is acknowledged that the clinical evaluation may be imprecise.

3.2 Histopathological evaluation

The cervical and lumbar enlargements of the spinal cord and specific structures at five levels of the brain should be examined (6) (see Appendix 3). The cervical and lumbar enlargements should each be divided equally into six blocks. The blocks should be dehydrated and embedded in paraffin wax; 15 µm sections should be cut and stained with gallocyanin. Alternatively, 5 µm sections will be suitable for hematoxylin and eosin (H&E) staining or Nissl staining (gallocyanin, cresyl violet), as well as for immunohistochemistry techniques. One section, consisting of two hemisections, should be cut from each block.

Tissue blocks 3–4 mm thick should be taken from the brain by making the following frontal cuts:

Block I: the corpus striatum at the level of the optic chiasma;
Block II: the thalamus at the level of the mamillary bodies;
Block III: the mesencephalon at the level of the superior colliculi;
Block IV: the pons and cerebellum at the level of the superior olives;
Block V: the medulla oblongata at the midlevel of the inferior olives.

These blocks should be dehydrated and embedded in paraffin wax and 15 µm sections cut and stained with gallocyanin. Alternatively, 5 µm sections will be suitable for H&E staining or Nissl staining (gallocyanin, cresyl violet), as well as for immunohistochemistry techniques. A single section, consisting of two hemisections, should be cut from each block.

Sections should be examined microscopically and numerical scores assigned to each hemisection of the cervical and lumbar enlargements, and to each anatomical structure (see Appendix 3) within each hemisection of the brain blocks, according to the following grading system:
1 (minimal): 1–3 small, focal inflammatory infiltrates. A few neurons may be changed or lost;

2 (moderate): more extensive focal inflammatory infiltrates (neuronal changes or loss affects no more than one third of neurons);

3 (severe): neuronal changes or loss of 33–90% of neurons, with moderate focal or diffuse inflammatory infiltration;

4 (overwhelming): more than 90% of neurons are changed or lost, with variable, but frequently severe, inflammatory infiltration.

Each brain block contains several anatomical structures, which contribute in different ways to the assessment of a test sample. For example, certain structures differentiate more reproducibly than others between acceptable and unacceptable yellow fever seed lots and vaccines (6). These are called “discriminator areas”, whereas structures that are more susceptible to yellow fever virus replication are called “target areas”. Though both rhesus and cynomolgus monkeys are acceptable, the discriminator and target areas are different for the two species. The major difference is that in cynomolgus monkeys the cervical and lumbar enlargements are target areas whereas in rhesus monkeys they are discriminator areas. The footnotes to the worksheets provided in Appendix 3 indicate in more detail the discriminator and target areas for the two species. The worksheets also list other anatomical structures that will be present in the brain sections but that are not included in the evaluation of a test sample because they are rarely affected (spared areas).

Three separate scores should be calculated for each monkey: (a) discriminator areas only; (b) target areas only; and (c) discriminator plus target areas. These three scores should be calculated as shown in the sample worksheets provided in Appendix 3.

Overall mean scores should also be calculated for each group of monkeys as the arithmetic mean of individual monkey scores for discriminator areas only, and for discriminator plus target areas. Both of these overall mean scores should be considered when determining virus seed lot acceptability. For the histological criterion of the neurotropism test to be satisfied, both of the overall mean scores for the test monkeys should not be significantly greater (at the 5% significance level) than the overall mean scores for the monkeys injected with the reference virus.

Both the clinical and histological criteria of the neurotropism test should be satisfied in order for the virus seed lot to meet the requirements for use in production.

It is acknowledged that clinical observations may be more subjective than histological scoring.
Manufacturers are encouraged to explore the possible use of telemetry to render the assessment more objective.

Any failure to meet the statistical criteria should result in failure of the batch. Any exception made to this rule should be rare and would only be acceptable after a thorough investigation of the conducting of the tests, including a review of historical in-house data. Clinical observation should be included in the review and the record of the ultimate decision even if the findings do not meet the statistical criteria for a pass. However, any decision to ignore the statistical evaluation of clinical signs should be a rare and exceptional event involving close discussion with the NRA.

Authors and acknowledgements

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References


Annex 3

Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations

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Guidance documents published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes regulatory considerations for national regulatory authorities (NRAs) and for manufacturers of biological products.
Abbreviations

AESI  adverse events of special interest
COVID-19  coronavirus disease 2019
DNA  deoxyribonucleic acid
DNase  deoxyribonuclease
dsRNA  double-stranded RNA
GMP  good manufacturing practice(s)
HPLC  high-performance liquid chromatography
ICH  International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IU  International Unit(s)
IVT  in vitro transcription
LNP  lipid nanoparticle
mRNA  messenger RNA
NRA  national regulatory authority
ORF  open reading frame
PCR  polymerase chain reaction
PEG  polyethylene glycol
PEGylation  polyethylene-glycol-ylation
RNA  ribonucleic acid
RT-PCR  reverse transcription polymerase chain reaction
sa-mRNA  self-amplifying mRNA
SARS-CoV-2  severe acute respiratory syndrome coronavirus 2
tRNA  transfer RNA
UTR  untranslated region
WHO  World Health Organization
1. Introduction

Although the immunostimulatory effects of RNA have been known since the early 1960s (1), the possibility of using direct in vivo administration of in vitro transcribed messenger RNA (mRNA) to temporarily introduce genes expressing proteins (including antigens) was demonstrated in 1990 following the direct injection of “naked” nucleic acids (2). Subsequent improvements in stabilizing mRNA, increasing the feasibility of manufacturing RNA-based products and decreasing RNA-associated inflammatory responses have led to significant advances in the development of mRNA vaccines and therapeutics (3–6). There are several reasons why the mRNA platform has emerged at the forefront of vaccine technology. Among these are the rapid speed at which mRNA candidate vaccines can be constructed and manufactured, and the need to rapidly develop vaccines against emerging pathogens, such as zoonotic influenza virus strains, Zika virus and most recently severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19).

A number of publications have now discussed some of the safety, production and regulatory issues associated with this new technology (7–14). In addition, the rapidity with which clinical trials have progressed for COVID-19 candidate vaccines, their approval or authorization by NRAs and subsequent widespread use have created a pressing need for WHO guidance on evaluating the quality, safety and efficacy of mRNA products used for the prevention of infectious diseases in humans. Such evaluations must take into account: (a) the inherent immunological, physiochemical and structural properties of mRNA; (b) the need for special formulations such as lipid nanoparticles (LNPs) to ensure in vivo stability and efficient delivery; and (c) the novel cell-free enzymatic manufacturing process. Because detailed information is not yet available on the methods used for production, controls are not yet standardized for safe and efficacious mRNA vaccines, and certain details remain proprietary and thus not publicly available, it is not feasible to develop specific international guidelines or recommendations at this time. Consequently, flexibility in the scientific approach to regulating mRNA vaccines is currently needed. The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of mRNA vaccines, should be discussed with and approved by the NRA on an individual case-by-case basis. Nevertheless, the key principles described in this document are applicable to the class of preventive mRNA vaccines against infectious diseases for human use in general and are intended to provide guidance until more detailed information becomes available. For mRNA vaccines that target diseases for which there are existing vaccines and corresponding WHO guidance, it may be appropriate to consider the relevant sections of this document for issues specific to mRNA
vaccines in conjunction with the corresponding Part B (nonclinical evaluation) and Part C (clinical evaluation) of the respective WHO Recommendations and Guidelines for guidance on issues specific to the evaluation of vaccines against that disease (15).

Any given manufacturer’s mRNA vaccines might potentially be viewed as a platform technology in which the coding region can readily be changed without necessarily having to change the manufacture or control of the resulting new product (except for antigen-specific tests for identity, potency and stability). However, this will depend on the resulting characteristics of the final vaccine. If significant changes are made to the final vaccine, resulting in changes to the critical quality attributes as well as subsequent cellular interaction, then further consideration of the manufacturing process, controls and testing of the product will be required.

The WHO Expert Committee on Biological Standardization discussed these and related issues at its meetings in August and December 2020, and expressed its support for the development of a WHO guidance document on regulatory considerations in the evaluation of mRNA vaccines, which could be updated as more scientific and clinical data on this novel product type became available (16, 17).

2. Purpose and scope

This document provides information and regulatory considerations regarding key aspects of the manufacture and quality control, and nonclinical and clinical evaluation, of preventive mRNA vaccines against infectious disease for human use. Sufficient information should be provided as is phase-appropriate, and is expected to increase as product development advances. Although the most advanced vaccines of this type are COVID-19 vaccines and are used as examples in the text, the document should not be taken as providing guidance specific only to COVID-19 vaccines. However, in light of the current COVID-19 pandemic and corresponding speed of mRNA vaccine development, the document is intended to provide special considerations for this type of preventive mRNA vaccine as rapidly as possible. It should nevertheless be noted that there remain gaps in the scientific understanding of the types and amount of immunogenicity that any given mRNA vaccine might need to achieve for it to be successful, broadly relevant and durably efficacious against the disease it is intended to prevent. Each vaccine will therefore need to be evaluated in terms of its own benefits and risks.

Because mRNA vaccines are novel and differ from other types of vaccines (even other nucleic acid vaccines such as plasmid DNA vaccines), a short introduction to mRNA-vaccine-specific topics is provided where deemed
useful. Due to the novelty of mRNA vaccines and their manufacturing process, a comprehensive approach has been taken to ensure that all relevant aspects can be considered by manufacturers when developing this type of product, and by regulators when evaluating such products.

The scope of the current document is limited to mRNA and self-amplifying mRNA (sa-mRNA) packaged in LNPs for in vivo delivery of the coding sequences of a target antigen relevant to active immunization for the prevention of an infectious disease. It is acknowledged that mRNA and sa-mRNA products in formulations other than LNPs are also in development, and parts of this document may be applicable to those products as well.

Replicating agents, viral vectors and RNA replicons packaged in viral proteins or encoded by plasmid DNA are outside the scope of this document. In addition, mRNA and sa-mRNA products intended for therapeutic purposes (that is, products for the treatment, mitigation or cure of diseases, including infectious diseases, as opposed to active immunization for their prevention) are also outside the scope of this document. In addition, mRNA products expressing monoclonal antibodies (whether serving as passive immunization for disease prevention or therapy) are also outside the scope of this document. It may be the case that some aspects discussed in section 6 and its subsections do apply to mRNA-based therapeutic products (including those expressing monoclonal antibodies), as the manufacturing steps of such products may be similar to those described for vaccines. However, because the nonclinical and clinical evaluations of such therapeutic products would need to be based on their therapeutic indication, it is not feasible to include regulatory considerations for them within this document.

As there may be a need to develop multivalent mRNA vaccines or to change the existing vaccine strain for some pathogens (for example, influenza viruses or SARS-CoV-2), specific considerations are provided in this document where appropriate. In addition, any general WHO guidance of relevance should also be consulted; a number of WHO documents providing such guidance are listed below in section 4.

Because regulatory pathways for emergency use authorization vary and not all NRAs have such pathways, approval for emergency use is also outside of the scope of the document. However, suggestions are provided, where possible, for rapid vaccine development in the case of priority pathogens during public health emergencies (see sections 7.3 and 8.3 below).

This document has been developed in light of the available knowledge to date. Given that this is a dynamic field, both in terms of vaccine manufacturing technologies and clinical-trial design, this document should be read in conjunction with other relevant recent guidance, including WHO disease-specific guidelines and recommendations, if available.
3. Terminology

The definitions given below apply to the terms as used in this document. These terms may have different meaning in other contexts.

**Adjuvant:** a substance intended to enhance the relevant immune response and subsequent clinical efficacy of a vaccine.

**Biological (or biological product):** a medicine produced by a biological system, as opposed to strictly chemical reactions. These include traditional biologicals (such as live vaccines) and biotechnologically produced medicines (such as monoclonal antibodies or subunit vaccines such as human papillomavirus vaccines). In other documents, these may be referred to as biologics or biological medicines.

**Candidate vaccine:** an investigational vaccine that is in the research and clinical development stages and has not been granted marketing authorization or licensure by a regulatory agency in the country in which such authorization or licensure will be sought.

**Design of experiments:** a structured, organized method for determining the relationship between factors affecting a process and the output of that process.

**Drug product:** see final vaccine.

**Drug substance:** the purified mRNA before final formulation. It is prepared as a single homogeneous production batch, kept in one or more containers designated as such and used in the preparation of the final dosage form (final vaccine or drug product).

**Double-stranded RNA (dsRNA):** some viruses have genomes comprising fully double-stranded RNA along their entire length rather than in distinct segments (such as the secondary structure of mRNA). If present, such dsRNA is sensed by intracellular receptors and can activate innate immune responses. Depending on the manufacturing method, dsRNA can be generated as a by-product during the in vitro transcription (IVT) manufacturing process for some mRNA vaccines, though some segments may be single stranded. This type of dsRNA is an impurity that should be removed from the mRNA during the manufacturing process, or its amount in the product at least determined and controlled. If the manufacturing method does not produce dsRNA, then the control of this as an impurity is unnecessary.

**Engineering run:** a manufacturing campaign conducted to engineer manufacturing methods in order to improve or confirm those methods for use in good manufacturing practice (GMP)-compliant production. The materials made in such a campaign are not intended for use in humans.

**Excipient:** a constituent of a medicine other than the active drug substance, added in the formulation for a specific purpose. While most excipients are considered inactive, some can have a known action or effect in certain
circumstances. The excipients must be declared in the labelling and package leaflet of the medicine to ensure its safe use. In the context of the current document, the lipids that form the LNPs are excipients but the LNPs if formed separately from the mRNA are defined as intermediates in the production of the drug product.

**Final formulated bulk:** an intermediate in the manufacturing process of the final vaccine, consisting of a homogeneous preparation of the final formulation of drug substance(s) and excipients at the concentration to be filled into final containers. Alternatively, the final formulated bulk may be stored at a higher concentration and diluted immediately prior to filling. In the context of this document, the term refers to mRNA formulated with LNPs and other excipients as needed. Note that if more than one drug substance is to be combined (as in a multivalent or combination vaccine), their mixing would occur as part of the preparation of this final formulated bulk.

**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 final formulated bulk in one continuous working session. A final formulated bulk might be filled into more than one final lot.

**Final vaccine (or drug product):** a final dosage form (for example, a vialled frozen or liquid suspension or lyophilized cake) that contains one or more drug substances (active ingredient) typically formulated with excipients and packaged for use. In the context of this document, the term refers to a preparation of mRNA formulated with LNPs and other excipients that is filled into final containers. If filled in concentrated form or lyophilized, a diluent is needed. Otherwise, the final containers should be filled at the concentration for the clinical dose (though each container might contain multiple doses). Also referred to as “finished product” in other documents.

**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.

**Immunogenicity:** the capacity of a vaccine to elicit a measurable adaptive immune response against a target antigen(s).

**In vitro transcribed mRNA:** the product of a manufacturing process whereby mRNA is generated in vitro from a linear DNA template using a DNA-dependent RNA polymerase enzyme (for example, a T7, T3 or Sp6 phage RNA polymerase) and nucleoside triphosphates or modified nucleoside triphosphates.

**Lipid nanoparticle (LNP):** a delivery formulation consisting of various lipid components to ensure that the mRNA is stabilized and encapsulated, for example, to avoid extracellular degradation and to facilitate its uptake into cells and release into the cytosol. The lipid components may include, but are not limited to, an ionizable/cationic lipid, a helper lipid (for example, phospholipids
and/or cholesterol) and a lipid(s) modified for example by polyethylene-glycol-ylation (PEGylation). The LNPs and/or lipid components may also have adjuvant activity.

**Marketing authorization or approval:** a formal authorization for a medicine (including vaccines) to be marketed. Once an NRA approves a marketing authorization application for a new medicine (different NRAs may use different terms for such applications), the medicine may be marketed and may be available for physicians to prescribe and/or for public health use (also referred to as product (drug or biological) licensing, product authorization or product registration). Once authorized or approved, the new medicine must be manufactured, controlled and labelled as described in the authorization or approval file.

**Messenger RNA (mRNA):** a single-stranded RNA molecule that is translated in the cytoplasm of a cell into the protein that it encodes. It contains one or more open reading frames (ORFs) that encode the protein (in the case of vaccines, the target antigen), flanking untranslated regions (UTRs), a 5′ cap (or alternative) and a 3′ sequence such as a poly(A) tail.

**Mode-of-action and mechanism-of-action:** the manner in which the adaptive immune response elicited by the vaccine protects against the pathogen at the cellular (mode) or molecular (mechanism) level – for example, neutralization by neutralizing antibodies, opsonization by opsonizing antibodies or cytotoxicity by T cells.

**Modified nucleosides:** naturally occurring modified nucleosides (such as pseudouridine) that can be substituted for the usual nucleoside (in this case, uridine) when making mRNA vaccines, with a resultant potential decrease in inflammatory activity and/or increased in vivo stability. Another type of modification is methylation. Nucleosides used to manufacture mRNA vaccines might also contain unnatural modifications.

**mRNA integrity:** the proportion of the mRNA that is the correct size and contains the 5′ cap and poly(A) tail. In addition, the correct sequence of the mRNA should be confirmed.

**Novel excipient:** an excipient (for example, a lipid) not used before in any medicine approved or licensed for human use or, if previously used in an approved or licensed medicine for human use, then not using the same route of administration (and/or present at a higher concentration) as that approved or licensed. The word “novel” is used in this same way to describe other terms used in this document.

**Platform technology:** a group of technologies used as a base upon which other applications, processes or technologies are developed. In the context of mRNA vaccines, a given manufacturer might have one or more platforms on which they will develop vaccines (or therapeutics) against various diseases
(separate individual vaccines or a combination vaccine) or pathogen strains against the same disease (separate monovalent or mixed multivalent vaccines). The term could also be applied to a particular drug-delivery system (such as LNPs containing the mRNA) where identical lipids, concentrations, methods of preparation and purification and so on are used. Use of the term “platform technology” would be considered appropriate when: (a) the manufacturing methods are essentially unchanged (but may be optimized for each specific candidate vaccine); (b) the test methods (except for identity, potency and stability) and acceptance criteria are unchanged; (c) the immunomodulatory compounds or elements are unchanged; and (d) compliance with GMP is unchanged. One implication of the use of platform technology to develop new candidate vaccines is that the experience and knowledge gained, data generated (manufacturing, control, stability and nonclinical) and validation of unchanged methods can all be used as supportive data for the more rapid assessment and development of a new candidate vaccine. Clinical and nonclinical data from the platform in terms of safe starting doses or tolerable doses might also be supportive of initiating clinical trials of the new candidate vaccine at doses already known to be tolerable with the platform. If aspects of the platform technology have been changed, along with the mRNA sequence, then justification should be provided as to why data generated with the original platform should be considered supportive of the new candidate vaccine. Because the production and control methods used for mRNA vaccines are not yet standardized between manufacturers, information from other manufacturers would not be supportive of a platform technology. Such information may be considered to be similar to that for a product class and evaluated as being supportive if justification is provided and compelling. Furthermore, flexibility in the scientific approach to regulating mRNA vaccines is justified because of the current lack of standardization even in the face of platform technology use. As always, an individual case-by-case approach is justified and should be discussed and agreed with the relevant NRA(s).

**Self-amplifying mRNA (sa-mRNA):** an mRNA vaccine that in addition to encoding the desired antigen(s) also encodes nonstructural proteins of certain viruses (such as alphaviruses), either on the same molecule as the antigen or on a separate molecule. When expressed intracellularly, these ORFs produce the proteins of the viral replication machinery thus enabling the cell to produce multiple copies of the mRNA encoding the antigen protein. The goal of sa-mRNA is to increase the in vivo potency of the mRNA vaccine by increasing the amount of protein antigen made. Other designations have been given to this form of mRNA vaccine but in this document the term sa-mRNA will be used.

**Target antigen(s):** the protein(s) or portion thereof, encoded within the mRNA of an mRNA vaccine, for which an immune response to vaccination is expected to result in protection against one or more pathogens or strains.
That is, protection against disease (or infection) caused by a pathogen(s) may be conferred by the resulting immune response against the target antigen(s) following vaccination.

**Therapeutic**: a treatment given after a disease or condition (or signs or symptoms thereof) is evidenced, in contrast to the prevention of disease before exposure (or in rare cases following exposure but before the onset of signs or symptoms) to the infectious pathogenic organism has occurred. Although preventive vaccines are not considered to be therapeutic in this document, it is acknowledged that the definition of therapeutic in some regulatory jurisdictions may differ. Therapeutics as defined here are outside the scope of the current document.

**Transfer RNA (tRNA)**: an RNA molecule used by ribosomes and that acts as an adaptor involved in translating the codons of the mRNA into a protein.

### 4. General considerations

As with all vaccines, the intended clinical use of the mRNA vaccine should be described, including the pathogen targeted, the target antigen(s) chosen, disease to be prevented and the target population(s). Given the novel structure and manufacturing of mRNA candidate vaccines (in contrast to other already licensed vaccine types with which regulators are familiar), consideration should be given to the following when evaluating mRNA vaccines for their quality, safety and efficacy:

- In particular, the relevant biological characteristics of the specific mRNA technology used should be described – including for example the capability of the given mRNA to trigger innate immune responses as well as target-antigen-specific responses; the quality, quantity and bias of the immune responses (for example, type 1 T-helper (Th1) or Th2 cell phenotype); and in vivo stability. To justify the vaccine design, all available information on the type of immunity (protective and immunopathogenic) considered relevant to the specific pathogen and disease should also be described.

- The rationale for the selection of the target antigen(s) or parts thereof and of any proteins (for example, cytokines) that are encoded, as well as their contribution to the proposed mode- or mechanism-of-action (proposed protective process) of the vaccine, should be clearly described. Likewise, the rationale for the selection of any coding sequences added to or any modification of the target antigen, such as those to ensure the folding of the target antigen into a particular conformation, should be provided. The complete
annotated sequence identifying all ORFs (including any unexpected ORFs) and all other sequence elements (including their justification for use) should be provided. Justifications for the use of any specific or specially designed noncoding sequence (including poly(A) tail) and of structural elements (such as the chosen 5' cap structure or alternative) should be provided. With regard to sa-mRNA, any viral replicon genes encoded in the vaccine construct to allow amplification of the mRNA in human cells after delivery should be described in detail. The anticipated function and purpose of each gene sequence encoded in the mRNA should be indicated, as well as those of specific noncoding and structural elements, explaining their contribution to the overall mode- or mechanism-of-action.

- The formulation of the final vaccine product and all excipients (including all components used for the generation of LNPs) should be described. An appropriate rationale for the proposed composition of the final vaccine and inclusion of excipients should be provided. Information on the method of production of the LNPs and the final vaccine (drug product) including information on the critical quality attributes of the intermediates and final product, their in-process controls and any sterilization procedure should also be provided. Toxicological and immunogenicity data on the LNP should also be provided.

- For each novel excipient (see **Terminology** for definition) detailed information on the rationale for its inclusion, the method of production (including details and controls on the starting materials, intermediates and raw materials) and data from nonclinical and/or human clinical studies on its safety and, if required by a given NRA, on its safety pharmacology (see section 7.2.d below) should be provided.

- The intended dosing, the route of administration, and a description and justification of any novel administration device as well as any required diluent should be provided. Relevant compatibility studies should be performed where necessary.

- Although any given manufacturer's mRNA vaccine product may be considered to be produced by a platform technology if only the target antigen sequence is changed, the control, nonclinical testing and clinical development of each vaccine should be considered individually, and any special features of that candidate vaccine taken into account. Early consultation with the NRA(s) will be key to ensuring the efficient development of any given candidate vaccine.
With regard to the development of combination or multivalent candidate vaccines, noting the development of precedents might be helpful. Relevant examples might include: (a) seasonal influenza virus vaccines, which are both multivalent and undergo annual strain changes; (b) human papillomavirus vaccines such as the quadrivalent vaccine that was changed after initial approval to a nonavalent (that is, nine-valent) vaccine, trivalent poliomyelitis vaccines, multivalent rotavirus vaccines and multivalent pneumococcal vaccines, which are used against different strains that cause the same (or related) disease(s); or (c) diphtheria and tetanus-toxoid-containing vaccines or measles, mumps and rubella vaccines, which are combination vaccines used against different disease targets. Available guidance on the development of combination vaccines against multiple diseases may also be considered.

The current document should be read in conjunction with other relevant WHO guidelines such as:

- WHO guidelines on nonclinical evaluation of vaccines (18);
- Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (19);
- Guidelines on clinical evaluation of vaccines: regulatory expectations (20);
- WHO good manufacturing practices for pharmaceutical products: main principles (21);
- Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (22);
- WHO good manufacturing practices for biological products (23);
- WHO good manufacturing practices for sterile pharmaceutical products (24);
- Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (25);
- **WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products** (26);
- Guidelines on stability evaluation of vaccines (27);
- Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (28);
- Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (29);
Guidelines for independent lot release of vaccines by regulatory authorities (30);

Guidelines on procedures and data requirements for changes to approved vaccines (31); and


5. Special considerations

The mRNA of vaccines that are currently the most advanced in terms of clinical development or that are currently in widespread use against COVID-19 is produced enzymatically rather than biologically within a cell. This approach thus differs from the production of most other biologicals with which manufacturers and regulators are familiar (1, 33). Manufacturing either starts with linearized DNA plasmids that have been produced in bacteria (similar to the way in which biologicals such as plasmid DNA vaccines are produced) or with a linear DNA molecule produced enzymatically using the polymerase chain reaction (PCR) or other synthetic methods. Regardless of whether the manufacture of the RNA starts with a linearized molecule generated from a plasmid DNA or from an already linear DNA sequence, mRNA production occurs enzymatically in vitro by means of a DNA-dependent RNA polymerase that transcribes the linear DNA template into an mRNA molecule. The mRNA sequence generally consists of the usual elements of cellular mRNA, such as the coding region, 5′ and 3′ untranslated regions (UTRs) that regulate mRNA translation, a 5′ cap and a 3′ poly(A) tail.

The nucleotides used in manufacture may contain naturally occurring nucleosides or modified or synthetic nucleosides (3, 8). Examples of alterations that might be made to the naturally occurring nucleoside include the use of pseudouridine or N1-methylpseudouridine in place of uridine (3, 4, 34). In addition, altering or optimizing codon use (without changing the encoded amino acids) may impact in vivo stability and enhance in vivo translation of the mRNA in humans (for example, for translation by transfer RNAs (tRNAs) more frequently found in human cells). Alternatively, codons may be selected for more infrequent tRNAs in order to slow translation of the protein, thus permitting desired protein folding. Some changes to the mRNA are designed both to increase its in vivo stability and to moderate activation of the innate immune system (4). Depending on the clinical indication, it may be desirable to decrease innate immune responses that might lead to inflammatory reactogenicity in vivo (3, 4, 34). For some preventive vaccines, some of the innate immune response may be considered useful for augmenting the desired adaptive immune response, and the mRNA may be designed accordingly. The gene sequence encoding the target antigen should contain start and stop codons and be flanked by 5′ and 3′ UTRs.
and generally have a 5’ cap and a 3’ poly(A) tail. The cap can be added to the mRNA enzymatically or during in vitro transcription (IVT) using appropriate cap analogues. Likewise, the 3’ poly(A) tail can be encoded in the DNA template or added enzymatically after IVT. These design features can impact the critical quality attributes, the manufacturing methods and the control testing of the mRNA drug substance(s) and/or the final vaccine drug product.

Of relevance to considerations of the safety and efficacy of mRNA vaccines are the structures adopted by the RNA in the vaccine product. Unlike DNA, which is normally double stranded, RNA is often represented diagrammatically as being single stranded. However, depending on its sequence, RNA can form a complex structure consisting of short double-stranded segments with various single-stranded loops in between. The reason this is relevant is that double-stranded RNA (dsRNA) is a form taken by the genome of some RNA viruses and can induce cells to trigger immune reactivity as an innate response to viral infection. However, endogenous cellular mRNA does not induce such an effect despite containing partial double-stranded segments. The in vivo effects, including potential triggering of innate immunity, of an mRNA candidate vaccine should therefore be characterized and addressed in the vaccine design, nonclinical studies and clinical trials.

RNA-based products can take different forms. The most advanced candidate vaccines and the widely used COVID-19 vaccines take the form of mRNA encoding the target antigen (35, 36). Because mRNA (and RNA in general) is subject to degradation by nucleases, the most advanced mRNA candidate vaccines and widely used COVID-19 vaccines at the time of writing are formulated in LNPs, which aids in vivo stability and delivery (33, 37–43). There are different types of LNPs depending on their composition, the types of lipids employed and the manufacturing process used (44). Some may not yet have been employed for the delivery of mRNA (45–48). Other stabilizing and delivery systems using polymer and polypeptide, as well as other lipid-based systems or combination of polymer and lipid-based systems, may be developed for mRNA delivery in the future. These drug delivery systems could also be surface modified for tailored cellular interactions, where necessary.

It is important to note that the drug substance is the mRNA(s). The lipids which form the LNPs are excipients of the final vaccine or drug product. The manufacture of LNPs from the different lipids is part of the drug product manufacturing process. It is acknowledged that some LNPs, depending on their composition, may also have immunomodulatory effects (47–50) and some lipids may act as adjuvants without being formulated as LNPs. Nonetheless, vaccine adjuvants, which are immunomodulating to the vaccine, are also considered to be excipients. Similarly, as discussed above, RNA itself can be immunomodulating. Consequently, both components (the mRNA and the lipids in the LNPs) may
contribute to the mode-of-action of the vaccine and the implications of this need to be considered in the critical quality attributes and in the nonclinical and clinical evaluations.

Some candidate vaccines may contain various mRNAs encoding different antigens. Examples include multiple antigens from the same pathogen, the same antigen representing different strains or serotypes of the same pathogen, or multiple antigens from different pathogens. Such vaccine development is not without precedent – as discussed above in section 4. As with other combination or multivalent vaccines, each mRNA would be considered to be a separate drug substance that will be combined into a final drug product. Also, as with other combination or multivalent vaccines, the amount of mRNA for each target antigen, the expression efficiency of each and the resulting immune responses, must be balanced against the other(s) in order to avoid interference with the expression of (and thus immune responses to) all the target antigens, and to ensure the necessary strain- or antigen-specific immune responses to the total vaccine. Furthermore, consideration should be given to ensuring an adequate dose of mRNA for each target antigen using appropriate nonclinical toxicity studies to evaluate the highest tolerable total mRNA and LNP doses, and if applicable, justified by previous clinical experience. See sections 7.3 and 8.1 below for further discussion of this point.

Additional consideration should also be given to the manufacture, control and stability of combination or multivalent vaccines to ensure appropriate quality control of each drug substance and the drug product and to ensure the suitability of the analytical procedures used to control each mRNA component (that is, each drug substance) in the final vaccine.

Interactions between the mRNA and the LNPs may vary depending on the length and secondary structure of the mRNA, as well as the lipid composition of the LNPs. Therefore, the particle size, morphology, surface properties (for example, charge) and encapsulation efficiency of the resultant LNPs containing the mRNA could vary when a different mRNA is used. Consideration of the critical quality attributes and physicochemical properties of both the mRNA and the LNPs is therefore necessary to provide an understanding of the desirable properties of the final vaccine.

Some candidate products contain the components needed to permit the mRNA vaccine to be self-amplifying (so-called self-amplifying mRNA or sa-mRNA) (8, 38, 51). These products include the gene sequences for replicon proteins (to date, from alphaviruses) in addition to the target antigen, either on the same or a different mRNA molecule. As a result, the mRNA coding for the antigen can be replicated in vivo, leading to increased expression of the target antigen. Current sa-mRNAs are also formulated in LNPs (38). There may be implications for vaccine safety and efficacy due to the design of the sa-mRNA
if the target antigen is encoded either on a separate mRNA molecule or on the same molecule as the replicon gene sequences. For example, the particle size and morphological characteristics of the LNPs may vary depending on the size of the mRNA encapsulated. In addition, the total amount of mRNA needed to achieve the same level of efficacy may vary between the two designs due to differences in the degree of expression efficiency, as well as in the amount of dsRNA, the innate immune response, the half-life of the mRNA and sa-MRNA, and so on – all of which could result in a different safety profile, and hence all of which needs to be considered in the vaccine design and evaluation.

In contrast to viral replicons (which are packaged in viral structural proteins) sa-mRNAs are delivered in LNPs or other delivery systems. This means that the cells that take up sa-mRNAs and those that take up viral replicons are likely to differ as viral replicons enter their host cells via the viral receptor, while sa-mRNAs depend on a formulation for intracellular delivery (38). RNA-based products can also be contrasted with both viral-vectored vaccines and with live viral vaccines (for RNA viruses) by their lack of genes encoding the structural proteins of the virus being used as the vector or live vaccine. Thus, there are various similar products in development, the differences between which are largely dictated by biology or design. Other similar technologies include circular RNA products that are in development, mRNAs that use an internal ribosome entry site (IRES) instead of a cap and RNA encapsulated in other drug-delivery systems using polymer and polypeptide systems (or a combination of polymer and lipid-based systems).

As described above in sections 1 and 2, and in order to develop WHO guidance as rapidly as possible, the scope of the current document is limited to mRNA or sa-mRNA encapsulated in LNPs.

It should also be noted that in the case of current mRNA vaccines, various cell types at the site of injection take up the mRNA. However, future delivery systems may be designed that selectively target the mRNA to specific cell types or tissues – for example, through the use of surface-modified LNPs in which a targeting ligand/motif could be attached to the LNP surface. Any changes to the physicochemical properties that result in different innate immunostimulatory effects may have further implications for the safety or efficacy of the mRNA or sa-mRNA vaccine but these are beyond the scope of the current document.

6. Manufacture and control of mRNA vaccines

All mRNA vaccines are regulated as biologicals and, as with other biologicals, adequate control of the starting and raw materials and excipients and of the manufacturing processes is as important as that of the final product. Regulatory considerations therefore place considerable emphasis on the control strategy
for the vaccine manufacturing process as well as on the comprehensive characterization and release testing of the drug substance and the final vaccine itself.

The general guidance contained in WHO good manufacturing practices for pharmaceutical products: main principles (21), Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (22), WHO good manufacturing practices for biological products (23) and WHO good manufacturing practices for sterile pharmaceutical products (24) should be applied to the design, establishment, operation, control and maintenance of manufacturing facilities for mRNA vaccines. The primary guidance on GMP requirements for mRNA vaccines used to prevent infectious diseases is provided in WHO good manufacturing practices for biological products (23). This document advises that for recombinant or biotechnology products, GMP compliance is expected from the starting materials through to the filled, finished product. WHO guidance should also be applied to the control of mRNA vaccine filled in the final form, the keeping of records and retained samples (for future studies and needs), labelling, distribution and transport, as well as storage and expiry dating for mRNA vaccines (27–29). Quality control during the manufacturing process relies on the implementation of quality systems, such as GMP to ensure the production of consistent commercial vaccine lots with product characteristics similar to those of lots shown to be safe and efficacious in clinical trials.

Throughout the process, a number of in-process monitoring and/or control tests (with acceptable limits) should be established through a risk-based approach (including tests to measure critical and non-critical quality attributes, as applicable) in order to allow quality to be monitored for each batch or lot from the beginning to the end of production. Release specifications and characterization methods should be agreed with the NRA(s) as part of the clinical trial authorization/approval or marketing authorization/approval. The drug substance and drug product release specifications for marketing authorization/approval should be set based on the testing of product resulting from the commercial manufacturing process as well as the results obtained for the lots used in clinical trials. Such release specifications and characterization methods should cover critical attributes that can provide reassurance on the consistent quality required to provide a safe and effective vaccine. This will include methods to assess content, identity, purity, potency, quality and safety attributes, and stability.

mRNA vaccines for use in clinical trials should also be prepared under GMP conditions appropriate 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; under emergency conditions, and based on benefit–risk assessment, it may be acceptable to consider using starting materials that were not prepared in complete compliance...
with GMP. Appropriate attention needs to be given to ensuring the quality and correct identity of all materials used in production and control. Particular attention should be given to the sourcing of components of animal (including human) derivation. Attention should also be given to ensuring freedom from, or control of, potential adventitious agents supported by relevant evidence and risk assessment. Many of the general requirements for the quality control of biological products, such as tests for endotoxin, stability and sterility, should also be applied to mRNA vaccines. The commercial specifications should be defined on the basis of the results of tests on lots that have been shown to have acceptable performance in clinical studies. Additional controls specific to mRNA or sa-mRNA vaccines formulated in LNPs should be described, including controls for raw materials and excipients and in-process controls for manufacturing intermediates.

It should be 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 an assessment of the risks derived from the drug product and the manufacturing process. This would include, for example, identification of 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 processes and tests, results of testing of vaccine lot(s) (and if applicable, for a clinical trial application, placebo or other comparator) to be used in the proposed clinical trial and results of preliminary stability testing. As with all vaccines, for pivotal clinical trials the level of detail provided on the quality (manufacturing and controls) of an mRNA vaccine would be expected to increase substantially.

While not every mRNA vaccine can be viewed as being made based on a platform technology, a given manufacturer’s technology might to some extent be viewed this way. In other words, if essentially no changes are made to the manufacturing processes (other than process optimization for each candidate vaccine), tests (except for identity or potency) or specifications, then a new candidate mRNA vaccine might be supported by data from an earlier candidate mRNA vaccine or licensed product. For example, this could be the case when the only changes made are to the sequence and these changes do not change the size or secondary structure of the resultant new mRNA or its interaction with the LNP. Supportive data might include data gained on the manufacturing processes, tests, specifications, stability and nonclinical and clinical safety.

Details of any changes made to product composition (for example, change in the mRNA sequence, enhanced valency, change in excipients or addition of preservatives) or manufacture (for example, change in process, site or scale) during the development of clinical lots should be provided. Depending on the way in which the final product composition was changed (for example, addition of novel
excipients) new nonclinical studies might be warranted (see section 7 below). For changes to the manufacturing process (such as scale-up or change to the purification process) the comparability of the resulting drug substance and drug product with those produced using the previous process should be evaluated. Such comparability studies might be based on immunogenicity data from animal models, results from physicochemical analyses, studies of process and product-related impurities, and/or stability data. The WHO Guidelines on procedures and data requirements for changes to approved vaccines (31) should be consulted in this regard. All changes made to the product post-approval should follow the requirements listed in these same Guidelines (31); other relevant guidance may also be considered such as the ICH Harmonised Guideline on pharmaceutical product lifecycle management (52).

Defined recombinant nucleic acids used as active drug substances in vaccines, whether of biological or synthetic origin, could be assigned an international nonproprietary name (INN) upon request (53, 54).

6.1 General manufacturing overview

Vaccines based on mRNA represent a new type of biological product and are manufactured differently from traditional biologicals. Most such biologicals are propagated or produced in a cell-based (or organism-based) system whereas the mRNA component is manufactured enzymatically via IVT. The production process normally involves the use of a linear DNA template to direct DNA-dependent RNA transcription with recombinant enzymes and nucleoside triphosphates. The choice of sequence or structure not only of the ORF but also of the UTRs, the cap and the poly(A) tail length should be justified.

Generally, once the mRNA has been transcribed the template DNA is digested using deoxyribonuclease (DNase) prior to purification of the mRNA. If the cap and poly(A) elements are not added during the IVT process, or if a longer poly(A) tail is required, these can be added enzymatically following synthesis but prior to purification (8, 34, 55, 56). In addition to removing the DNA template, the unattached caps, unincorporated nucleotides and the enzymes (such as RNA polymerase) used in production, all process-related and product-related impurities (for example, dsRNA and incorrectly sized mRNA molecules) should also be removed to the extent feasible. Attention should also be paid to the removal of enzymes possibly involved in DNA template generation, such as DNA polymerase and restriction enzymes (if not controlled at the level of the DNA template). The methods of purification and their purposes should be described and justified. Any purification processes – such as protein digestion with proteinase(s) as an impurity-reduction step – should be validated at the appropriate phase of development (see section 6.4 below).

In most cases, the purified mRNA would be considered to correspond to what is termed for other vaccines “the purified bulk antigen” – even though
the mRNA is not the actual antigen but instead mimics the transcript encoding the antigen. This could also be thought of as the bulk biological substance or bulk active substance and is referred to in this document as the drug substance in order to use terminology familiar to most manufacturers and regulators to describe the active biological element of the vaccine.

As would be expected for any vaccine, a flowchart of production should be provided that indicates each process step, the samples taken at that process step and the in-process control tests for which the samples are taken. The process flowchart should also clarify the steps in the process at which manufacturing reaches the stages of drug substance, final formulated bulk and final filled vaccine (drug product), and at which steps in the flowchart samples are being taken for in-process control and release testing. The tests carried out at each of these steps should also be indicated. The duration of storage of the concentrated purified mRNA (drug substance) or any intermediates (such as the final formulated bulk) that are held or stored should be supported by hold-time/stability studies. As with any vaccine, an agreed-upon number of lots of the drug product should be placed on a stability programme.

The mRNA (drug substance) is not suitable for clinical use unless it is protected and delivered by a given formulation (the preparation of which is part of drug product manufacture). The formulations chosen for the most advanced mRNA vaccines so far are based on LNPs. Although there are other approaches to encapsulating mRNA-based products, the current document only covers systems that use LNPs. The formulation both stabilizes the mRNA and facilitates its entry into cells and release into the cytosol, which could be achieved by either active or passive uptake. The LNPs may also provide an adjuvant activity (47, 49, 50). In order to protect the mRNA from degradation by nucleases, it is incorporated into the LNPs to make it inaccessible to such nucleases – however, the LNPs must also release the mRNA once inside the target cell. The LNPs must also be of a suitable size range with desirable surface properties for optimal uptake by target cells. Hence, product development data concerning the optimization of both the formulation and the manufacturing process should be provided. For example, consideration should be given to the concentrations of the different lipids, the mRNA–lipid ratio, pH of buffers/solvents, mRNA encapsulation efficiency, and the flow rate and mixing rate of the lipids and mRNA, as well as the thawing temperature of the different components, as these will all have an impact on the quality of the final vaccine (drug product). In this way, the process of encapsulation into the LNPs can be carefully controlled and the production methods and control measures adequately described and suitably validated.

Although sa-mRNA contains the coding sequences (viral nonstructural genes) for additional proteins that permit its in vivo amplification (but not its packaging, which requires viral structural genes), the method of manufacture in
which IVT is followed by purification and formulation in LNPs is essentially the same as that described above. For sa-mRNA with the additional coding sequence on the same molecule as the target antigen coding sequence the control measures required for the manufacturing processes might also be similar or the same as those for an mRNA vaccine. However, if the replicon genes are encoded on a separate mRNA molecule, then additional manufacturing processes and quality controls may be needed to ensure that the required mRNAs are adequately encapsulated in the same LNPs, and these additional processes should be described. The molar ratio of the two encapsulated mRNAs should be provided and justified, and the method of validation described. The degree of expression efficiency might also vary between the two approaches (for example, using two mRNAs as opposed to a single one) and this might have implications for the expected safety and efficacy of the vaccine design due to differences in the amount of dsRNA, the innate immune responses elicited, the half-life of the mRNA and so on. If the separate mRNA molecules are encapsulated separately and not mixed prior to encapsulation, this would also need to be described and may involve additional manufacturing processes and quality controls to ensure adequate final mixing and an appropriate ratio of the two (or more) mRNAs. Likewise, for multivalent mRNAs the mixing step(s) either before or after encapsulation need to be described and controlled appropriately. For sa-mRNA in which the mRNA encoding the replicon and the mRNA expressing the target antigen are encoded on different molecules, it will be important that these two RNAs are co-encapsulated in order for them to be taken up by the same cell in vivo. Therefore, if the two RNAs are encapsulated separately and then mixed, a justification for this approach will be required.

The key quality control points should include:

a. Starting and raw materials and excipients – including, but not limited to: (a) a linear DNA template, which could be enzymatically or synthetically generated (for example, by PCR) or a plasmid DNA that has been linearized (generally by restriction endonucleases); (b) nucleotides; (c) enzymes (for example, DNA-dependent RNA polymerase (which is usually the T7 RNA polymerase), capping enzyme, 2’O-methyltransferase, poly(A) polymerase, DNase and proteinase K); (d) buffers; (e) solvents; (f) column resins (if column chromatography is used in purification); and/or (g) lipids. The linear DNA template is considered to be the starting material for the manufacture of the drug substance. The other listed items (along with any not listed but also used in manufacture) would be considered to be raw materials. Excipients are those raw materials that are present as inactive ingredients in the final drug product/vaccine. For the manufacturing of excipients,
compliance is expected with WHO Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (22).

- In particular, any animal-derived (including human-derived) starting or raw materials or excipients, or any starting or raw materials or excipients that were themselves produced using animal-derived (including human-derived) raw materials should be subject to control by appropriate sourcing, by control testing and by risk assessment. Materials of animal origin (including human origin) should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (26).

- Attention should be given to ensuring freedom from or control of potential adventitious agents supported by relevant evidence and risk assessment.

b. In-process controls of the manufacturing processes and intermediates – including the processes used to manufacture the bulk mRNA substance (drug substance), as well as the formulation (the LNP manufacture and encapsulation steps), final formulated bulk and filling of the final formulated bulk (drug product); also including either controls for or validation of the consistency of LNP formulation (regarding, for example, their size and polydispersity), consistency of mRNA encapsulation, and removal of partial mRNAs and dsRNA impurities.

c. Release of the mRNA vaccine drug substance and final filled vaccine (drug product) following manufacture.

d. Process validation – processes should be validated to demonstrate the consistent manufacturing of the commercial final drug product with the desired quality profile (see section 6.4 below).

Analytical methods that might be considered for assessing some of these key quality control points are discussed in the literature – though detailed analytical procedures and acceptance criteria for tested attributes are not yet standardized or in the public domain. As of the time of publication of the current document, these are considered by manufacturers to be proprietary and confidential. The methods shown in Table 1 may be considered as examples of possible means for characterization or control at various key quality control points (57–59).

For clinical trial use, mRNA candidate vaccines should be manufactured under GMP conditions appropriate for the stage of clinical development. It is expected that clinical trial material should be released on the basis of meeting appropriate quality control standards. Full compliance with GMP would be
expected for clinical trial material used in pivotal trials and for commercial manufacture (18, 19).

Any manufacturing changes made during clinical development, particularly if made following completion of pivotal safety and efficacy trials but prior to seeking licensure, need to be described and justified. A comparative analysis with the clinical efficacy lots should be made. For post-approval changes, compliance with the WHO Guidelines on procedures and data requirements for changes to approved vaccines (31) would be expected, though other relevant guidance might also be considered such as the ICH Harmonised Guideline on pharmaceutical product lifecycle management (52).

Table 1

**Examples of possible methods for characterization or control at various key quality control points, by potential use(s)**

<table>
<thead>
<tr>
<th>Examples of possible method</th>
<th>Potential use(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA template sequencing; mRNA sequencing</td>
<td>Identity</td>
</tr>
<tr>
<td>Quantitative reverse transcription PCR (qRT-PCR)</td>
<td>Identity and quantification</td>
</tr>
<tr>
<td>Ultraviolet spectroscopy; fluorescein-based assays</td>
<td>Quantification and purity</td>
</tr>
<tr>
<td>Agarose or acrylamide gel electrophoresis, including capillary electrophoresis</td>
<td>RNA quantification, RNA size, RNA integrity, LNP surface charge and percentage encapsulation</td>
</tr>
<tr>
<td>Chromatographic assays such as size-exclusion, anion-exchange, affinity or reverse-phase</td>
<td>Quantity of mRNA, quantity of lipids, quality of mRNA and nanoparticle integrity</td>
</tr>
<tr>
<td>Mass spectrometry</td>
<td>Quantity and nanoparticle integrity</td>
</tr>
<tr>
<td>dsRNA blot; tests for percentage capping; percentage transcripts with (and size of) poly(A) tail</td>
<td>Purity and other quality attributes</td>
</tr>
<tr>
<td>Cell-free translation or cell-based expression systems</td>
<td>Potency and expression of correct protein</td>
</tr>
<tr>
<td>Light-scattering techniques such as dynamic or static light-scattering analysis; nanoparticle tracking analysis; electron microscopy; size-exclusion chromatography</td>
<td>Particle size distribution (purity, consistency, safety)</td>
</tr>
<tr>
<td>Laser Doppler electrophoresis; dynamic light-scattering analysis</td>
<td>Particle surface characterization (including size, polydispersity and zeta potential)</td>
</tr>
</tbody>
</table>
Table 1 continued

<table>
<thead>
<tr>
<th>Examples of possible method</th>
<th>Potential use(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron microscopy; atomic force microscopy; X-ray diffraction; differential scanning</td>
<td>Physicochemical characterization (including surface and morphological properties)</td>
</tr>
<tr>
<td>calorimetry analysis</td>
<td></td>
</tr>
<tr>
<td>Tests for sterility, endotoxin content</td>
<td>Safety attributes</td>
</tr>
<tr>
<td>pH determination; gravimetric, azeotropic or titrimetric method to test residual moisture</td>
<td>Quality attributes</td>
</tr>
</tbody>
</table>

6.2 **General information and description of vaccine construct and composition**

Information should be provided that describes the mRNA drug substance and the formulated mRNA vaccine in terms of its design, sequence and construction, its composition (for example, lipids and other excipients), and the quantities of each excipient used. The rationale for and function of the chosen excipients should also be provided in the description. Where relevant, information on the structure and molecular weight of the lipids employed and on their role in the vaccine formulation should be included.

6.2.1 **mRNA sequence and arrangement of elements**

a. The annotated sequence of the DNA template should be provided. The sequence and position or length of all elements contained within the mRNA, including start and stop codons, flanking UTRs, regulatory elements (for example, promoter for the RNA polymerase) and 5′ cap and 3′ poly(A) tail, should be provided, as well as the ORF for the target antigen. If any additional proteins are encoded (such as those for a self-amplifying construct or a cytokine) their sequence should be provided (see points d and e below). The presence and function of any additional sequences included in the construct should be described.

b. Because vaccine mRNA can be manufactured containing nucleosides that are naturally occurring or modified or synthetic, the sequence information should include the specific nucleosides used.

c. Additionally, if sequences are changed from the native sequence in order to optimize codons, these changes should be described and justified. Codons may be altered for several reasons, including to better match the frequency of the appropriate tRNAs in human cells, to attain a specific secondary or tertiary mRNA structure, to
reduce innate immune responses or to increase the in vivo stability of the mRNA.

d. As noted above, in addition to coding for the target antigen(s), sa-mRNA also codes for a viral RNA-dependent RNA polymerase complex. Such a construct constitutes a replicon with the result that multiple copies of the mRNA coding for the antigen can then be made in vivo upon delivery to and uptake by the cells of the vaccine recipient, thus potentially increasing the potency of the vaccine. The sequences for any such replicon should be provided and their functions explained. If the replicon is encoded on a separate mRNA molecule from the target antigen, then the manufacture and control of each component should be illustrated and narratively described. Generally, these coding sequences are present on the same molecule but if separated, then additional controls may be required and should be described.

e. If an mRNA vaccine includes sequences that code for any other immunomodulator (such as a cytokine) or non-coding sequences intended to act as an immunomodulator, then such sequences and information on their purpose should be provided.

6.2.2 Formulations and components

a. Batch formula: the batch formula for commercial production should be provided. The amounts of each component in a single vaccine dose should be listed. The total volume of a batch should be defined. If more than one mRNA molecule (drug substance) is included in the drug product (final vaccine), this should be described, including whether the different mRNA molecules are encapsulated in a single LNP at the same time or encapsulated separately in LNPs that are subsequently mixed.

b. Chemical nature and formulation: the mRNA is formulated principally for increased in vivo stability and to aid cellular uptake. While several potential types of delivery agents exist (such as protamine complexes, cationic liposomes and lipid-, polymer- or lipid/polymer-based nanoparticles) the mRNA vaccines currently in use or in the most advanced clinical trials are encapsulated into LNPs. Characterization of these formulations, both chemically and in terms of the physical attributes of the structural formulation (such as nanoparticles), is required and should address characteristics such as the consistency and stability of the formulation and final product. Considerations of the quality of the
lipids and critical quality attributes of the drug product should also be included. Sufficient characterization of the mRNA-LNP and of its uptake into target cells should be provided. This may include an understanding of the surface chemistry, size, polydispersity, shape, charge and protein-binding properties of the resultant mRNA-LNP in order to ensure that adequate protection of the mRNA and the required stability of the vaccine are achieved. Where the LNPs are shown to have inherent immunomodulatory effects, relevant data on the potential benefits and drawbacks should be presented. Thus any characteristics of the formulation that might impact the safety, immunogenicity and efficacy of the vaccine should be described and their effects (positive or negative) should be considered during formulation development.

c. **Additional immunomodulators or adjuvants**: the mRNA might also encode specific immunomodulatory molecules such as cytokines. Furthermore, a separate adjuvant or immunomodulatory (stimulatory or suppressive) compound not encoded in the mRNA might be added to the formulation or as part of the LNP. As a general principle regarding vaccines formulated with adjuvants, a demonstration of the contribution of such an addition to vaccine immunogenicity should be provided (19). Quality aspects of the separate adjuvant, if included, should also be addressed and described.

d. **Additional peptides/proteins**: if additional peptides/proteins are included to target the mRNA to antigen-presenting cells or other specific cell types or to increase the release of the mRNA from the endosome, the sequence and function of these additions need to be described and evidence provided of their function to support their proposed mechanism-of-action.

e. **Additional excipients (such as preservatives)**: the composition, necessity for and (in the case of preservatives) the preservative efficacy of such additional excipients should be described and shown not to adversely affect the properties of the LNP.

### 6.3 Control of starting and raw materials and excipients

As with any vaccine, appropriate attention needs to be given to the sourcing and quality of all materials used in production (22). The raw materials should be procured from vendors/suppliers approved by the manufacturer through the internally defined quality systems. Suppliers of such materials should be managed by an appropriate qualification programme.
6.3.1 Quality of starting and raw materials and excipients

The starting and raw materials and excipients, including those used to produce the mRNA (such as the DNA template, nucleotides (which may contain modified nucleosides), enzymes, buffer, solvents, any columns for purification and so on) and the lipids in the LNP should be described. Information should be provided on their provenance, quality, control, stability and role, including the point at which each material is used in the manufacturing process. The materials should be suitable for use in GMP production, and reference to internationally accepted pharmacopoeias or details on their specifications should be provided.

The processes used for the derivation of the linear DNA template and raw materials should also be described. Although the starting material for the production of mRNA vaccines is the linear DNA template, that template may be derived from upstream materials such as a DNA plasmid propagated in a recombinant cell bank (see section 6.3.1.1 below).

With respect to the LNPs, the source and quality of the lipids used in their manufacture (especially of novel lipids present in LNPs that have not previously been studied nonclinically or clinically) should be sufficiently detailed to permit meaningful assessment of their safety and quality. Suitable specifications should also still be provided for any such excipient not considered to be novel. In the case of novel excipients (for example, cationic lipids) details of the manufacturing process and control of the novel lipids (including the starting materials and intermediates) should be provided, where feasible. This will include information on and relevant justification of the proposed starting materials and any intermediates used in the synthesis of the novel excipients. Consideration should be given to performing a nitrosamine risk assessment on the (cationic) lipid(s), if relevant.

Details of the manufacturing site(s) and manufacturing process, along with the required process controls and specifications of the starting materials, raw materials (for example, enzymes, buffers and solvents), intermediates and final excipients (for example, lipids and salts) should be provided. Consideration should also be given to the use and control of solvents, and to the potential for contamination with elemental impurities (60–62). Where the recycling of materials/solvents is proposed, this should be justified and appropriately controlled. The level of impurities associated with the excipients should also be suitably controlled and justified. Any purification and isolation steps should be detailed. To assure the quality of the proposed novel excipients, their manufacturer should also have available relevant information on the analytical methods used for the characterization, stability monitoring and batch analyses of the materials. Since inclusion of a PEGylated lipid plays a critical role in providing in vivo stability and enhancing the cellular interaction of LNPs (42), adequate controls (for example, of molecular weight, polydispersity and mole percent) should be in
place for the PEGylated lipid. For the manufacturing of excipients, compliance is expected with WHO Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (22).

6.3.1.1 Quality of linear DNA derived from plasmid as starting material

As stated above, the linear DNA template is considered to be the starting material for the GMP production of the mRNA vaccine. If the linear DNA is prepared from plasmid DNA, then the procedures for establishing the cell banks and the manufacture of the plasmid DNA should be performed in accordance with the requirements for the production of material for use in subsequent GMP manufacture.

A cell bank system should be established, described and tested for microbial purity (freedom from bacterial and fungal contamination) and identity. The genetic stability of the seed bank must be demonstrated. If the poly(A) tail is encoded in the plasmid DNA, then that region on the DNA plasmid in particular should be tested for the rate of recombination. A purification process also needs to be in place to reduce impurities from the DNA plasmid (such as RNA, host-cell DNA, protein, lipids and polysaccharides). The manufacturing process needs to be set up in such a way as to minimize the risk of microbiological contamination.

Testing of DNA plasmids (if used to generate the linear DNA) and the linear template should include tests for genetic identity by sequencing, for integrity (including confirmation of the desired encoded antigen sequence and regulatory/controlling sequences) and for percentage linear DNA, as well as tests (using appropriate reference standards) for residual genomic DNA, RNA and protein, sterility or permissible bioburden, and endotoxin levels. In early development, testing might be carried out only on the DNA plasmid (if used) or on the linear DNA.

In early clinical development it may be acceptable to use well-qualified material on the understanding that greater control will be expected to support pivotal trials and commercial manufacture.

6.3.2 Release of starting and raw materials and excipients

As with any vaccine, certificates of compliance (if applicable) and certificates of analysis should be provided for all raw materials and a clear indication given of which testing is performed by the mRNA manufacturer or whether the material is accepted on the basis of the certificate of analysis provided by the manufacturer/vendor/supplier of the raw material. An internal policy should be defined based on criticality risk ranking for the in-house testing and release of raw materials used in the manufacturing process. Starting materials should be released in accordance with the requirements and specifications for use in subsequent GMP manufacture.
6.4 **Process development and in-process controls**

The development history of the commercial manufacturing process should be provided. Tests and acceptance criteria for alert and action limits for critical steps of the manufacturing process should be developed and justified to ensure, and provide feedback on, the control of the process. In cases where a well-established platform technology is being used, knowledge gained from the manufacture of approved products can be considered in the justification.

Validation of the manufacturing processes should demonstrate that they comply with their critical and key parameters and yield a product that consistently meets the predefined quality attributes. This should include demonstration of the reproducible and consistent clearance of process- and product-related impurities to levels acceptable for intended use in humans.

Process validation is not generally required for a candidate vaccine used in preliminary clinical trials, though critical steps such as aseptic processing and sterility of the drug product should be validated or appropriately demonstrated to be controlled during the manufacture of clinical materials.

6.5 **Product characterization**

A summary of the characterization of the mRNA (drug substance) and the final vaccine (drug product) should be provided in addition to in-process and lot-release testing. Rigorous characterization using a range of orthogonal chemical, molecular, physical and biological methods will be essential. Characterization refers to studies and analyses that are not performed routinely on every lot but which allow the manufacturer to gain important knowledge of the structure, performance and safety of their product in order to guide process and analytical test development and improvement. This is in contrast to the in-process and lot-release testing performed on every lot. Justification of the choice of analytical methods for the determination of various attributes should be considered, particularly when a different outcome would likely be obtained using alternative techniques – for example, particle size measurement using different methods. It is for this reason that orthogonal methods are recommended.

The sequences of the population of manufactured mRNA should be determined and the degree of consistency of the proper sequence defined. Consistency of manufacture is discussed further in section 6.6 below. The degree of consistency of the capping and polyadenylation processes should also be characterized and may need to be validated (see section 6.4 above). Demonstration of expression of the complete encoded protein(s) without truncated or alternative forms should be provided. In particular, if expression of truncated or alternative forms of the target antigen is demonstrated during characterization studies and these alternative forms would result in neo-antigens or unwanted immune
responses, then this may require a redesign of the mRNA sequence. The degree of consistency of encapsulation of the mRNA in the LNPs should also be addressed during characterization. Particle-uptake studies could assist in characterizing potential potency measures through identification of cell types that take up the particles, mode or mechanism of uptake, and efficiency of uptake, and thus guide selection of the type of cell-free or in vitro method that best allows for assessment of these activities. During characterization, it should be determined whether any of these characteristics should be controlled as critical quality attributes and/or stability-indicating attributes.

Certain aspects of the LNPs should be very carefully characterized. These include particle size as determined by different analytical techniques to explore the morphological and dimensional characteristics of the LNPs containing the mRNA. Information on the density and distribution of polyethylene glycol (PEG) within the LNPs would also be useful to help understand the surface properties of the mRNA-LNP. Measurement of surface charge (for example, zeta potential) should also be considered as a method for characterizing the LNPs. These, and other properties, will affect the in vivo stability, cellular interaction and immunological response properties of the product; such information would also help to confirm the consistency of the manufactured vaccine.

The immunogenicity elicited by the mRNA-encoded target antigen is a critical characteristic of the product that should be characterized in nonclinical studies as a means to understand the product. Additionally, if the LNPs have inherent immunomodulatory effects these should also be characterized. Whenever other immunomodulatory elements or genes are included in the mRNA, their contribution to the mode-of-action (for example, immunogenicity) of the mRNA-encoded target antigen should also be determined in nonclinical studies in order to justify their inclusion in the characterized product design (see section 7 below). Consideration of these aspects is important in gaining understanding and knowledge of the product in order to optimize its design and develop appropriate control methods.

Potential impurities that might be introduced by the starting materials, and potential product- or process-related impurities in the purified mRNA, should be described and investigated. Such impurities may include residual bacterial host-cell proteins (if used to manufacture the DNA template), endotoxins, residual bacterial host-cell RNA and chromosomal DNA (if bacteria were used to manufacture the DNA template), enzymes (such as DNA and RNA polymerases and restriction enzymes), unincorporated nucleotides, dsRNA, incomplete or differently sized RNA, and other materials used in the manufacturing process. Data should be provided on the impurities present in the purified mRNA in order to justify the specifications set for their maximum acceptable or lowest achievable levels. For impurities and residuals with known or potential toxic
effects, a toxicological risk assessment is expected to be carried out. Degraded mRNA may be assessed as part of analytical procedures such as polyacrylamide or agarose gel electrophoresis, high-performance liquid chromatography (HPLC) and/or capillary gel electrophoresis. The degree of consistency of the sequence and structure of the mRNA, and its expression of a consistent protein when transfected into cells in vitro, are important characteristics to be determined for the drug product.

Any potential impurities (both process- and product-related) that may arise from the lipids used in the formulation of the drug product should also be characterized and investigated. This will permit justification of the specification limits proposed so that these impurities are suitably controlled and are within the clinically determined acceptable range.

6.6 **Consistency of manufacture**

As with other biologicals, prior to seeking marketing authorization, a number of consecutive batches should be tested and analyzed using validated methods to determine the consistency of manufacture. Any differences between one batch and another outside the accepted range for the attributes tested should be noted and investigated. The data obtained from such studies, combined with product and process knowledge and evaluation of the criticality of variations in specific attributes, should be used as the basis for justification of the chosen specifications.

During preliminary clinical development few lots will have been made and demonstration of production consistency may be limited or not possible. The ability to demonstrate consistency will increase as manufacturing experience is gained during product development. Confirmation of the consistency of lots is generally done during advanced development (for example when the manufacturing process has been scaled up for commercial manufacture) but prior to submission of application(s) for marketing authorization. However, in some cases, scale-up for commercial manufacture may be undertaken while marketing authorization is being sought for clinical trial-scale material. Whenever changes to the manufacturing process are implemented, the comparability of lots, especially to those used in pivotal studies and made by 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 (31).

6.7 **Manufacture and control of bulk purified mRNA (drug substance)**

As stated above in section 6.1, an overview of the development and manufacture of the mRNA should include a justification for the selection of the target antigen
gene, other gene(s) contained in the mRNA sequence, UTRs, 5′ cap, 3′ poly(A) tail and regulatory elements used. Any gene expression or other optimization modifications should be described. Annotated sequences of the complete DNA template and mRNA should be provided. Both an illustrative and annotated flowchart and a narrative description of the manufacture, in-process controls and release tests should be provided. The detailed production and control procedures along with any significant changes in them that may affect the quality, safety and efficacy of the mRNA vaccine should be discussed with and approved by the NRA.

In the case of sa-mRNA, if the replicon and target antigen are expressed on separate mRNA molecules, this should be described and clearly illustrated in the provided flowchart, which should also include any additional manufacturing processes and/or quality control tests. For example, consideration should be given to controls such as the ratio of replicon-encoding mRNA molecules to target-antigen-encoding mRNA molecules, or to methods to ensure (or controls to determine whether or not) both molecules are encapsulated into the same LNP, if applicable. For sa-mRNA in which the mRNA encoding the replicon and the mRNA expressing the target antigen are encoded on different molecules, it will be important that these two RNAs are co-encapsulated in order for them to be taken up by the same cell in vivo. Therefore, if the two RNAs are encapsulated separately and then mixed, a justification for this approach will be required.

6.7.1 Control of bulk purified mRNA (drug substance)

Specifications for critical quality attributes for the identity (see section 6.7.1.1 below), purity (section 6.7.1.2), quantity and physical state (section 6.7.1.3), safety (section 6.7.1.4) and quality (section 6.7.1.5) of the bulk purified mRNA should be established and justified. Descriptions of the analytical methods used should be provided, the acceptance limits defined and assay validation information described. The results of testing of all batches produced at commercial scale should be summarized and provided. Specifications should also be established for stability under storage conditions.

Early in development, to support clinical trial authorization, results from testing batches made in accordance with GMP (21–24) and, if available, engineering runs performed to establish manufacturing procedures should be summarized and provided. Although specifications may be limited and have somewhat wide acceptance criteria in early development, these should be reviewed and tightened, when appropriate, as experience in the manufacturing process and analytical methods is gained. Not all of the tests conducted during product characterization need to be carried out on each batch of vaccine as release testing. Some tests are required only to obtain product and process knowledge on a limited series of batches to establish the methods and consistency of production.
Thus, a comprehensive analysis of the initial commercial-scale production batches should be undertaken to establish consistency with regard to the identity, purity, quality, safety and stability of the drug substance; thereafter, a limited series of tests may be appropriate for quality control, as agreed with the NRA.

As experience is gained in manufacturing consistency, post-approval changes might permit reducing the testing and the amount of supporting information required through the use of process validation, product characterization and/or a comparability protocol (31).

6.7.1.1 Identity

Each batch of bulk purified mRNA should be tested to confirm its identity. Confirmation of identity could include determination of the mRNA sequence by direct RNA sequencing, sequencing (or determining the presence or absence) of a reverse transcription PCR (RT-PCR) product or high-throughput sequencing. If identity is based on an RT-PCR amplicon that represents only a portion of the complete mRNA sequence (including accessory and regulatory regions), then the sequence chosen should be unique to that mRNA product and not be common to any others that might be manufactured in the same facility or using the same equipment. However, it might be more appropriate to sequence the entire mRNA as this approach could serve to address both identity and potentially purity, depending on the sequencing method used.

6.7.1.2 Purity and impurities

Each batch of bulk purified mRNA should be tested for purity and the result should be within the allowable limits established. The control of impurities should also address the materials introduced during manufacture, such as the DNA template, unincorporated nucleotides, unincorporated caps, enzymes, mRNA fragments and dsRNA. This may be achieved through process validation to establish the removal of process-related impurities or through release tests for the residual impurities. Consideration of the necessity of testing for dsRNA should take into account the design of the manufacturing process as not all processes produce dsRNA. The analyses should include sensitive and reliable assays for process- and product-related impurities, and strict upper (maximum allowable) limits should be specified for their content in the bulk purified mRNA. Chromatographic detection methods may be considered. Residual DNA template might be quantified by quantitative PCR. It is important that the techniques used to demonstrate purity and to measure impurities are based on as wide a range of physicochemical, biological and/or molecular properties as possible. Consideration of the results of methods such as forced degradation studies may guide decisions on which product-related impurities will need to be tested for and/or measured during production, at release and/or in stability protocols.
Tests for residual levels of process- or product-related impurities as part of quality control may be reduced or discontinued once production processes have been adequately validated for their suitable removal, and production consistency has been demonstrated, if agreed by the NRA. Plans and specifications for the periodic revalidation of processes should be described. Until the processes are validated, impurities should continue to be tested for and/or measured in a number of lots as agreed by the NRA. In the case of major changes to manufacturing, revalidation or continued measurement would be expected for the number of lots agreed with the NRA. Container-closure system compatibility, leachables and extractables should also be assessed and discussed in the application for marketing authorization.

6.7.1.3 Quantification and physical state
The integrity of the structure of the mRNA is considered to be a critical quality attribute for release of the mRNA. Thus, control is needed of mRNA integrity, 5′ capping efficiency, 3′ poly(A) tail presence or length, percentage intact mRNA, percentage mRNA fragments, percentage of dsRNA and so on. The need to measure 3′ poly(A) tail presence or length depends upon the way in which this sequence is added to the mRNA. If encoded in the DNA template, then all full-length mRNA should include the poly(A) tail but if it is added enzymatically after IVT, then it would be appropriate to address this attribute through testing or process validation. Likewise, the presence of dsRNA depends on whether the processes used are capable of producing it. Tests such as gel electrophoresis, PCR or chromatographic detection methods might be considered for these purposes. It should be borne in mind that quantification of the mRNA is the basis for vaccine dosing and the presence of intact mRNA is key to the mechanism-of-action of the vaccine. Thus, the methods used for quantifying the mRNA (for example, ultraviolet spectrophotometry) and for quantifying the intact mRNA (for example, gel electrophoresis) should be described.

6.7.1.4 Safety attributes
Relevant safety tests should be described. These may include tests for endotoxins along with testing either for bacterial and fungal sterility (including demonstration of lack of bactericidal or fungicidal activity of the test article) or microbial bioburden (including quantity, identification of microbe species and freedom from specified unwanted organisms). Such testing is generally not required by an NRA for the drug substance, but if required a test for pyrogenicity may be performed on the drug product (which may be the monocyte activation test). Animal testing should be avoided whenever alternative satisfactory testing is available and allowed. For scientific and ethical reasons, it is desirable to apply the 3Rs concept of “Replace Reduce Refine” to minimize the use of animals.
in testing and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation, as well as for other product tests. 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 (63). This test should therefore not be required or requested for either the drug substance or the drug product.

6.7.1.5 Additional quality attributes

Additional important quality attributes should be established and controlled (such as appearance, pH and, if relevant, viscosity).

6.7.1.6 Reference materials

An in-house reference preparation (that is, working standard) should be established for use in assay standardization and comparability assessment. Information on the reference standards or reference materials used for testing of the bulk purified mRNA 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 an adequate sample retained for use as a chemical and biological reference material. The reference material should be formulated in an appropriate form. Storage should be under conditions at which the reference material has been shown to be stable. A routine programme for monitoring the stability of the material should be implemented. A plan for replacing the initial reference material upon exhaustion should be agreed with the NRA.

In early development (for example, preliminary clinical trials) an engineering run batch or a batch from which the lot of mRNA vaccine evaluated in the pivotal nonclinical studies was made may serve as a reference until a suitable clinical trial batch has been identified and characterized for use as a reference in advanced development (for example, pivotal clinical trials) and commercial manufacture. Whatever approach is taken should be clearly described.

6.7.1.7 Stability

A stability assessment should be conducted in accordance with the WHO Guidelines on stability evaluation of vaccines (27). The types of studies conducted, the protocols followed, and the study results should all be summarized in an appropriate format such as tables or graphs along with a narrative document. The summary should include results as well as conclusions with respect to
appropriate storage conditions or shelf-life. Data on stability to support the shelf-life of the bulk (or stored intermediates) and any future extension of it should be based on long-term real-time stability studies under actual conditions. For the transportation of intermediates or drug substance, it is expected that shipment validation will be conducted at appropriate storage temperatures and conditions.

6.8  **Manufacture and control of final formulated vaccine (drug product)**

As stated above in section 6.1, an overview of the development and manufacture of the vaccine should include both an illustrative and annotated flowchart and a narrative description of the manufacture, in-process controls and release tests. The methods used to assure the proper formation of LNPs should be detailed. Any proposed hold-time of the bulk formulation or bulk LNPs should be appropriately specified and validated. Adequate consideration should be given to ensuring physicochemical stability and microbial control during such hold-times. The methods used for final formulation, fill and finish should also be described and suitably validated.

6.8.1  **Composition**

The final composition of the vaccine, including the active drug substance (mRNA) and all excipients (for example, lipids), should be described along with the quantity of the components in each presentation – particularly if marketing authorization is being sought for more than one dosage or dosage form. The function of each of the components should also be described.

6.8.2  **Manufacture and control of LNPs and encapsulation of mRNA**

The methods used to assure the proper formation of LNPs should be described. Appropriate product development data should be provided to support the rationale for their proposed formulation and manufacturing process. All critical quality attributes of the LNPs and final mRNA-LNPs should be investigated. Where suitable, a Design of Experiments (DoE) approach could be adopted. Their size and polydispersity, and in turn stability, are all influenced by both the flow dynamics of the lipid and aqueous phase and the shear stress induced during the manufacturing process. Thus, relevant studies that explore the critical processing parameters and their operational ranges optimal for mRNA-LNP formulation and stability of the final formulated vaccine should be performed. This will ensure that the product is consistently manufactured to the required quality. Any proposed hold-time of the bulk LNPs or bulk formulation should be appropriately specified and validated. If stored, these are important intermediates in the production of the final vaccine and should be controlled appropriately.
Adequate consideration should be given to ensuring physicochemical stability and microbial control during such hold-times.

The preparation of the lipids, the encapsulation of mRNA with the lipids into LNPs, dilution and any purification steps, and subsequent filling into suitable containers should be described and the process validated to meet the necessary in-process specifications. Various filtration techniques (for example, tangential flow filtration) should be considered for the removal of raw materials used in the preparation of LNPs. Specific attention should be given to minimizing the degradation of the mRNA during encapsulation into the LNPs and under manufacturing conditions known to influence the stability of the LNPs and final mRNA-LNP vaccine product (for example, the impact of thawing of the mRNA and the freezing rate of the LNPs or mRNA-LNPs). Likewise, if lyophilized, the conditions for freeze-drying and reconstitution should be considered and justified. If applicable, the diluent or reconstitution solution should be described.

Suitable controls for the LNPs should also be specified and would typically include: (a) identity, quantity and purity (including impurities) of the lipids; (b) particle size and distribution (polydispersity); and (c) RNA encapsulation efficiency/proportion encapsulated. In some cases, the surface properties (for example, charge), lipid molar ratio, or cationic lipid to mRNA ratio (for example, nitrogen to phosphate ratio) may also need to be specified to ensure consistency and stability of the product.

It will also be important to consider the subsequent impact that any change made to the mRNA drug substance (for example, change in sequence, length or secondary structure) may have on the critical quality attributes of the LNPs (for example, particle size and distribution, morphology, and surface properties) and ultimately on the final vaccine product (for example, percentage of encapsulation and cellular interaction/uptake). Relevant developmental data are expected to demonstrate product consistency and to support the product optimization process. Likewise, if platform data are intended to support development of new candidate vaccines, the impact of the new mRNA drug substance on the critical quality attributes of the final vaccine product should be determined.

6.8.3 Manufacture of final vaccine (drug product), filling and containers

An annotated flowchart should be provided that illustrates the manufacturing steps from the bulk purified mRNA (drug substance) to the final vaccine (drug product). The chart should include all steps (that is, unit operations) such as dilution of the final formulated bulk, identification of materials and intermediates, and in-process and quality control tests. A narrative description of each process step depicted in the flowchart should be provided. Information should also be included on, for example, its scale, buffers and other additives, major equipment and process controls (including in-process tests and critical process operational parameters
with acceptance criteria that are justified by relevant development data). Details of the sterilization process and microbial control should also be included.

The general guidance concerning filling and containers provided in WHO good manufacturing practices for biological products (23) should be applied to vaccine filled in the final form. The aseptic fill process of the mRNA-LNP should be adequately validated to ensure all critical quality attributes are maintained and meet the required specifications. Care should be taken to ensure that the materials of which the containers and closures (and, if applicable, the transfer devices) are made do not adversely affect the quality of the vaccine. To this end, a container-closure integrity test and assessment of extractables and/or leachables for the final container-closure system are generally required for the qualification of containers and may be needed as part of stability assessments.

If multi-dose vaccine vials are used and the vaccine does not contain a preservative, then their use should be time restricted, as is the case for reconstituted vaccines such as bacillus Calmette–Guérin (BCG) and measles-containing vaccines (32). In addition, the multi-dose container should prevent microbial contamination of the contents after opening. Relevant simulation studies (for example, multi-puncture tests) of the container-closure system may be required to demonstrate the suitability of the proposed system. Multi-dose vials should be designed to meet the label claim, with acceptable overfill to allow for correct dosing. Multi-dose vaccine vials should be evaluated for the maximum anticipated vial septum punctures to assess the risk of compromising vial integrity and the potential for vial contamination. The extractable volume of multi-dose vials should be validated. If multi-dose vaccine vials are supplied as concentrate, an additional compatibility study should be conducted using the proposed reconstitution solutions and an appropriate post-dilution hold-time should be established. The pre-dilution and post-dilution specifications should be set out and justified. Manufacturers should provide the NRA with adequate data demonstrating the stability of the product under appropriate conditions of storage, distribution and during use.

When a final vaccine contains more than one mRNA species (for example, in a combination or multivalent vaccine, or an sa-mRNA consisting of separate mRNAs) there may be additional considerations in the manufacture of that final vaccine. One such consideration will be ensuring the appropriate ratio of the different mRNAs in the formulation to optimize the expression of each and to minimize immune interference (in the case of combination or multivalent vaccines). Another consideration will be whether the mRNAs will be mixed prior to encapsulation in the LNPs or whether each mRNA will be separately encapsulated into LNPs and then a mixture of the two or more mRNA-LNPs prepared. In either case, the approach selected should be described and justified with relevant data.
6.8.4 **Control of final vaccine (drug product)**

Samples should be assessed from each final vaccine lot. All tests and specifications should be approved by the NRA. Specifications for the final vaccine should be established and justified by the manufacturer. As a principle, the final specifications should be defined on the basis of the relevant batch data on lots that have been shown to have acceptable performance in clinical studies. Descriptions of analytical methods and acceptance limits for the vaccine should be provided, including information on method validation. It is recommended that testing should include an assessment of identity (see section 6.8.4.1 below), purity (section 6.8.4.2), content (section 6.8.4.3), safety (section 6.8.4.4), additional quality attributes (section 6.8.4.5) and potency (section 6.8.4.6). Stability will also need to be established to justify the requested expiry dating.

Although specifications may be limited and have somewhat wide acceptance criteria in early development, these should be reviewed and tightened, when appropriate, as experience in the manufacturing process and analytical methods is gained.

A summary of the results of the testing of all lots produced at commercial scale should be provided. Early in development, to support clinical trial authorization, results from testing lots made in accordance with GMP (21–24) and, if available, engineering runs performed to establish manufacturing procedures should be summarized and provided.

Not all of 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 obtain product and process knowledge on a limited series of lots to establish consistency of production, as discussed in sections 6.4–6.6 above. Several consecutive lots of vaccine, in final dosage form, should be tested and analysed using validated methods to confirm manufacturing consistency. Any statistically significant or scientifically meaningful differences between one lot and another should be noted and investigated. The data obtained from such studies, as well as clinical trial outcomes with various lots, alongside product and process knowledge and evaluation of the criticality of variations in specific attributes, should be used as the basis for defining the vaccine specifications and acceptance criteria to be used for routine lot release. Thus, a comprehensive analysis of the initial commercial production lots should be undertaken to establish consistency with regard to the identity, purity, strength/content/quantity, safety, additional quality parameters, potency and stability of the mRNA vaccine but thereafter a more limited series of tests may be appropriate, if agreed with the NRA.

When a final vaccine contains more than one mRNA species (for example, in a combination or multivalent vaccine, or an sa-mRNA consisting of separate mRNAs) there may be additional considerations in the control of that final vaccine. Some of these considerations will be based on the approach taken in manufacture.
– for example, whether the mRNAs were encapsulated together at the same time as a mixture or were encapsulated separately and then the different mRNA-LNPs mixed. This may then affect the size, charge and polydispersity of the LNPs. In addition, validating the consistency of mixing is crucial to ensuring that each dose contains the appropriate ratio of each of the mRNAs. Ensuring the proper ratios in the total mRNA content of the final vaccine will be critical as the total mRNA content is the basis for dosing. Identity testing should address the inclusion of each mRNA, while still differentiating the vaccine from other products made in the facility. If one drug substance or component (for example, the mRNA encoding the replicon) is used in more than one vaccine or product made in the facility, then such identity testing will also be crucial in preventing mix-ups.

As experience is gained in manufacturing consistency, post-approval changes might permit reducing the testing and amount of supporting information required through the use of process validation, product characterization and/or a comparability protocol (31).

6.8.4.1 Identity
Each lot of vaccine should be subjected to an appropriate test to confirm the identity of the final product and distinguish it from other products made in the same facility or using the same equipment. If the vaccine contains more than one mRNA species (for example, in a combination or multivalent vaccine, or an sa-mRNA consisting of separate mRNAs), then the identity of each mRNA should be confirmed. Confirmation of mRNA identity by sequence analysis should be considered (see section 6.7.1.1 above).

6.8.4.2 Purity and impurities
The purity of each lot of final vaccine should be assessed and shown to be within the specified limits. Consideration should be given to potential impurities resulting from any component of the delivery system and to controlling aspects of impurity such as oxidation and degradation in the final vaccine. It is unlikely that a single test will be sufficient to detect all potential impurities. Tests for mRNA integrity, particle size, lipid/polymer impurities and the proportion/efficiency of mRNA encapsulated in the LNPs should be considered. Container-closure system compatibility, leachables and extractables should also be assessed and discussed in the application for marketing authorization (see also section 6.7.1.2 above).

6.8.4.3 Content, strength or quantity
mRNA vaccines are dosed based on quantity of the mRNA by weight. Therefore, in addition to assessing potency (see section 6.8.4.6 below), a quantification method for the mRNA should be described (see section 6.7.1.3 above). If the
vaccine contains more than one mRNA species (for example, in a combination or multivalent vaccine, or an sa-mRNA consisting of separate mRNAs), then the quantity of each mRNA should be measured and confirmation made that the ratio of each mRNA to the other is as intended and the total mRNA dose has not been exceeded.

### 6.8.4.4 Safety attributes

Each lot of final vaccine should be tested for bacterial and fungal sterility (including demonstration of lack of bactericidal or fungicidal activity of the test article). If the vaccine is to be administered by a non-parenteral route, then omission of the sterility test and inclusion of an appropriate alternative microbial bioburden test needs to be appropriately justified. Further, 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 (which may be the monocyte activation test). Animal testing should be avoided whenever alternative satisfactory testing is available and allowed. For scientific and ethical reasons, it is desirable to minimize the use of animals in testing, and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation and other product control tests. 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 (63). This test should therefore not be required or requested.

### 6.8.4.5 Additional quality attributes

Other important quality attributes should also be established and controlled. These can include appearance (including presence of both visible and sub-visible particulate matter), extractable volume and pH. Depending on the product characteristics, the control of other attributes such as osmolality or viscosity may also be important. For the final vaccine (drug product), additional attributes should include lipid/polymer identification and content, nanoparticle size, mRNA–lipid ratio and polydispersity index.

With respect to nanoparticle size, multiple point control should be adopted similar to the control of nanoparticle-based therapeutic products, and the test used for measurement of particle size should be specified, as the results will be dependent upon the analytical method employed. The degree of encapsulation of the mRNA in the LNP should also be regarded as a critical quality attribute as non-encapsulated mRNA is considered to be unstable. Confirmation should be provided that the structure of the final product does not change due to freeze-
thawing and dilution. Techniques such as gel or capillary electrophoresis and/or HPLC already being performed for purity or for identity may also be useful in assessing some quality attributes.

Other tests (such as a test for residual moisture if the vaccine is lyophilized) may be required to confirm the physical characteristics of the product as well as the formulation. Validation of the analytical methods used should be described to assure the control of the identified critical quality attributes of the drug product.

6.8.4.6 Potency

The potency of each lot of the final vaccine should be determined using a suitably quantitative and validated functional method(s). Different tests may be required to control various aspects of potency (including functionality), which will likely be disease specific. Immunogenicity in the vaccine recipient is a complex function of the final vaccine properties, including delivery to target cells by its formulation as well as expression of the mRNA-encoded protein(s) (which may include a self-amplifying replicon component). Thus, potential in vitro potency assays may include cell-based transfection systems or cell-free assays. Such methods would demonstrate that the correctly sized protein of correct identity is expressed from the mRNA. However, because potency should be analyzed on the basis of not only the product type (in this case mRNA vaccines) but also the clinical indication of the disease to be prevented, it is not possible to indicate a particular assay method that should be used to measure potency. Scientific justification for the potency test(s) selected to control the product should be provided and correlated with clinical performance, as with all quality control tests.

When a new candidate vaccine against a new strain(s) is developed, consideration should be given to ensuring that the potency assay(s) used is valid for the strain change.

The potency specifications for mRNA vaccines should be set based on the minimum dose used to demonstrate efficacy in clinical trials plus human immunogenicity data. An upper limit should also be defined based on available human safety data.

Animal-based assays tend to be highly variable and difficult to validate. Consideration should therefore be given to the use of appropriate in vitro alternative methods for potency evaluation. It is envisaged that, as with plasmid DNA vaccines, a combination of biochemical or biophysical measures (such as nucleic acid quantity and mRNA integrity) might be used to establish and monitor the potency of mRNA vaccines. Manufacturers are encouraged to work towards the goal of employing in vitro assays that are suitably quantitative and assess function. However, it needs to be acknowledged that these measures only account for the mRNA and not the impact of any formulation, adjuvant, immunomodulators and so on, and the potency assessment of mRNA vaccines
will thus need to be considered on an individual case-by-case basis. Therefore, discussing appropriate potency measures and reaching agreement with the NRA is advised.

6.8.4.7 Reference materials

A suitable lot of the final vaccine that has been clinically evaluated should be fully characterized in terms of its chemical composition, purity, biological activity and full sequence, and retained for use as an internal reference material. This material should be used as the basis for evaluation of product quality for commercial production lots (see also section 6.7.1.6 above).

In the future, national standards may be prepared and provided by the NRA while international standards may become available from WHO. Should such international standards become available it will be important to calibrate the internal or national reference material against them. In this way, comparisons can be made in a more reliable way whenever new reference materials need to be prepared. In addition, the expression of results in a common unit (such as IU), when appropriate, will also allow for the comparison of test results obtained from different laboratories, and for different products against the same pathogen based on the same or similar technologies (for example, different COVID-19 mRNA vaccines).

6.8.4.8 Stability testing, storage and expiry date

The relevant guidance provided in WHO good manufacturing practices for biological products (23), WHO good manufacturing practices for sterile pharmaceutical products (24) and WHO Guidelines on stability evaluation of vaccines (27) appropriate for the respective mRNA vaccine should apply. Furthermore, the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (29) might also apply. The statements concerning storage temperature and expiry date that appear on the primary and secondary packaging should be based on experimental evidence and should be submitted to the NRA for approval. For guidance regarding vaccine vial monitors, the WHO Getting started with vaccine vial monitors and related WHO guidance should be consulted (64, 65).

6.8.4.8.1 Stability

Adequate stability studies form an essential part of vaccine development. To support commercial use, the stability of the final product in the container proposed for use should therefore be determined and the results used to set a shelf-life under appropriate storage conditions. Attributes that are stability-indicating should be measured and these may include appearance (including visible and sub-visible particulate matter), mRNA quantity, vaccine potency, mRNA integrity,
degree of encapsulation, particle size, polydispersity and impurities associated with the mRNA and lipids. The attributes to be measured should be described and specifications defined and justified. Real-time stability studies should be undertaken for this purpose – though accelerated stability studies at elevated temperatures may provide additional and complementary supporting evidence for the stability of the product and confirm the stability-indicating nature of the assays used to determine stability. If long-term storage (for example, > 6 months) at temperatures above freezing is being considered, this may require additional analytical testing to assess potential lipid oxidation or other such changes and the resultant impact of these changes on potency.

In addition, accelerated and stress testing data as well as platform data can be taken into account to support the shelf-life. Stability data that support clinical use, such as data on stability at elevated temperatures for short-term storage and dispensing, should be generated. For multi-dose vials, in-use stability data will be needed to provide assurance of the required microbial quality and stability of the vaccine under in-use conditions (32).

During initial clinical development limited stability information would be expected. For example, some regulators accept 3 months of real-time stability of the lot of final vaccine to be used in the proposed clinical trial in the containers that will be used for the clinical trial, or one produced in the same manner in the same container type and size and meeting the same specifications, at the time of application for clinical trial authorization, but this should be agreed with the NRA. Initial clinical development may also be supported by including results from predictive stability modelling utilizing an accelerated stability assessment programme. Likewise, stability data on a platform technology can be supportive for new candidate vaccines based on that platform.

If deep-freeze conditions are recommended for long-term storage, then alternative short-term storage conditions (such as frozen and/or refrigerated) should be explored to support vaccine distribution and dispensing. Similarly, temperature excursion studies or transportation simulation studies may also be expected. Container-closure system compatibility with storage stability (including with regard to leachables and extractables) should be assessed and discussed. The stability assessment should comply with WHO Guidelines on stability evaluation of vaccines (27). Consideration should be given to the development of vaccine formulations that are more thermostable to improve their global utility.

### 6.8.4.8.2 Storage conditions

Storage conditions should be validated. 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 the vaccine administration stage in the fully enrolled clinical trial.

6.8.4.8.3 Expiry date

The expiry date should be defined on the basis of the shelf-life of the final container supported by real-time stability studies and should be approved by the NRA. The expiry date should be based on the date of blending of the final formulated 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.

6.9 Records

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (21) should apply, as appropriate to the level of development of the candidate vaccine.

6.10 Retained samples

A sufficient number of samples should be retained for future studies and needs. These needs may include but are not limited to manufacturing investigations or development, nonclinical studies or future bridging clinical trials. A vaccine lot used in a pivotal clinical trial may serve as a reference material and a sufficient number of vials should be reserved and stored appropriately for that purpose. Advanced planning is required to enable the retention of an appropriate number of containers of the pivotal clinical trial lot.

6.11 Labelling

The guidance on labelling provided in WHO good manufacturing practices for biological products (23) should be followed as appropriate. The label of the carton enclosing one or more final containers, or the leaflet accompanying the container, should include, at a minimum and as agreed with the NRA:

- the common and trade names of the vaccine;
- INN, if applicable;
- the names and addresses of the manufacturer and distributer;
- lot number;
nature and content of the drug (active) substance;
product composition, including list of excipients;
a statement that specifies the nature and content of adjuvant contained in one human dose, if any;
dosage form and appearance;
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;
a statement on the trace amounts of any other residuals of clinical relevance;
the temperature recommended during storage and transport;
container-closure information;
the expiry/retest date;
any special dosing schedules;
any special instructions for in-use handling – for example, necessity for gloves to prevent exposure of product to RNases when handling multi-dose vials, or stability on mixing of contents; and
contraindications, warnings and precautions, and information on concomitant vaccine use and on known adverse events.

6.12 Distribution and transport
The guidance provided in WHO good manufacturing practices for biological products (23) appropriate for the vaccine should apply. Further guidance is provided in WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (28). Shipments should be maintained within specified temperature ranges, as applicable, and packages should contain cold-chain monitors, if applicable (29).

7. Nonclinical evaluation of mRNA vaccines
The nonclinical evaluation of candidate mRNA vaccines should be considered on a product-specific basis taking into account the intended clinical use. The design, conduct and analysis of nonclinical studies including selection of appropriate studies relating to the “pharmacology” (in the case of vaccines, immunogenicity
and proof-of-concept) and toxicology of the product should be based on the following WHO guidelines:

- WHO guidelines on nonclinical evaluation of vaccines (18); and
- WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (19).

There are several potential concerns that need to be considered during the safety and proof-of-concept evaluations of mRNA vaccines. Because of the novelty of this product type, numerous issues are addressed below which may be relevant to any given mRNA vaccine. However, there may also be future additional concerns that come to light that would need to be taken into consideration when appropriate. Not all of these issues will necessarily be relevant to any given mRNA vaccine, depending on its design. However, it is incumbent upon the vaccine developer/manufacturer to provide evidence demonstrating the proof-of-concept (for example, immunogenicity and, if an appropriate animal model is available, challenge protection) and safety of their candidate vaccine. The types, design and number of studies expected should be agreed with the NRA.

7.1 Pharmacology/immunology-proof-of-concept

In addition to the types of studies discussed in the WHO guidelines above (18, 19), additional issues that the NRA might expect nonclinical studies to address may include:

a. Durability of immune responses or immune cell phenotypes that suggest durability, particularly those that are proposed to be related to the candidate vaccine's induction of protection. To assess the durability of immune responses, characterization of immune cell phenotypes and/or cytokine expression could be helpful in investigating persistence and memory responses.

b. Induction of innate immune responses by RNA (such as induction of type I interferon), which have been reported to decrease translation of the target antigen or that could affect the need for (or timing of) boosts or subsequent doses.

7.2 Safety/toxicity in animal models

In addition to the expectations outlined in the WHO guidelines listed above (18, 19), consideration should be given to whether studies need to be designed to address the following:
a. **Biodistribution and persistence**: developing a database of evidence about this potential concern will permit the more rapid development of future candidate vaccines (3, 66–71). This potential issue may also depend on whether the vaccine migrates to specific cells or tissues. Nonclinical studies that address whether the mRNA and the LNPs (or lipid components) distribute away from the tissue into which the vaccine was administered, into which tissues they distribute and how long they persist may be expected by the NRA. Agreement on these studies should be sought from the NRA.

b. **Inflammation**: RNA is inflammatory via a number of pathways, particularly via the innate immune system with its numerous sensors for RNA. In mRNA vaccines, both the mRNA molecules and the LNPs (which enable successful delivery and cellular uptake) have properties that can influence and trigger the innate immune system (72, 73). While some of this activity may be beneficial for the immune response to the vaccine, it will be important to monitor for both systemic and local toxicity and inflammatory responses. Nonclinical study design needs to take into account any immune responses, reactogenicity or toxicities that might predict immune indicators (72, 73) of serious adverse events or adverse events of special interest (AESI) in humans. Additionally, other components added to aid delivery, such as PEG, although relatively benign, can also influence the physicochemical properties and thus the safety profile (74–77). It is therefore important to understand the overall product profile including the formulation and how physicochemical properties (which may vary) can influence inflammation and the safety profile. The choice of animal model will, as always, be critical, recognizing that anti-RNA innate immune responses in animal models are generally significantly milder than those observed in humans.

c. **Unexpected and serious toxicities from modified nucleosides**: some antivirals and anti-cancer drugs that contained specific unnatural nucleoside analogues with altered conformation have caused mitochondrial toxicities, resulting in myopathy, polyneuropathy, lactic acidosis, liver steatosis, pancreatitis, lipodystrophy and even fatality. However, some of these clinically observed toxicities were not observed in the nonclinical animal models used. While the modified nucleosides used in the most advanced mRNA vaccines (against COVID-19) are naturally occurring, future candidate vaccines may contain modifications that are unnatural. Thus, particularly for mRNA vaccines that
include unnatural nucleoside modifications that have not already been well characterized in other developed nucleic-acid-based products, careful consideration will need to be given to how these potential toxicities might be observed in appropriate animal models and nonclinical studies during safety evaluation (78–80).

d. Novel lipids and novel LNPs: because the lipids used to formulate the LNPs affect the overall charge of the particle, when using LNPs made with novel lipids or when the LNPs are themselves modified (for example, altered ratios or modified processes) and these LNPs have not previously been nonclinically and clinically tested in mRNA products encapsulated in LNPs, then evaluation of the toxicity of the new formulation containing the novel lipids (or any novel excipients) may be required. Furthermore, the NRA may require that the genotoxicity and systemic toxicity of the novel lipid component be assessed, similar to the expectations for novel adjuvants set out in the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (19) and/or those for new chemical entities in the ICH guideline S2 (R1) (62).

e. Novel formulations: likewise, for formulations (other than LNPs) containing novel excipients, data on and assessment of the systemic toxicity and genotoxicity of the formulation may be expected.

It should be noted that early theoretical concerns during plasmid DNA vaccine development regarding the potential for integration of vaccine nucleic acids into the host genome do not apply to mRNA vaccines for the following reasons:

- The only known mechanism by which RNA can integrate into the host genome requires the presence of a complex containing reverse transcriptase and integrase.

- Further, the design of candidate mRNA vaccines should be considered so that they do not include specific RNA-binding sites for primers required for the reverse transcriptase to initiate transcription. In addition, the RNA would have to be relocated to the nucleus after reverse transcription for the resulting product to be integrated.

- Finally, the vaccine mRNA degrades within a relatively short time once taken up by the body’s cells, as does the cell’s own mRNA. During that entire time, the mRNA vaccine is expected to remain in the cytoplasm, where it will be translated and then degraded by normal cellular mechanisms.

Therefore, nonclinical studies do not need to be performed to specifically address integration or genetic risks for mRNA vaccines.
As with any vaccine that is anticipated to be used widely in pregnant women or women of childbearing potential, the guidance provided in section 4.2.2 of the WHO Guidelines on nonclinical evaluation of vaccines (18) and section D.2.3 of the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (19) should be consulted. The necessity for such studies will be based on the target population for the given clinical indication of the vaccine. Often, if required, these studies are performed during or after pivotal clinical trials have been performed with the candidate vaccine produced using commercial manufacturing methods and scale. As a result, data should be available at the time of filing for marketing authorization on populations that include women of childbearing potential, and such data should be evaluated prior to the intentional enrolment of pregnant women in clinical studies.

If clinical data from similar candidate vaccines based on the same platform technology are available, then agreement should be reached with the NRA on whether or not such data are scientifically sufficient to preclude the need for further nonclinical studies. If nonclinical safety data from similar candidate vaccines based on the same platform technology are available, agreement should also be reached with the NRA on whether or not such data are scientifically sufficient to preclude the need for further nonclinical safety studies. Likewise, nonclinical safety data (and if available, clinical data) from a monovalent drug product formulation can support the clinical development of a multivalent drug product formulation (for example, for different strains of the same disease antigen) or combination vaccine (different disease antigens) in cases where the same LNP with the same molar and mRNA–lipid ratios is used, and where the sum of all the mRNAs in the multivalent drug product formulation will be no more than the highest dose shown to be safe in the monovalent nonclinical safety study.

7.3 Accelerating nonclinical evaluation in the context of rapid vaccine development against a priority pathogen during a public health emergency

In the case of the rapid development of vaccines against a priority pathogen during a public health emergency and when the new candidate vaccines are based on a given manufacturer’s platform technology, consideration may be given to an abbreviated nonclinical programme as follows:

- Where changes are made to the sequence of the target antigen encoded in an mRNA vaccine that has already been clinically tested (for example, in the case of a pandemic influenza strain when a seasonal or other potential pandemic strain antigen has been tested, or where a variant SARS-CoV-2 spike protein arises), where the same LNPs are used (that is, same lipid composition and mRNA–
lipid ratio, and where the total amount of mRNAs and LNPs per dose remain equal to or below that clinically tested) and where an approved manufacturing process is used, then, depending on NRA requirements, the nonclinical programme might be limited to an immunogenicity study (or studies) or a challenge-protection study (or studies) in a relevant animal model, if available. As much safety information as feasible should be collected during these immunogenicity or challenge-protection studies on the understanding that such nonclinical proof-of-concept studies are generally performed without full compliance to good laboratory practices (GLP). If safety information on veterinary vaccines expressing related antigens is available, this might also be useful and should be provided. Any other information concerning the safety of the platform technology used should also be provided for NRA consideration, for example, prior toxicology and biodistribution study data.

- Where the LNPs have been tested clinically with an unrelated mRNA such that the target antigen is novel (that is, not related to another antigen that has been clinically tested), then the approach of limiting nonclinical studies to an immunogenicity or challenge-protection study might not be sufficient. The decision regarding what type of nonclinical safety/toxicology information should be required might be guided by consideration of what and how much is known about the natural disease in terms of its pathology. If the natural disease is associated with immunopathology due to cross-reactivity, molecular mimicry, autoimmunity, allergenicity or immunity-associated disease enhancement, then toxicology studies would likely be needed to ensure that the novel target antigen was not associated with these effects. It should be noted that it may not be possible to investigate autoimmunity in nonclinical studies (18). Where natural disease is not associated with immunopathology or where little is known about the natural disease, discussion with the NRA should be undertaken on how the nonclinical programme might be abbreviated.

- Finally, where the LNPs and the encoded target antigen (and hence the mRNA structure and sequence) are both novel, nonclinical evaluation may be more complex and more extensive studies may be required; thus, discussion with the NRA should also be undertaken and it may not be possible to significantly abbreviate the nonclinical programme. However, it may be possible to initiate clinical studies while some of the required nonclinical studies are being performed in parallel with (or slightly ahead of) clinical development.
Decisions on abbreviating the nonclinical programme should always take into account what is already known about related and previously tested products, particularly if based on the same platform technology. If clinical data from a related product(s) are available, these data are likely to be more meaningful for evaluating the safety of the candidate vaccine in humans than data from any given animal model or from an in vitro human model.

8. Clinical evaluation of mRNA vaccines

The clinical evaluation expectations for clinical trial authorization or marketing authorization will be driven by the disease against which the mRNA vaccine is being or has been developed and the vaccine mode-of-action (or mechanism-of-action). If an immune correlate of protection has been identified this may change the expectations compared to what might be expected in the absence of such a correlate. Clinical studies should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (25) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20). Post-marketing pharmacovigilance is also discussed in the latter guidelines. Furthermore, these same guidelines provide considerations in evaluating dosing regimens, clinical development plans, boosting, collection of safety data, designs for pivotal efficacy trials (including potential end-points), standardizing immunogenicity assays (including use of IS and reporting of data in IU) and immunobridging to infer efficacy (20). Considerations for studies during pregnancy are also discussed in these same guidelines.

Clinical trials should capture safety, immunogenicity and efficacy data, as expected for any other type of vaccine, but with particular consideration given to the potential concerns outlined below as these may be more relevant for mRNA vaccines than for other types of vaccines that might already be licensed and with which regulators might be more familiar.

8.1 Safety and immunogenicity evaluation

Sufficient data should be obtained from preliminary clinical studies to permit evaluation of the following safety and immunological aspects that may be particularly relevant to mRNA vaccines:

a. Adverse immune effects

Transient decreases in lymphocytes (Grades 1–3) a few days after vaccination were reported in the interim human clinical trial results of an mRNA COVID-19 vaccine, with lymphocytes returning to baseline within 6–8 days in all participants and with no associated clinical observations (81). Such transient drops have
been observed for other vaccines and have resulted in no significant deleterious effect on the immune response (82, 83). Because RNA induces type 1 interferons, which have been associated with the transient migration of lymphocytes into tissues, the phenomenon of any effect on lymphocyte counts in blood may need specific attention in preliminary clinical trials (69, 84–86). Nonetheless, because this phenomenon may be important for the immune response to the candidate vaccine, it may be important to observe whether changes in leukocyte counts and subsets are associated with any adverse clinical signs or symptoms. Thus, the monitoring of appropriate reactogenicity parameters in the immediate post-vaccination period is paramount. Further general guidance on safety evaluation is provided in section 7 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20).

b. Types and scope of immune responses
In addition to the type and scope of immunogenicity described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20), in studies in which immunogenicity is measured, additional facets of the safety and immunogenicity of mRNA vaccines may include:

- whether the mRNA candidate vaccine biases towards certain types of immune responses, depending on what is known about the natural disease and the vaccine mode-of-action. To date, two clinical studies of COVID-19 mRNA vaccines have noted a Th1-type bias (37, 43). This information may be useful for predicting and understanding the impact of the immune responses for a particular disease.

- as with any new vaccine, any instances or evidence of AESI as defined in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20) or of any other novel adverse event should be captured in clinical trials and in post-marketing evaluation. If so, then investigations should be conducted into associations and potential causes, such as whether unwanted immune responses against vaccine components (such as RNA or lipids) are generated or, if pre-existing in the vaccine recipient, are increased or exacerbated. Alternatively, epitope mimicry due to the responses to the expressed antigen(s) may need to be investigated.

Consideration should also be given to the total dose of mRNA (especially if the vaccine is a multivalent or combination vaccine, or an sa-mRNA vaccine
where separate mRNAs are used) and to the total dose of LNPs with regard to the maximally tolerable dose determined during the development of an mRNA vaccine. For platform technologies, a maximally tolerable dose for a given population may be suggested by the dose previously determined for vaccines (or candidate vaccines) produced using that platform.

If boosting following a primary dose or series is being considered due to waning effectiveness, careful evaluation of any increased frequency or severity of local or systemic reactions should be performed. As with all vaccines, it is recommended that a careful exploration of dose, timing and number of immunizations (primary series and boosters if needed), and kinetics and durability of immune responses be performed in preliminary clinical trials to guide the design of the efficacy trial(s). Discussion of these issues can be found in section 5.5 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20). In certain situations, a determination that booster doses are needed might not be made until post-marketing data have been collected (for example, indicating waning immunity or protection). The above Guidelines (20) also discuss the boosting of licensed vaccines in section 5.6.1.2.3, which addresses the situation in which an alternative posology to the licensed product may need to be developed for the booster. Waning protection and the necessity for booster doses is then discussed in section 6.3.8. (20). Differences between the vaccine and circulating strains, including the potential need to add or replace strains, are briefly discussed in section 5.3.3 and other sections that discuss influenza vaccines, for which strain changes are frequently made.

It should be noted that during clinical trials or widespread use of COVID-19 mRNA vaccines, immunologically relevant adverse events of particular note (such as anaphylaxis or anaphylactoid reactions) have been observed (87, 88). Anaphylaxis is known to occur very rarely with all vaccines and is not unique to mRNA vaccines. It is not yet known what aspect of the formulation is associated with immunological adverse events and it is advised, as with other vaccines, that individuals with known allergies to specific vaccine components should not be vaccinated with vaccines containing such components (89–92). Myocarditis and pericarditis have also been observed during COVID-19 mRNA vaccine pharmacovigilance and appear to be associated – though the biological mechanism and associated vaccine component have not yet been identified (93, 94). It should further be noted that recent publications by several regulatory authorities provide useful relevant information, including publications by the European Medicines Agency (71, 95), the Medicines and Healthcare products Regulatory Agency (89, 96) and the US Food and Drug Administration (92, 97).

In line with the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20), the establishment and implementation of active pharmacovigilance plans are recommended. In the specific case of COVID-19
or other vaccines deployed in the context of a public health emergency, consideration should also be given to running a public awareness campaign on potential adverse events. As with any new vaccine, all adverse events potentially associated with COVID-19 vaccines are currently being further assessed as part of pharmacovigilance activities.

Given the short period for and limited scope of safety studies as part of the efficacy studies that have led to the current widespread use of COVID-19 mRNA vaccines, and the still unknown long-term safety impacts of mRNAs formulated with LNPs in large human populations, it will be important to continue monitoring and recording rare adverse events that have an unknown relationship with the use of such vaccines. Regulatory agencies should analyze such data for vaccines made by different manufacturers to provide a better clinical understanding and a more precise safety profile for mRNA vaccines in the current formulation designs. Furthermore, manufacturers and public health agencies should consider conducting post-introduction vaccine effectiveness studies, addressing questions of effectiveness among specific at-risk groups, the duration of protection, and effectiveness against both infection and transmission. As stated above, this is a rapidly evolving area and significant new data are emerging on an ongoing basis.

When international standards expressed in IU are available for standardizing the immune assays used in clinical evaluation of the vaccine, they should be used to calibrate internal standards or other working reference materials, and results should be reported in IU to improve the comparability of results across vaccines, across studies and across different assay platforms.

8.2 Efficacy evaluation

Efficacy evaluation will depend upon the disease against which the candidate vaccine is intended to protect, and the clinical indication determined in clinical trials. Factors that should be considered in the evaluation of vaccine efficacy are described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20).

It should be noted that in countries in which COVID-19 mRNA vaccines are currently being widely used, the use of placebo controls in trials requires special consideration. The ethical considerations regarding the conducting of ongoing COVID-19 vaccine trials with placebo controls were discussed in open public meetings held in December 2020 (98, 99). Trial design issues (including the selection of appropriate comparators) are discussed in the above WHO Guidelines (20). Further guidance is also provided in the outcome document of a WHO Expert consultation on the use of placebos in vaccine trials (100). As with all candidate vaccines, both the scientific merits and ethical considerations should inform the trial design and decisions must be made in the current benefit–risk context of the country in which regulatory authorization is being sought (101, 102). In addition,
WHO has now published more than 70 Guidelines and Recommendations for vaccines against specific diseases, any one or several of which may provide relevant guidance on the evaluation of any given mRNA vaccine (15).

8.3 **Efficacy evaluation in the context of a public health emergency in which immune-escape and other variants arise**

As discussed in section 5.6.2 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20) it may be feasible to consider immunobridging between the manufacturer’s original candidate vaccine (or subsequently marketed vaccine) and a variant candidate vaccine in order to infer efficacy of the variant mRNA candidate vaccine based on a manufacturer’s given platform technology in which clinical end-point efficacy has been demonstrated for the original candidate (or marketed) vaccine. The immunobridging may have to be supported by justification of how comparable antibody titres for the prototype and variant vaccines would translate into similar efficacy. Consideration must be given to the following scenarios: (a) the variant candidate vaccine will replace the original candidate vaccine; or (b) the variant and original candidate vaccines will be combined (that is, in a bivalent or multivalent vaccine) or administered simultaneously or in sequence. Collection of comparative safety data during such immunobridging studies will also be expected. Overall, the considerations for immunobridging studies may depend upon factors such as the disease, pathogen and induced immune response(s) – trial designs and data requirements should thus be decided on an individual case-by-case basis.

In the specific case of COVID-19 vaccines, consideration may be given to the guidance provided by WHO (103, 104), the European Medicines Agency (71, 95), the Medicines and Healthcare products Regulatory Agency (89, 96), the US Food and Drug Administration (92, 97) and other regulatory authorities (105–107).

Currently, mRNA vaccines against influenza viruses are in development and any proposed strain changes may have to take into consideration current practices for inactivated or live attenuated influenza virus vaccines. The WHO recommendations to assure the quality, safety, and efficacy of influenza vaccines (human, live attenuated) for intranasal administration (108) and WHO Recommendations for the production and control of influenza vaccine (inactivated) (109) should be consulted.

**Authors and acknowledgements**

The preliminary draft of this WHO document was prepared by Dr M.A. Liu, consultant, ProTherImmune, the USA; and Dr H-N. Kang, World Health Organization, Switzerland. This preliminary draft was then subject to expert working group review during the period 1–15 October 2020 and comments
received from: Dr. E. Grabski and Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr. K. Karikó, Dr A. Kuhn, Dr U. Blaschke, Dr C. Blume and Dr J. Diekmann, BioNTech, Germany; Dr D. Kaslow, PATH Vaccine Development Global Program, the USA; Professor S. Lu, University of Massachusetts Medical School, the USA; Professor E.E. Ooi, Duke-NUS Medical School, Singapore; Dr K. Peden, United States Food and Drug Administration, the USA; Professor P. Roy, London School of Hygiene & Tropical Medicine, the United Kingdom; Dr S. Sankarankutty, Health Sciences Authority, Singapore; Dr J. Ulmer, GSK (formerly); Dr F. Verdier, Sanofi Pasteur, France; Dr M. Watson and Dr C. Vinals, Moderna, the USA; and Dr T.Q. Zhou, World Health Organization, Switzerland.

The first draft was then prepared by a WHO drafting group comprising Dr R. Sheets, consultant, the USA; Dr M.A. Liu, consultant, ProTherImmune, the USA; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr K. Peden, United States Food and Drug Administration, the USA; Dr S. Sankarankutty, Health Sciences Authority, Singapore; and Dr T.Q. Zhou, World Health Organization, Switzerland. The resulting draft document was then posted on the WHO Biologicals website during December 2020 and January 2021 for a first round of public consultation. Comments were received from: Dr S. Acharya and Dr F. Atouf, US Pharmacopeia, the USA; Dr A. Adisa, Therapeutic Goods Administration, Australia; Dr L. Bisset, Dr A. Cook, Dr V. Ganeva and Dr K-W. Wan, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr R.M. Bretas, Agência Nacional de Vigilância Sanitária, Brazil; Dr G. Cirefice and Dr C. Milne, European Directorate for the Quality of Medicines & HealthCare, France; Dr I. Feavers, consultant, the United Kingdom; Dr D. Feikin, IVB/WHO (on behalf of the Covid-19 Vaccine Effectiveness working group); Dr G. Frank, Biotechnology Innovation Organization, the USA; Dr E. Griffiths, consultant, the United Kingdom; Dr R.A. Hafiz, Saudi Food & Drug Authority, Saudi Arabia; Dr W. Jaroenkunthum, Department of Medical Sciences, Thailand; Dr K. Karikó, Dr A. Kuhn, Dr U. Blaschke, Dr C. Blume, Dr C. Lindemann and Dr J. Diekmann, BioNTech, Germany; Dr D.C. Kaslow, PATH Vaccine Development Global Program, the USA; Dr M. Kucuku, National Agency for Medicines and Medical Devices, Albania; Dr U. Loizides and Dr R.G. Balocco, International Nonproprietary Names Programme and Classification of Medical Products, World Health Organization, Switzerland; Professor S. Lu, University of Massachusetts Medical School, the USA; Dr I. Mahmood Al-Sabri, Ministry of Health, Oman; Professor S.F. Malan, University of the Western Cape, South Africa; Dr J. Maslow, GeneOne Life Science Inc., the USA; Dr S. M. Morales Sánchez, National Food and Drug Surveillance Institute, Colombia; Professor E.E. Ooi, Duke-NUS Medical School, Singapore; Dr C. Pohl, Schlosspark-Klinik, Germany; Dr I. Prawahju, National Agency for Drug and Food Control, Indonesia; Dr R. Rabe, Norwegian Medicines Agency, Norway; Professor M.
Rizzi, University of Piemonte Orientale, Italy; Dr J.S. Robertson, consultant, the United Kingdom; Dr N. Rose and Dr S. Schepelmann, National Institute for Biological Standards and Control, the United Kingdom; Professor P. Roy, London School of Medicine & Tropical Hygiene, the United Kingdom; Dr M. Savkina, Federal State Budgetary Institution Scientific Centre for Expert Evaluation of Medicinal Products, Russian Federation; Dr L. Tesolin, Dr K. Brusselmans and Dr W. van Molle, Sciensano, Belgium; Dr J. Ulmer, independent consultant, the USA; Dr L. Viviani, Humane Society International, Italy; Dr A.L. Waddell, WHO temporary advisor, the United Kingdom; Dr M. Li and Dr W. Wei, Center for Drug Evaluation, National Medical Products Administration, China; Dr K. Weisser, Paul-Ehrlich-Institut, Germany; and Dr G. Zenhäusern, Swiss Agency for Therapeutic Products, Switzerland. Dr P. Barbosa, International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) provided the consolidated comments of subject matter experts from: GSK Vaccines, Belgium; Pfizer Vaccines, the USA; and Sanofi Pasteur, France.

Taking into consideration the comments received, the second draft document was prepared by Dr R. Sheets, consultant, the USA; Dr M.A. Liu, consultant, ProTherImmune, the USA; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr K. Peden, United States Food and Drug Administration, the USA; Dr S. Sankarankutty, Health Sciences Authority, Singapore; Dr K-W. Wan, Medicines and Healthcare products Regulatory Agency, the United Kingdom; and Dr T.Q. Zhou, World Health Organization, Switzerland. The second draft was then reviewed at a WHO informal consultation on regulatory considerations for evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases, held virtually on 20–22 April 2021 and attended by: Dr I.G. Al Gayadh and Dr R.A. Hafiz, Saudi Food & Drug Authority, Saudi Arabia; Dr P. Aprea, Administración Nacional de Medicamentos, Alimentos y Tecnología Medica, Argentina; Dr M. Ayiro, Pharmacy and Poisons Board, Kenya; Dr C. Bae, Ministry of Food and Drug Safety, Republic of Korea; Dr K. Bloom, University of the Witwatersrand and South African Medical Research Council, South Africa; Dr K. Bok and Dr B.S. Graham, National Institutes of Health, the USA; Dr R. Bose, Central Drugs Standard Control Organization, India; Dr J. Fernandes, Health Canada, Canada; Dr E. Grabski and Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr S. Alireza Hosseini, Food and Drug Administration, the Islamic Republic of Iran; Dr N. de Jesus Huertas Mendez, Instituto Nacional de Vigilancia de Medicamentos y Alimentos, Colombia; Mrs T. Jivapaisarnpong, King Mongkut’s University of Technology Thonburi, Thailand; Professor F. Krammer, Icahn School of Medicine at Mount Sinai, the USA; Dr M.A. Liu, consultant, ProTherImmune, the USA; Dr J. Lu, National Medical Products Administration, China; Professor S. Lu, University of Massachusetts Medical School, the USA; Dr A. Marti and Dr T. Schochat, Swiss Agency for Therapeutic Products,
Switzerland; Dr T. Matano, National Institute of Infectious Diseases, Japan; Dr C. Milne, European Directorate for the Quality of Medicines & HealthCare, France; Dr P. Minor, consultant, the United Kingdom; Dr W. van Molle and Dr L. Tesolin, Sciensano, Belgium; Professor E.E. Ooi, Duke-NUS Medical School, Singapore; Dr M. Page, Dr N. Rose and Dr S. Schepelmann, National Institute for Biological Standards and Control, the United Kingdom; Dr K. Peden, United States Food and Drug Administration, the USA; Dr S. Pumiamorn, Ministry of Public Health, Thailand; Dr J.S. Robertson, independent expert (representative of the International Nonproprietary Names Programme), the United Kingdom; Professor P. Roy, London School of Hygiene & Tropical Medicine, the United Kingdom; Professor K. Ruxrungtham, Chulalongkorn University, Thailand; Dr S. Sankarankuttty, Health Sciences Authority, Singapore; Dr Y. Sheler, Central Drugs Standard Control Organization (HQ), India; Dr R. Sheets, consultant, the USA; Dr R. Shivji, European Medicines Agency, Netherlands; Ms G.S. Silveira, Agência Nacional de Vigilância Sanitária, Brazil; Dr V.G. Somani, Central Drugs Standard Control Organization, India; Dr K-W. Wan, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr J. Wang and Dr Y. Wang, National Institutes for Food and Drug Control, China; Dr W. Wei, Center for Drug Evaluation, National Medical Products Administration, China; and Professor D. Weissman, Penn Center for AIDS Research, the USA. 

Representatives of other organizations: Dr S.K. Acharya, United States Pharmacopeia, the USA; Dr N. Jackson, Coalition for Epidemic Preparedness Innovations, the United Kingdom; Dr D. Kaslow, PATH Vaccine Development Global Program, the USA; and Dr C. Vinals, the Biotechnology Innovation Organization, the USA. 

Representatives of the Developing Countries Vaccine Manufacturers Network (DCVMN): Dr M.M. Ahasan, Incepta Vaccine, Bangladesh; Dr D.T. Dat, Vabiotech, Viet Nam; Dr C. Roy, Bharat Biotech, India; Dr W. Wijagkanalan, BioNet-Asia Co., Ltd, Thailand; and Mr A. Wong, Walvax Biotechnology, China. 

Representatives of the IFPMA: Dr D. Boyce, Pfizer, Ambler, PA, the USA; Dr S. Chiyukula and Dr S. Simons, Sanofi, Cambridge, MA, the USA; Dr R. Forrat, Sanofi, Lyons, France; Dr S. Gregory and Dr G. Maruggi, GSK, Rockville, MD, the USA; Dr S. Lockhart, Pfizer, New York, NY, the USA; and Dr F. Takeshita, Daiichi Sankyo, Japan. 

Individual manufacturers/other industries: Dr T. Class, Translate Bio, the USA; Dr S. Gould, Charles River Laboratories, Lyons, France; Dr A. Kuhn (20 and 21 April), Dr R. Rizzi (20 and 21 April), Dr C. Lindemann (22 April) and Dr E. Lagkadinou (22 April), BioNTech SE, Germany; Dr K. Lindert, Arcturus Therapeutics, the USA; Dr J. Maslow, GeneOne Life Science Inc., the USA; Dr J.M. Miller and Dr D. Parsons, Moderna, Inc., the USA ; Dr F. Neske and Dr L. Oostvogels, CureVac AG, Germany; Mr Y. Park, GeneOne Life Science Inc., Republic of Korea; and Dr B. Ying, Suzhou Abogen Biosciences Co., Ltd. P.R., China. 

WHO staff: Dr C. Ondari, Dr I. Knezevic, Dr T.Q. Zhou, Dr M. Friede, Dr B. Giersing, Ms
E. Sparrow, Dr U. Loizides, Dr R.G. Balocco and Dr D. Wood, World Health Organization, Switzerland; and Dr S. Escalante and Dr J. Shin, WHO Regional Office for the Western Pacific, Philippines.

Based on the outcomes of the above informal consultation, the document WHO/BS/2021.2402 was prepared by Dr R. Sheets, consultant, the USA; Dr M.A. Liu, consultant, ProTherImmune, the USA; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr K. Peden, United States Food and Drug Administration, the USA; Dr S. Sankarankutty, Health Sciences Authority, Singapore; Dr K-W. Wan, Medicines and Healthcare products Regulatory Agency, the United Kingdom; and Dr T.Q. Zhou, World Health Organization, Switzerland. Document WHO/BS/2021.2402 was then posted on the WHO Biologicals website for a second round of public consultation from July to September 2021 and written comments received from: Dr S. Acharya and Dr F. Atouf, United States Pharmacopeia, the USA; Dr L. Bisset, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr E. Cocuzzi, Health Canada, Canada; Dr T. Class, Translate Bio, the USA; Dr S. Dubey, Dr J. Nussbaum, Dr L. Plitnick, Dr J. Prescott, Dr J. Wei and Dr Y. Zhang, Merck Vaccines, the USA; Dr M. Li and Dr S. Jin, Center for Drug Evaluation, National Medical Products Administration, China; Dr S. Gould, Charles River Laboratories, France; Dr Z. Guo, Chinese Pharmacopoeia Commission, China; Dr A. Gudjonsson, Dr G. Abrahamsen and Dr T.K. Andersen, Norwegian Medicines Agency, Norway; Dr F. Neske, CureVac, Germany; A.A. Zuniga, A.M. Santana Puentes, Instituto Nacional de Vigilancia de Medicamentos y Alimentos, Colombia; Dr S. Muchakayala, Touchlight Genetics Ltd, the United Kingdom; Dr S. Wendel, Hospital Sírio-Libanès Blood Bank, Brazil; Dr G. Sanyal, Dr P-A. Gilbert and Dr D. Robinson, Bill & Melinda Gates Foundation, the USA; Dr J. Wang, National Institutes for Food and Drug Control, China; Dr I. Zadezensky, Moderna, the USA; and A. Kuhn, U. Blaschke, K. Karikó, D. Theisen, B. Vallazza, C. Lindemann and E. Lagkadinou, BioNTech SE, Germany. Dr P. Barbosa, IFPMA, provided the consolidated comments of subject matter industry experts.

Further changes were made to document WHO/BS/2021.2402 by the WHO Expert Committee on Biological Standardization.

References


Annex 3


3 October 2021).


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 diagnosis. 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. 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: https://www.who.int/health-topics/Biologicals#tab=tab_1.

At its meetings held via video conference on 18–22 October 2021, 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

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<tr>
<th>Material</th>
<th>Unitage</th>
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<tr>
<td><strong>Biotherapeutics other than blood products</strong></td>
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<td>Follicle-stimulating hormone (human, recombinant) for bioassay</td>
<td>137 IU/ampoule</td>
<td>Third WHO International Standard</td>
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<tr>
<td><strong>Blood products and related substances</strong></td>
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</tr>
<tr>
<td>Ferritin (human, recombinant)</td>
<td>10.5 μg/ampoule Expanded uncertainty limits = 10.2–10.8 μg/ampoule (95% confidence; k = 2.23)</td>
<td>Fourth WHO International Standard</td>
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<tr>
<td><strong>In vitro diagnostics</strong></td>
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</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em> (H37Rv) DNA for NAT-based assays</td>
<td>$6.3 \log_{10}$ IU/vial</td>
<td>First WHO International Standard</td>
</tr>
<tr>
<td>Varicella zoster virus DNA for NAT-based assays</td>
<td>$7.0 \log_{10}$ IU/vial</td>
<td>First WHO International Standard</td>
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<tr>
<td>Anti-Lassa virus immunoglobulin G</td>
<td>25 IU/ampoule for neutralizing antibody, 250 IU/ampoule for anti-GP binding IgG, 250 IU/ampoule for anti-NP binding IgG</td>
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<tr>
<td>Anti-Lassa virus immunoglobulin G</td>
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<td>First WHO International Reference Panel</td>
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<tr>
<td>Anti-thyroid peroxidase antibodies</td>
<td>555 IU/ampoule</td>
<td>First WHO International Standard</td>
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8 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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<tr>
<th>Material</th>
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<td><strong>Vaccines and related substances</strong></td>
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<td></td>
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<tr>
<td>Diphtheria antitoxin (equine)</td>
<td>57 IU/ampoule</td>
<td>Second WHO International Standard</td>
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SELECTED WHO PUBLICATIONS OF RELATED INTEREST

WHO Expert Committee on Biological Standardization
WHO Technical Report Series, No. 1030, 2021 (xvii + 269 pages)

WHO Expert Committee on Biological Standardization
Seventy-first report.
WHO Technical Report Series, 1028, 2021 (xii + 102 pages)

WHO Expert Committee on Biological Standardization
Seventieth report.
WHO Technical Report Series, No. 1024, 2020 (xvi + 227 pages)

WHO Expert Committee on Biological Standardization
Sixty-ninth report.
WHO Technical Report Series, No. 1016, 2019 (xv + 251 pages)

WHO Expert Committee on Biological Standardization
Sixty-eighth report.
WHO Technical Report Series, No. 1011, 2018 (xvi + 380 pages)

WHO Expert Committee on Biological Standardization
Sixty-seventh report.

WHO Expert Committee on Biological Standardization
Sixty-sixth report.
WHO Technical Report Series, No. 999, 2016 (xix + 267 pages)

WHO Expert Committee on Biological Standardization
Sixty-fifth report.
WHO Technical Report Series, No. 993, 2015 (xvi + 262 pages)

WHO Expert Committee on Biological Standardization
Sixty-fourth report.
WHO Technical Report Series, No. 987, 2014 (xviii + 266 pages)

Website: https://www.who.int/health-topics/Biologicals#tab=tab_1

To purchase WHO publications, please contact:
WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland;
email: bookorders@who.int; order online: http://apps.who.int/bookorders
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 brought to the attention of the Committee at its meetings held via video conference in October 2021. 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, the following two documents were adopted on the recommendation of the Committee: (a) Amendment to the WHO Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines; and (b) Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations.

Subsequent sections of the report provide information on the current status, proposed development and establishment of international reference materials in the areas of: biotherapeutics other than blood products; 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 two WHO documents adopted on the advice of the Committee are then presented as part of this report (Annexes 2 and 3). Finally, all new and replacement WHO international reference standards for biological products established during the October 2021 meeting are summarized in Annex 4. The updated full catalogue of WHO international reference standards is available at: https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/catalogue.